2025 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 15, Issue 4: 210-219; July 30, 2025

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2025.24

EFFECT OF TEMULAWAK (*Curcuma xanthorrhiza*) POWDER IN REDUCING PROTEOLYSIS IN FERMENTED TOTAL MIXED RATION WITH OKARA FOR RUMINANTS

Tekad Urip Pambudi SUJARNOKO[1], Martin Saputra LIM[2], Dwi BUDIONO[3], Novia Amalia SHOLEHA[1], Mochamad Dzaky ALIFIAN[4], Tri UJILESTARI[4], and Mohammad Miftakhus SHOLIKIN[4]✉

[1]*Chemical Analysis Study Program, Vocational School, Institut Pertanian Bogor (IPB) University, Bogor 16680, Indonesia*
[2]*Graduate School of Animal Nutrition and Feed Science, Faculty of Animal Science, Institut Pertanian Bogor (IPB) University, Bogor 16680, Indonesia*
[3]*Paramedic Veterinary Study Program, Vocational School, Institut Pertanian Bogor (IPB) University, Bogor 16680, Indonesia*
[4]*Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Bogor 16915, Indonesia*

✉Email: mohammad.miftakhus.sholikin@brin.go.id

Supporting Information

RESEARCH ARTICLE

PII: S222877012500024-15

Received: May 07, 2025

Revised: July 20, 2025

Accepted: July 21, 2025

ABSTRACT: High protein content in silage feed often triggered excessive proteolysis caused by proteases derived from both plants and spoilage bacteria, which reduced the nutritional quality for ruminants. Temulawak (*Curcuma xanthorrhiza*) is an herbal plant with antibacterial and antioxidant properties, which have been reported to reduce proteolysis in previous studies. This study aimed to evaluate the effects of temulawak powder (TP) as an anti-proteolysis additive in the fermentation of total mixed ration-based okara silage (TMRO-silage) and its impact on silage characteristics. Additionally, the study examined the effects of drying temperature on temulawak extract optimization. Temulawak was dried at 50, 65, and 80 °C, followed by the measurement of phenol, flavonoid, and ferric reducing antioxidant power (FRAP) content. The TMRO-silage was composed of commercial feed and okaras (1:1 w/w), supplemented with 0-1% temulawak in increments of 0.25% (5 treatments and 4 replications). Fermentation lasted for 14 days. Proximate and *in vitro* analyses (control vs. temulawak treatments) were conducted to assess silage quality. Drying temulawak at 65 °C significantly ($P < 0.01$) increased phenol (16.9 µg quercetin equivalent, QE), flavonoid (23.3 µg QE), and FRAP (30.1 µg QE) content per dry weight. Temulawak supplementation significantly reduced ammonia levels and increased the crude protein content of TMRO-silage ($P < 0.01$). Moreover, it decreased ammonia concentration in the rumen ($P < 0.01$), improved dry matter and organic matter digestibility ($P < 0.05$), and notably reduced methane production per total gas volume ($P < 0.05$). In conclusion, temulawak effectively preserves the quality of complete feed silage, enhances rumen metabolism, and mitigates methane emissions.

Keywords: Antibacterial activity, Antioxidant properties, Fermentation, Proteolysis, Silage quality.

INTRODUCTION

Ensuring high-quality feed for ruminants, particularly beef cattle, requires a high-protein intake, commonly achieved through high-protein silage. This need becomes even more critical when the silage has a high digestibility rate (Li et al., 2021). High-protein silage, such as alfalfa or leguminous plants, serves as a key commodity. Similarly, tofu byproducts still contain a significant amount of protein. Tofu byproduct, or okara, is a soybean-processing residue rich in protein (9.9-32.8%), fat (6.2-22%), crude fiber (4.1-23.4%), and essential minerals such as calcium, iron, and copper (Kamble and Rani, 2020; Ginting et al., 2024). However, its high moisture content (74-80.3%) makes it highly susceptible to microbial spoilage, which can degrade its nutritional quality and cause an unpleasant odor (Kamble and Rani, 2020). Fermenting okara with microorganisms such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus rhamnosus* enhances its nutritional profile. This process increases the concentration of small peptides (from 7.35% to 39.58%), enriches its amino acid and organic acid content, and improves digestibility (Suwarsito and Purbomartono, 2018; Kamble and Rani, 2020; Heng et al., 2022). As a result, fermented okara becomes a more stable and nutrient-dense alternative protein source for animal feed, and potentially for functional food applications. However, the absence of a controlled ensiling system presents several challenges, particularly the risk of excessive proteolysis (Hadidi et al., 2023). Therefore, supplementation with temulawak, an herbal additive containing bioactive compounds, became necessary to inhibit proteolysis. Reports also indicated its potential to improve ruminant production parameters (Adli et al., 2024; Sujarnoko et al., 2020).

Proteolysis occurs when proteins in the silage break down into non-protein nitrogen compounds, reducing the silage's nutritional value (Jayanegara et al., 2019; Okoye et al., 2023). This process is often exacerbated by plant protease activity

Citation: Sujarnoko TUP, Lim MS, Budiono D, Sholeha NA, Alifian MD, Ujilestari T, and Sholikin MM (2025). Effect of temulawak (*Curcuma xanthorrhiza*) powder in reducing proteolysis in fermented total mixed ration with okara for ruminants. *Online J. Anim. Feed Res.*, 15(4): 210-219. DOI: https://dx.doi.org/10.51227/ojafr.2025.24

and spoilage microorganisms, including *Clostridium* sp., *Enterobacteria*, *Pseudomonas*, molds (*Aspergillus* sp. and *Fusarium* sp.), and yeasts (*Candida* sp. and *Saccharomyces* sp.) (Ahangari et al., 2021; He et al., 2024; Snyder et al., 2024). These microorganisms not only degrade high-protein silage quality but also produce mycotoxins, such as those from *Aspergillus* sp. and *Fusarium* sp., which are toxic (Ekwomadu et al., 2021; Navale et al., 2021). Therefore, incorporating additives, such as *Curcuma xanthorrhiza* (temulawak), has become a primary strategy to mitigate these issues.

