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Comparative Analysis of Commercial Chinese Bean Sauce (Chunjang) from Korea and China Based on Antioxidant Activity

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ABSTRACT: In this study, the antioxidant effects of seven types of commercial Chunjang from China (C1～3) and Korea (K1～4) were compared for their ability to protect against H_2O_2-induced DNA damage. Outputs included total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH RSA), oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant potential (TRAP), and comet assays. TPC was the highest in C3 (1,250.8 mg gallic acid equivalent/100 g). C1 exhibited a significantly higher DPPH RSA IC_{50} (7.5 mg/mL) and ORAC concentration (8.22 μM Trolox equivalent) compared with all other samples, and C1 and K1 exhibited the highest TRAPs. H_2O_2-induced DNA damage was effectively protected by Chunjang, with a higher observed in C2, C3, and K1 (24.2～25.3 μg/mL) compared with the other samples (28.3～30.0 μg/mL). Our results showed that commercial Chunjang contains polyphenol and antioxidant activities. The differences between the samples might be attributed to different origins, materials, and processing methods.

Keywords: antioxidant activity, comet assay, Korean and Chinese commercial Chunjang, polyphenol

INTRODUCTION

Chunjang is a traditional Chinese fermented soybean paste that is produced via the fermentation of soybeans and salt by naturally occurring bacteria and subsequent caramelization. Chunjang has been consumed for more than a century as a seasoning ingredient, and is used as a seasoning ingredient to prepare Jajang noodles, which is popular worldwide (Imm et al., 2009).

However, most commercial Chunjang that is used in Chinese restaurants in Korea is not prepared by traditional fermentation. It is produced by adding caramel after preparing a paste consisting of soybean and wheat. Consumers consider caramel to be an artificial additive and not a healthy food ingredient. Accordingly, caramel-containing foods are considered unhealthy. Consumers desire to eat healthy food is reflected in the food market; hence, the sale of organic Chunjang has begun recently.

Caramel is a natural pigment used as a food additive that is consumed in large amounts worldwide (Tsai et al., 2009). Caramel possesses 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging activities, metal chelating activity, and a power-reducing effect; in particular, the brown pigment of caramel is likely to contribute to the antioxidant activity (Rhee and Kim, 1975; Manzocco et al., 2000; Phongkanpai et al., 2006; Tsai et al., 2009; Woo et al., 2011). Hence, it is meaningful to compare the antioxidant activity of organic Chunjang with that of Chunjang prepared with caramel. Additionally, a few studies have been performed on Chunjang, which is the main ingredient in Jajang noodles. Thus far, only studies on Chinese bean sauce cultures (Son and Kim, 2012) and the formation and destruction of biogenic amines in Chunjang and Jajang (Bai et al., 2013) have been conducted.

Therefore, the purpose of this study was to compare the antioxidative activities of Chunjang supplemented with caramel with organic Chunjang marketed in Korea and China.

MATERIALS AND METHODS

Sample preparation

Three kinds of Chinese (C1～3) and 4 kinds of Korean (K1～4) Chunjang samples were purchased from local markets. Among these, C1, K1, and K2 are Chunjang

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containing caramel, and C2, C3, K3, and K4 are organic Chunjang. The purchased samples were refrigerated until the end of the analysis. For the extraction of Chunjang, 5 g of Chunjang was added to 100 mL of ethanol, extracted for 72 h, and then concentrated to prepare the stock solution. Chunjang was redissolved in dimethyl sulfoxide (DMSO) to a final concentration of 50 mg/mL, and stored at −20°C until use.

Measurement of total phenolic contents (TPC)

TPC of Chunjang was determined according to the method of Park et al. (2011). Chunjang was mixed with 2 mL of 1 N Folin-Ciocalteu reagent and incubated at 25°C. Then, 2 mL of 10% Na_2CO_3 was added and the mixture was incubated at room temperature. The absorbance was measured at 690 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Tecan Austria GmbH., Grödig, Austria). TPC was expressed in gallic acid equivalents (GAE).

