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# Factors Affecting Lipid Oxidation In Full-fat Soy Flour

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**Abstract**

Corsoy 79 soybeans were ground into 8-(coarse) and 24-mesh (fine) full-fat soy flours. From the particle size analysis, the 8-mesh full-fat soy flours were found to have larger values for geometric mean diameter and geometric standard deviation. However, the distribution moduli of coarse and fine soy flours were similar and indicated soybeans were nearly "brittle". Development of hydrolytic and oxidative rancidities of coarsely and finely ground full-fat soy flours were followed from grinding to 24 hrs later. No increases in peroxide value and conjugated dienes in the oil and hexanal content in the headspace of the flour were observed when the moisture was 10.7% or less. At 14.9% moisture and above, lipid oxidation increased with increased moisture content and storage time. Free fatty acid contents increased slightly at all moisture contents. However, hydrolysis did not exceed 0.06% over the moisture range of 4 to 18%, which is of little practical significance. Fine grinding increased oxidative and hydrolytic rancidities, especially at 14.9% moisture and above. these findings indicate that raw soybeans can be ground to full-fat soy flours and stored up to 24 hrs without undergoing significant lipid and flavor deterioration if the moisture content is 11% or less.

Key words: full-fat soy flour, lipid deterioration, moisture content

## Introduction

Soy flours are used for functional properties, as well as nutritional[1]. Full-fat soy flur is often used for enrichment of bread[2] and in baby foods, low calorie foods, meats, beverages, soups and sauces. In such products, full-fat soy flours partially replace more expensive and scarce ingredients, such as egg, milk and meat[3].

Lo[4] used full-fat soy flours to prepare a soy beverage. The soy flour was suspended in water to provide a beverage having substantially the same consistency as bovine milk. Another procedure to utilize full-fat soy flours to prepare a soy beverage was developed by Mustakas *et al.*[5]. Recently, Johnson *et al.*[6] developed a new method for processing aqueous extracts from soybeans, which used steam infusion cooking. This process has become known as rapid-hydration hydrothermal cooking (RHHTC). This process is unique in that it involved a simple method to produce a soybean beverage from full-fat soy flours with very high yield and minimum off-flavor.

Crushing or macerating raw soybean tissue triggers lipolysis through release of lipolytic enzymes such as lipase[7] and lipoxygenase[8] and mixing them with substrate[9]. Upon hydration, oxygen can diffuse into the tissue, enzyme and substrate may gain mobility, and oxidation occurs[10]. Both lipase and lipoxygenase are active at very low relative humidities (10 and 15%, respectively)[7,8]. These enzymes are responsible for off-flavor problems which hinder consumer acceptability of soybean products and the marketability of soy foods. Furthermore, Wolf[12] indicated that the significance of lipoxygenase action is less certain when soybeans are processed under low moisture conditions as in the commercial extraction of oil. Mustakas *et al.*[13] reported that inactivation of lipoxygenase was a key step in the preparation of good-flavored full-fat soy flours. In addition to flavor considerations, lipid hydroperoxides can lead to loss of color and taste of soy protein products[14].

The concentration of hexanal and other fatty acid oxidation products in the headspace vapors of soy flours and crude oils can be readily and quantitatively determined by gas liquid chromatography and, thus, used to evaluate the quality of soybeans[15,16]. Wilkens and Lin[17] observed some 80 volatile com-

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pounds contributing to the flavor of soymilk, but hexanal predominated accounting for 25% of the total peak area. Hexanal and other volatile carbonyls collectively impart a grassy, beany flavor to soymilk. Other authors have used hexanal as an indicator of rancidity in soy foods and soybean oil.[15,18] Hexanal content was shown to closely follow the development of off-flavor[19], and to be a simple and rapid yet effective analytical tool for measuring oxidative deterioration[20].

The objective of this study was to determine how quickly full-fat soy flours must be processed. Moisture content and particle size were expected to affect the extent of hydrolytic and oxidative rancidity of full-fat soy flours during shoart-term storage.

## Materials and Methods

### Selection of soybeans

Corsoy 79 soybeans grown at Ames, IA during 1984 were used in this study. Protein, moisture, crude oil and ash content were determined by AACC standard procedures 46-11, 44-15A, 30-25 and 08-01, respectively[21].