Curcuma xanthorrhiza has the potential to reduce protein degradation by inhibiting the growth of spoilage microorganisms (Yogiara et al., 2020; Septama et al., 2022; Nurcholis et al., 2024). It acts as an antimicrobial agent that suppresses pathogenic bacteria like *Clostridium* sp., *Enterobacteria*, and *Pseudomonas*, as well as mycotoxin-producing fungi such as *Aspergillus* sp. and *Fusarium* sp. Consequently, the conversion of protein into non-protein nitrogen and ammonia decreases. Additionally, temulawak contains curcuminoids and xanthorrhizol, which can reduce protease activity from both plants and spoilage microbes, thereby minimizing protein degradation (Seseogullari-Dİrihan et al., 2018; Al-Amin et al., 2024). Furthermore, yeast and *C. xanthorrhiza* supplementation in goats fed a high-PUFA diet reduced methane production and rumen protozoa populations while improving nutrient metabolism efficiency *in vitro* (Sulistyowati, 2014).

This study hypothesizes that the secondary metabolites of temulawak can inhibit proteolysis during the fermentation of total mixed ration-based okara silage (TMRO-silage) containing tofu by-products by reducing the protease activity of plants and spoilage microorganisms. This study introduces a novel application of temulawak as a natural additive in complete feed silage, which has not been widely explored in previous research. It not only acts as an antimicrobial agent to suppress the growth of spoilage bacteria and mycotoxin-producing molds but also serves as an anti-proteolytic agent that helps preserve protein content in the silage. This study aims to evaluate temulawak's effectiveness in reducing proteolysis during the fermentation of tofu by-product-based TMRO-silage, optimize its drying method to enhance bioactive metabolite content, and analyze its impact on silage quality, feed digestibility, and methane gas production in the rumen.

MATERIALS AND METHODS

Preparation temulawak powder

Fresh temulawak (*C. xanthorrhiza*) was harvested from Dramaga District, Bogor (6.5829°S, 106.7338°E) following a three-month cultivation period, consistent with local agronomic practices. The rhizomes were sliced into 3 mm-thick pieces and dried in an oven at 50°C, 65°C, and 80°C until they reached a stable weight. The dried temulawak was then ground and passed through a 40-mesh sieve. The resulting powder was extracted using an ultrasonic Branson 1510 with an oscillation frequency of 40 kHz in an aqueous-acetone solution (70:30 v/v). The extract was then analyzed for total phenolic content, flavonoid content, and antioxidant activity using the ferric reducing antioxidant power (FRAP) method.

Determination of total phenolic, total flavonoid, and FRAP

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, following Makkar's protocol (Makkar, 2003). First, 10 µL of 10% Folin-Ciocalteu reagent was added to the sample and incubated for 5 minutes. Then, 20 µL of 7.5% Na_2CO_3 was introduced into the mixture, which was subsequently incubated in a dark room for 30 minutes. The absorbance of the resulting solution was measured at a wavelength of 750 nm using a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH). Gallic acid was used to construct a standard curve for determining the TPC of temulawak powder, expressed as mg gallic acid equivalent (GAE) per gram of dry weight (DW). The standard curve was prepared using seven concentration levels: 0, 50, 100, 150, 200, 250, and 300 ppm. The TPC was calculated using the equation $y = 0.006x + 0.198$, where y represents the absorbance value and x denotes the gallic acid concentration.

The total flavonoid content (TFC) was determined using $AlCl_3$ colorimetric method, following the protocol described by Chang (Chang et al. 2002). First, 250 µL of the sample extract was mixed with 75 µL of 5% $NaNO_2$ and incubated for 5 minutes. Then, 150 µL of 10% $AlCl_3$ was added, followed by additional 5-minute incubation. Afterward, 500 µL of 1 M NaOH was introduced into the mixture, and the final volume was adjusted to 2 mL with distilled water. The absorbance of the solution was measured at 510 nm using a nano-spectrophotometer (SPECTROstar Nano, BMG LABTECH). Quercetin was used to generate the standard curve, and TFC was expressed as mg quercetin equivalent (QE) per gram of dry weight (DW). The standard curve was prepared with quercetin concentrations of 0, 20, 40, 60, 80, and 100 ppm. The TFC was calculated using the equation $y = 0.005x + 0.102$, where y represents the absorbance value, and x denotes the quercetin concentration. The antioxidant capacity of temulawak powder was determined using the Ferric Reducing Antioxidant Power (FRAP) assay, following Benzie and Strain (1996). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio. Then, 100 µL of the sample extract was mixed with 900 µL of the FRAP reagent and incubated at 37°C for 30 minutes. The absorbance of the resulting solution was measured at 593 nm using a nano-spectrophotometer

(SPECTROstar Nano, BMG LABTECH). A standard curve was prepared using $FeSO_4 \cdot 7H_2O$ at concentrations of 0, 200, 400, 600, 800, and 1000 µM. The FRAP value was expressed as mg Trolox equivalent (TE) per gram of DW and calculated using the equation y = 0.004x + 0.123, where y represents the absorbance value, and x denotes the Fe^{2+} concentration.

Preparation of fermented okaras for complete feed preservation

This study examined the effect of temulawak powder dosage (0%, 0.25%, 0.5%, 0.75%, and 1% DM) on complete feed supplemented with fermented okaras, using a fresh okara-to-complete feed ratio of 1:1. The fermentation process lasted 14 days. Fresh okaras were sourced from a home-based tofu industry in Dramaga District, Bogor. The complete feed was a commercial product from Agro Apis Palacio Company in Bubulak. The feed ingredients were formulated and analyzed for proximate composition at the Feed Science and Technology Laboratory, Faculty of Animal Science, IPB University. Proximate components were analyzed following AOAC (2023) methods, including dry matter (934.01), crude protein (990.03), crude fiber (962.09), crude fat (920.39), and ash (942.05); nitrogen-free extract was calculated by difference. Table 1 presents the composition and nutritional content.

Table 1 - Feed composition and nutritional content

Ingredient	Level (% DM)
Okara	12
Palm kernel meal	25
Casava dreg	17
Coffee husk	22
Copra meal	10
Pollard	8
Molasses	5
Urea	1
Nutrition content (%)	
Dry matter	56.5
Crude protein	16.9
Crude fiber	18.5
Non-nitrogen extract	52.3
Crude fat	5.28
Ash	7.04

***In vitro* model of Theodorou (1994) and gas production assessment**

The fermented okara and complete feed included a control group without temulawak powder (TMRO-0) and with treatment groups supplemented with 0.25% to 1% temulawak powder (TMRO-0.25, TMRO-0.5, TMRO-0.75, TMRO-1), were oven-dried at 60°C for 24 hours. The samples were then incubated *in vitro* with a rumen fluid and buffer mixture following the Theodorou method (Theodorou et al. 1994). Rumen inoculum from beef cattle was collected at a government-operated slaughterhouse in Bogor, West Java. The facility complied with animal welfare standards established by the National Research and Innovation Agency (BRIN). A 500 mg sample was placed into a 125 ml serum bottle, followed by the addition of 15 ml of rumen fluid and 60 ml of buffer mixture. The treatment allocations followed a randomized complete design. Incubation was conducted in four replicates, with four bottles per replicate.

