Effect of gamma irradiation on physiological activity of Korean soybean fermented foods, *Chungkookjang* and *Doenjang*

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Abstract

Effects of gamma irradiation on the physiological activity of Korean soybean fermented foods were investigated. *Chungkookjang*, the whole cooked soybean product and *Doenjang*, soybean paste were purchased and irradiated at 5, 10 and 20 kGy of absorbed doses. The physiological activity was evaluated by angiotensin converting enzyme (ACE) inhibition, xanthine oxidase (XOase) inhibition, tyrosinase inhibition and radical scavenging ability and results indicated that at 10 kGy or below did not show any significant change on physiological activities by irradiation.

Keyword : gamma irradiation; soybean; physiological activity, fermented food

1. Introduction

Soybean based fermented foods such as the whole cooked soybean fermented product (*Chungkookjang* in Korea, *Natto* in Japan) and soybean paste (*Doenjang* in Korea, *Miso* in Japan) are very popular in Korea. *Chungkookjang* has been made traditionally with whole cooked soybean by fermenting with *Bacillus* species without salt addition. *Doenjang* has been traditionally prepared by mixing, then fermenting a

moldy cooked soybean (*Meju*), and 20% of salt is added in a container (porcelain pot).

The microorganisms present in *Chungkookjang* and *Doenjang* are usually *Bacillus*, yeast and acid-forming bacteria. Kim et al. (2000) reported that the number of *Bacillus* cell decreased about 5 log cycles with 10 kGy of irradiation dose using *Chungkookjang* .

The evaluated D_{10} value of *Bacillus* was 1.78 kGy and was nearly eliminated by 20 kGy of irradiation. Moreover, the number of *Bacillus* survived even at 10 kGy of gamma irradiation decreased significantly during 6 weeks of storage because of serious cell damage. The number of *Bacillus* cells included in *Doenjang* also decreased by 2 log cycles with the irradiation dose of 10 kGy, having 5.26 kGy of D_{10} value. Yeast and Lactobacillus can be eliminated at 5 kGy in *Doenjang* (Byun et al., 2001). Byun et al. (2001) reported that gamma irradiation technology has positive effects in preventing decay by sterilizing microorganisms and by improving the safety and shelf-stability of food products.

Korean fermented soybean foods have various physiological effects. *Chungkookjang* is known to have a fibrinolytic enzyme that may have a role to remove blood clots (Kim et al., 1996; Sumi et al., 1987; Lee et al., 1998). *Chungkookjang* also presented anticancer, blood pressure reduction, and hypocholesterolemic effect in serum (Yoo et al., 1997). *Doenjang* has antimutagenic effect on the mutagenesis of AFB_1 on *Salmonella typhimurium* strains TA98 and TA100 (Park, 1996) and also a reduction of blood pressure (Shin et al., 1995; Hwang et al., 1997). Recently, several trials to use the irradiation technology in processing Korean soybean fermented foods to improve quality and microbial safety(Kim et al., 2000). However, concerns are addressed on the effect of irradiation on the physiological activity of those foods.

The objective of this study was to investigate the effect of irradiation on physiological activity of Korean soybean fermented foods.

2. Materials and Methods

2.1. Sample preparation

The whole cooked soybean fermented product (*Chungkookjang*) and soybean paste (*Doenjang*) purchased from Myungga Food Co. (Chungbook, Korea). Samples were irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Canada). The

source strength was approximately 100 kCi with a dose rate of 70 Gy min^{-1} at 15±0.5℃. The absorbed doses applied in this study were 5, 10 and 20 kGy and the actual doses were within ±2% of target dose.

2.2. Extraction

Irradiated or nonirradiated samples (control, 10 g each) were extracted with distilled water (100 ml) at room temperature for 2 hours, centrifuged at 10,000 rpm for 15 min, filtered, and the filterate was diluted by 10 times of its volume with distilled water.