### Adjustment of moisture content

Two 4-kg samples of soybeans were adjusted to the desired level of moisture by mixing calculated amounts of distilled water and soybeans in air-tight bags, and allowing several days for the absorbed moisture to equilibrate within the beans at 5-6℃. Also, 8-kg samples of soybeans were dried to various moisture contents in an open-top drum drier equipped with a blower motor and a temperature controller. The drying temperature was less than 41℃[22]. Samples were ground and moisture content was determined by the vacuum oven method[21].

### Grinding and storage of soy flour

Soybeans were ground through 8- and 24-mesh screens using a Fitzmill (Model D, Fitzpatrlck Co., Elmhurst, IL). The flour (300 g) was immediately placed in 1-quart Mason jars, sealed and stored at 25℃ for 5 sec and 6, 12 and 24 hrs until analyzed. The Mason jars were about two-thirds full and oxygen should not be limiting.

### Particle Size Analysis

The U.S. standard sieve series were used for sizing. Particle size distributions were determined by using methods of the ASAE[23]. The size of particles for each sample was reported in terms of geometric mean diameter (dgw) and geometric standard deviation (sgw) by weight. From a log-log plot of "percent finer than" against screen size for each sample, product size modulus (k) and distribution modulus (a) were calculated[24].

### Crude oil extraction

Samples of full-fat soy flours (300 g each) were transferred to 1-L brown-colored separatory funnels with a wide-opened top. The separatory funnel was loosely plugged with glass wool at the exit. Chloroform-methanol (2 : 1 v/v) was used to extract free and bound fat. Crude oils were stored in glass vials flushed with $N_2$ gas at 5℃ and lipid analysis was conducted as quickly as possible after extraction.

### Measurement of lipid oxidation

The free fatty acid (FFA) contents of crude oils were determined using AOCS method Ca5a-40[25]. The sample size was 28.2 g of crude oil. Peroxide value (PV) and conjugated dienoic acid (CD) were also determined by AOCS official methods Cd8-53 and Ti la-64, respectively[25]. To calculate percent conjugated dienoic acid , a $k_o$ value of 0.07 was chosen.

### static headspace capillary gas chromatography of soy flours

Headspace volatiles, especially hexanal concentration, were determined in soy flour samples. A 100 *ml* bottle was filled with 50 *ml* of boiling distilled water and placed into a boiling water bath. A 6 g sample of soy flour was suspended and 1 *ml* of daily fresh internal standard, 4-heptanone (Aldrich Chemical Company, Inc., Milwaukee, WS) was added. The concentration of 4-heptanone was 25 ppm. The bottle was sealed with a septum secured by an aluminum cap. After 30 min passed in a boiling water bath, the bottle was placed in a 5℃ refrigerator until analyzed. Before injection, the bottles were warmed to 37℃ and equilibrated for 3 hrs using a shaking water bath (Model 127, Fisher Scientific, Springfield, NJ). A 1 *ml* sample of the headspace was introduced into a Varian Model 3700 gas chromatograph (varian Associates, Inc., Walnut Creek, CA) equipped with a Durabond DB-5 fused silica capillary column, 30 m×0.32 mm, 1 micron film thickness (J & W Scientific, Rancho Cordova, CA) in a split mode (20:1). The column temperature was programmed at the rate of 5℃/min from 40℃ (zero hold time) to 125℃ (5 min hold time). A hydrogen flow rate of 30 ml/min, nitrogen flow rate of 30 ml/min (column: 1.5 ml/min; makeup: 28.5 ml/min) and air flow rate of 300 ml/min were used. A 10 ml gas tight syringe (Hamilton Company, Reno, NV) was used and cleaned at a syringe cleaner (Hamilton Company, Reno, NV) after each run. The results were expressed as the peak area ratio of hexanal to 4-heptanone.

### Statistical analysis

Data were analyzed by using a Statistical Analysis System[26] program package. The General Linear Models (GLM) procedure was used to determine the main and interaction effects. Significant differences among treatment means were determined by Duncan's multiple range test or the least significant difference (LSD) procedure. Probability levels of $p \leq 0.05$ were considered.

