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Note

Physicochemical Properties of Yanggaeng with Added Tempeh Powder

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ABSTRACT: In this study, we investigated the physicochemical and antioxidative properties of the traditional Korean confectionery, Yanggaeng, when various amounts of tempeh powder (TP) were added. We replaced a portion of the white bean paste in Yanggaeng with TP at percentages of 0% (CON), 2% (TP2), 4% (TP4), and 6% (TP6) by total weight. The proximate composition results showed that TP6 exhibited the highest crude ash and crude protein contents, but its moisture content and carbohydrate content were the lowest compared to the CON. Tempeh addition altered the colorimetric properties by increasing the L^* value, b^* value, and browning index; however, tempeh addition did not alter the a^* value. The results also showed that tempeh addition gradually decreased the pH of Yanggaeng. The Brix value was the highest in TP2; in TP4 and TP6, the Brix value gradually decreased, and these formulations exhibited lower Brix values than the CON. Furthermore, tempeh addition gradually induced antioxidative capacities, as evidenced by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activities. The results of this study demonstrate that the addition of tempeh to Yanggaeng alters its physicochemical properties and antioxidative capacity.

Keywords: antioxidative capacity, physicochemical properties, tempeh, Yanggaeng

INTRODUCTION

Yanggaeng is a traditional Korean confectionery made from bean paste combined with agar and sugar (Choi and Lee, 2015). Over the years, Yanggaeng has maintained its reputation as a beloved snack for people of all ages thanks to its delightful sweetness and its soft, chewy texture. Consequently, Yanggaeng is frequently packaged as a gift and enjoyed during special occasions in Korea, such as the Lunar New Year (Yoon et al., 2018). Its compact packaging makes Yanggaeng a versatile choice, meaning Yanggaeng can serve as an alternative food in scenarios such as providing an energy boost for the elderly (Park et al., 2022), or as a convenient snack to replenish energy during physical activity (Kim et al., 2014). However, due to its high sugar content, excessive consumption of Yanggaeng can lead to health problems. A diet rich in refined sugars can elevate the risk of metabolic syndrome, including conditions like dyslipidemia, insulin resistance, cardiovascular disease, diabetes, and obesity (Macdonald, 2016).

Tempeh, originating from Indonesia, is a fermented soybean product created through the action of the mold species *Rhizopus oligosporus* (Aryanta, 2020). Tempeh has served as an affordable source of high-quality protein for Indonesians (Subali et al., 2023) for over three centuries (Shurtleff and Aoyagi, 2022). In addition to its excellent nutrient density, tempeh is a complete protein source, containing all nine essential amino acids, which sets it apart from most other plant-based proteins (Reese, 2021). Tempeh also boasts a high concentration of biologically functional compounds, including polyphenolic compounds, particularly isoflavone aglucone, making it an antioxidant-rich fermented food (do Prado et al., 2022). Furthermore, tempeh is low in saturated fats and cholesterol compared to common animal-based protein sources such as meat and eggs. Notably, tempeh also contains prebiotic content, which may promote intestinal health, and is a good source of calcium (Reese, 2021).

Microbial fermentation leads to the hydrolysis of protein compounds, facilitating the efficient absorption of fer-

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mented protein products in the digestive system. Therefore, fermented foods such as tempeh are easily digested, absorbed, and utilized by the human body. The molds (*Rhizopus* sp.) involved in the tempeh-generating process break down complex nutrients into smaller molecules. Bean proteins exposed to *Rhizopus* sp. generate isoflavone and aglucone as metabolites (Liu et al., 2023). Two controlled studies conducted by Su et al. (2023) showed that consistent consumption of tempeh could lower the blood pressure, reduce *de novo* adipogenesis, and prevent type 2 diabetes. A peer-reviewed study by Lecerf et al. (2020) suggested that the consumption of soybean-based products was associated with a reduced risk of type 2 diabetes due to the low glycemic index and high isoflavone content of soybeans. Nitric oxide, synthesized from arginine, an amino acid compound found in tempeh, has been shown to reduce fat mass in obese and diabetic rats (Jobgen et al., 2006). Nitric oxide also plays a role in glucose and glycogen regulation, insulin release, and diabetes prevention (Bahadoran et al., 2020) by increasing intestinal glucagon-like peptide-1 secretion (Douglas et al., 2015; Boer et al., 2020).

