

## Effects of Blanching Conditions on the Quality of Immatured Soybeans during Frozen Storage

Joohun Hong, Dongho Bae\* and Yonghee Choi

*Department of Food Science and Technology, Kyungpook National University, Taegu 702-701, Korea*

*\*Department of Agricultural Chemistry, Konkuk University, Seoul 143-701, Korea*

### 냉동저장동안 풋콩의 품질에 영향을 끼치는 Blanching 조건

홍주헌 · 배동호\* · 최용희

경북대학교 농과대학 식품공학과, \*건국대학교 농과대학 농화학과

#### Abstract

Blanching conditions for immatured soybeans were optimized by analyzing the effects of various time/temperature blanch treatment on the activities of peroxidase and lipoxygenase, the stability of vitamin C and color, and moisture content in immatured soybeans for the purpose of minimizing quality deterioration during frozen storage. Blanching at 96°C for 70 sec led to maximum inactivation of lipoxygenase in the immatured soybeans, while any blanching conditions tested in this study were not enough to inactivate peroxidase. Blanching at 82°C for 60 sec resulted in the highest amount of vitamin C remaining in the immatured soybeans after blanching. Hunter -a/b ratios of immatured soybeans blanched at 82°C for 60 sec and hue values ( $\Delta E$ ) of the immatured soybeans blanched at 76°C for 60 sec showed the closest values to those of fresh products. The changes in moisture content of immatured soybeans was not so significant after blanching. In conclusion, it was suggested that immatured soybeans be blanched at 96°C for 70 sec to minimize lipoxygenase activity and resulting quality deterioration, while blanching at 82°C for 60 sec was recommended to stabilize vitamin C and color.

Key words : blanching, putkong, immatured soybean, frozen storage, lipoxygenase

#### Introduction

Traditionally, immatured soybean (*Glycine max*,

Corresponding author : Young-Hee Choi Dept. Food Sci & Technol, Kyungpook National University, Sankyuk-Dong, 1370, Taegu 702-701, Korea

Putkong) has been widely consumed in diets in Korea, and it's demand has increased recently for versatile utilization with expansion of the food service industry. For example, the consumption of immatured soybeans has highly increased in Japan as an appetiser and the shortage of supplies is

supplemented mostly by imports from Taiwan. Although our domestic immatured soybeans are of reasonably good quality due to the climatic and geographic advantages, the export of them to foreign countries is limited for some reasons [1]. One of the reasons is that the production of immatured soybeans has not been industrialized and the proper storage technique has not been developed. This has resulted in their supplies being available only during the harvest period (from early July to mid August). Therefore, studies on storage technique for immatured soybean are needed to increase the ability to compete with foreign products. One of the simplest and widely used techniques for crops is the frozen storage. Most raw vegetables can be stored only a short time even at  $-20^{\circ}\text{C}$ . This is due to the changes in texture, color, flavor and nutritional quality that occur as a result of action of several enzymes that are still active after harvest. Thus, most vegetables require a mild heat treatment or blanch to inactivate enzymes and stabilize quality prior to and during the frozen storage. However, physicochemical changes that occur in the heat-processed vegetables have been reported by several investigators [2-6]. According to them, blanching of vegetables prior to freezing has some advantages as well as a number of disadvantages. The advantages include the stabilization of texture, color, flavor and nutritional quality; the destruction of microorganisms; and the wilting of vegetables. The disadvantages include some losses in texture, color, flavor and nutritional quality by the heating process; formation of a cooked taste; some losses of soluble solids; and the adverse environmental impact because of the need for large amounts of a water and energy. Therefore, there are trade-offs in

blanching, and thus, process optimization is critical.

The objective of this study was to optimize blanching conditions for immatured soybeans by analyzing the effects of various time/temperature blanch treatment on the activities of peroxidase and lipoxygenase, the stability of vitamin C and color, and moisture content in immatured soybeans and to minimize quality deterioration during frozen storage for the purpose of providing immatured soybeans of good quality around the year.

## Materials and Methods

### Sources of materials and sample preparation

Immatured soybean varieties used in this study were Seoklyang and Miwon harvested from a farm in Kyungpook National University in August, 1996. Immatured soybeans were sorted into 800g lots and water-blanch at various temperatures (Table 1) used most commonly and commercially [3, 7, 8]. The immatured soybeans in Lot 5 were not blanch and served as a control. After blanching, immatured soybeans were cooled immediately in ice water, drained, frozen at  $-40^{\circ}\text{C}$  and packed in plastic bags for storage at  $-20^{\circ}\text{C}$ . After 2 days of the frozen storage, samples were analyzed for their enzyme activities, vitamin C contents, color and moisture contents.

