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ARTICLE

# Characteristics of Gouda Cheese Supplemented with Korean Traditional Yakju

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**Abstract**

The quality properties of Gouda cheese supplemented with Korean traditional Yakju (*Acanthopanax senticosus* or *Pueraria thunbergiana* wines) were investigated. Yakju was added in the process of Gouda cheese preparation, and proximate composition, lactic acid bacterial population, pH, water-soluble nitrogen, sensory characteristics and proteolysis were determined. The electrophoretic patterns of cheese proteins, the target functional components and thiobarbituric acid values of the cheeses also were analyzed. The sensory characteristics including appearance of the cheeses were not affected by supplementing Yakju. Significantly higher amounts of crude ash, minerals and polyphenols were observed in the cheese supplemented with Yakju compared to the control cheese. The results suggest that the Gouda cheese prepared with Yankju has functional and additional nutrient values without changing cheese characteristics.

**Key words**: Gouda cheese, yakju, quality property, medicinal wine, supplementation

## Introduction

Gouda cheese is a wheel-shaped Dutch cheese that has been traditionally produced with unpasteurized raw milk (Van Slyke and Publow, 1913), and it was regularly exported to the eastern and northern European countries. Today, it is manufactured also in the EU, United States, and Japan (Herbst, 2007). In Korea, Gouda cheese was introduced to dairy farmers through cheese making education in 1997, and has been currently produced and commercialized by farmstead cheese factories and small-scale cheese makers (Bae and cho, 2006). Functional food markets have been growing worldwide including in Korea over the past decade. Since Korean traditional liquor is brewed with special herbal medicine, it is considered effective in curing various diseases such as private treatment methods. Korean traditional liquor was verified to have cell toxicity effects on DLD-1, one of colorectal cancer cell lines (Kim *et al.,* 2004). One of the most popular functional beverages consumed in Korea is medicinal-wine. Medicinal-wine has been traditionally consumed as a functional alcoholic beverage in Korea (Kim *et al*., 2000). Fruit beverage manufacturers have produced commercial medicinal-wines supplemented with functional ingredients, and Yakjus comprise 0.5% (10,270 kL/year) of the alcoholic fruit beverage market in Korea. Various medicinal plants such as *Acanthopanax senticosus* and *Pueraria thunbergiana* have been used to produce Yakjus in Korea.

*Acanthopanax senticosus* (*Gasiogapi*) is a species of woody shrub in the family Araliaceae (Kim, 1997). It is a plant in the genus *Acanthopanax* spread in the highlands of Korea, Manchuria, Japan, China and Siberia, which has thin and long thorns on the entire stem (Nationwide Oriental Medicine College Medicinal Plants Professors). It is often colloquially referred to as Siberian ginseng. It has been reported to have antioxidant (Kim *et al.,* 2009; Lin and Huang, 2000), anti stress (Davydov and Krikorian, 2000), anticancer (Hacker and Medom, 1984; Yoko *et al.,* 2000), and anti allergic properties (Jeong *et al.,* 2001; Yi *et al.,* 2001; Yoon *et al.,* 2002).

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*Pueraria thunbergiana* (Arrow root, *kudzu*) is a perennial deciduous tendril plant in the pea family Fabaceae in terms of systematic botany. It is located in the temperate and tropical zones including Northeast Asia, South America and the United States (Oh, 1990). *Pueraria thunbergiana* survives well, so it grows naturally in the fields and mountains of all parts of Korea (Kim *et al.,* 1996). The roots of *Pueraria thunbergiana* have been used as ingredients to make starch and tea and as medicine for a long time. In this way, *Pueraria thunbergiana* has a wide range of uses (Oh, 1990).

In this study, the effects of adding Korea traditional liquor, Yakju, to natural cheese like Gouda cheese on the physicochemical and other properties of the cheese were examined in order to develop Korean type natural cheese with western cheese quality.

## Materials and Methods

**Yakjus**

Yakjus made of *Acanthopanax senticosus* (AS) or *Pueraria thunbergiana* (PT) were purchased at local markets in Suncheon, Korea.