Immediately after sample preparation, the serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals. The incubation process lasted for 24 hours at 39°C, with gas production recorded throughout. After incubation, the supernatant was collected to measure total volatile fatty acids (VFA) and ammonia concentration, following the Jayanegara method (Jayanegara et al., 2016). The remaining residue was weighed to determine *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD).

Data analysis

The assessment of drying temperature on the chemical characteristics of temulawak extract followed a completely randomized design (CRD) with different treatments. In the drying experiment, the effect on flavonoid content, phenol levels, and ferric reducing antioxidant power (FRAP) was evaluated. Temulawak was dried at three different temperatures: 50°C (T50), 65°C (T65), and 80°C (T80), with each treatment replicated four times. The average drying time was then presented graphically. In the silage experiment, the addition of temulawak powder followed a CRD. The treatments consisted of temulawak powder at 0% (TP0), 0.25% (TP0.25), 0.5% (TP0.5), 0.75% (TP0.75), and 1% (TP1) of the total TMRO-silage weight. If the ANOVA results were significant ($p < 0.05$), further analysis was conducted using Duncan's multiple range test. A graphical illustration was employed to observe the dynamic changes in ammonia production in TMRO-silage following temulawak powder addition. In the *in vitro* experiment, the treatments included a control diet consisting of complete feed and okaras (TP0) and a diet supplemented with 1% DM temulawak (TP1). Each treatment was replicated four times. Data analysis was conducted using an independent t-test in SPSS 16 to compare the means between the two groups. The t-test statistic (t) was calculated using the formula where X_1 and X_2 represent the mean values of groups TP0 and TP1, respectively; s_1^2 and s_2^2 denote their variances; n_1 and n_2 indicate the sample sizes of each group. A significance level (α) of 0.05 was used to determine statistical differences between the treatments. Gas production was fitted to the following non-linear regression model: $V_t = (a + b) \times (1 - e^{-ct})$,

where V_t represents the volume of gas production (ml) at time t, t is the incubation period (h), a + b denotes the gas production potential, e is the base of the natural logarithm, and c is the gas production rate constant. The model's fit was assessed using the coefficient of determination (R^2) and mean square error (MSE), both of which were reported to validate the regression accuracy.

RESULTS AND DISCUSSION

Impact of thermal drying conditions on bioactive compound stability in temulawak

Drying is one of the most common methods for preserving herbal materials (Calín-Sánchez et al., 2020). Using an oven at a specific temperature influences the drying rate (Torki-Harchegani et al., 2016). Additionally, oven temperature affects the quality of the active compounds produced (Abd Ghani et al., 2023). Optimizing drying temperature is essential to maintaining the quality of bioactive compounds in temulawak. Temulawak is an herbal plant with antibacterial and antioxidant properties and can influence rumen metabolism (Rosidi et al., 2016; Sujarnoko et al., 2023). The effect of temperature on drying speed is shown in Figure 1. Drying at 50°C takes approximately 25 hours to reach a stable dry weight. At 65°C, the material reaches a stable dry weight in 9 hours, while at 80°C, it achieves equilibrium after 7 hours of drying (Figure 1).

Oven temperature significantly influenced ($P<0.01$) total flavonoid content, with the highest levels observed at 65°C, followed by 50 and 80°C (Table 2). The lower flavonoid content at 80℃ resulted from excessive heat exposure, which degraded a significant portion of the flavonoids. High temperatures caused structural damage to the functional groups of flavonoids (Zhang et al., 2023). For instance, rutin, naringin, and luteolin degrade at 130°C after two hours, while high temperatures also inhibit anthocyanin biosynthesis (Chaaban et al., 2017; Yang et al., 2024). Drying at 50°C preserved more flavonoid content than at 80°C because lower temperatures minimized functional group degradation (Sharma et al., 2015; Červenka et al., 2018). However, drying at 65°C resulted in significantly higher flavonoid content than at 50℃, likely due to prolonged heat exposure enhancing flavonoid release.

Phenols are among the compounds in temulawak that exhibit antibacterial and antioxidant properties (Abd Rashid et al., 2022). Drying temulawak at 80°C significantly reduces the total phenol content compared to drying at 50 and 65°C (Table 2). This reduction occurs because phenolic groups degrade and oxidize when exposed to high temperatures, leading to a significant decline in total phenol content. Heat exposure causes structural damage to these compounds (Cheng et al., 2014; Wang et al., 2021). In contrast, drying at 50 and 65°C does not result in a significant difference in total phenol content. This stability likely occurs because the temperatures used do not exceed the degradation threshold of phenols (Levén and Schnürer, 2005). Temulawak is an herbal plant with antioxidant properties that enhance its ability to absorb free radicals (Rosidi et al., 2016). Drying at 65°C increases its antioxidant activity compared to drying at 80°C (Table 2). This occurs because high temperatures accelerate the thermal degradation of antioxidant compounds, reducing temulawak's ability to neutralize free radicals (Levén and Schnürer, 2005; Peron et al., 2017). Meanwhile, drying at 50°C results in lower antioxidant activity than at 65°C. This may be due to prolonged exposure to heat and oxygen, which promotes oxidation (Poljšak and Fink, 2014; Bahrololoumi et al., 2022).

