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Exploring the hypoglycemic potential of fresh, *semangit*, and *bosok* tempe: A comparative metabolite profile

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Abstract

Tempe, a traditional Indonesian fermented soybean product made with *Rhizopus* spp., is classified based on fermentation duration into fresh (two days), *semangit* (five days), and *bosok* (seven days) varieties, fermented at room temperature (28–30°C). Longer fermentation is believed to enhance its antidiabetic properties. The aim of this study was to compare the metabolite profiles and hypoglycemic activities of fresh, *semangit*, and *bosok* tempe made from germinated and non-germinated soybeans. Diabetic rat models were used to assess the effects of these tempe types on body weight, blood glucose levels, serum insulin, pancreatic β-cell count, and glycogen content in liver and muscle tissues. Metabolomic profiling was conducted using gas chromatography-mass spectrometry (GC-MS), followed by principal component analysis (PCA) to assess the influence of fermentation stage and germination. Fresh tempe, especially from germinated soybeans, had the highest moisture content. Fermentation duration significantly influenced color, texture, and pH, with *bosok* tempe showing the most notable changes. Tempe and gliclazide significantly reduced blood glucose in diabetic rats in vivo, with *semangit* and *bosok* tempe restoring levels close to normal. However, weight loss was not reversed. *Bosok* non-germinated tempe induced the highest insulin levels among tempe treatments and improved β-cell count and density to levels comparable with gliclazide. Glycogen stores in the liver and muscle were significantly restored by tempe, with *bosok* non-germinated tempe showing the greatest effect. GC-MS profiling identified 154 metabolites, of which 63 were annotated. Fermentation and germination shifted the metabolite profile, with *bosok* non-germinated tempe showing the highest diversity, including amino acids, sugars, and amines. PCA separated samples by fermentation stage, highlighting metabolite accumulation with prolonged fermentation. The findings revealed that *bosok* tempe from non-germinated soybeans had the highest abundance of bioactive metabolites, including isoflavones, which likely contributed to its superior antioxidant and hypoglycemic potential compared to other tempe types.

Keywords: Diabetes mellitus, tempe, germination, hypoglycemic, metabolites

Introduction

Tempe or *tempeh* is a traditional Indonesian fermented soybean product produced with the aid of *Rhizopus* spp. mold. It is widely consumed across Indonesia and is classified into three types

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based on fermentation duration: fresh, *semangit*, and *bosok* tempe. Fresh tempe undergoes fermentation for two days, characterized by white hyphae and pale grayish-yellow soybean seeds [1]. *Semangit* tempe, fermented for five days, exhibits darker hyphae with dark yellow soybean seeds [2]. *Bosok* tempe, with a fermentation period of seven days, presents blackish hyphae and darkened soybean seeds. In Javanese-Indonesian culinary practices, *semangit* and *bosok* tempe are often incorporated as natural seasonings and alternatives to monosodium glutamate flavoring [3].

Tempe is hypothesized to have beneficial effects on diabetes mellitus (DM). Tempe has been reported to inhibit α-glucosidase and α-amylase, enzymes involved in carbohydrate metabolism [1]. Additionally, tempe has antioxidant properties, suggesting its potential to reduce oxidative stress in DM patients [1,4]. In vitro and in vivo studies further indicate that tempe consumption may lower blood glucose levels [3-7]. The bioactive compounds responsible for these effects include insulinotropic amino acids (leucine, alanine, isoleucine, phenylalanine, lysine, and arginine), aglycone isoflavones (daidzein and genistein), and bioactive peptides [5,8].

Extended fermentation is thought to enhance the bioactivity of tempe. A five-day fermentation period has been associated with increased levels of isoflavones, aglycones, and phenolic compounds [9]. It is hypothesized that a seven-day fermentation process may further augment hypoglycemic and antioxidant activities [2], although no prior studies have systematically investigated this. Additionally, the use of germinated soybeans in tempe production may enhance its hypoglycemic potential. Germinated soybeans contain higher levels of phenolic compounds, free isoflavones, and insulinotropic amino acids than non-germinated soybeans [10,11]. Tempe made from germinated soybeans has also been reported to exhibit increased antioxidant activity, α-amylase and α-glucosidase inhibition and elevated levels of bioactive compounds compared to tempe from non-germinated soybeans [2]. Therefore, the aim of this study was to evaluate metabolite profiles and compare the hypoglycemic activity of fresh, *semangit*, and *bosok* tempe. Additionally, this study also assessed tempe characteristics (proximate analysis, color, texture, pH) and their effects on body weight (BW), blood glucose, serum insulin, pancreatic β-cells, and glycogen levels in liver and muscle tissues in a diabetic rat model.