**Preparation of Gouda cheese**

Gouda cheese was prepared as described previously (Hill, 2007; Mistry *et al*., 1996) with some modifications. Raw milk was obtained from the dairy farm of Suncheon National University, Suncheon, Korea. AS or PT medicinal wines, equivalent to 4.0% total in milk volume, were supplemented to fresh raw milk, and the milk containing AS or PT wines was pasteurized at 63°C for 30 min and cooled to 32°C. The milk was held in a vat, inoculated with Probat 505 starter (*Lactococcus lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris, L. lactis* subsp. *lactis* biovar. *diacetylactis, Leuconostoc mesenteroides* subsp. *cremoris,* Danisco, Denmark) at an inoculant concentration of 1.0% ($10^{10}$ CFU/mL) and incubated for 45 min. After incubation, rennet (Naturen 290, Christian Hansen, Denmark) was supplemented at 19 mL/100 kg of milk to coagulate the milk. Curds were formed, cut into 10 mm square pieces and agitated for 30 min. Then the whey (30%) was drained, and the same volume of hot water (~75°C) was supplemented to the curds to dilute the lactose concentration in the whey and to keep a final temperature of 38°C. After 1.0 h of agitation, half of the remaining whey was removed and the curd was pressed with the same weight of the curd for 1.0 h. When the pH of the cheese reached 5.3, it was pressed with twice the curd weight for 2.0 h, immersed in 20% brine for 8 h/kg and ripened at 14°C with a 90-95% relative humidity for 15 wk.

**Enumeration of lactic acid bacteria (LAB)**

The numbers of LAB were monitored every 3 weeks during ripening. Samples were mixed with sterilized saline at a ratio of 1:2 and homogenized (M. Zipper GmbH, Germany) at maximum speed for 2 min. According to the method of Andrew (1983), 1.0 mL of homogenized sample was aseptically diluted with sterilized saline and spread on MRS agar (BBL/Difco, USA). The MRS agar plates were incubated at 37°C for 48 h and the colonies were counted.

**pH measurement**

The cheese samples were homogenized with sterile saline at the ratio of one to two and measured using the pH meter (IQ Scientific Instruments Inc, USA).

**Water soluble nitrogen (WSN)**

In order to measure the total protein decomposition level while ripening the cheese, change in WSN (water soluble nitrogen) was measured according to the method of Bütikofer (1993), and Hull *et al.* (2006).

That is, the sample for measuring its change was homogenized and centrifuged (Supra 25K, Hanil Science Industrial, Korea)as shown in the pH measurement and then the filtrate (Whatman No.2) was colored according to the method of Hull (1947). Next the content of WSN was measured in 570 nm by using the UV- Spectrophotometer (Smart Plus Spectrophotometer Co., Korea). The content of a nitrogenous compound was calculated according to the linear regression equation obtained by making tyrosine as a standard substance.

**Gel electrophoresis of casein**

Polyacrylamide gel electrophoresis of a casein sample was performed according to the method of Laemmli (1970) and Creamer (1982). The cheese sample was prepared by an addition of 6 ml of TCA (12.0%) to the cheese (0.3 g), sedimentation, and filtration with a filter paper (Whatman No. 42). The filtrates were dissolved with 0.076 M Tris-citrate buffer (pH 9.0) at ca. 30 mg/mL. 40 μL of solution dialyzed with electrode buffer for 48 h were boiled in the SDS sample buffer (×5) for 3 min to denature the proteins. The boiled samples were carefully loaded up to 10 μL and the current level was adjusted to 30 mA for a 1.5-mm-thick gel and electrophoresis started. The gel was prepared at a pH of 8.8 and

15%. Whole casein (Bio-Rad Laboratories, Hercules, CA, USA) was used as a standard protein marker. After the electrophoresis had been completed, the gel was stained with Coomassie brilliant blue Gel Stain, then bleached, and photographed.

**Determination of polyphenol content**

To determine the polyphenol content in the cheeses, extracts (100 mL) were obtained by adding ethanol to the homogenized samples. Extracts (5 mL) obtained after discarding the first 20 mL were discarded and mixed with 5 mL of ferrous tartrate stock solution (100 mg $FeSO_4$ $7H_2O$ + 500 mg/100 mL $H_2O$ Rochelle salt), and the pH was adjusted to 7.5 with 0.066 M $Na_2HPO_4$ $2H_2O$ + 0.066 M $KH_2PO_4$. The absorbency of the treated extracts was then measured at a wavelength of 540 nm using a spectrophotometer.