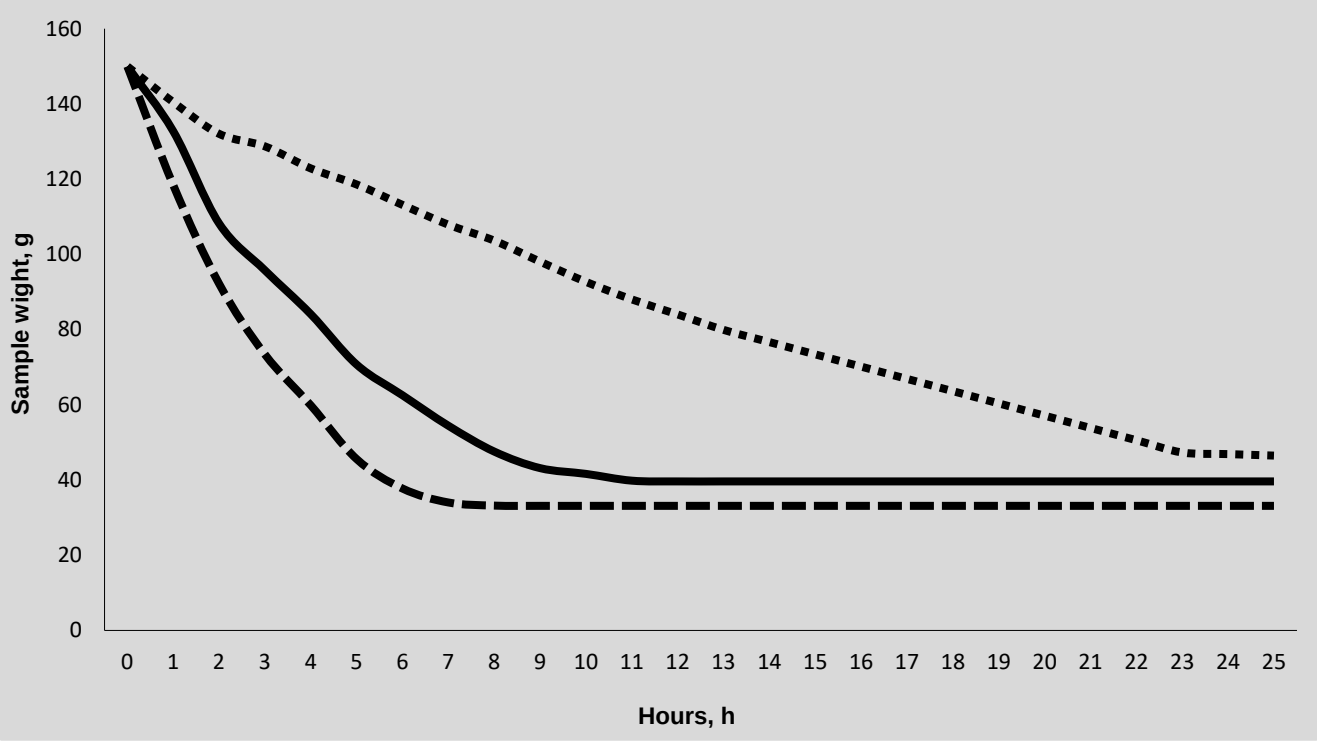

Figure 1 - Oven temperature influenced the sample weight (g) of temulawak powder over time (h), where 50°C (solid), 65°C (small dashed), 80℃ (long dashed).

Table 2 - Flavonoid content, phenol levels, and FRAP under drying treatments at 50, 65, and 80 °C in temulawak powder.

Drying temperature (°C) / Flavonoid content	50 °C	65 °C	80 °C	P value
Total flavonoid (µg QE/g DW)	18.1 ± 1.45[b]	23.3 ± 1.72[a]	14.5 ± 1.71[c]	<0.01
Total phenol (µg GAE/g DW)	17 ± 1.36[a]	16.9 ± 1.73[a]	12.4 ± 1.63[b]	0.01
FRAP (mg TE/g DW)	19.7 ± 2.25[b]	30.1 ± 2.42[a]	9.18 ± 2.91[c]	<0.01

Different superscripts within the same row are significantly different at P<0.05. FRAP=ferric reducing antioxidant power, GAE=gallic acid equivalent, QE=quercetin equivalent.

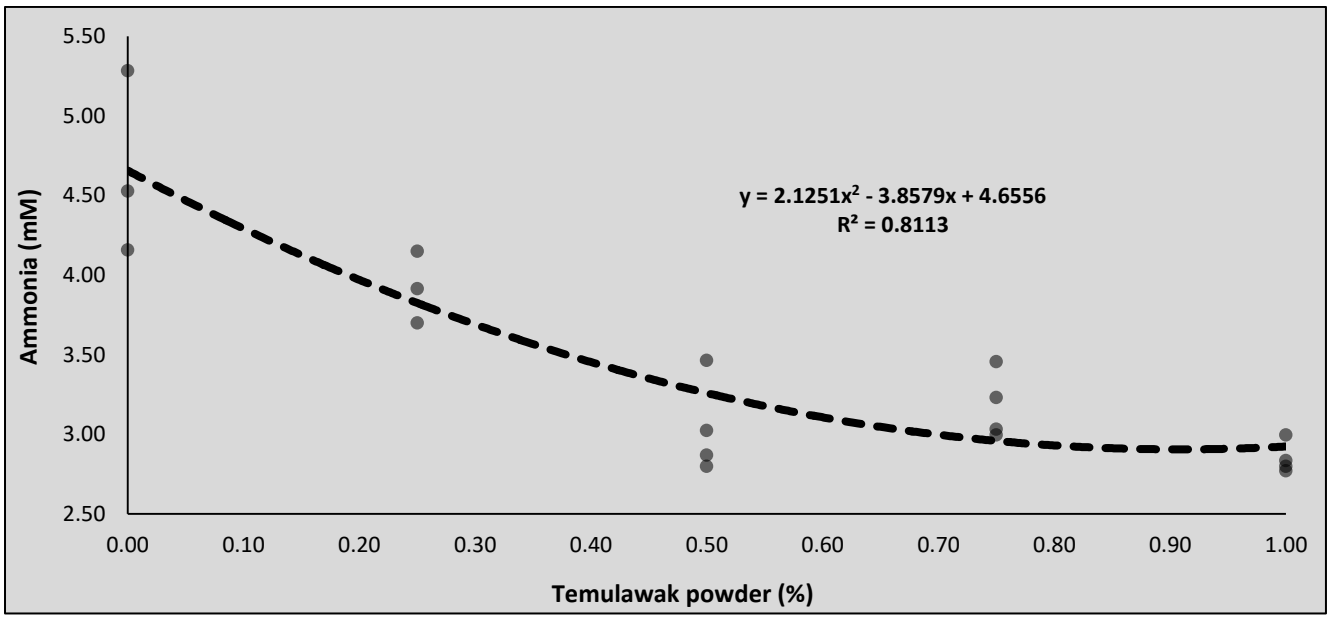

Figure 2 - The impact of temulawak powder addition (%, x-axis) to TMRO-silage on ammonia production (mM, y-axis).