### Proximate analysis

The contents of moisture, protein, lipid, carbohydrate and ash were determined by the AOAC Official Methods [9].

### Activities of peroxidase and lipoxygenase

Enzymes were extracted using sucrose/phosphate buffer as mentioned by Wisseman and Lee

**Table 1. Blanching conditions for immatured soybeans**

| Lot No. | Blanch temperature (°C) | Blanch time (sec.) |
|---------|-------------------------|--------------------|
| 1       | 96                      | 70                 |
| 2       | 82                      | 60                 |
| 3       | 76                      | 60                 |
| 4       | 71                      | 180                |
| 5*      | —                       | —                  |

\* regarded as control group

[10]. Frozen immatured soybeans (100g) were added to a prechilled homogenizer (Nissei, AM-7) with 200mL of chilled 0.05M  $\text{Na}_2\text{HPO}_4$  containing 0.4M sucrose (pH 6.5). After homogenization for 2.5 min, samples were filtered through cheesecloth. Tween-20 (Sigma Chem. Co.) was added to 0.25% (v/v), samples were stirred for 1 hr at 4°C, and then centrifuged at  $20,000 \times g$  for 30 min. Supernatant was filtered through glasswool, and aliquots of the filtrates were taken to measure the activities of peroxidase and lipoxygenase using a spectrophotometer (Spectronic, Genesys 5).

Peroxidase activity was measured as the change in absorbance at 470 nm using guaiacol and  $\text{H}_2\text{O}_2$  as substrates [11]. The substrate solution of 0.5% (v/v) guaiacol in 0.1M  $\text{K}_2\text{HPO}_4$  (pH 6.0) was stirred for 30 min and 0.008% (v/v) of 30%  $\text{H}_2\text{O}_2$  was added. Enzyme solution (50  $\mu\text{L}$ ) was added to 2.5mL substrate in a cuvette and then absorbance was measured. One unit of peroxidase activity was defined as a change of 0.1 absorbance unit per min per g. Then, peroxidase activity of the sample in each lot was compared with control (Lot 5), which had not been heated and was assumed to have 100% peroxidase activity.

Lipoxygenase was measured at 234 nm with a linoleic acid substrate solution based on the method of Chen and Whitaker [12]. An 0.01M

stock solution of linoleic acid (99%) with 1.0N NaOH and Tween-20 in distilled water was prepared fresh. Before assay this substrate stock solution (0.01 M) was diluted 5 fold with 0.2M  $\text{Na}_2\text{HPO}_4$  (pH 7.0). The substrate/buffer solution was flushed with nitrogen gas for 10 min and allowed to equilibrate at 25°C for 10 min before use. For the assay, 100  $\mu\text{L}$  enzyme extract was added to 3.0mL linoleic acid substrate in a cuvette and mixed, and then the change in absorbance at 234 nm was determined. One unit of lipoxygenase activity was defined as that amount of enzyme that produced a change of absorbance of 1.0 per min per g. Then, lipoxygenase activity of the sample in each lot was compared with the sample in Lot 5 that had not been heated, and was assumed to have 100% lipoxygenase activity.

### Vitamin C content

Vitamin C analysis was conducted according to a modified method of Omaye et al. [13]. A 50 g sample of immatured soybeans was added to 250 mL of chilled 5% metaphosphoric acid in a homogenizer (Nissei, AM-7). After homogenization for 3 min, sample was filtered through Whatman #2 paper and then diluted to 1:2 with 5% metaphosphoric acid before analysis.

A 250mL of 2, 6-dichlorophenolindophenol sodium salt stock dye solution containing 0.15 g of 2, 6-dichlorophenolindophenol sodium was prepared for further uses. 0.6mL of diluted immatured soybean extract was placed in a small test tube, 0.3mL of citrate/acetate buffer containing p-chloromercuribenzoate was added, and then each sample was analyzed individually. Diluted 2, 6-dichlorophenolindophenol sodium salt stock dye solution (0.3 mL) was added to each test tube and

Table 2. Proximate compositions of immatured soybeans

|           | Moisture (%) | Protein (%) | Fat (%)  | Carbohydrate (%) | Ash (%)  |
|-----------|--------------|-------------|----------|------------------|----------|
| Seoklyang | 60.2±2.58*   | 23.6±1.52   | 1.7±0.10 | 12.6±0.88        | 2.0±0.23 |
| Miwon     | 66.9±3.91    | 19.7±1.07   | 1.9±0.09 | 10.2±0.63        | 1.3±0.11 |

\*Mean ± standard deviation (n=3)

the absorbance was determined at 520 nm against a water blank. A few crystals of vitamin C were added in order to bleach the dye by reducing it completely and the absorbance was determined again, which served as a blank for sample. A standard curve ranging from 0~24mg of vitamin C per 1mL of 5% metaphosphoric acid, was run at each trial.