**Proximate composition analysis**

AOAC methods (1990) were used to determine the moisture, protein, and fat content in the cheeses.

**Analysis of thiobarbituric acid (TBA) value**

Cheeses that had completely ripened were stored in a refrigerator for 0-5 wk and the rancidity was measured. Cheese samples (20 g) were supplemented to 50 mL of 2.0 M phosphoric acid with 20% TCA to obtain extracts. Residues of the extracts were diluted in 40 mL of distilled water, homogenized, and filtered with Whatman no. 1 paper. Then, the filtrates (5 mL) were mixed with 2-thiobarbituric acid and maintained for 15 h at room temperature. Next, the absorbency was read at 530 nm using a visible spectrum spectrophotometer (Model 20D', Milton Roy, Ivyland, USA).

**Sensory evaluation**

The cheeses were tested in triplicate by a highly trained panel of seven assessors at Kansas State University°Øs Sensory Analysis Center, KS, USA. A 0-15 scale was used and the answers to 27 questions were evaluated (Otremba *et al*., 2000; Shin *et al.*, 2000).

**Statistical analysis**

The data was analyzed using the ANOVA procedure in Statistical Analysis Systems software (SAS Institute, Cary, NC, USA). Fisher's least significant difference test was used to determine differences in chemical compositions and polyphenol content between cheeses supplemented with medicinal wines and those without supplemented wine. The significance was detected at the 95% confidence level ($p \leq 0.05$).

## Results and Discussion

**Appearance**

Fig. 1 show the appearance of the Gouda cheeses made with Korean traditional Yakju matured for 15 wk. There were no significant differences in the appearance of the cheeses between the Control and sample cheeses; however, the cheese supplemented with wines tended to become slightly dark yellow in color. It was considered that dark colored Gouda cheese can be favored by market consumers.

**Composition**

The chemical composition and mineral composition of the Gouda cheese supplemented with Yakjus is shown in Table 1 and Table 2. The moisture contents were similar among the cheeses; the control cheese was at a range to 36.2%, AS or PT wines had the contents of 35.4 and


**Fig. 1. Appearance of Gouda cheese supplemented with traditional Korean Yakju.** 1, Control Gouda cheese; 2, *Acanthopanax senticosus* (AS) wine supplemented cheese; 3, *Pueraria thunbergiana* (PT) wine supplemented cheese.

**Table 1. Chemical composition of the Gouda cheese supplemented with traditional Korea Yakju**

| Component (%) | Wine-supplemented cheeses[1] | | |
|---|---|---|---|
| | Control | AS wine | PT wine |
| Moisture | 36.2 ± 0.40 | 35.4 ± 0.20 | 36.3 ± 0.36 |
| Crude ash | 3.3 ± 0.11 | 4.7 ± 0.05 | 4.0 ± 0.15 |
| Crude protein | 27.2 ± 0.20 | 29.8 ± 0.35 | 29.5 ± 0.05 |
| Crude fat | 33.3 ± 0.30 | 30.7 ± 0.15 | 30.2 ± 0.15 |

Mean±SD ($n$=15).
Means in each column with different superscripts differ significantly ($p<0.05$)
[1]Contral, Gouda cheesse; Aswine, *Acanthopanax senticosus* (AS) wine supplemented chees; PT win, *Pueraria thunbergiana* (PT) wine supplemented cheese

36.3%. The control cheese had a crude protein content of 27.2%, while the cheese made with AS or PT wines had the contents of 29.8 and 29.5%. The crude fat content of the control cheese was 33.3%, and that with Yakju ranged from 30.7-30.2%. The crude ash content was lower in the control cheese compared with those containing Yakju. Cheese supplemented with AS wine had the highest crude ash (4.7%) among the cheese samples. The mineral content (Fe, mg) was significantly higher in checse containing AS wine and PT wine compared with those of Yakju. This result was partly caused by the high amount of crude ash and minerals (5951 mg of K/100 g and 286 mg of Mg/100 g) in AS wine (Kim *et al*., 2006).