Effect of temulawak powder on TMRO-silage

Preserving high-protein feed ingredients during the rainy season presents a significant challenge (Wang et al., 2019). In the silage process, spoilage bacteria such as *Clostridium* sp. and *Escherichia coli* deaminate protein chains, converting them into ammonia. This reaction makes it more difficult to lower the silage pH (Jayanegara et al., 2019). Temulawak, a rhizome with antibacterial properties, can help prevent protein degradation during silage production (Rahmat et al., 2021). Adding 0.5% temulawak powder to TMRO-silage effectively inhibited deamination, as indicated by a significant decrease in ammonia levels ($P < 0.01$; Table 3). Lower ammonia levels help stabilize silage pH during storage (Jayanegara et al., 2019). Temulawak reduces ammonia content by using its phenolic compounds to inhibit spoilage bacteria (Sadarman et al., 2019; Figure 2). Additionally, tannins in temulawak bind to proteins, further limiting deamination (Kondo et al., 2006). Adding 1% temulawak powder effectively prevented crude protein loss, significantly increasing the feed protein percentage on TMRO-silage ($P < 0.05$; Table 4). Soluble carbohydrates in the feed are converted into lactate, carbon dioxide, and water, which leads to a relative increase in protein content within the silage. Phenols can inhibit microbial activity, while tannins help protect proteins from degradation (Jayanegara et al., 2019; Sujarnoko et al., 2020). However, temulawak powder did not significantly lower the pH, which remained within the acceptable range for silage (pH 4-4.5). The addition of temulawak powder effectively preserved the dry matter content of TMRO-silage, as indicated by the significantly higher dry matter values in the treatment group compared to the control ($P < 0.01$; Table 4). The preservation process for complete feed and tofu by-products improved because the nutrients were protected from spoilage. Additionally, nutrient degradation was prevented (Rahmat et al., 2021; Abd Rashid et al., 2022).

Effect of adding temulawak powder on *in vitro* rumen fermentation

The addition of 1% temulawak powder to TMRO-silage significantly reduced rumen ammonia levels compared to the other treatment ($P < 0.01$; Table 5). This reduction is likely due to temulawak's phenolic and flavonoid compounds, which can inhibit protease activity and microbial proliferation (Landis-Piwowar et al., 2008; Hernández-Rodríguez et al., 2019). The decrease in ammonia production tends to lower pH levels since ammonia, which has a basic nature due to its ($-NH_2$) group, is reduced (Calsamiglia et al., 2008). Additionally, a strong correlation exists between phenolic content and lactic acid bacteria populations (Pianpumepong and Noomhorm, 2010). Temulawak powder also increases total gas production

and digestibility (IVDMD and IVOMD). However, an important finding is the reduction in methane production per total gas ($P < 0.05$; Table 5). Previous *in vitro* studies also reported methane reduction with other phenolic-containing compounds (Sujarnoko et al., 2020; 2023). The positive correlation between temulawak powder and rumen fermentation characteristics is primarily attributed to its phenolic and flavonoid content. However, drying methods should be optimized to ensure the best residual compound retention. Future research should explore not only the application of temulawak powder in high-protein silage preservation, such as TMR-silage with okaras, but also in other high-protein silage applications, including fermented food products.

Table 3 - Influence of the temulawak powder on the ammonia production (mM) of TMRO-silage

Parameters	TMRO-0	TMRO-0.25	TMRO-0.5	TMRO-0.75	TMRO-1	P-value
Ammonia (mM)	4.52 ± 0.66^{c}	3.91 ± 0.23^{b}	3.1 ± 0.37^{a}	2.9 ± 0.35^{a}	2.84 ± 0.13^{a}	0.01

Different superscripts within the same row are significantly different at P<0.05. TMRO-silage=total mixed ration-based okara silage; TP: temulawak powder; TMRO-0=TMRO-silage+0%TP; TMRO-0.25=TMRO-silage+0.25TP; TMRO-0.5=TMRO-silage+0.5%TP; TMRO-0.75=TMRO-silage+0.75%TP, TMRO-1: TMRO-silage+1%TP.

Table 4 - Influence of the temulawak powder on pH and proximate composition of TMRO-silage

Parameters	TMRO-0	TMRO-0.25	TMRO-0.5	TMRO-0.75	TMRO-1	P-value
pH	4.32 ± 0.02	4.3 ± 0.07	4.3 ± 0.03	4.33 ± 0.03	4.3 ± 0.05	0.34
Dry matter (%)	54.1 ± 0.64^{a}	56 ± 0.7^{b}	57.4 ± 0.63^{c}	57.2 ± 0.75^{c}	55.3 ± 0.76^{b}	<0.01
Crude protein (%)	15.2 ± 0.1^{a}	15.5 ± 0.65^{ab}	15.7 ± 0.32^{ab}	15.8 ± 0.52^{b}	17.1 ± 0.41^{c}	<0.01
Ether extract (%)	6.72 ± 0.25^{b}	5.56 ± 0.22^{a}	7.8 ± 0.19^{c}	7.07 ± 0.16^{b}	7.67 ± 0.46^{c}	<0.01
Crude fiber (%)	22.3 ± 0.49^{b}	22.6 ± 0.92^{b}	21.9 ± 0.84^{b}	21.7 ± 1.16^{ab}	20.4 ± 0.82^{a}	0.03
Ash (%)	8.74 ± 0.26^{a}	8.77 ± 0.45^{a}	9.03 ± 0.65^{a}	9.17 ± 0.67^{a}	10.1 ± 0.83^{b}	0.03
NFE (%)	47 ± 46^{c}d	47.6 ± 0.5d	45.5 ± 0.43^{ab}	46.3 ± 0.86^{bc}	44.7 ± 0.53^{a}	<0.01

Different superscripts within the same row are significantly different at P<0.05. TMRO-silage=total mixed ration-based okara silage; TP: temulawak powder; NFE=nitrogen-free extract. TMRO-0=TMRO-silage+0%TP; TMRO-0.25=TMRO-silage+0.25TP; TMRO-0.5=TMRO-silage+0.5%TP; TMRO-0.75=TMRO-silage+0.75%TP, TMRO-1: TMRO-silage+1%TP.