Methods

Study design and setting

This study was an experimental investigation using a completely randomized design (CRD), conducted from June 2024 to February 2025. The primary experiment was conducted at the Seafast Center Laboratory for Experimental Animals, IPB University, Bogor, Indonesia, with histological analyses performed at the Histology Laboratory, School of Veterinary Medicine and Biomedical Sciences, IPB University. A total of 45 male Sprague Dawley rats were used, sourced from the Food and Drug Supervisory Agency in Jakarta. Eligible rats were randomly assigned to nine groups: six tempe-based dietary treatment groups, two diabetic controls, and one non-diabetic control group. DM was induced in rats using streptozotocin, and dietary intervention lasted four weeks. Various outcome parameters, including blood glucose, insulin levels, BW, organ histology, and metabolite profiles, were assessed to determine the effects of different tempe-based diets.

Animals, eligibility, sampling strategy and randomization

A total of 45 male Sprague Dawley rats (*Rattus norvegicus*) were obtained from the Food and Drug Supervisory Agency (BPOM), Jakarta, Indonesia. Based on the Federer formula, a minimum of three rats per treatment group was required. To account for potential mortality or other factors that could lead to data loss, five rats per group were allocated. All animals used were 2.5–3.0 months old and weighed between 240–250 g at the start of the experiment. Rats that were unhealthy or did not meet the specified age or weight criteria were excluded. Eligible rats were randomly assigned to nine groups, consisting of eight diabetic groups and one non-diabetic control group. The variation in BW among rats within each group was maintained within 10 g, and the variation among groups did not exceed 5 g.

Acclimatization and housing

The rats were housed individually in cages under controlled conditions, with adequate lighting and ventilation, a 12-hour light–dark cycle, a stable temperature of 22–25°C, and relative humidity maintained at 50–60%. A seven-day acclimatization period was carried out to allow adaptation to the new environment. During this period, a standard laboratory diet was provided, and clean drinking water was made available *ad libitum*. Health status and general behavior were monitored daily to ensure the animals' suitability for the experiment.

Diabetes mellitus model induction

A rat model of DM was employed in this study. Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin at a dose of 40 mg/kg BW, dissolved in 1 mL of citrate buffer (0.1 M, pH 4.5). Prior to injection, the rats were fasted for eight hours. Following streptozotocin administration, sugar water was provided to prevent hypoglycemia resulting from pancreatic β-cell damage. Three days after injection, BW and blood glucose levels were measured using blood collected from the tail vein. Rats with blood glucose levels exceeding 250 mg/dL were classified as diabetic and included in subsequent analyses. In cases where diabetic conditions were not achieved, blood glucose levels were reassessed on day 6.

Study groups and interventions

The dietary intervention was carried out over a period of four weeks. Each rat was provided with 25 g of ration per day and had *ad libitum* access to drinking water. The study included six treatment groups that received tempe-based diets and three control groups that received a standard diet. Diabetic rats in the treatment groups were fed tempe flour-based diets, categorized according to the type of soybean (germinated or non-germinated) and the stage of fermentation. The six treatment groups included: *d*iabetic treated *f*resh tempe flour from *g*erminated soybean (DFG), *d*iabetic treated *f*resh tempe flour from *n*on-*g*erminated soybean (DFNG), *d*iabetic treated *semangi*t tempe flour from *g*erminated soybean (DTG), *d*iabetic treated *semangi*t tempe flour from *n*on-*g*erminated soybean (DTNG), *d*iabetic treated *bosok* tempe flour from *g*erminated soybean (DBG), and *d*iabetic treated *bosok* tempe flour from *n*on-*g*erminated soybean (DBNG). The control groups comprised three distinct sets: a *n*egative *c*ontrol (NC) group consisting of non-diabetic rats fed a standard diet, a *p*ositive *c*ontrol (PC) group consisting of diabetic rats receiving a standard diet, and a drug control group consisting of diabetic rats treated with oral gliclazide at a dose of 3.6 mg/kg BW/day along with the standard diet (called *p*ositive *c*ontrol *g*liclazide *g*roup (PCGL)).