**Lactic acid bacteria (LAB)**

Populations of LAB were significantly higher in the cheeses supplemented with Yakjus than in the Control (Fig. 2). Cheese supplemented with PT wine had slightly higher numbers of LAB than those made with AS wine in the beginning of ripening. The LAB population in the cheese with AS wine was low at the initial ripening time, but after 6 wk, it was higher than those of the Control cheese, which was due to the biologically active substances that originated from the wine.

**pHs**

The pH values (5.10-5.29) of the cheese supplemented with wines were slightly lower than that of the Control cheese. Cheeses with and without wines changed to normal pH of standard Gouda cheese (pH 5.20). The increase of the pH during ripening may be caused by decarboxylation and deamination of amino acids in the cheeses (Bachmann *et al.,* 1999).

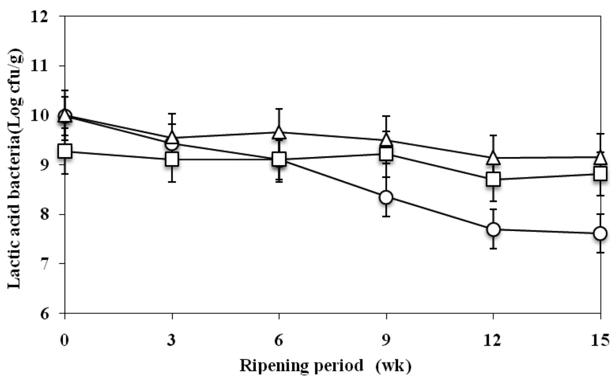


**Fig. 2. Changes in LAB count of wine supplemented Gouda cheese during ripening.** ○-○, Control Gouda cheese; □-□, AS wine supplemented cheese; △-△, PT wine supplemented cheese

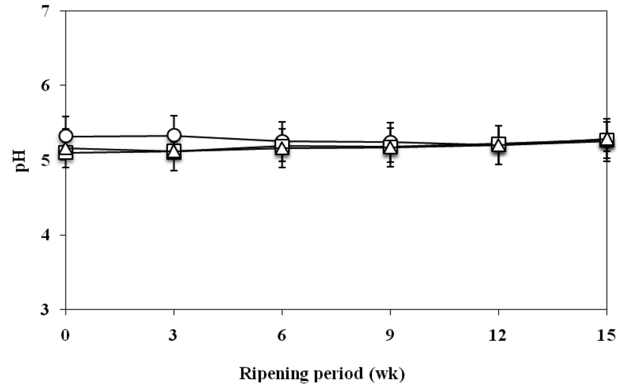


**Fig. 3. Changes in pH of wine supplemented Gouda cheese during ripening.** ○-○, Control Gouda cheese; □-□, AS wine supplemented cheese; △-△, PT wine supplemented cheese

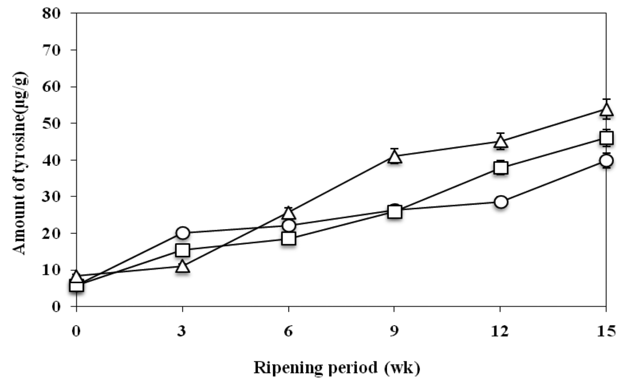


**Fig. 4. Changes in water-soluble nitrogen (WSN) content of wine supplemented Gouda cheese during ripening.** ○-○, Control Gouda cheese; □-□, AS wine supplemented cheese; △-△, PT wine supplemented cheese

**Water-soluble nitrogen (WSN)**

The content of WSN in the cheeses was changed rapidly during the ripening period (Fig. 4), resulting from proteolysis. Higher amounts of WSN were observed in the Control cheese until 3 wk, but the amount became lower after 9 wk compared to the cheeses supplemented with wines. The WSN content in cheeses with Yakjus increased continuously for 12 wk, whereas the WSN content in the Control cheese did not. The increase of WSN in the cheese generally comes from casein degradation, and it usually depends on the proteases produced by the lactic starters and the nitrogen associated with the rennet used in cheese manufacturing (Galan *et al*., 2008). It was also noted that protease produced by LAB in cheeses causes constant protein degradation (Sallami *et al*., 2004). Apparently, the compounds in Yakjus also promoted continual degradation and increased WSN throughout.