Measurement of DPPH radical scavenging activity (RSA)

DPPH RSA was measured according to the method of Mensor et al. (2001), with a few modifications. Briefly, 80 μL of 0.2 mM DPPH ethanol solution was added to 20 μL of sample solution at different concentrations (1～10 mg/mL), and was allowed to react at room temperature. The control consisted of 20 μL DMSO and 80 μL 0.2 mM DPPH. The mixtures were incubated at room temperature for 10 min and then the absorbance was measured by ELISA at 492 nm. The half maximal inhibitory concentration (IC_{50}) value is the extraction concentration at which the amount of DPPH radicals is reduced by 50%. A low IC_{50} value is indicative of a strong DPPH-scavenging activity.

Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out on a FLUOstar OPTIMA fluorescence plate reader (BMG Labtech, Ortenberg, Germany) with fluorescence filters (excitation wavelength 485 nm/emission wavelength 535 nm) according to the method by Park et al. (2011). The results were obtained from calculations based on the difference in the area under the fluorescence decay curves between the blank and each sample. ORAC activity was were expressed in μmol of Trolox equivalents (TE).

Total radical trapping antioxidant potential (TRAP)

TRAP was determined following a modified version of the photometric method described by Rice-Evans and Miller (1994). Twenty microliters of each sample (2.5～20 mg/mL) were added to tubes containing phosphate buffered saline (PBS), 2.5 mM metmyoglobin, and 150 μM ABTS, and samples were mixed by vortexing. Reactions were initiated by adding 250 μL to 75 μM H_2O_2, and the absorbance was measured at 734 nm using a spectrophotometer (UV-1601; Shimadzu Corporation, Kyoto, Japan).

DNA damage determination by alkaline comet assay

The effect of Chunjang on HepG2 cell DNA was measured using the alkaline comet assay. HepG2 cells (2×10^5 cell/mL) were incubated with varying concentrations of Chunjang for 30 min at 37°C. For oxidative stimulation, cells were re-suspended in PBS with 200 μM H_2O_2 for 5 min on ice. After each treatment, cells were centrifuged at 500 *g* for 5 min and washed with PBS. DMSO (1%) without an oxidative stimulus was used as the negative control (NC). After treatment, cells were mixed with 75 mL of 0.7% low melting agarose and added to slides precoated with 0.5% agarose. The slides were then immersed in lysis solution [2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Tris, and 1% sodium laurylasarcosine; 1% Triton X-100 and 10% DMSO] for 1 h at 4°C. The slides were then placed into an electrophoresis tank containing 300 mM NaOH and 10 mM Na_2EDTA (pH 13.0) for 20 min. For DNA electrophoresis, an electric current of 25 V/300±3 mA was applied for 20 min at 4°C. The slides were washed three times with a neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4°C and then treated with ethanol for 5 min. Samples were stained with 20 mL of ethidium bromide (20 mg/mL) and measured through fluorescence microscopy (LEICA DM LB, Bensheim, Germany) using image analysis software (Komet version 5.0, Kinetic Imaging Ltd., Liverpool, UK). The percentage of fluorescence in the tail (tail intensity; 50 cells from each of the two replicate slides) was determined.

Statistical analyses

All results are presented as mean±standard deviation (SD), and statistical analyses were performed using the SPSS (ver. 23.0, SPSS Inc., Chicago, IL, USA) package for Windows. Mean values were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range post-hoc tests. *P*-values <0.05 were considered statistically significant. Pearson's correlation was used to determine the correlation of the results.

RESULTS AND DISCUSSION

Total polyphenolic content of Chunjang samples

The results of the TPC for the seven types of Chunjang are presented in Fig. 1. Regardless of origin and organic matter content, all Chunjang samples were shown to contain total polyphenols (119.5～1,250.8 mg GAE/100 g). Xu et al. (2015) also reported that the TPC of commercial fermented soy products was 236～1,237 mg GAE/

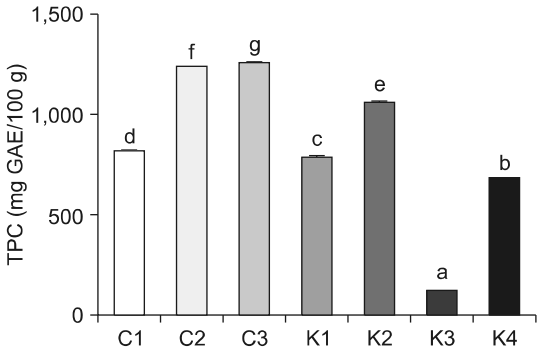

Fig. 1. Total polyphenolic content (TPC) of commercial Chunjang samples [Chunjang from China (C1~3) and Korea (K1~4)]. Bars with different letters (a-g) indicate a statistical difference according to the Duncan's multiple range test (P<0.05). GAE, gallic acid equivalent.