2.3. Angiotensin converting enzyme (ACE) inhibitory activity

ACE inhibitory activity was assayed by the method of Cushman and Cheung (1970) with some modification. Hip-His-Leu (10 mM, Sigma Co. Ltd., St. Louis, USA) and the prepared sample was dissolved in a 100 mM potassium phosphate buffer (pH 8.3) containing 300 mM NaCl, and incubated at 37℃ for 30 min. The ACE inhibitory ratio(%) was calculated by observing the spectrometric reading at 228 nm by a spectrophotometer (UV-1601PC, Shimadzu Co., Kyoto, Japan).

2.4. Xanthine oxidase (XOase) inhibitory activity

The XOase inhibitory activity was assayed spectrophotometrically at 292 nm as described by Marcocci et al. (1994). The reaction mixture was prepared with 0.1 M potassium phosphate buffer (pH 7.5), 2 mM xanthine and 0.2 unit xanthine oxidase. Xanthine oxidase activity was expressed as percent inhibition of xanthine oxidase, calculated as (1-B/A)100, where A is the change in absorbance without sample, and B is the change in absorbance with sample.

2.5. Tyrosinase inhibition activity

Sample (0.2 ml) was added to the reaction mixture containing 10 mM L-3,4-dihydroxyphenyl-alanine (L-DOPA, Sigma, St. Louis, USA) solution, 1/15 M sodium phosphate buffer (pH 6.8) and mushroom tyrosinase solution (100 unit/ml). The reaction mixture was incubated at 25℃ for 15 min. The amount of dopachrome

produced in the reaction mixture was determined at 475 nm by the spectrophotometer.

2.6. Scavenging effects of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The free radical scavenging effect was estimated according to the method of Blois (1958) with some modification. Sample (1 ml) was added into 0.2 mM DPPH radical (1 ml). The mixture was shaken and stood for 30 min at room temperature and measured at 517 nm with the spectrophotometer.

3. Results and Discussion

3.1. ACE inhibitory activity

The role of ACE is blood pressure regulation, conversion of angiotensin Ⅰ to the potent vasoconstrictor angiotensin Ⅱ, and inactivation of the vasodilator bradykinin Fig. 1 shows the ACE inhibitory activities (% ratio) of gamma irradiated *Chungkookjang* and *Doenjang* diluted by 10 times with distilled water. ACE inhibitory effect of *Chungkookjang* was higher than that of *Doenjang*, showed 64%, 56%, 56% and 52% at nonirradiated, 5, 10 and 20 kGy of irradiation, respectively. There was no difference found among irradiation doses in *Chungkookjang*. However, *Doenjang* at 20 kGy of irradiation had lower ACE inhibitory activity than 0 or 5 kGy of irradiation. These results indicated that gamma irradiation below 10 kGy has no effect on peptide, known to act as an ACE inhibitor.

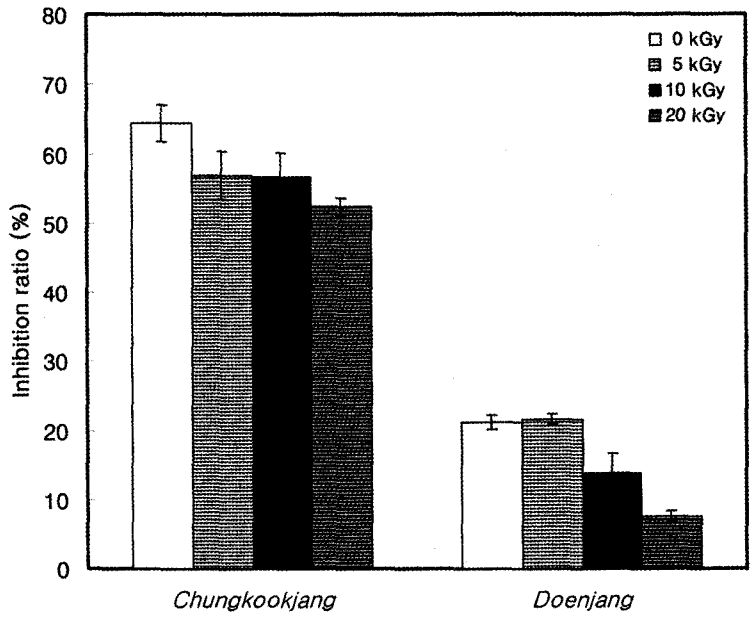

Fig. 1. Angiotensin converting enzyme (ACE) inhibitory effects of irradiated Korean soybean fermented foods, *Chungkookjang and Doenjang*.

