

Fermented Wheat Grain Products and *Kochujang* Inhibit the Growth of AGS Human Gastric Adenocarcinoma Cells

Su-Ok Kim¹, Chang-Suk Kong¹, Jeung-Ha Kil¹, Ji-Young Kim¹, Min-Soo Han² and Kun-Young Park^{1†}

¹Department of Food Science and Nutrition, Pusan National University, Busan 609-735, Korea

²R&D Center, Haechandle Foods Co. LTD, Chungnam 320-833, Korea

Abstract

The growth inhibitory effect of wheat grain, fermented wheat grain products, red pepper powder and *kochujang* was examined in AGS human gastric adenocarcinoma cells. The *kochujang* samples were prepared by mixing red pepper powder and each fermented wheat grain products, such as first fermented wheat grain (FFWG), second fermented wheat grain (SFWG) and final fermented wheat grain (FiFWG). The methanol extract of FiFWG showed the highest growth inhibitory effects. In order to investigate the effects of fermented wheat grain products at each stage of fermentation on anticancer activity, the *kochujang* prepared with red pepper powder and each of FFWG, SFWG and FiFWG were fermented until reaching pH 5.1. The *kochujang* adjusted to pH 5.1 by fermentation increased the growth inhibitory effect; however, the *kochujang* prepared with FiFWG showed the highest effect. Increased fermentation time increased the growth inhibitory effect of *kochujang* when prepared with FiFWG. These results suggested that the anticancer effect of the *kochujang* on the gastric cancer cells was affected by the fermentation periods of the wheat grains. The well-fermented end products of the wheat grains seem to be one of the major components that showed anticancer activity of *kochujang*.

Key words: *kochujang*, fermented wheat grain, AGS human gastric cancer cells, MTT assay

INTRODUCTION

Kochujang, a fermented red pepper soybean paste, is one of the famous traditional foods in Korea. It is prepared with red pepper powder, boiled grains, salt, and *koji* (fermented soybeans or wheat grains) and has played an important role in providing specific taste and flavor in Korean foods. The unique hot, sweet, salty and savory tastes, color, and flavors of *kochujang* are produced by the actions of microorganisms such as *koji* mold, bacteria, and yeasts during the fermentation process (1-4).

Generally, traditional *kochujang* is prepared with glutinous rice, *meju* (fermented soybean blocks), red pepper powder and salt, which is fermented for 6 to 18 months. However, in commercial *kochujang*, fermented products are commonly made from various materials such as soybean, glutinous rice, barley or wheat grains by inoculating with *Aspergillus oryzae*, which is substituted for *meju* (3).

Recently, commercial *kochujang* sales increased and the use of homemade products have decreased correspondingly (5). However, there are few studies on the biological functions of commercially-made *kochujang*. A few studies have reported on the anti-obesity and can-

cer preventive effects of traditional *kochujang* (6,7). Rhee et al. (8) reported that the fermentation process of traditional *kochujang* increased anti-obesity activity. The traditional *kochujang* and its ingredients had higher antimutagenic and anticancer activities, and *meju* and glutinous rice seemed to be especially potent ingredients (9-13). However, the anticancer effects of fermented wheat grains, which are used in commercially prepared *kochujang*, have not been reported.

In this study, the growth inhibitory effects of wheat grains, fermented wheat grain products, red pepper powder and the *kochujang* were examined in AGS human gastric adenocarcinoma cells. The effects of fermentation of wheat grains and fermentation time of *kochujang* with final fermented wheat grain (FiFWG) on the inhibitory activity of *kochujang* were evaluated by MTT assay.

MATERIALS AND METHODS

Ingredients and preparation of fermented wheat grain products

Red pepper powder, wheat grains, wheat flour, salt and *Asp. oryzae* (*koji*) were obtained from Haechandle

†Corresponding author. E-mail: kunypark@pusan.ac.kr
Phone: +82-52-510-2809. Fax: +82-51-514-3138

Co. LTD. (Chungnam). The three kinds of fermented wheat grain products, first fermented wheat grain (FFWG), second fermented wheat grain (SFWG) and final fermented wheat grain (FiFWG), were prepared as follows: *Koji* was prepared with the wheat flour by inoculation of *Asp. oryzae* and incubating at 35°C for 3 days. FFWG was prepared with *koji*, steamed wheat grains and salt. The mixture was matured in the presence of *Zygosaccharomyces rouxii* at 30°C for a week and followed by fermentation for 30~40 days, these were called SFWG and FiFWG, respectively.

Preparation of *kochujang*

The *kochujang* samples were prepared by mixing each fermented wheat grain product and red pepper powder (4:1 ratio) and followed by sterilizing at 70°C for 8 min. The commercial *kochujang* prepared with the FiFWG showed pH 5.1. The *kochujang* prepared with FFWG and SFWG were fermented at 30°C until they also reached pH 5.1, taking 7 and 8 days, respectively. The *kochujang* was prepared with FiFWG and was fermented for 30 days to evaluate the effect of further fermentation.