In response to the modern consumer's preference for functional foods that promote health, several studies have been conducted to explore the addition of functional ingredients to Yanggaeng. The goal is to decrease the sugar content while boosting the protein levels in Yanggaeng. Recent initiatives focused on elevating the protein content of Yanggaeng have explored the incorporation of ingredients such as lentil beans (Noh et al., 2016), mealworms (Lee et al., 2021), and dry shrimp (Park et al., 2022). Numerous studies have also focused on enhancing the health-promoting properties of Yanggaeng by incorporating ingredients that are rich in functional and nutritional components. In this study, various concentrations of tempeh were added to Yanggaeng to create a novel high-protein snack. The proximate composition, physicochemical properties, and radical scavenging capacities of the different Yanggaeng were investigated.

MATERIALS AND METHODS

Preparation of tempeh-treated Yanggaeng

Frozen tempeh was imported from Bumi Food (Frozen Food: Tempeh, BumiFood Industry) and mechanically ground using an automated blender (HR2904, Philips Co.). The resulting powdery product, tempeh powder (TP), was used for further formulation. To prepare the Yanggaeng mixture, 10 g of agar (Thehadam) was mixed with 400 g of water and the mixture was left to stand for 10 min before being heated over medium heat for approximately 2 min. Various concentrations of TP were added to create mixtures containing 0% (CON, *w/w*), 2% (TP2, *w/w*), 4% (TP4, *w/w*), or 6% (TP6, *w/w*) TP, as summarized in Table 1. TP was added to the water-agar mixture, followed by the inclusion of white bean paste (Daedoo Food) and white sugar (Cheiljedang). The mixture was gently stirred over low heat for 10 min. Subsequently, the heated mixture was poured into a square-shaped mold with a thickness of 2 cm and left to solidify at room temperature for 2 h.

Proximate analysis of Yanggaeng

The proximate characteristics of Yanggaeng, including crude ash, crude fat, moisture, crude protein levels, and carbohydrates, were assessed according to a well-established analytical method described previously (Bae et al., 2019; Ha et al., 2019).

Chromaticity of Yanggaeng

The L^*, a^*, and b^* values of Yanggaeng were determined using a spectropolarimeter (LC100, Tintometer Ltd.), and the browning index (BI) was calculated from the assessed L^*, a^*, and b^* values, following a previously described method (Kim et al., 2021; Jang et al., 2023a).

pH and Brix of Yanggaeng

Two grams of Yanggaeng was suspended in 20 mL of distilled water and centrifuged at 3,000 *g* for 10 min (Hanil Science Co., Ltd.), then the supernatant was collected. Subsequently, the pH and Brix value were determined using a pH meter (Orion Star, Thermo Fisher Scientific), and a Brix meter (HI 96801, Hanna Instruments), respectively, as described previously (Jang et al., 2023a, 2023b; Lee et al., 2023).

Antioxidant capacity of Yanggaeng

The total flavonoid contents (TFC) and total phenolic contents (TPC) were determined using established peer-reviewed methods (Kim et al., 2021). Quercetin and gallic acid were employed as reference materials for TFC and TPC measurement, respectively, and the results were reported as quercetin equivalents (mg QE/g) and gallic acid equivalents (μg GAE/g), respectively. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical

Table 1. Tempeh powder-added Yanggaeng formulations

Ingredient (g)	CON	TP2	TP4	TP6
White bean paste	500	490	480	470
Water	400	400	400	400
White sugar	100	100	100	100
Agar powder	10	10	10	10
Tempeh powder	0	10	20	30

CON, Yanggaeng prepared with 0% tempeh powder; TP2, Yanggaeng prepared with 2% tempeh powder; TP4, Yanggaeng prepared with 4% tempeh powder; TP6, Yanggaeng prepared with 6% tempeh powder.