3.2. Xanthine oxidase inhibitory activity

Xanthine oxidase (EC 1.2.3.2) catalyses the metabolism of hypoxanthine and xanthine to uric acid. The overproduction and/or underexcretion of this acid causes the incidence of hyperuricemia such as gout (Kong et al., 2000). The *Chungkookjang* had lower xanthine oxidase inhibitory activity than that of *Doenjang*, and 20 kGy irradiation sample had significantly lower than other irradiation doses. Xanthine oxidase inhibitory effects of *Doenjang* were about 18% in nonirradiated or 5 and 10 kGyirradiated sample, but 20 kGy of irradiation reduced to 6-8%. Thus, the irradiation dose of soybean based fermentation foods should be limited below 10 kGy, if XOase inhibitory effect is considered.

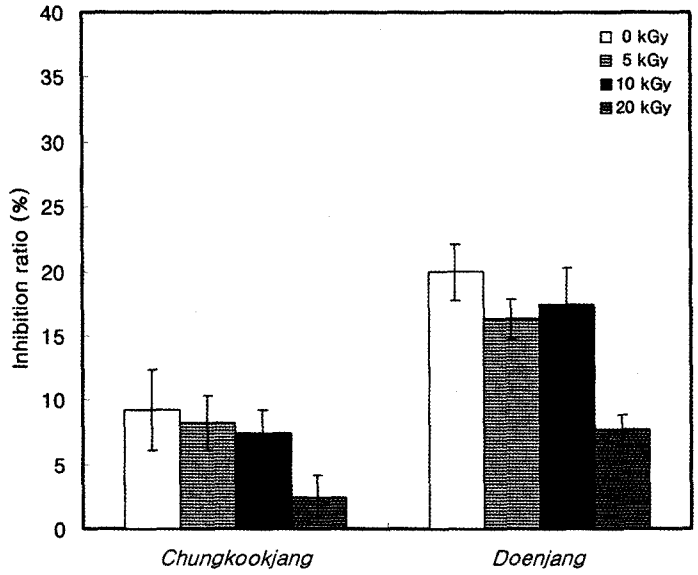

Fig. 2. Xanthine oxidase (XOase) inhibitory effects of irradiated Korean soybean fermented foods, Chungkookjang and Doenjang.

3.3. Tyrosinase inhibitory activity

The melanin pigment in human skin is a major defense mechanism against ultraviolet light to the skin, but darkened skin color, which is the result of increased and redistributed epidermal melanin, could be a serious aesthetic problem (No et al., 1999). Tyrosinase was mainly responsible for melanin biosynthesis (melanogenesis) in animals and enzymatic browning (melanosis) in plants. Tyrosinase inhibition (% ratio) of nonirradiated and irradiated *Chungkookjang* has no difference among irradiation doses Similarly, the tyrosinase inhibitory activity of *Doenjang* was about 30% and was not different between samples.

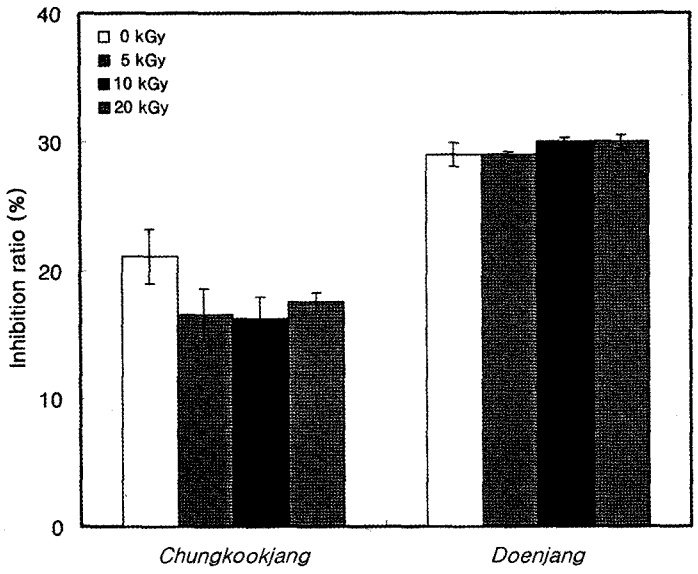

Fig. 3. Tyrosinase inhibitory effects of irradiated Korean soybean fermented foods, *Chungkookjang and Doenjang*