## Results and Discussion

### Particle size analysis

From the log-probability plot of cumulative percent by weight against particle size for full-fat soy flours ground through 8- and 24-mesh screens, the plots were relatively straight lines indicating that the distributions were evenly spread throughout the sieves. It was of interest to note that the distribution of full-fat soy flours ground through the 8-mesh screen consisted of coarser particles with a wider range of sizes than soybeans ground through the 24-mesh screen.

Two important distribution parameters, the geometric mean diameter (dgw) and the geometric standard deviation (sgw), were obtained from each flour listed in Table 1. The dgw of flours ground through the 8-mesh screen (609.8 micron) was larger than that of flours ground through the 24-mesh screen (293.7 micron). The sgw is a measure of the dispersion of particle sizes relative to the dgw. The sgw of flours ground through the 8-mesh screen was larger than that of flours ground through the 24-mesh screen (2.05 versus 1.44). Therefore, the soy flours ground though the 8-mesh screen had a wider distribution of particle sizes and were less uniform in size.

Another important distribution parameter, distribution modulus, was obtained from the log-log plot of cumulative weight versus particle size. The distribution modulus should be constant for a product ground through different screen sizes. Therefore, the distribution modulus has the same value whether it refers to breakage of a single particle or to the entire product. Hansen and Stewart[27] stated that the distribution modulus of 1.00 indicates complete "brittleness", Table 1 showed the distribution moduli had 1.24 for flours ground through the 8-mesh screen and 1.22 for flours ground through the 24-mesh screen. Therefore, under the conditions of this experiment soybeans approached complete "brittleness".

### Determination of lipid oxidation

Corsoy 79 soybeans whose original moisture content was 10.7% were treated to adjust their moisture content by tempering or drying and then ground into the flours with two different particle size distributions. These flours had 4.0, 6.8, 7.6, 10.7, 14.9 and 17.5% moisture.

Free fatty acid contents were determined in soy flour stored up to 24 hrs at different moisture levels (Figure 1). In general, lipase activity increased with increased moisture content and storage time, but at all moisture contents hydrolytic activity was low and of little practical significane. The hydrolysis of triglycerides in full-fat soy flours did not exceed 0.06% free fatt acid over the moisture range of 4.0 to 17.5% during the 24-hr storage period. This amount of hydrolysis in not organoleptically significant. Fritsch[28] indicated that at least 2% free fatty acid level in soybean oil is necessary for an adverse effect upon the odor or the flavor of foods.

**Table 1. Particle size distribution parameters of full-fat soy flours**

| Screen mesh size | Geometric mean diameter dgw (micron) | Geometric standard deviation sgw (micron) | Distribution modulus a | Size modulus k (micron) |
|---|---|---|---|---|
| 8 | 609.8 | 2.05 | 1.24 | 1,300 |
| 24 | 293.7 | 1.44 | 1.22 | 535 |

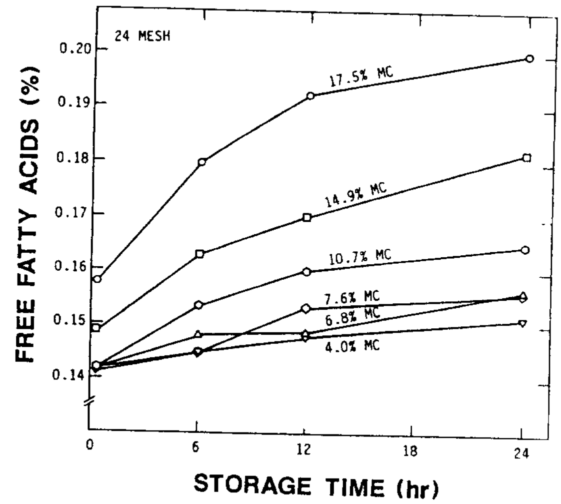


**Fig. 1. Effect of moisture content (MC) on hydrolytic rancidity of oil in full-fat soy flours during short-term storage (LSD was 0.02)**