### Color measurement

L, a and b color values were measured using a Minolta Chromameter (Model CR-200, Minolta Co., Japan). After standardization on a white background, L, a and b values were measured on the immatured soybeans in Lot 1~5 [14]. Color difference values ( $\Delta E$ ) were calculated as follows;

$$\Delta E = \sqrt{(L - L_{ref})^2 + (a - a_{ref})^2 + (b - b_{ref})^2}$$

The color of the immatured soybeans in Lot 5 was used as a reference.

### Results and Discussion

The proximate compositions of immatured soybeans are summarized in Table 2. Fresh immatured soybeans were high in protein and carbohydrates, and contained a moderate amounts of fat and ash. Seoklyang contained the higher amounts of protein, carbohydrates and ashes than Miwon, while Miwon was higher in moisture and fat. Blanching at 82°C for 60 sec resulted in the highest amount of vitamin C remaining in immatured soybeans after blanching. The -a/b ratios of

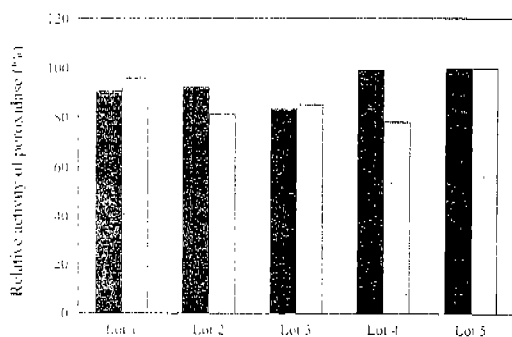
immatured soybeans blanched at 82°C for 60 sec were similar to those of the fresh products, while hue values ( $\Delta E$ ) of immatured soybeans blanched at 76°C for 60 sec were similar to those of the fresh product. Because the visual freshness and consumer acceptance of vegetables are highly affected by the yellow and green color of vegetables, the -a/b ratio is considered more reliable than the  $\Delta E$  value. And the changes in moisture content of immatured soybeans was not so significant after blanching.

### Activities of peroxidase and lipoxygenase

Conventionally, vegetables are blanched to inactivate peroxidase because it appears to be one of the most heat-stable enzymes in plants. It has been generally accepted that if peroxidase is destroyed then it is quite unlikely that other enzymes will have survived [15]. Thus, peroxidase activities of immatured soybeans blanched under various water-blanching conditions have been compared in Fig. 1. Peroxidase activity of Seoklyang tended to be influenced more by blanching temperature than time. This result was in accordance with the report of Dietrich et al. [16]. Remaining peroxidases in all the lots exhibited high residual activities after blanching. According to Dietrich et al. [16], even heating at 100°C for 6 min and blanching at 71°C for 3 min inactivated only 70% and 50% of peroxidase in brussels sprouts and green peas, respectively. In this study, the lowest peroxidase activity (Miwon in Lot 4) found in this study was as high as 78.2% of the

unblanched sample. This result indicated that the tested blanching conditions in this study were not enough to inactivate peroxidase in immatured soybeans. As mentioned previously, peroxidase is one of the widely used indicators of adequate blanching. There were, however, problems associated with the use of peroxidase as an indicator. Burnette [17] noted that peroxidase had not been shown to be directly responsible for quality deterioration during the frozen storage of vegetables, and thus heating to peroxidase inactivation may lead to over-blanch, impairing the quality of the frozen product. Therefore, it was concluded that the high peroxidase activity in these blanched immatured soybeans was not a serious problem as far as the quality stabilization of the products was concerned during the frozen storage.

One criterion for choosing the enzyme used as



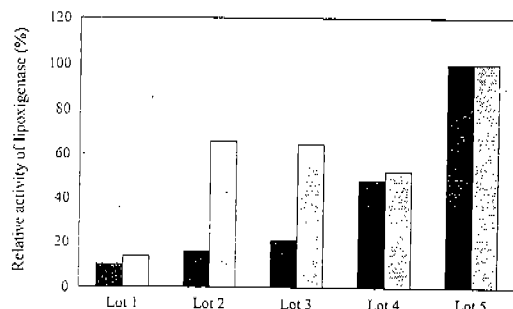
**Fig. 1. Peroxidase activities in blanched immatured soybeans under various conditions.**

■ Seoklyang □ Miwon

the index for proper heat treatment is that its role in the quality deterioration of the frozen foods. Some researchers [18, 19] suggested that lipoxygenase should receive more attention as the index for the proper heat treatment instead of

peroxidase. Wagenknecht and Lee [18] reported that lipoxygenase was implicated in the loss of chlorophyll and production of off-flavor in frozen green peas.