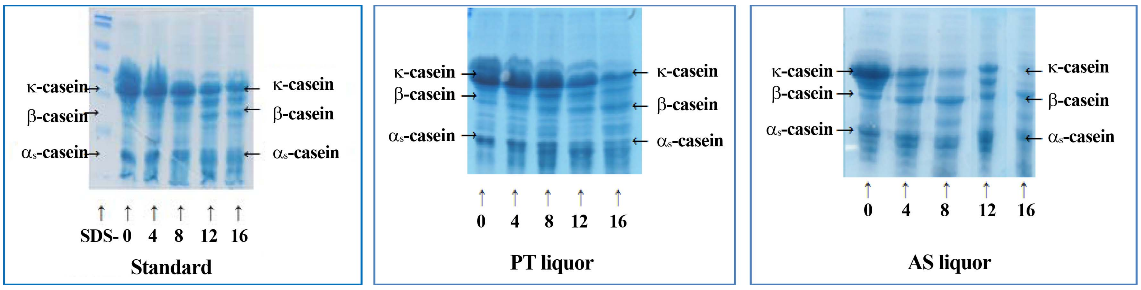


**Fig. 5. Proteolytic activity of Gouda cheese supplemented with traditional Korean Yakju during ripening PT wine.**

### Casein proteolysis

Fig. 5 shows the electrophoretic patterns of the cheese proteins during ripening. The samples were obtained at 4-wk intervals for 16 wk and analyzed by SDS-PAGE as described previously (Andrews, 1983). Several bands that were displayed on the gel indicate that the cheeses with wines were ripened with significant degradation compared to the Control cheese. This result was similar to the report by Fox *et al.* (Fox and Stepaniak, 1993). It is well established that $\alpha_s$-casein, β-casein and k-casein were gradually hydrolyzed as the cheese ripened and the $\alpha_s$-casein proteolysis occurrence was almost similar. In this study, $\alpha_s$-casein was not detected after 8 weeks of ripening, implying proper degradation patterns in the cheese samples.

### Determination of polyphenols, anthocyanin and flavonoids content

Table 3 shows the amounts of polyphenols, anthocyanin and flavonoids in the Yakjus used in this study. AS wine had higher amounts of polyphenols (2.1 mg%) and flavonoids (36.3 mg%) than PT wine, but the amount of anthocyanin (0.3 mg%) was similar. The polyphenol content in the cheeses made with or without Yakjus is listed in Table 3. The contents of polyphenols in cheese made with wines were comparatively high; 19.91-20.05 mg/100 g for the cheese during the initial 9 wk. Its contents remained at these levels until the end of ripening.

**Table 2. Mineral composition of Gouda cheese supplemented with Korea traditional Yakju**

| Mineral Component | Wine-supplemented cheeses | | |
|---|---|---|---|
| | Control | AS wine | PT wine |
| Fe | 1.4±0.05 | 4.0±0.15 | 3.2±0.20 |
| Mg | 7.0±0.15 | 37.6±0.15 | 19.7±0.05 |
| Ca | 542.5±0.35 | 346.6±1.24 | 272.3±5.30 |

Mean±SD ($n$=15), Means in each column with different superscripts differ significantly ($p$<0.05).

**Table 3. Polyphenol, anthocyanin and flavonoid contents in Korea traditional Yakju** (mg%)

| | Polyphenol | Anthocyanin | Flavonoids |
|---|---|---|---|
| AS wine | 2.1±0.05 | 0.3±0.35 | 36.3±0.20 |
| PT wine | 1.9±0.11 | 0.3±0.26 | 21.3±0.28 |

Mean±SD ($n$=15), Means in each column with different superscripts differ significantly ($p$<0.05).