Table 5 - *In vitro* characteristics of TMRO-silage added with temulawak powder

Parameters	TMRO-0	TMRO-0.25	TMRO-0.5	TMRO-0.75	TMRO-1	P-value
Ammonia (mM)	12.3 ± 1.14^{b}	11.4 ± 0.87^{b}	10.3 ± 0.79^{ab}	9.7 ± 0.53^{a}	9.03 ± 0.62^{a}	<0.01
pH	6.99 ± 0.17^{b}	6.97 ± 0.14^{b}	6.91 ± 0.21^{b}	6.87 ± 0.07^{b}	6.81 ± 0.83^{a}	0.01
VFA (mM)	125 ± 5.63	123 ± 6.01	120 ± 5.99	118 ± 6.3	115 ± 6.46	0.07
IVDMD (%)	49.6 ± 0.85^{a}	51.0 ± 0.56^{ab}	50.5 ± 0.48^{b}	50.8 ± 0.38^{b}	51.1 ± 0.6^{b}	0.03
IVOMD (%)	47.2 ± 0.89^{a}	47.8 ± 0.75^{ab}	48.2 ± 0.86^{b}	48.3 ± 0.71^{b}	48.9 ± 0.78^{b}	0.03
Total gas (mL)	113 ± 2.95^{a}	115 ± 2.53^{ab}	118 ± 2.16^{b}	117 ± 2.01^{b}	119 ± 1.97^{b}	0.03
Methane (ppm)	72,166 ± 6,135	70,135 ± 5,345	69,140 ± 4,866	68,450 ± 3,852	68,114 ± 3,313	0.26
MTG (ppm/mL)	631 ± 54.7^{b}	611 ± 47.2^{b}	595 ± 41.6^{ab}	582 ± 35.7^{ab}	576 ± 30.6^{a}	0.05

Different superscripts within the same column are significantly different at P<0.05. TMRO-silage=total mixed ration-based okara silage; TP: temulawak powder; NFE=nitrogen-free extract. TMRO-0=TMRO-silage+0%TP; TMRO-0.25=TMRO-silage+0.25TP; TMRO-0.5=TMRO-silage+0.5%TP; TMRO-0.75=TMRO-silage+0.75%TP, TMRO-1: TMRO-silage+1%TP. VFA=volatile fatty acid; IVDMD=*in vitro* dry matter digestibility; IVOMD=*in vitro* organic matter digestibility; MTG=methane per total gas.

CONCLUSION

Adding 1% dry matter temulawak powder effectively reduces the risk of protein degradation during the optimal storage of total mixed ration-based okara silage (TMRO-silage). It also improves the nutritional characteristics of TMRO-silage. Additionally, incorporating 1% temulawak powder enhances *in vitro* digestibility and protects feed protein from deamination. This dosage optimally reduces methane relative to total gas production while increasing total gas production. This benefit is likely related to the higher levels of total phenols, flavonoids, and ferric reducing antioxidant power (FRAP) in temulawak processed at 65 °C.

DECLARATIONS

Corresponding author

Mohammad Sholikin serves as the corresponding author of the article. E-mail: mohammad.miftakhus.sholikin@brin.go.id

Data availability

The data are available upon request from the corresponding author via the provided contact information.

Ethical considerations

Rumen donors were obtained from slaughtered beef cattle, based on approval from the relevant institutional ethics committee (Approval No. 078/KE.02/SK/10/2022).

Authors' contribution

The authors contributed equally to data analysis and manuscript writing. T.U.P.Sujarnoko led the conceptualization. M.S.Lim conducted data curation. M.S.Lim and D.Budiono carried out formal analysis. T.U.P.Sujarnoko secured the funding and performed validation. N.A.Sholeha conducted the investigation and visualization. N.A.Sholeha and M.D.Alifian developed the methodology. D.Budiono and M.D.Alifian administered the project. N.A.Sholeha provided the resources. M.S.Lim, T.Ujilestari, and M.M. Sholikin developed the software. T.U.P. Sujarnoko and M.M. Sholikin provided supervision. M.S.Lim, T.Ujilestari, and M.M.SHOLIKIN drafted the original manuscript, while T.Ujilestari and M.M.SHOLIKIN reviewed and edited the manuscript.

Consent to publish

Each author reviewed and approved the final version of the manuscript.

Acknowledgements

I wish to express my sincere appreciation to all factory owners and staff of Agro Apis Palacio.

Funding sources

The authors acknowledge the financial support from the Institut Pertanian Bogor (IPB University), Bogor, Indonesia Vocational School Grant Program awarded to Tekad Urip Pambudi Sujarnoko and his team.

Competing interests

The authors declare no competing interests.

REFERENCES

Abd Ghani A, Abd Rashid NY, Abd Razak DL, Jamaluddin A, Abdul Manan M, and Mansor A (2023). The effect of heat treatments on the bioactive compounds, antioxidant activity, and cosmeceutical properties (anti-pigmentation and anti-ageing) of fermented broken rice. Food Research, 6: 155–162. DOI: https://doi.org/10.26656/fr.2017.6(S2).009

Abd Rashid SNA, Hasham R, Abd. Rashid ZI, Cheng KK, Aziz AA, Shafin N et al. (2022). Formulation and characterization of the physicochemical, antioxidant activity and sensory attributes of curcuma-based herbal drink. Materials Today: Proceedings, 57: 1061–1066. DOI: https://doi.org/10.1016/j.matpr.2021.09.272

Adli DN, Sholikin MM, Ujilestari T, Ahmed B, Sadiqqua A, Harahap M A, et al. (2024). Effect of fermentation of herbal products on growth performance, breast meat quality, and intestinal morphology of broiler chickens: A meta-analysis. Italian Journal of Animal Science, 23(1): 734–750. DOI: https://doi.org/10.1080/1828051X.2024.2351441

Ahangari H, Yazdani P, Ebrahimi V, Soofiyani S R, Azargun R, Tarhriz V, et al. (2021). An Updated review on production of food derived bioactive peptides: Focus on the psychrotrophic bacterial proteases. Biocatalysis and Agricultural Biotechnology, 35: 102051. DOI: https://doi.org/10.1016/j.bcab.2021.102051

Al-Amin M, Fazalul Rahiman S S, Khairuddean M, and Muhamad Salhimi S (2024). (R)-(−)-Xanthorrhizol inhibits the migration and invasion of triple-negative breast cancer cells by suppressing matrix metalloproteinases via the nf-κb signaling pathway. Planta Medica, 90(10): 785–791. DOI: https://doi.org/10.1055/a-2339-2633

AOAC (2023). Association of Official Analytical Chemists. Official Methods of Analysis, 21th Edition. New York DOI: https://doi.org/10.1093/9780197610145.002.001

Bahrololoumi A, Shaafaey M, and Dargazany R (2022). Constitutive modeling of the cross-linked polymers during the trio-aging mechanism: Effects of water, oxygen, UV. ASME 2022 International Mechanical Engineering Congress and Exposition, 2022: IMECE2022-95558. DOI: https://doi.org/10.1115/IMECE2022-95558

Benzie IFF, Strain JJ. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Analytical Biochemistry, 239(1): 70–76. DOI: https://doi.org/10.1006/abio.1996.0292

Calín-Sánchez Á, Lipan L, Cano-Lamadrid M, Kharaghani A, Masztalerz K, Carbonell-Barrachina A A, et al. (2020). Comparison of traditional and novel drying techniques and its effect on quality of fruits, vegetables and aromatic herbs. Foods, 9(9): 1261. DOI: https://doi.org/10.3390/foods9091261