Lipoxygenase activities of immatured soybeans blanched under various water-blanching conditions are described in Fig. 2. The lipoxygenase activities of Seoklyang were decreased by increasing blanching temperature. Thus, heating at 96°C for 70 sec led to the almost complete inactivation of lipoxygenase, with which Miwon also showed the lowest lipoxygenase activity. This result was in accordance with the report of Chen and Whitaker [12]. They also found that blanching at 60°C for 6 min resulted in a 10 times higher lipoxygenase activity in peas than blanching at 96°C for 70 sec. Therefore, it was suggested that immatured soybeans be blanched at 96°C for 70 sec to minimize lipoxygenase activity.



**Fig. 2. Lipoxygenase activities in blanched immatured soybeans under various conditions**

■ Seoklyang □ Miwon

### Vitamin C content

Vitamin C contents in immatured soybeans blanched under various conditions are described in Fig. 3. Heating at 96°C for 70 sec caused the highest loss of vitamin C. Heating at 71°C for 180 sec also

caused a higher loss of vitamin C than heating at 76°C or 82°C for 60 sec. According to Fennema [20], vitamin C was lost during water blanching primarily by leaching due to its solubility in water.

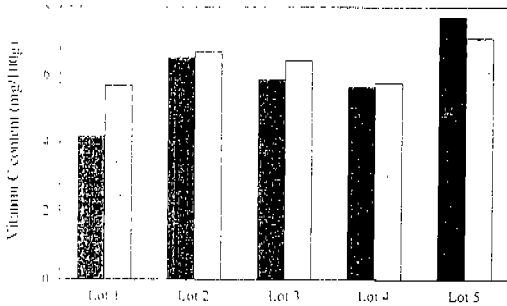


Fig. 3. Vitamin C contents in blanched immature soybeans under various conditions.

■ Seokkyang □ Miwon

Therefore, as blanching time increased from 60 sec (Lots 2 and 3) to 70 sec (Lot 1) and 180 sec (Lot 4), the loss of vitamin C was significant. Similar results were reported by Ponne et al. [8]. According to them, a 'dry' blanching method such as the microwave method appeared to result in about ten times higher vitamin C retention whereas in the conventional water blanching step much vitamin C was lost. However, the vitamin C loss in this study was not so severe as in the

report of Ponne et al. [8]. In addition, vitamin C losses could also be expected to occur from the thermal degradation and due to the action of ascorbic acid oxidase. In fact, the vitamin C content of Miwon in Lot 1 was a little lower than that in Lot 4 as expected. However, the vitamin C content of Seokkyang in Lot 1 was significantly lower than that in Lot 4. These results implied that the heat degradation susceptibility of Seokkyang was higher than that of Miwon.

### Color measurements

Hunter values (L, a and b) and  $\Delta E$  (the total color value, hue) of samples, and -a/b ratio were estimated and presented in Table 3. Some differences in the trends of Hunter value changes with blanching were observed between the varieties. Seokkyang tended to darken in color with the decreased L values, and became less yellow with the decreased b values and more green with more negative values of a after blanching. Miwon tended to lighten in color with the increased L values, and became more yellow with the increased b values and more green with more negative values of a. The lowest hue values ( $\Delta E$ ) were found on Seokkyang and Miwon in Lot 3. The -a/b ratio was calculated from Hunter values. The -a/b ratios of Seokkyang and Miwon in Lot 2