Both the standard and tempe-based diets were formulated in accordance to the Association of Official Analytical Collaboration (AOAC) guidelines [12]. The standard diet included casein as the primary protein source due to its 100% digestibility, while the tempe-based diets utilized the respective tempe flours as protein sources. To ensure nutritional adequacy, additional components were incorporated into the tempe-based rations, including cornstarch (carbohydrate source), corn oil (fat source), and commercially available vitamins and minerals. Each diet was formulated to contain 10% crude protein, 8% crude fat, 5% moisture, 1% crude fiber, 1% vitamins, 5% minerals, and 70% carbohydrates.

Fresh, *semangit*, and *bosok* tempe preparation

Tempe production was conducted following the standard procedure employed at Rumah Tempe Indonesia, Bogor, Indonesia. Two types of soybeans—germinated and non-germinated—were used. The germination procedure was adapted from a previous study [13]. Soybeans were first sorted and soaked in water for two hours, then drained. Germination was carried out by storing the soybeans in a dark environment at room temperature (28°C) for 28 hours, with periodic watering every three hours. Once radicles of approximately 0.5 cm in length had emerged, the germinated soybeans were considered ready for tempe production. Non-germinated soybeans underwent sorting and washing, followed by a 2-hour soaking step. This initial soaking stage was omitted for germinated soybeans. After soaking, both types of soybeans were boiled at 100°C for 30 minutes, then subjected to a second soaking period lasting 18 hours to promote natural fermentation by environmental lactic acid bacteria. After fermentation was initiated, the soybeans were dehulled and split using a mechanical processor, followed by thorough washing to

remove sour odors. The soybeans were then rinsed with 100°C water, drained, and spread on a clean surface to cool to room temperature.

The main fermentation process was initiated by adding 1 g of inoculum (*Rhizopus* spp.-based tempe starter) per kg of soybeans. The inoculated soybeans were then packed into perforated food-grade plastic bags (20×10 cm) with holes spaced at 2×2 cm intervals. Fermentation was carried out at 28–30°C with a relative humidity of 80% for different durations to produce distinct types of tempe. Fresh tempe was obtained after two days of fermentation, *semangit* tempe after five days, and *bosok* tempe after seven days. This process yielded six distinct tempe types: fresh germinated tempe (FGT), fresh non-germinated tempe (FNT), *semangit* germinated tempe (SGT), *semangit* non-germinated tempe (SNT), *bosok* germinated tempe (BGT), and *bosok* non-germinated tempe (BNT).

Tempe characteristic evaluation

The six types of tempe produced were evaluated for their proximate composition, color, texture, and pH. Proximate composition analysis included moisture content (oven method), ash content (furnace method), protein (Kjeldahl method), fat (Soxhlet method), and carbohydrate content (by difference), following the AOAC 2019 guidelines [14]. Color analysis was conducted using a Minolta CR-300 chromameter (Konica Minolta Sensing Americas Inc., Ramsey, USA). The color characteristics were assessed using L* (lightness), a* (red-green axis), b* (yellow-blue axis), C* (chroma), and h° (hue angle). Texture analysis was performed using a texture analyzer, where the results were expressed as hardness and slicing power values. The pH measurement of the tempe samples was conducted using a pH meter to determine acidity levels.

Study measurements and outcomes

Throughout the four-week dietary intervention, multiple parameters were measured. Blood glucose levels were recorded every four days using a glucose measurement kit, while BW was monitored every two days. Rat termination was conducted via anesthesia (ketamine 70 mg/kg BW and xylazine 20 mg/kg BW) followed by exsanguination through intracardiac puncture. Following rat termination, additional data were collected from various organs. Serum insulin levels were analyzed post-termination using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co., Ltd., Wuhan, China). Pancreatic beta cell count and density were assessed using immunohistochemical staining. Glycogen deposits in the liver and muscle tissues were evaluated using Periodic Acid-Schiff (PAS) staining.