### Analysis of thiobarbituric acid

The TBA values of the cheeses supplemented with AS or PT wines were periodically measured for 9 mon after ripening (Fig. 6). The TBA levels of all cheeses were similar during the initial storage time of 2 mon. The TBA value of cheese supplemented with PT wines decreased slightly thereafter, and it was not changed until 9 mon, whereas the value of the cheese supplemented with AS wine increased rapidly after 7 mon. The TBA values of the cheeses with AS or PT wine ranged between 5 and 20 mmol/100 g, after 9 mon of storage. However, the value of the Control cheese increased dramatically after 3 to 6 mon but decreased gradually thereafter. After 9 mon of storage, the TBA value reached 40 mmol/100 g. These results showed that antioxidant materials i.e. polyphenols,

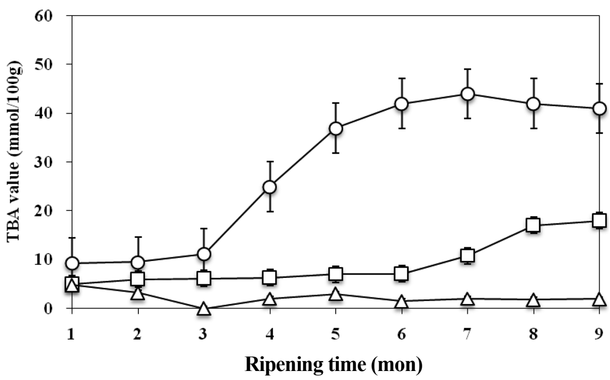


**Fig. 6. Changes in Thiobarbituric acid (TBA) values of wine supplemented Gouda cheese during ripening.** ○-○, Control Gouda cheese; □-□, AS wine supplemented cheese; △-△, PT wine supplemented cheese

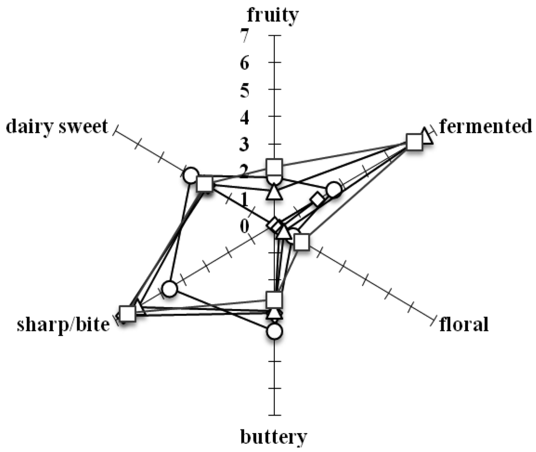


**Fig. 7. Sensory evaluation of Gouda cheese supplemented with Korea traditional Yakju (○-○), imported Gouda cheese (◇-◇), with AS wine (□-□) and PT wine (△-△).**

retarded the oxidation process for the cheeses with Yakju. It is suggested that adding Yakjus to cheeses can directly impact the shelf life of the cheese products.

### Sensory evaluation

The sensory attributes of the test Gouda cheeses made were compared with imported Gouda cheese after 14 wk of the ripening period (Fig. 7). Cheese flavor, such as the Thage *et al.* (2004) reports that leucine, and the valine amino acids were important functional flavors. A fermented taste and a sharpness/bitterness were predominant in the cheeses supplemented with AS or PT wine. However, typical flavors of dairy sweetness and fruity, buttery, floral tastes were not affected by the addition of Yakju.

The results of this study indicate that adding AS or PT wine to Gouda cheese provides several benefits; adding the wines increased the lactic acid bacterial populations in the cheese by forming biologically active substances. The higher populations of LAB in the cheeses produced more proteases, resulting in a higher level of WSN. Furthermore, the polyphenols in Yakjus possibly retard cheese oxidation and prolong the shelf-life of the products. Therefore, it is concluded that the quality of Gouda cheese was not affected by adding Korean traditional wines. Also various components in the wines had functionalities for the cheese products.

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