100 g, similar to the results of this study. Antioxidant activity in food is related to TPC (Xu and Chang, 2007). Polyphenols are of considerable interest for the human diet due to their antioxidant properties (Popa et al., 2019). The TPC significantly differed between the samples; C3 (1,250.8±8.8 mg GAE/100 g) possessed the highest TPC, followed by C2 (1,226.3±3.3 mg GAE/100 g) > K2 (1,051.8±12.0 mg GAE/100 g) > C1 (809.8±6.0 mg GAE/100 g) > K1 (778.6±16.4 mg GAE/100 g) > K4 (675.3±3.2 mg GAE/100 g) > K3 (119.5±0.0 mg GAE/100 g). For the Chunjang samples from China, TPC was significantly higher in organic Chunjang (C2 and C3) than normal Chunjang (C1). Whereas for samples from Korea, the TPC of organic Chunjang (K3 and K4) was lower than that of normal Chunjang (K1 and K2). The nutritional value of organic crops is slightly higher than that of conventional crops (Mie et al., 2017), especially in relation to phenolic compounds, vitamins, and minerals. However, organic passion fruits possess a lower TPC than conventionally grown variants (de Oliveira et al., 2017). According to a study by Brenna et al (2009), caramel contains variable amounts of reducing compounds, as demonstrated using the Folin-Ciocalteu method. Hence, the TPC of Chunjang is thought to be influenced by organic matter, as well as the caramel color.

Antioxidant activities of Chunjang samples

The results of the DPPH RSA are shown in Fig. 2. All Chunjang samples exhibited dose-dependent DPPH RSA. The DPPH RSA of Chunjang at high concentrations (10 mg/mL) ranged from 28.2% (K3) to 78.2% (K1). The IC_{50} values calculated based on DPPH RSA did not significantly differ between samples C1 (7.5±0.0 mg/mL), C2 (7.6±0.0 mg/mL), C3 (7.6±0.1 mg/mL), K1 (7.7±0.0 mg/mL), and K2 (8.0±0.0 mg/mL). The DPPH RSA IC_{50} of K3 and K4 were significantly higher than that of the other samples, which is probably due to the TPC.

The ORAC activities of the Chunjang samples are shown in Fig. 3. ORAC activity was confirmed in all Chunjang samples, and was concentration dependent. At

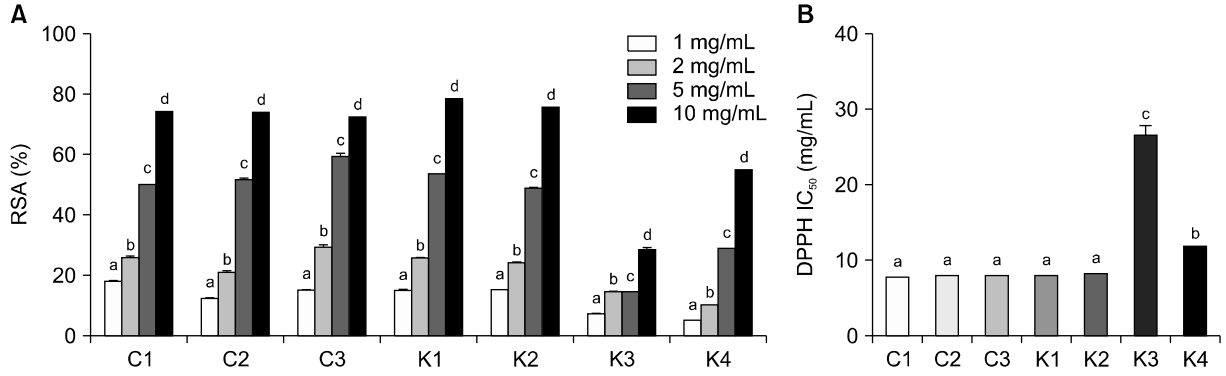

Fig. 2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) (A) and the half maximal inhibitory concentration (IC_{50}) (B) of commercial Chunjang samples [Chunjang from China (C1~3) and Korea (K1~4)]. Bars with different letters (a-d) indicate a statistical difference according to the Duncan's multiple range test (P<0.05).