For tempe characterization, data collected included proximate composition, color characteristics (L*, a*, b*, h°, C*), texture parameters (hardness and sliceability), and pH. Additionally, the metabolite profile of tempe samples was determined using gas chromatography-mass spectrometry (GC-MS).

Blood glucose, body weight (BW), and blood serum insulin analysis

Blood glucose levels were measured every four days using the GlucoDr glucometer kit (All Medicus, Anyang-si, South Korea). Blood was collected from the tail tip, which was first disinfected with 70% alcohol. A lancet needle was used to make a small incision, and gentle massage was applied to facilitate blood flow. The blood sample was applied to a glucometer test strip until the capillary channel was filled, after which the strip was inserted into the glucometer for immediate glucose measurement (mg/dL).

BW was recorded every two days using a precision scale until day 28. Daily food and water intake were monitored by measuring the amount provided and subtracting the residual quantity.

Serum insulin level was analyzed using an ELISA kit (Elabscience Biotechnology Co., Ltd., Wuhan, China) following the manufacturer's protocol. For each well, 50 µL of sample solution (comprising 10 µL of serum and 40 µL of diluent) was added. After stopping the reaction with 50 µL of stop solution, absorbance was measured at 450 nm within 15 minutes.

Immunohistochemical staining of insulin pancreatic β-cells

Pancreatic β-cells were identified via immunohistochemical staining using Cu, Zn-SOD (Sigma Aldrich, Darmstadt, Germany) as explained previously [15,16]. Tissue sections were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide to block endogenous

peroxidase. Sections were incubated with 60 µL of insulin monoclonal antibody, followed by 60 µL of Trekkie Universal Link secondary antibody and 60 µL of Trekk Avidin-HRP (Biocare Medical, Pacheco, California, USA). Diaminobenzidine (DAB) substrate was used for color development, and hematoxylin was applied for counterstaining. After dehydration and mounting with Entellan, β-cells were visualized under a light microscope and analyzed using ImageJ software (National Institutes of Health, Maryland, USA). Cell density was calculated per 1000 µm² of tissue area.

Glycogen content assessment

Liver and muscle glycogen content were evaluated using PAS staining as explained previously [17]. Briefly, tissue sections were deparaffinized, rehydrated through graded ethanol, and rinsed in distilled water. Sections were treated with 1% periodic acid for ten minutes, followed by Schiff reagent (9 minutes for liver; 15 minutes for muscle). Liver sections were additionally soaked in sulfite water. After washing in warm and room-temperature distilled water, counterstaining was done with hematoxylin for three minutes. Dehydration was completed through ethanol and xylene, and sections were mounted using Entellan (Merck, Darmstadt, Germany). Microscopic images were captured for analysis. Observations were conducted using a light microscope, and images were captured using a microscope-mounted camera.

Tempe metabolite profile analysis using a metabolomic approach

Metabolite profiling of tempe samples was performed using GC-MS following derivatization to enhance volatility [17]. Briefly, freeze-dried samples (10 mg) were extracted using a methanol: ultrapure water: chloroform solution (5:2:2) containing ribitol (200 µL/mL) as an internal standard, then lyophilized. The powdered extract was derivatized with 100 µL of 2% methoxyamine in pyridine (30°C, 90 min), followed by silylation with 50 µL of N-methyl-N-(trimethylsilyl)trifluoroacetamide (37°C, 30 min). Derivatized samples were transferred to GC-MS vials for analysis.

To ensure data reliability, a pooled quality control (QC) sample and blanks were included. GC-MS data were converted to AIA format and processed using MS-DIAL ver. 4.00 (Riken, Kanagawa, Japan) with the GC/MS-5MP library, and validated against an in-house database and GL Sciences DB (InertCap 5MS-NP, Kovats RI MSP file; GL Sciences Inc., Tokyo, Japan).