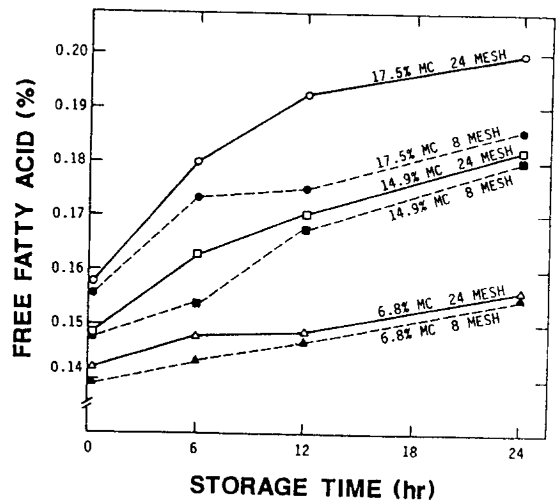


**Fig. 2. Effect of particle size in hydrolytic rancidity of oil in full-fat soy flours at different moisture contents (MC) during short-term storage (LSD was 0.02)**

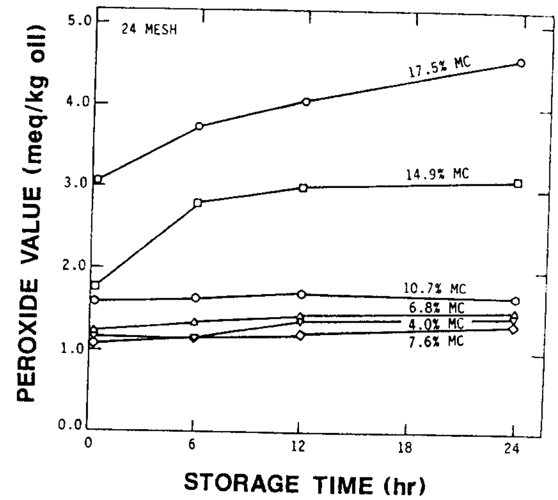


**Fig. 3. Effect of moisture content (MC) on peroxide value of oil in full-fat soy flours during short-term storage (LSD was 0.34)**

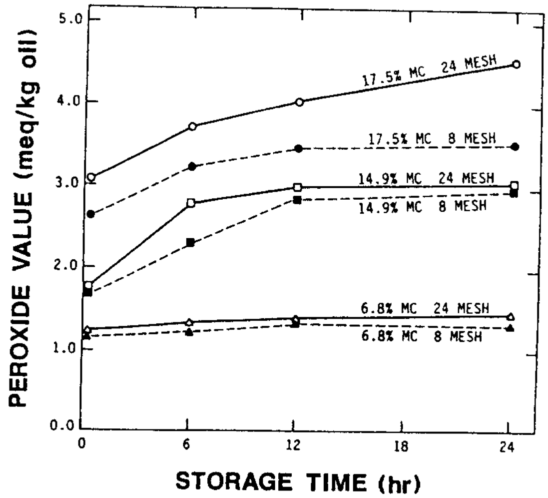


**Fig. 4. Effect of particle size on peroxide value of oil in full-fat soy flours at different moisture contents (MC) during short-term storage (LSD was 0.43)**

Figure 2 illustrates the effect of particle size on free fatty acid contents of crude oils in the 8- and 24-mesh full-fat soy flours. Grinding to small particle sizes slightly increased hydrolytic rancidity. Fine grinding increased the rate of hydrolysis more at higher moisture contents than at lower moisture contents. Fine grinding destroys the natural compartmentalization of cells. Oil is protected in spherosomes from lipolytic enzymes in the cytoplasm. Fine grinding more extremely breaks these structures down and provides more mixing of enzymes with substrates.

## Enzyme-catalyzed oxidation

The primary product of lipid oxidation, hydroperoxides, was determined in the 8- and 24-mesh full-fat soy flours stored for 24 hrs at different moisture levels (Figures 3 and 4). Peroxide values increased with storage time especially at 14.9% moisture and higher. However, oxidative rancidity, as indicated by peroxide values, was not practically significant over