Calsamiglia S, Cardozo P W, Ferret A, and Bach A (2008). Changes in rumen microbial fermentation are due to a combined effect of type of diet and pH. Journal of Animal Science, 86(3): 702–711. DOI: https://doi.org/10.2527/jas.2007-0146

Červenka L, Červenková Z, and Velichová H (2018). Is air-drying of plant-based food at low temperature really favorable? A meta-analytical approach to ascorbic acid, total phenolic, and total flavonoid contents. Food Reviews International, 34(5): 434–446. DOI: https://doi.org/10.1080/87559129.2017.1307389

Chaaban H, Ioannou I, Chebil L, Slimane M, Gérardin C, Paris C, et al. (2017). Effect of heat processing on thermal stability and antioxidant activity of six flavonoids. Journal of Food Processing and Preservation, 41(5): e13203. DOI: https://doi.org/10.1111/jfpp.13203

Cheng Y, Xu Q, Liu J, Zhao C, Xue F, and Zhao Y (2014). Decomposition of five phenolic compounds in high temperature water. Journal of the Brazilian Chemical Society, 25(11): 2102-2107. DOI: https://doi.org/10.5935/0103-5053.20140201

Ekwomadu T I, Akinola S A, and Mwanza M (2021). Fusarium mycotoxins, their metabolites (free, emerging, and masked), food safety concerns, and health impacts. International Journal of Environmental Research and Public Health, 18(22): 11741. DOI: https://doi.org/10.3390/ijerph182211741

Ginting E, Elisabeth DAA, Khamidah A, Rinaldi J, Ambarsari I, and Antarlina SS (2024). The nutritional and economic potential of tofu dreg (okara) and its utilization for high protein food products in Indonesia. Journal of Agriculture and Food Research, 16: 101175. DOI: https://doi.org/10.1016/j.jafr.2024.101175

Hadidi M, Orellana Palacios JC, McClements DJ, Mahfouzi M, and Moreno A (2023). Alfalfa as a sustainable source of plant-based food proteins. Trends in Food Science & Technology, 135: 202–214. DOI: https://doi.org/10.1016/j.tifs.2023.03.023

He Y, Degraeve P, and Oulahal N (2024). Bioprotective yeasts: Potential to limit postharvest spoilage and to extend shelf life or improve microbial safety of processed foods. Heliyon, 10(3): e24929. DOI: https://doi.org/10.1016/j.heliyon.2024.e24929

Heng X, Chen H, Lu C, Feng T, Li K, and Gao E (2022). Study on synergistic fermentation of bean dregs and soybean meal by multiple strains and proteases. LWT, 154: 112626. DOI: https://doi.org/10.1016/j.lwt.2021.112626

Hernández-Rodríguez P, Baquero L P, and Larrota H R (2019). Flavonoids: Potential Therapeutic Agents by Their Antioxidant Capacity. In: M. R. S. Campos (Editor), Bioactive Compounds, p. 265–288 (Bg). DOI: https://doi.org/10.1016/B978-0-12-814774-0.00014-1

Jayanegara A, Sujarnoko T U P, Ridla M, Kondo M, and Kreuzer M (2019). Silage quality as influenced by concentration and type of tannins present in the material ensiled: A meta-analysis. Journal of Animal Physiology and Animal Nutrition, 103(2): 456–465. DOI: https://doi.org/10.1111/jpn.13050

Jayanegara A, Dewi S, Laylli N, Laconi E, Nahrowi, and Ridla M (2016). Determination of cell wall protein from selected feedstuffs and its relationship with ruminal protein digestibility *in vitro*. Media Peternakan, 39: 134–140. DOI: https://doi.org/10.5398/medpet.2016.39.2.134

Kamble DB and Rani S (2020). Bioactive components, *in vitro* digestibility, microstructure and application of soybean residue (Okara): A review. Legume Science, 2(1): 1–9. DOI: https://doi.org/10.1002/leg3.32

Kondo M, Kita K, and Yokota H (2006). Evaluation of fermentation characteristics and nutritive value of green tea waste ensiled with byproducts mixture for ruminants. Asian-Australasian Journal of Animal Sciences, 19(4): 533–540. DOI: https://doi.org/10.5713/ajas.2006.533

Landis-Piwowar KR, Milacic V, and Dou QP (2008). Relationship between the methylation status of dietary flavonoids and their growth-inhibitory and apoptosis-inducing activities in human cancer cells. Journal of Cellular Biochemistry, 105(2): 514–523. DOI: https://doi.org/10.1002/jcb.21853

Levén L and Schnürer A (2005). Effects of temperature on biological degradation of phenols, benzoates and phthalates under methanogenic conditions. International Biodeterioration & Biodegradation, 55(2): 153–160. DOI: https://doi.org/10.1016/j.ibiod.2004.09.004

Li R, Zheng M, Jiang D, Tian P, Zheng M, and Xu C (2021). Replacing alfalfa with paper mulberry in total mixed ration silages: Effects on ensiling characteristics, protein degradation, and *in vitro* digestibility. Animals, 11(5): 1273. DOI: https://doi.org/10.3390/ani11051273

Makkar H P S (2003). Quantification of Tannins in Tree and Shrub Foliage. Springer Netherlands, Dordrecht, pp. 49-51 DOI: https://doi.org/10.1007/978-94-017-0273-7

Navale V, Vamkudoth K R, Ajmera S, and Dhuri V (2021). *Aspergillus* derived mycotoxins in food and the environment: Prevalence, detection, and toxicity. Toxicology Reports, 8: 1008–1030. DOI: https://doi.org/10.1016/j.toxrep.2021.04.013

Nurcholis W, Rahmadansah R, Astuti P, Priosoeryanto B P, Arianti R, and Kristóf E (2024). Comparative analysis of volatile compounds and biochemical activity of *Curcuma xanthorrhiza* Roxb. essential oil extracted from distinct shaded plants. Plants, 13(19): 2682. DOI: https://doi.org/10.3390/plants13192682

Okoye C O, Wang Y, Gao L, Wu Y, Li X, Sun J, et al. (2023). The performance of lactic acid bacteria in silage production: A review of modern biotechnology for silage improvement. Microbiological Research, 266: 127212. DOI: https://doi.org/10.1016/j.micres.2022.127212

Peron D V, Fraga S, and Antelo F (2017). Thermal degradation kinetics of anthocyanins extracted from Juçara (*Euterpe edulis* Martius) and "Italia" grapes (*Vitis vinifera* L.), and the effect of heating on the antioxidant capacity. Food Chemistry, 232: 836–840. DOI: https://doi.org/10.1016/j.foodchem.2017.04.088