Table 2. Proximate compositions of Yanggaeng treated with tempeh powder (%)

Proximate composition	CON	TP2	TP4	TP6
Moisture	44.59±0.25[NS]	45.83±0.75	48.32±0.66	44.31±0.23
Crude ash	0.04±0.02[ab]	0.06±0.03[ab]	0.01±0.05[b]	0.09±0.03[a]
Crude fat	0.43±0.30[NS]	0.84±0.61	1.06±0.59	0.30±0.16
Crude protein	6.77±0.84[c]	9.76±3.21[bc]	13.56±1.66[ab]	14.33±1.90[a]
Carbohydrate[1)]	48.17±1.13[a]	43.50±2.11[ab]	41.07±2.06[b]	40.96±1.92[b]

CON, Yanggaeng prepared with 0% tempeh powder; TP2, Yanggaeng prepared with 2% tempeh powder; TP4, Yanggaeng prepared with 4% tempeh powder; TP6, Yanggaeng prepared with 6% tempeh powder.
Data are presented as mean±SD (n=3). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.
Means with different letters (a-c) in the same row are significantly different at $P<0.05$. NS, not significant.
[1)]Carbohydrate = 100 − (moisture + crude ash + crude fat + crude protein).

Table 3. Hunter's colorimetric properties of Yanggaeng treated with tempeh powder

	CON	TP2	TP4	TP6
L^*	42.27±0.15[d]	45.17±0.21[b]	48.13±0.57[a]	43.47±0.12[c]
a^*	−0.70±0.20[NS]	−0.53±0.06	−0.77±0.06	−0.60±0.00
b^*	2.50±0.20[d]	5.07±0.06[c]	5.73±0.21[b]	7.53±0.06[a]
BI	4.74±0.75[c]	10.74±0.23[bc]	11.19±0.40[ab]	17.54±0.19[a]

CON, Yanggaeng prepared with 0% tempeh powder; TP2, Yanggaeng prepared with 2% tempeh powder; TP4, Yanggaeng prepared with 4% tempeh powder; TP6, Yanggaeng prepared with 6% tempeh powder.
Data are presented as mean±SD (n=3). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.
Means with different letters (a-d) in the same row were significantly different at $P<0.05$. NS, not significant.
L^*, lightness; a^*, redness; b^*, yellowness; BI, browning index.

scavenging capacities were also determined following previously published protocols (Kim and Yook, 2022; Lee et al., 2022).

Statistical analysis

The data were expressed as the mean and standard deviation from three independent measurements. The values were ranked and analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test, performed using the XLSTAT 2012 (Addinsoft, Inc.). The significance threshold for statistical differences was set at $P<0.05$.

RESULTS AND DISCUSSION

Proximate composition of Yanggaeng

The moisture, crude ash, crude fat, crude protein, and calculated carbohydrate content of the Yanggaeng are presented in Table 2. The moisture and crude fat content of the Yanggaeng did not vary significantly with the addition of tempeh. The crude ash content was lowest in TP4 and highest in TP6; however, considering the overall Yanggaeng composition, the statistical difference was negligible. As expected, the addition of tempeh, a protein-rich source, significantly and gradually increased the crude protein content of the Yanggaeng. The addition of a plant protein-rich source as a food additive in Yanggaeng generally leads to an elevation in crude protein content (Hu et al., 2022). Meanwhile, as the additional protein content replaced the white sugar content, the carbohydrate content gradually decreased (Gillespie et al., 2023) in the tempeh-added Yanggaeng.

Colorimetric properties of Yanggaeng

Table 3 presents the colorimetric properties of the various Yanggaeng. With an increase in TP, the overall L^* (lightness) values of Yanggaeng significantly increased, resulting in enhanced color contrast (Fig. 1 and Table 3). Specifically, the L^* values for Yanggaeng treated with TP2, TP4, and TP6 were 45.17, 48.13, and 43.47, respectively, while the control (no TP) showed an L^* value of 42.27. Meanwhile, increased tempeh addition significantly improved the overall a^* (redness) values of the Yanggaeng (Fig. 1 and Table 3). The a^* values for the CON, TP2, TP4, and TP6 were −0.70, −0.53, −0.77, and −0.60, respectively. Among these, TP2 exhibited the highest a^* value, while TP4 displayed the lowest a^* value. Furthermore, when using the acquired L^*, a^*, and