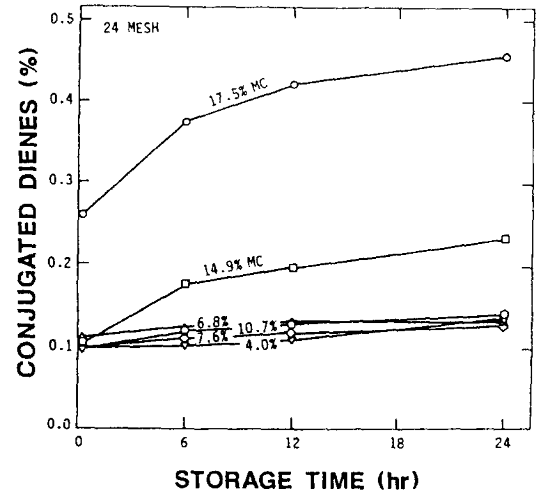


**Fig. 5. Effect of moisture content (MC) on conjugated dienes of oil in full-fat soy flours during short-term storage (LSD was 0.02)**

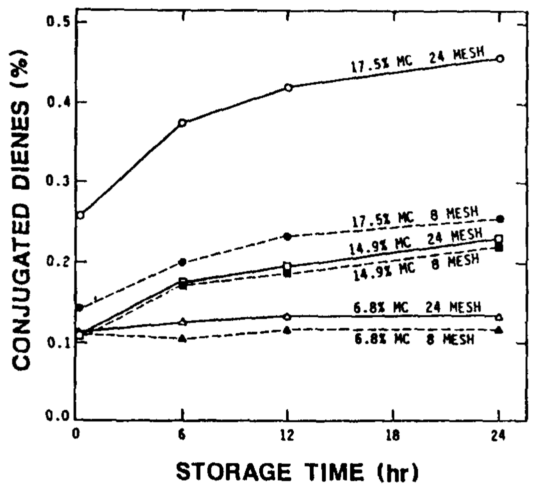


**Fig. 6. Effect of particle size on conjugated dienes of oil in full-fat soy flours at different moisture contents (MC) (LSD was 0.02)**

the 24-hr storage time at moisture contents of 10.7% or less. Figure 4 shows that fine grinding increased oxidative rancidity. This effect was more statistically significant at the 5% level over the 24-hr storage time at the moisture content of 17.5%. The results (Figures 3 and 4) indicate full-fat soy flours with 10.7% moisture content or less can be stored for at least 24 hrs without incurring significant increases in hydroperoxides.

Additional evidence of oxidative changes during

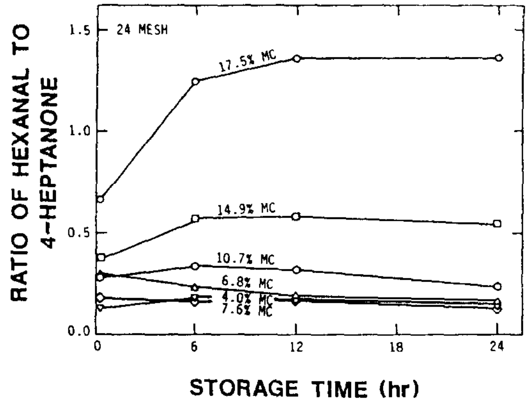


**Fig. 7. Effect of moisture content (MC) on hexanal content of full-fat soy flours during short-term storage (LSD was 0.19)**

storage of full-fat soy flours with different moisture contents was obtained by measuring diene conjugation in extracted oil. Compared to the peroxide value, the conjugated diene hydroperoxide method was faster, was simpler, required no chemical reagents, did not depend upon a chemical reaction for color development and was conducted on smaller samples. As shwon in Figure 5, conjugated dienes increased initially with increased storage time and moisture. The production of conjugated dienes was pronounced at 14.9% moisture or higher. Conjugated diene contents changed little in soybeans with 10.7% moisture and less.

More conjugated dienes were observed in the 24-mesh flour than the 8-mesh flour. At high moisture content (17.5%), fine grinding greatly accelerated conjugated diene formation. However, at 14.9% moisture and less, the increased production of conjugated dienes due to fine grinding was small (Figure 6).

### Headspace analysi of volatiles

The hexanal peak, which was of interest, was identified on the basis of relative retention time to a hexanal standard. Heptanone was used as an internal standard, which eluted after the hexanal peak. Heptanone was found to be a reliable internal standard and no interactions with the volatiles from the homogenates were observed. The peak ratio of hexanal to heptanone was highly reproducible.