Methanol extracts of the samples

Wheat grain, fermented wheat grain products, red pepper powder and *kochujang* were freeze-dried and powdered. Twenty folds of methanol was added to the powdered samples and extracted three times with shaking. The methanol extracts were evaporated using a vacuum evaporator, concentrated, then dissolved in phosphate buffered saline (PBS).

Cell culture

RPMI 1640 medium, fetal bovine serum (FBS), trypsin-EDTA and penicillin-streptomycin were purchased from GIBCO Co. (Gaithersburg, MD, USA). AGS human gastric adenocarcinoma cells were obtained from Korea Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in RPMI 1640 medium and supplemented with 1% penicillin-streptomycin and 10% heat-inactivated FBS at 37°C in a humidified atmosphere with 5% CO₂. The medium was changed two or three times each week.

MTT assay

After six or seven days, the cultured cancer cells were washed with phosphate buffered saline (PBS). The cells were dissociated with 0.05% trypsin-0.02% EDTA and 180 µL of the cell suspensions (1×10^4 cells/mL) were seeded in each well of 96-well microtitre plates with various concentrations of each sample (20 µL). After 72 hours incubation, 20 µL of MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide] solution (5 mg/mL PBS) was added. After an additional 4 hours of incubation, the supernatant medium was carefully removed.

The formazan dye was solubilized by adding 150 µL DMSO to each well followed by gentle shaking. The optical densities were read on a microplate reader at 540 nm (14-16).

Statistical analysis

Statistical analyses were performed by analysis of variance. Significant differences between treatment means were determined by using Duncan's multiple range tests (17). Significance of difference was defined at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Effects of the fermented wheat grain products and *kochujang*

The inhibitory effects of methanol extracts from wheat grain, fermented wheat grain products, red pepper powder and the commercially prepared *kochujang* on the growth of AGS human gastric adenocarcinoma cells were examined (Fig. 1). The methanol extract of FiFWG showed the highest inhibitory activity. Wheat grain showed lower inhibitory effects than fermented wheat grain products. The fermented wheat grain products were more effective on the growth inhibition of the cancer cells. As fermentation of wheat grains proceeded, there were significant increases in the inhibitory activity. That is, FFWG had

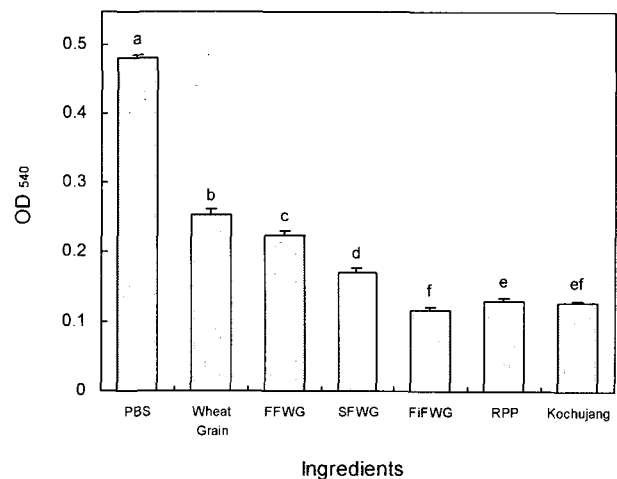


Fig. 1. Comparison of the inhibitory effects of methanol extracts (5 mg/mL) from wheat grain, fermented wheat grain products, red pepper powder and *kochujang* on the growth of AGS human gastric adenocarcinoma cells in 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. FFWG: First fermented wheat grain (*Asp. oryzae*). SFWG: Second fermented wheat grain (yeast). FiFWG: Final fermented wheat grain (more fermented for 30~40 days). RPP: Red pepper powder. Means with the different letters (a~f) are significantly different ($p < 0.05$) by Duncan's multiple range test.

a 10% greater inhibitory effect than wheat grain, SFWG had a greater effect than FFWG, and FiFWG was more effective than SFWG. Red pepper powder also inhibited the growth of AGS human gastric cells. FiFWG and red pepper powder were the major ingredients which had anticancer effects in commercial *kochujang*. In the preparation of *kochujang*, *koji* is usually made from soybean or the mixture of rice and soybean. Several studies indicated that soybean (18,19), *meju* (12,13), *doenjang* (20) produced from fermented soybean and *meju*, and the traditional *kochujang* (13,21) with *meju* showed the higher antimutagenicity and anticancer effects than commercial *kochujang*. The whole grains reduced the tumor incidence and cardiovascular disease because of containing dietary fiber, fermented carbohydrates such as resistant starch and oligosaccharide, phytochemicals such as phenolic compounds and phytoestrogen, etc. (22). Some bioactive constituents of the fermented wheat grain products may be produced from these materials by microbes during fermentation.