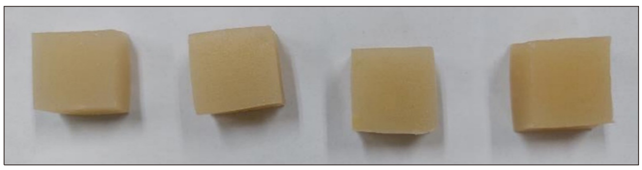

Fig. 1. External features of Yanggaeng made with tempeh powder. CON, control (no added tempeh powder); TP2, Yanggaeng prepared with 2% tempeh powder; TP4, Yanggaeng prepared with 4% tempeh powder; TP6, Yanggaeng prepared with 6% tempeh powder.

b^* (yellowness) measurements to calculate the BI, it was observed that the b^* values for TP2, TP4, and TP6 were 5.07, 5.73, and 7.53, respectively, which were all significantly higher than the b^* value of the control (2.50). A similar trend was observed in the BI values, which were 10.74, 11.19, and 17.54 for TP2, TP4, and TP6, respectively, which were all significantly higher than the BI value of the control Yanggaeng (4.74).

The increased b^* and BI values of the Yanggaeng samples with added tempeh can be attributed to the inherent browning characteristics of tempeh. It is possible that the darker color is generated by the Maillard reaction (Kathuria et al., 2023) due to the additional protein content of tempeh and the sugars in Yanggaeng. If the Maillard reaction is enhanced in tempeh-added Yanggaeng, then the flavor and aroma would also be altered (Zhang et al., 2020). Alternatively, the increased b^* and BI values in the tempeh-added Yanggaeng may have been caused by an elevation in anthocyanin levels in the soybeans during microbial fermentation, which is a strong characteristic of tempeh (Anggraini and Mirantana, 2022). Therefore, physical modifications by fermentation in tempeh may create a red to reddish-brown color (Zheng and Tian, 2006), and the b^* and BI values may be affected. Further studies are needed to confirm whether the addition of tempeh darkens the color of Yanggaeng, as we used white bean paste, which may brown easily. If the browning of Yanggaeng is not widely accepted by consumers, then we may consider using black bean paste to minimize browning when adding tempeh to Yanggaeng.

pH and Brix values of the Yanggaeng

Table 4 shows the pH and Brix values of the formulated Yanggaeng. With an increase in the amount of tempeh-added, the overall pH of the Yanggaeng was significantly reduced. The pH was 6.69 in the control, and with the addition of tempeh, the pH gradually decreased to 6.62, 6.48, and 6.42 in the TP2, TP4, and TP6 samples, respectively. During microbial fermentation, pH is typically reduced due to the production of acidic metabolites (Zheng and Tian, 2006), including organic acids and lactic acid, as well as GABA (γ-aminobutyric acid) (Guo, 2013). Since tempeh undergoes fermentation during its production, it may have an acidic nature, which is why the addition of tempeh lowers the pH when used as a food additive. This change in pH may also enhance the textural properties, for example by improving the chewiness and softness, as demonstrated in a pilot study conducted by Jang (2023a). Further analysis of the textural properties is necessary in the follow-up study.

The addition of tempeh had an intriguing effect on the Brix value of the Yanggaeng. The Brix value was highest in TP2 (3.00), and was significantly higher than that of the control (2.70). However, TP4 (2.20) and TP6 (1.87) exhibited significantly lower Brix levels compared to the control. In our experimental recipe, a portion of the white bean paste was replaced by TP. In the control, there may have been a significant amount of undissolved white bean paste, which could explain why the relative Brix may have been higher in TP2. In the case of TP4 and TP6, despite having a higher amount of tempeh compared to the other groups, it exhibited a lower Brix, possibly because the Maillard reaction decreased the Brix in TP4 and TP6 (Buedo et al., 2000).