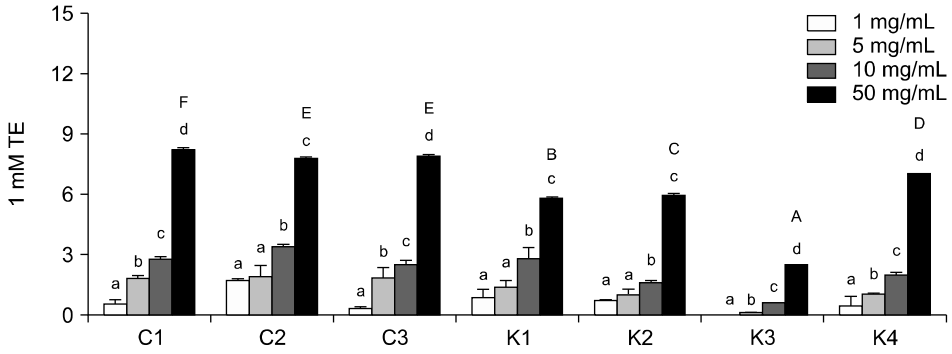

Fig. 3. Oxygen radical absorbance capacity (ORAC) activity of commercial Chunjang samples [Chunjang from China (C1~3) and Korea (K1~4)]. Bars with different small letters (a-d) indicate a statistical difference for various concentrations of the same sample and different capital letters (A-F) indicate a statistical difference at 50 mg/mL for each sample according to the Duncan's multiple range test (P<0.05). TE, Trolox equivalent.

a concentration of 50 mg/mL, ORAC activity ranged from 2.4 mM TE (K3) to 8.2 mM TE (C1). The Chunjang from China (C1～3) showed higher ORAC activities than Chunjang from Korea (K1～4). Similar to the results of TPC, K3 showed significantly lower ORAC activity than the other samples.

The TRAP assay results are shown in Fig. 4. All Chunjang samples showed concentration-dependent TRAP activity. TRAP activity at the highest concentration (20 mg/mL) was highest for samples C1 [1.13 mM Trolox equivalent antioxidant capacity (TEAC)] and K1 (1.12 mM TEAC), followed by K2 (1.03 mM TEAC) > C3 (0.99 mM TEAC), C2 (0.97 mM TEAC), and K4 (0.95 mM TEAC) > K3 (0.83 mM TEAC). To summarize, all the Chunjang samples showed antioxidant activity, and there was a difference in the activity of Chunjang depending on the types of antioxidant activity. Chung et al. (2015) reported that the antioxidant activity of natural fermented rice vinegar varies depending on the type, content, fermentation method, and aging of raw materials. According to Zhang and Hamauzu (2003) and Sun et al. (2007), differences in antioxidant activity in samples are due to differences in the antioxidant compounds in them. Thus, it is expected that there will be additional components that affect the antioxidant activity of Chunjang, including polyphenols. Brenna et al. (2009) reported that melanoidin compounds in caramel contribute to the antioxidant powers of some foodstuff. As a result, it appears that Chunjang can possess additional antioxidant activity from the caramel added during Chunjang production. However, the limitations of this study include that only commercial Chunjang samples were analyzed and therefore the materials used during the production and processing methods could not be controlled.

Protective effects of Chunjang samples against H_2O_2-induced DNA damage

Chunjang was protective against H_2O_2-induced DNA damage in a dose-dependent manner (Fig. 5A). This is thought to be due to the antioxidant compounds and antioxidant activity of Chunjang. In other studies samples containing antioxidant components and antioxidant activity show protection against DNA damage (da Silva et al., 2011; Kumar Salar et al., 2017; Shameem et al., 2017). Festa et al. (2001) and Cheng et al. (2013) used the comet assay to investigate the protective effect of an-