Hexanal contents in the headspace of soy flour slurries increased at higher flour moisture contents and with smaller partcle size. A drastic increase in hexanal contents was noted at 14.9 to 17.5% moisture

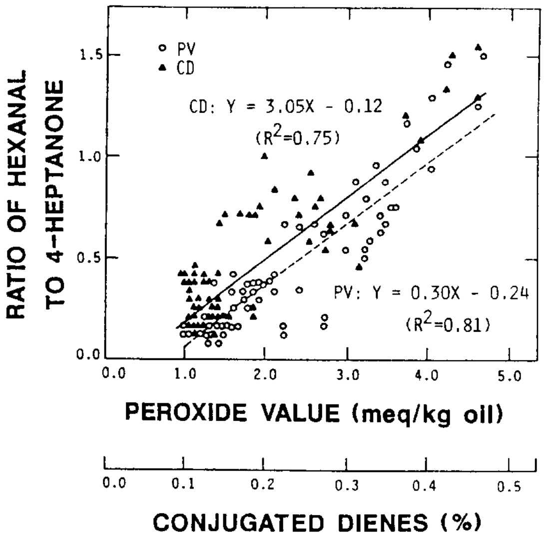


**Fig. 8. Relationship between hexanal content in full-fat soy flours to peroxide value and conjugated dienes in the oil**

and this increase was significant during 6 hrs of storage. However, hexanal content did not significantly increase during storage of soy flours at moisture contents of 10.7% and below. Therefore, raw full-fat soy flours can be stored at 10.7% moisture or less without significant lipid deterioration (Figure 7).

Soybeans possess high lipoxygenase activity and the enzyme is distributed throughout the cotyledons. In the dry bean, oxygen is apparently limiting and enzyme-substrate contact is limited by substrate immobility or compartmentalization and, thus, lipoxygenase is apparently inactive. However, upon hydration, oxygen can diffuse into the tissue, enzyme and substrate may gain mobility and oxidation occurs. When cell structures are disrupted during grinding, conditions can be ideal for oxidation. Finer particles provide more surface area to accelerate the reaction. However, based on the results of this study a critical moisture content of 11% must be exceeded for this mechanism to proceed.

### Relationship between peroxide value and conjugated diene content in extracted oil and headspace volatiles

Peroxide value and conjugated diene measurements were compared to gas chromatographic analysis of headspace volatiles from the aqueous dispersion of full-fat soy flours (Figure 8). Linear relationships between them were observed. Their regression coefficients were 0.75 and 0.81 for conjugated dienes and peroxide value, respectively.

Recently, gas chromatographic analysis of headspace volatile compounds has been widely used to evaluate the quality of soybeans[16] and soybean oils[15, 18]. They also concluded that headspace gas chromatographic analysis of volatiles provides a sensitive method to evaluate oxidative deterioration of beans and crude oils.

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## 전지 대부분의 유지산화에 미치는 인자

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Corsoy 79 대두를 8-와 24-mesh 입자 크기의 전지 대부분으로 분쇄하였다. 입자크기 분석으로 8 mesh 전지 대부분은 기하학적 평균 크기와 표준편차가 24 mesh 전지 대두분 보다 컸다. 분포계수는 두 전지 대두분 모두 동일하여서 거의 완벽하게 원하는 입자 크기로 분쇄됨을 알 수 있었다. 대두의 분쇄 시작부터 그 후 24시간의 짧은 저장기간 동안 두 전지 대부분에서 유지를 추출하여 가수분해와 산화에 의한 유지의 변패를 측정하였다. 유지의 과산화물가나 conjugated diene의 증가는 없었으며, 수분함량 10.7% 이하에서 hexanal 함량증가도 없었다. 수분함량 14.9% 이상에서 유지산패는 수분함량과 저장기간의 증가로 그 값이 증가하였다. 대두의 수분함량이 4%에서 18%로 증가함에 따라서 가수분해에 의한 산패 즉 유리지방산가는 0.06 %만이 증가하였으므로 실질적으로 분쇄후 24시간 저장동안 전지대부분 변질에 어떠한 영향도 주지 못하였다. 이러한 결과에서 원료대두의 수분함량이 11%이하이면 전지 대부분으로 분쇄하고 그 후 24시간동안 저장하여도 유지나 향미의 변패에 어떠한 영향도 주지 못함을 알 수 있었다.