Metabolite identification was performed using multivariate statistical analyses, including principal component analysis (PCA) for unsupervised data processing and orthogonal projections to latent structures-discriminant analysis (OPLS-DA) for supervised modeling. OPLS-DA was applied to evaluate metabolites associated with hypoglycemic activity, specifically those linked to increases in β-cell number, insulin levels, and glycogen content in liver and muscle. The resulting model described the relationship between metabolite profiles and diabetic model control, with intensity values as the X variable and biological activity as the Y variable. Model construction and validation were conducted using SIMCA-P+ version 14.1 (Umetrics, Umeå, Sweden). Validation criteria included R2X, R2Y, and Q2Y values >0.5, a CV-ANOVA $p<0.05$, and intercepts lower than R2Y and Q2Y values, indicating a reliable and predictive model.

Statistical analysis

Tempe characteristic data were analyzed using two-way analysis of variance (ANOVA), followed by Duncan's post hoc test at a 5% significance level. For in vivo analyses, data were subjected to one-way ANOVA, also followed by Duncan's post hoc test at the same significance level. All statistical analyses were conducted using SPSS software version 22.0 (IBM Corp., Armonk, USA).

Results

Characteristics of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans

The proximate composition of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans is presented in **Table 1**. Fresh tempe exhibited the highest moisture content compared to *semangit* and *bosok* tempe, regardless of the soybean treatment. Germination

significantly increased the moisture content of tempe (p<0.001), with fresh germinated tempe displaying the highest levels. In contrast, no significant difference was observed between *bosok* germinated tempe and *bosok* non-germinated tempe.

Table 1. Proximate composition of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans

Tempe groups	Compositions				
	Moisture (%wb)	Ash (%db)	Fat (%db)	Protein (%db)	Carbohydrates (%db)
FGT	64.52±0.42^{e}	2.42±0.25	12.97±1.78	47.94±2.04	36.67±3.72
FNT	59.56±0.56^{d}	2.46±0.08	13.09±1.69	48.45±1.67	36.00±3.43
SGT	56.63±0.79^{c}	2.26±0.17	13.30±1.53	48.95±2.21	35.49±3.85
SNT	54.17±1.10^{b}	2.44±0.36	15.13±4.19	50.16±3.93	32.27±7.79
BGT	47.67±0.98^{a}	2.36±0.38	15.62±4.05	50.44±4.15	31.58±8.54
BNT	46.64±1.07^{a}	2.48±0.20	16.25±4.64	50.55±4.19	30.72±9.02
p-value*	<0.001	0.896	0.710	0.865	0.798

%db: percent dry basis; %wb: percent wet basis; BGT: *bosok* germinated tempe; BNT: *bosok* non-germinated tempe; FGT: fresh germinated tempe; FNT: fresh non-germinated tempe; SGT: *semangit* germinated tempe; SNT: *semangit* non-germinated tempe
Different letter superscripts (a, b, c, d, and e) on the same column indicate significant differences
*Statistically significant at p=0.05, using ANOVA