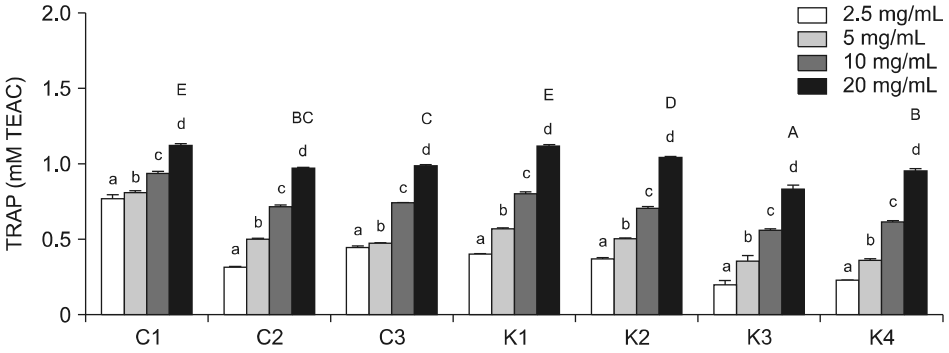

Fig. 4. Total radical trapping antioxidant potential (TRAP) value of commercial Chunjang samples [Chunjang from China (C1～3) and Korea (K1～4)]. Bars with different small letters (a-d) indicate a statistical difference for various concentrations of the same sample and different capital letters (A-E) indicate a statistical difference at 20 mg/mL for each sample according to the Duncan's multiple range test (P<0.05). TEAC, Trolox equivalent antioxidant capacity.

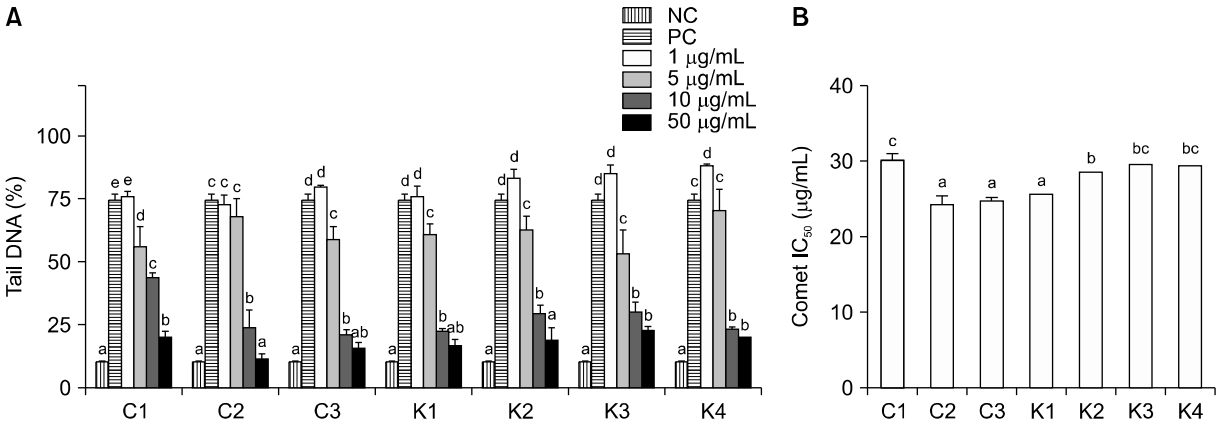

Fig. 5. DNA protective effect of commercial Chunjang samples [Chunjang from China (C1～3) and Korea (K1～4)] against H_2O_2-induced DNA damage (A) and Comet IC_{50} (B). Bars with different letters (a-c) indicate a statistical difference (P<0.05) according to the Duncan's multiple range test.

tioxidants against oxidative damage to DNA. The mechanism for the protective effect of Chunjang against oxidative stress-induced DNA damage can be explained as follows: hydroxyl radicals generated by H_2O_2 target DNA, resulting in fragmentation, base loss, and strand breaks (Rhaese and Freese, 1968). The polyphenol compounds in Chunjang donate hydrogen atoms or electrons to eliminate H_2O_2 and inhibit the formation of hydroxyl radicals. Therefore, the results of this study show that Chunjang is protective against oxidative DNA damage. This may be attributed to the polyphenolic component in Chunjang. The protective effect of Chunjang against H_2O_2-induced DNA damage was highest in sample C2 (24.2 mg/mL), followed by samples C3 (24.7 mg/mL)> K1 (25.3 mg/mL)> K2 (28.3 mg/mL)> K3 (29.5 mg/mL)> K4 (29.1 mg/mL)> C1 (30.0 mg/mL) (Fig. 5B). Visual photographs of comet-shaped DNA isolation for all samples at a concentration of 50 μg/mL are presented in Fig. 6.