Table 3. Color values of immature soybeans blanched under various conditions

| Type      | Lot No. | L                | a                 | b                | $\Delta E$ | -a/b |
|-----------|---------|------------------|-------------------|------------------|------------|------|
| Seokkyang | Lot 1   | 64.36 $\pm$ 0.60 | -21.12 $\pm$ 0.64 | 43.56 $\pm$ 0.47 | 4.89       | 0.48 |
|           | Lot 2   | 63.03 $\pm$ 1.04 | -15.54 $\pm$ 0.35 | 34.25 $\pm$ 0.73 | 7.14       | 0.45 |
|           | Lot 3   | 65.38 $\pm$ 1.65 | -17.20 $\pm$ 0.69 | 37.50 $\pm$ 0.48 | 3.50       | 0.46 |
|           | Lot 4   | 60.11 $\pm$ 0.49 | -17.43 $\pm$ 0.40 | 38.30 $\pm$ 0.38 | 6.48       | 0.46 |
|           | Lot 5   | 66.03 $\pm$ 0.59 | -17.35 $\pm$ 0.52 | 40.94 $\pm$ 1.30 | —          | 0.42 |
| Miwon     | Lot 1   | 64.31 $\pm$ 1.54 | -19.70 $\pm$ 0.36 | 38.42 $\pm$ 0.86 | 3.18       | 0.51 |
|           | Lot 2   | 63.31 $\pm$ 0.67 | -19.09 $\pm$ 0.64 | 41.65 $\pm$ 0.61 | 4.46       | 0.46 |
|           | Lot 3   | 61.41 $\pm$ 0.17 | -17.73 $\pm$ 0.82 | 40.14 $\pm$ 0.48 | 2.65       | 0.44 |
|           | Lot 4   | 63.04 $\pm$ 0.96 | -19.50 $\pm$ 0.47 | 41.71 $\pm$ 0.08 | 4.64       | 0.47 |
|           | Lot 5   | 62.30 $\pm$ 1.13 | -17.35 $\pm$ 0.61 | 37.67 $\pm$ 0.41 | —          | 0.46 |

resembled the fresh product mostly. Francis and Clydesdale [21] noted that there was a good correlation between the ratio of  $-a/b$  and the visual color. Actually, immatured soybeans blanched at 82°C for 60 sec visually looked fresh.

### Moisture content

Moisture content varied very slightly in immatured soybeans from Lot 1 to 5. Seokyang varied from 66.2% to 70.7% moisture while Miwon varied from 55.7% to 63.0% moisture (Fig. 4). Some moisture losses in blanched immatured soybeans had been expected because of protein shrinkage resulting from heat denaturation. However, blanched immatured soybeans unexpectedly contained slightly larger amounts of moisture than fresh ones.

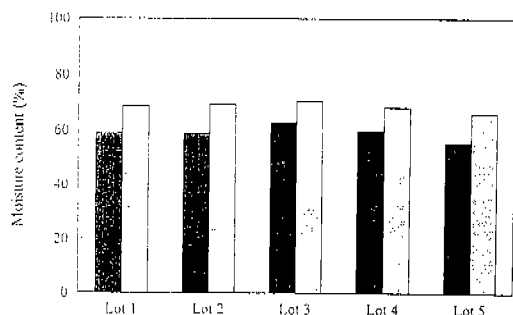


Fig. 4. Moisture contents in blanched immatured soybeans under various conditions.

■ Seokyang    □ Miwon

### Conclusion

Overall, heating at 96°C for 70 sec led to maximum inactivation of lipoxygenase in immatured soybeans, although it was not enough to inactivate peroxidase. Therefore, it was suggested that the immatured soybeans be

blanched at 96°C for 70 sec in order to minimize lipoxygenase activity and quality deterioration. As for vitamin C and color stabilities, blanching at 82°C for 60 sec was highly recommended.

The results of this study suggest the optimum conditions for minimizing quality and color deterioration, and vitamin C loss caused by blanching immatured soybeans. Further work is now needed to optimize frozen storage conditions and to develop new food uses of immatured soybeans for expanding the uses of our indigenous crops.

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### 요 약

본 연구에서는 석량, 미원 2품종의 풋콩을 실험재료로 하여 일반성분을 비교해보았고 가공·저장시 풋콩의 품질저하를 최소화 하는 blanching 조건을 찾고자 상업적으로 널리 이용되고 있는 여러 가지 blanching 조건에 따라 처리하였으며, 품질평가로는 수분함량, 색상, Vitamin C 함량의 변화와 peroxidase, lipoxygenase 활성을 살펴보았다. 일반성분은 두 품종 모두 수분, 단백질, 탄수화물이 대부분을 차지했으며 96°C에서 70초 동안 blanching한 경우가 lipoxygenase 불활성화에 가장 효과적이었는 반면, 어떤 blanching 조건에서도 peroxidase를 충분히 불활성화 시키지는 못했다. Vitamin C 함량과 색상의 변화는 82°C, 60초에서 처리한 경우가 가장 우수하였으며 풋콩의 수분함량은 다양한 조건에서 blanching을 했지만 뚜렷한 변화는 없었다. 결과적으로, 풋콩의

blanching 처리시 시간보다는 온도에 의해 많은 영향을 받을 수 있었다.

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