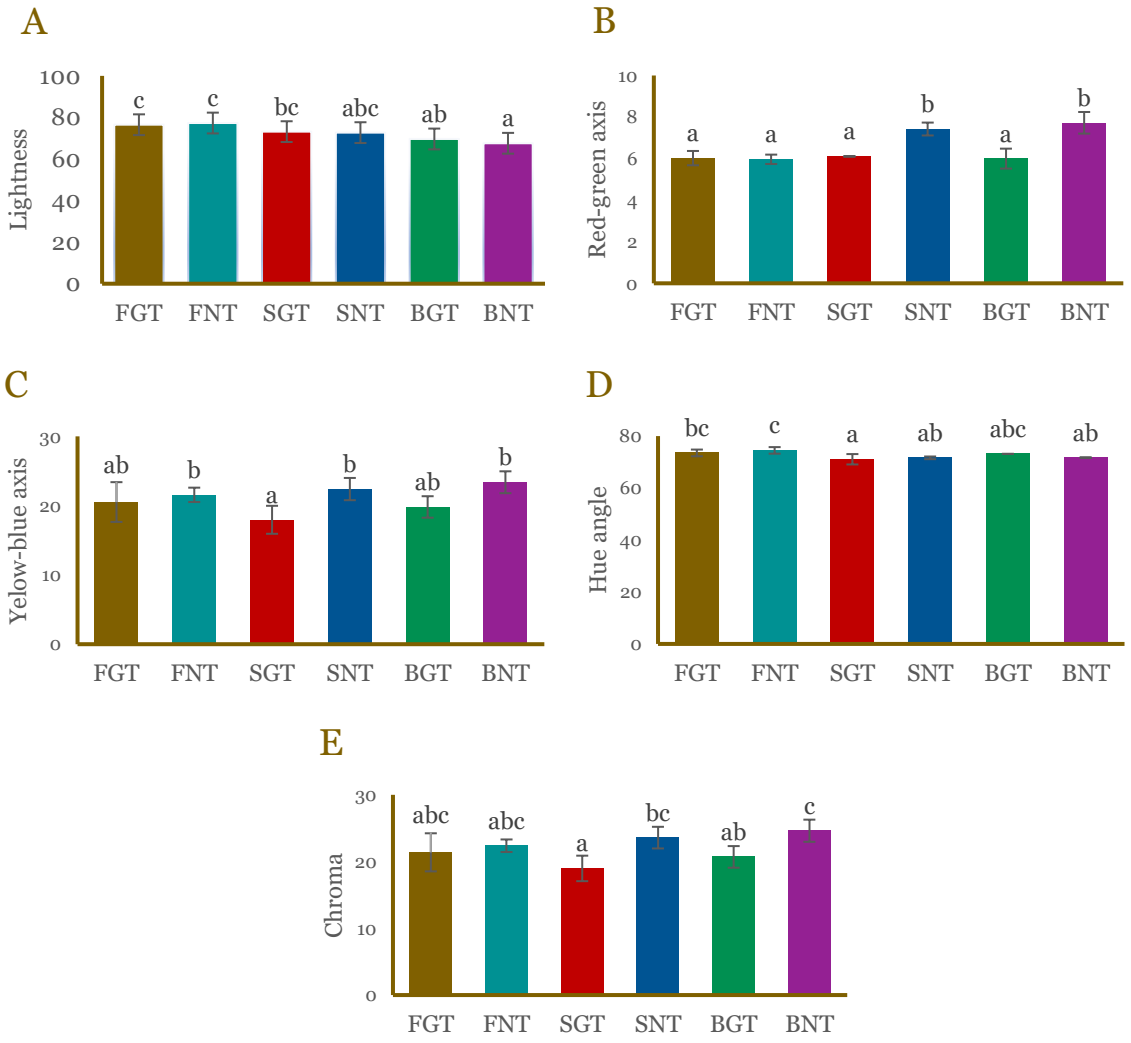

Figure 1. The color measurement of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans. (A) L* (lightness), (B) a* (red-green axis), (C) b* (yellow-blue axis), (D) h° (hue angle), (E) C* (chroma). The difference in letters between samples indicates a significant difference at p<0.05 from other groups. BGT: *bosok* germinated tempe; BNT: *bosok* non-germinated tempe; FGT: fresh germinated tempe; FNT: fresh non-germinated tempe; SGT: *semangit* germinated tempe; SNT: *semangit* non-germinated tempe.

The physical analysis revealed significant differences in color parameters across the different tempe types (**Figure 1**). Fermentation duration had a significant effect on brightness (L* value, p=0.007), redness (a* value, p<0.001), and yellowness (b* value, p=0.044). Fresh germinated and non-germinated tempe exhibited similar brightness levels (p=0.081) compared to *semangit* tempe from both treatments. In contrast, the redness of non-germinated *bosok* tempe and non-germinated *semangit* tempe showed no significant difference (p=0.324). Additionally, tempe from non-germinated soybeans had a more pronounced yellow hue compared to tempe from germinated soybeans (**Figure 1**).