Pianpumepong P and Noomhorm A (2010). Isolation of probiotic bacteria from turmeric (*Curcuma longa* Linn.) and its application in enriched beverages. International Journal of Food Science & Technology, 45(12): 2456–2462. DOI: https://doi.org/10.1111/j.1365-2621.2010.02337.x

Poljšak B and Fink R (2014). The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. Oxidative Medicine and Cellular Longevity, 2014(1): 1–22. DOI: https://doi.org/10.1155/2014/671539

Rahmat E, Lee J, and Kang Y (2021). Javanese Turmeric (*Curcuma xanthorrhiza* Roxb.): Ethnobotany, phytochemistry, biotechnology, and pharmacological activities. Evidence-Based Complementary and Alternative Medicine, 2021: 1–15. DOI: https://doi.org/10.1155/2021/9960813

Rosidi A, Khomsan A, Setiawan B, Riyadi H, and Briawan D (2016). Antioxidant potential of Temulawak (*Curcuma xanthorrhiza* Roxb.). Pakistan Journal of Nutrition, 15(6): 556–560. DOI: https://doi.org/10.3923/pjn.2016.556.560

Sadarman, Ridla M, Nahrowi, Sujarnoko T U P, Ridwan R, and Jayanegara A. (2019). Evaluation of ration based on soy sauce by-product on addition of acacia and chestnut tannin: An *in vitro* study. IOP Conference Series: Materials Science and Engineering, 546(2): 022020. DOI: https://doi.org/10.1088/1757-899X/546/2/022020

Septama AW, Tasfiyati AN, Kristiana R, and Jaisi A (2022). Chemical profiles of essential oil from Javanese turmeric (*Curcuma xanthorrhiza* Roxb.), evaluation of its antibacterial and antibiofilm activities against selected clinical isolates. South African Journal of Botany, 146: 728–734. DOI: https://doi.org/10.1016/j.sajb.2021.12.017

Seseogullari-Dİrihan R, Tekbas Atay M, Pashley D H, and Tezvergil-Mutluay A (2018). Inhibitory effect of curcuminoid pretreatments on endogenous dentin proteases. Dental Materials Journal, 37(3): 445–452. DOI: https://doi.org/10.4012/dmj.2017-116

Sharma K, Ko E Y, Assefa AD, Ha S, Nile SH, Lee ET, et al. (2015). Temperature-dependent studies on the total phenolics, flavonoids, antioxidant activities, and sugar content in six onion varieties. Journal of Food and Drug Analysis, 23(2): 243–252. DOI: https://doi.org/10.1016/j.jfda.2014.10.005

Snyder A B, Martin N, and Wiedmann M (2024). Microbial food spoilage: Impact, causative agents and control strategies. Nature Reviews Microbiology, 22(9): 528–542. DOI: https://doi.org/10.1038/s41579-024-01037-x

Sulistyowati E, Sudarman A, Wiryawan K G, and Toharmat T (2014). *In vitro* goat fermentation of PUFA-diet supplemented with yeast and *Curcuma xanthorrhiza* Roxb. Media Peternakan, 37(3): 175–181. DOI: https://doi.org/10.5398/medpet.2014.37.3.175

Sujarnoko TUP, Ridwan R, Nahrowi N, and Jayanegara A (2020). Extraction of tannin from Acacia (*Acacia mangium*) bark and its use as a feed additive for protecting *in vitro* ruminal degradation of tofu dregs. Advances in Animal and Veterinary Sciences, 8(7): 761-765. DOI: https://doi.org/10.17582/journal.aavs/2020/8.7.761.765

Sujarnoko TUP, Saputra Lim M, Budiono D, Amalia Sholeha N, Firmansyah D, Zahera R, et al. (2023). The effect of Temulawak (*Curcuma xhantorrhiza*) dose on tofu dreg plus complete feed silage with methane relative mitigation: *In vitro*. E3S Web of Conferences, 454: 02010. DOI: https://doi.org/10.1051/e3sconf/202345402010

Suwarsito S and Purbomartono C (2018). The effect of use of tofu dregs fermented on feed efficiency and growth of African cat fish (*Clarias gariepinus*). IOP Conference Series: Materials Science and Engineering, 434(1): 012128. DOI: https://doi.org/10.1088/1757-899X/434/1/012128

Theodorou MK, Williams BA, Dhanoa M S, McAllan AB, and France J (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology, 48(3–4): 185–197. DOI: https://doi.org/10.1016/0377-8401(94)90171-6

Torki-Harchegani M, Ghasemi-Varnamkhasti M, Ghanbarian D, Sadeghi M, and Tohidi M (2016). Dehydration characteristics and mathematical modelling of lemon slices drying undergoing oven treatment. Heat and Mass Transfer, 52(2): 281–289. DOI: https://doi.org/10.1007/s00231-015-1546-y

Wang D, Ding J, Wang B, Zhuang Y, and Huang Z (2021). Synthesis and thermal degradation study of polyhedral oligomeric silsesquioxane (POSS) modified phenolic resin. Polymers, 13(8): 1182. DOI: https://doi.org/10.3390/polym13081182

Wang H, Wang C, Li C, Xu X, and Zhou G (2019). Effects of phenolic acid marinades on the formation of polycyclic aromatic hydrocarbons in charcoal-grilled chicken wings. Journal of Food Protection, 82(4): 684–690. DOI: https://doi.org/10.4315/0362-028X.JFP-18-420

Yang J, Guo C, Chen F, Lv B, Song J, Ning G, et al. (2024). Heat-induced modulation of flavonoid biosynthesis via a LhMYBC2-Mediated regulatory network in oriental hybrid lily. Plant Physiology and Biochemistry, 214: 108966. DOI: https://doi.org/10.1016/j.plaphy.2024.108966

Yogiara, Mordukhova EA, Kim D, Kim W-G, Hwang J-K, and Pan J-G (2020). The food-grade antimicrobial xanthorrhizol targets the enoyl-ACP reductase (FabI) in *Escherichia coli*. Bioorganic & Medicinal Chemistry Letters, 30(24): 127651. DOI: https://doi.org/10.1016/j.bmcl.2020.127651

Zhang X, Li L, He Y, Lang Z, Zhao Y, Tao H, et al. (2023). The CsHSFA-CsJAZ6 module-mediated high temperature regulates flavonoid metabolism in *Camellia sinensis*. Plant, Cell & Environment, 46(8): 2401–2418. DOI: https://doi.org/10.1111/pce.14610