3.4. Free radical scavenging effect

No significant changes in the radical scavenging effect were observed in nonirradiated, 5, 10 and 20 kGy irradiated *Chungkookjang* and *Doenjang* (Fig. 4 and 5). These results indicate that the free radical scavenging effect was not influenced by gamma irradiation. Byun et al. (1999) reported that electron donating activity of Korean medicinal herbs was not influenced by gamma irradiation and agreed well with present results.

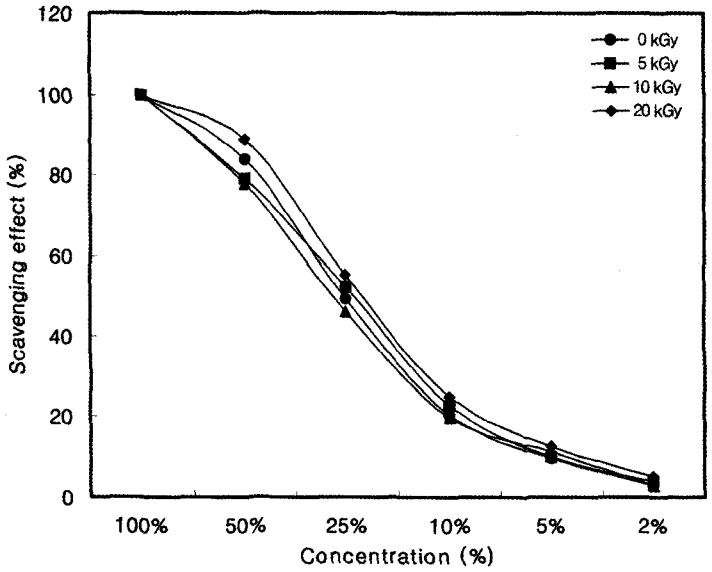

Fig. 4. 1,1-diphenyl-2-picrylhydrazyl radical(DPPH) scavenging effect of irradiated *Chunkookjang* extract.

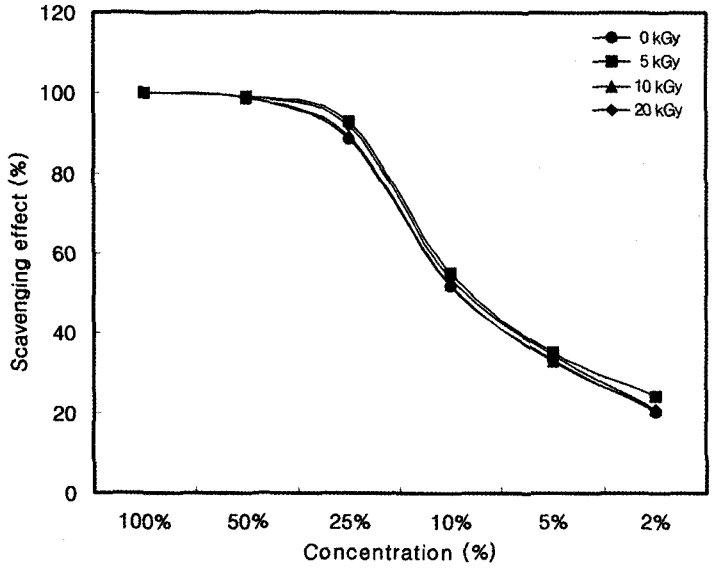

Fig. 5. 1,1-diphenyl-2-picrylhydrazyl radical(DPPH) scavenging effect of irradiated *Doenjang extract.*

4. Conclusion

Result showed that there is no difference in physiological activity among 0, 5 and 10 kGy-irradiated Korea soybean fermented foods (*Chungkookjang* and *Doenjang*). Therefore, gamma irradiation at 10 kGy or below is evaluated as an effective technique to maintain microbial safety of Korean fermented food without an adverse change in the physiological effects expected from the fermented food.

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