Antioxidative properties of Yanggaeng

Table 5 presents the TPC and ABTS radical scavenging activities of the tempeh-added Yanggaeng. Interestingly, there was an increasing trend in the TPC in the TP6 Yanggaeng compared to the other groups. This increase in TPC in TP6 may be attributed to microbial fermentation, which could enhance the level of phenolic compounds in tempeh, potentially leading to an elevation in TPC. The TPC values of the CON, TP2, and TP4 were 9.12, 9.13, and 9.69 μg GAE/g, respectively. Interestingly, the TPC value was far higher in TP6, measuring 16.11 μg GAE/g. Previous reports have clearly demonstrated that the hydrolysis of plant-based protein sources enhances the phenolic and/or flavonoid contents after enzymatic processing (Hu et al., 2022), as observed in our data.

Due to the production of TPC in TP6, we logically speculated that the TFC would also be elevated in tempeh-added Yanggaeng; however, flavonoids were not detectable in any of the samples (with a limit of quantification set at 100 mg QE/g; data not shown). This absence of detectable TFC could potentially be explained by the fact that white bean paste, a major component of Yanggaeng, is derived from kidney beans (*Phaseolus vulgaris*) with the outer skin removed (Kim et al., 2003). Functional ingredients, such as flavonols, are primarily found in the outer

Table 4. Variations in pH and Brix of Yanggaeng treated with tempeh powder

	CON	TP2	TP4	TP6
pH	6.69±0.02[a]	6.62±0.01[b]	6.48±0.01[c]	6.42±0.01[d]
Brix	2.70±0.00[b]	3.00±0.00[a]	2.20±0.06[c]	1.87±0.06[d]

CON, Yanggaeng prepared with 0% tempeh powder; TP2, Yanggaeng prepared with 2% tempeh powder; TP4, Yanggaeng prepared with 4% tempeh powder; TP6, Yanggaeng prepared with 6% tempeh powder.
Data are presented as mean±SD (n=3). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.
Means with different letters (a-d) in the same row are significantly different at $P<0.05$.

Table 5. Antioxidative activities of Yanggaeng treated with tempeh powder

	CON	TP2	TP4	TP6
TPC (μg GAE/g)	9.12±1.91[NS]	9.13±2.28	9.69±4.70	16.11±3.16
ABTS radical scavenging activities (inhibition %)	6.63±0.12[c]	6.60±0.40[c]	7.44±0.47[b]	12.27±1.44[a]

CON, Yanggaeng prepared with 0% tempeh powder; TP2, Yanggaeng prepared with 2% tempeh powder; TP4, Yanggaeng prepared with 4% tempeh powder; TP6, Yanggaeng prepared with 6% tempeh powder.
Data are presented as mean±SD (n=3). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.
Means with different letters (a-c) in the same row were significantly different at $P<0.05$. NS, not significant.
TPC, total phenol content; GAE, gallic acid equivalent; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

part of kidney beans. Therefore, the process of removing the skin to produce white bean paste could result in a significant loss of flavonoids, potentially rendering TFC undetectable in Yanggaeng. Consequently, we considered whether tempeh addition would enhance the radical scavenging capacities in Yanggaeng and measured the ABTS radical scavenging capacities. The CON and TP2 showed ABTS radical scavenging capacities of 6.63% and 6.60%, respectively, while TP4 and TP6 exhibited capacities of 7.44% and 12.27%, respectively, which were significantly higher than the capacities of either CON or TP2. Considering the TPC was only high in TP6, other unknown electron donors may act as antioxidants in tempeh-added Yanggaeng. Further in-depth studies are needed to identify which components enhance the radical scavenging activities. Potential components include isoflavon (Liu et al., 2023) and anthocyanin (Anggraini and Mirantana, 2022).

Conclusion

The physical and chemical properties of Yanggaeng with added TP vary depending on the concentration of addition. With the addition of tempeh, the crude protein content increased and carbohydrates decreased. The dark and brown color changed because L^*, a^*, and b^* values increased. Additionally, pH and brix also tended to decrease with the addition of tempeh. In the case of antioxidant activity, ABTS radical scavenging activities increased despite there being no significant increase in TPC. This research provides fundamental information to support the creation of innovative food items containing tempeh.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: A, IK, JHH. Analysis and interpretation: all authors. Data collection: A, JL, HJ, DK. Writing the article: A, JL, HJ, JHH. Critical revision of the article: all authors. Final approval of the article: all authors. Statistical analysis: A, JL, HJ, DK, JHH. Overall responsibility: JHH.

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