Correlation between TPC, antioxidant activity, and the protective effect against DNA damage

The correlation between total antioxidant activity and TPC was also calculated. TPC and DPPH RSA IC_{50} exhibited a strong negative correlation ($r=-0.866$, $P=0.000$). ORAC activity was positively associated with TPC ($r=0.806$, $P=0.001$) and negatively associated with DPPH RSA IC_{50} ($r=-0.871$, $P=0.000$). In this study, we found a weak positive correlation ($r=0.575$, $P=0.006$) between TRAP values and ORAC activity, and a negative correlation ($r=-0.784$, $P=0.000$) between TRAP values and DPPH RSA IC_{50}. Further, the protective activity of Chunjang against DNA damage (comet IC_{50}) was negatively correlated with TPC ($r=-0.661$, $P=0.01$). Similar to our results, other studies have shown strong linear correlations between TPC and results from one or more antioxidant activity assays (Jimenez-Alvarez et al., 2008; Song et al., 2010; Fu et al., 2011).

Consequently, the results of this study indicate that Chunjang, which contains organic food or caramel, possesses antioxidant activity. However different Chunjang samples, show significant differences in TPC, antioxidant activity, and protection against DNA damage. These differences are may be influenced by the origins, materials, processing methods, and fermentation of the different samples.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

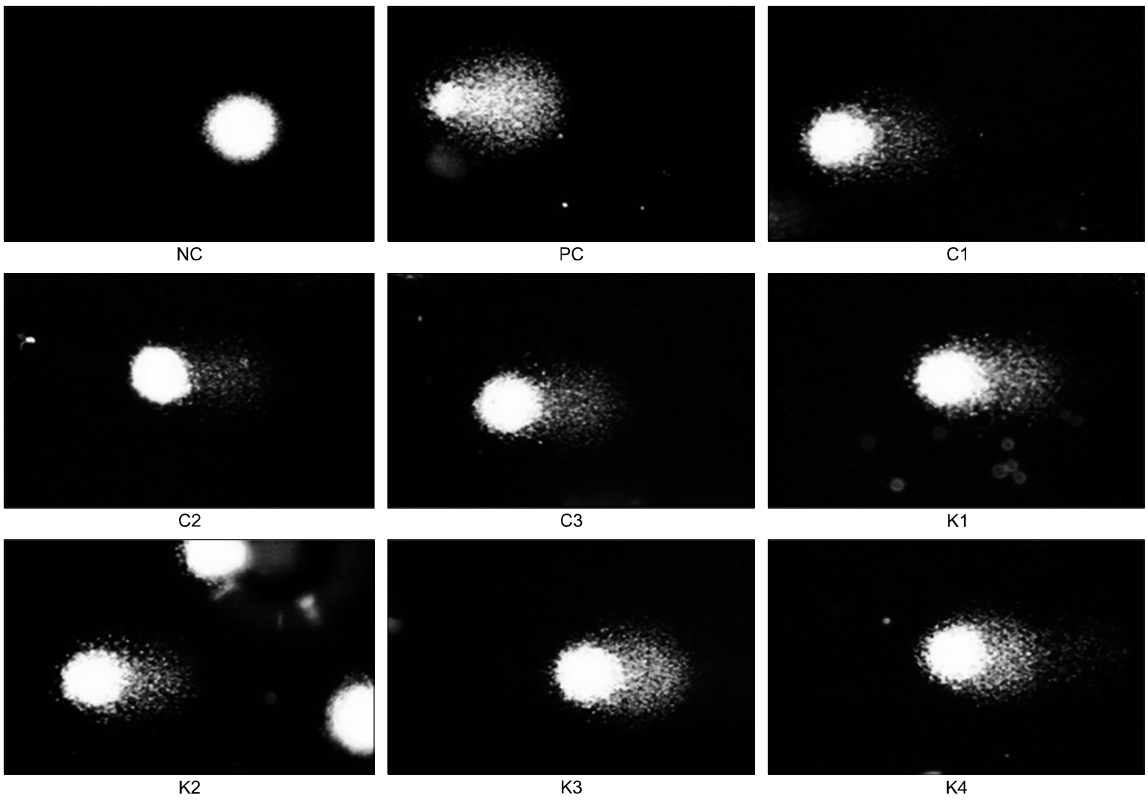

Fig. 6. A visual photograph of comet-shaped DNA isolation at the 50 μg/mL of commercial Chunjang samples [Chunjang from China (C1~3) and Korea (K1~4)].

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