Effects of *kochujang* with different fermentation stages of wheat grains

In order to examine the fermentation effect of *kochujang* prepared with each fermented wheat grain product on the growth of AGS human gastric adenocarcinoma cells, *kochujangs* were prepared by mixing red pepper powder and fermented wheat grain products such as FFWG, SFWG, and FiFWG (Table 1). The commercial *kochujang* was mostly prepared with FiFWG. The pH of *kochujang* at initial fermentation step was in the range of 5.1~5.4, and declined further as fermentation progressed (23). Therefore, the *kochujang* prepared with FFWG and SFWG were fermented at 30°C up to pH 5.1. When not fermented, the *kochujang* with FiFWG (inhibition rate 80%) showed a higher inhibitory effect than that with FFWG (63%) and SFWG (63%). The inhibitory activity of *kochujang* prepared with FFWG, which had only slightly fermented wheat grains, was not affected by the fermentation. The growth inhibitory effect of *kochujang*

Table 1. Inhibitory effects of *kochujang* (2.5 mg/mL) fermented up to pH 5.1 on the growth of AGS human gastric adenocarcinoma cells in 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay

	OD ₅₄₀ (level of sample, 2.5 mg/mL)	
	Non-fermentation	Fermentation to pH 5.1
Control	0.605 ± 0.005 ^{a1)}	
FiFWG + RPP	0.123 ± 0.030 ^d (80)	
FFWG + RPP	0.224 ± 0.017 ^b (63)	0.218 ± 0.018 ^b (64)
SFWG + RPP	0.224 ± 0.028 ^b (63)	0.179 ± 0.030 ^c (70)

¹⁾Means with the different letters are significantly different ($p < 0.05$) by Duncan's multiple range test.

prepared with SFWG was increased by fermentation. However, the two fermented *kochujang* showed lower inhibitory effects than that of *kochujang* with FiFWG.

Inhibitory effect of the fermented *kochujang*

Kochujang prepared with FiFWG, which had the greatest anticancer effect, was fermented for 30 days to observe the inhibitory effect of the fermented *kochujang* (Fig. 2). Increased fermentation time produced higher growth inhibitory effects of *kochujang*. There was no significant difference in the *kochujang* fermented for 15 and 30 days. Therefore, the anticancer effect of *kochujang* prepared with thoroughly fermented wheat grain product was increased by fermentation and its activity was maintained at the same level if fermented longer than 15 days. There were several indications that fermentation increased antimutagenicity and anticancer effects in *kochujang*. The traditional *kochujang* fermented for over 6 months exhibited greater antimutagenic activity than the commercial ones (13). The ripened traditional *kochujang* had a greater suppressive effect on tumor formation and lung metastasis in mice than *kochujang* without fermentation (21). *Meju* powder seemed to be the major antimutagenic component in traditional *kochujang* while fermented wheat grain product and red pepper powder were important ingredients in commercial *kochujang*. Further study is needed to identify whether the increased activity of commercially made *kochujang* was obtained by the fermentation of red pepper powder or fermented wheat grain product.

Among the ingredients in commercial *kochujang*,

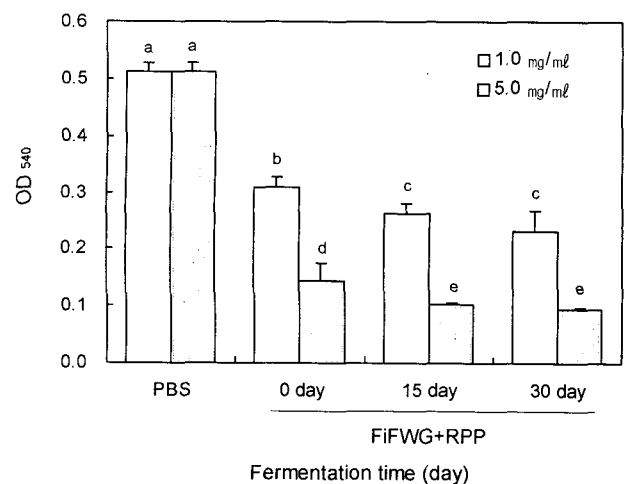


Fig. 2. Effect of fermentation time on the inhibitory effects of *kochujang* prepared with final fermented wheat grain on the growth of AGS human gastric adenocarcinoma cells in 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Means with the different letters (a~e) are significantly different ($p < 0.05$) by Duncan's multiple range test.

fermented wheat grain products had a greater inhibitory effect on the growth of AGS human gastric adenocarcinoma cells and these activities were increased by fermentation. Also, in preparation of *kochujang*, the fermentation time of wheat grains affected the inhibitory effect of *kochujang*, the *kochujang* prepared with final fermented wheat grain product had an excellent anti-cancer effect up to a certain fermentation time.

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