Fermentation duration significantly influenced tempe texture in hardness (p=0.001) and slicebility (p=0.003), as presented in **Figure 2**. *Bosok* tempe, regardless of soybean treatment, exhibited the highest hardness values (p=0.001), indicating increased firmness with prolonged fermentation. In terms of slicing power, non-germinated *bosok*, *semangit*, and fresh tempe required greater force compared to their germinated counterparts.

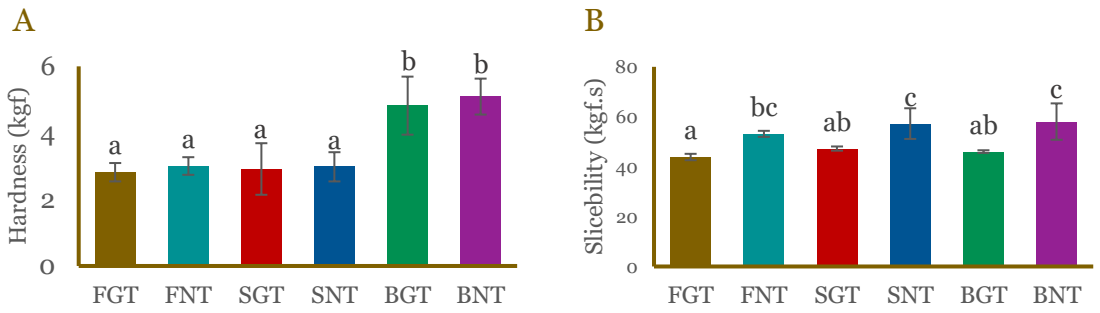

Figure 2. Texture analysis of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans: (A) tempe hardness and (B) tempe sliceability. The difference in letters between samples indicates a significant difference at p<0.05 from other groups. BGT: *bosok* germinated tempe; BNT: *bosok* non-germinated tempe; FGT: fresh germinated tempe; FNT: fresh non-germinated tempe; SGT: *semangit* germinated tempe; SNT: *semangit* non-germinated tempe.

Fermentation time also had a significant effect on tempe pH (p<0.001) (**Figure 3**). Non-germinated *bosok* tempe had the highest pH (p<0.001), whereas fresh tempe had the lowest pH compared to *semangit* and *bosok* tempe (p<0.001) (**Figure 3**).

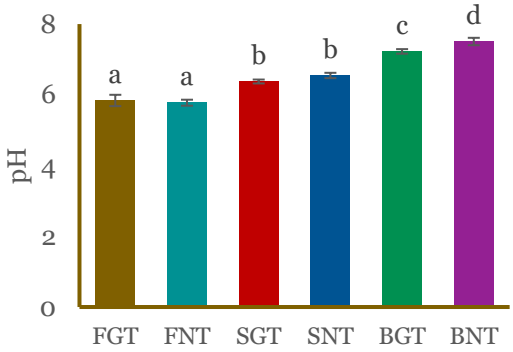

Figure 3. The pH value of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans. The difference in letters between samples indicates a significant difference at p<0.05 from other groups. BGT: *bosok* germinated tempe; BNT: *bosok* non-germinated tempe; FGT: fresh germinated tempe; FNT: fresh non-germinated tempe; SGT: *semangit* germinated tempe; SNT: *semangit* non-germinated tempe.

Effect of fresh, *semangit*, and *bosok* tempe on blood glucose, body weight (BW), and blood serum insulin levels

Based on blood glucose levels (**Figure 4A**), the normal rat group fed standard rations (NC) maintained the lowest and most stable blood glucose levels throughout the four-week treatment. In contrast, the diabetic rats fed standard rations (PC) exhibited persistently high blood glucose

levels, with only a slight reduction observed by the end of the study. Treatment with gliclazide (PCGL) and tempe flour significantly reduced blood glucose levels in DM rats, although neither reached the levels of the NC group. Notably, *semangit* and *bosok* tempe flour, from both germinated and non-germinated soybeans, effectively lowered blood glucose to levels comparable to normal rats by the end of the treatment period. In terms of BW (**Figure 4B**), NC rats maintained stable weight, whereas DM rats experienced weight loss during the observation period, stabilizing only toward the end of the study.

Serum insulin levels were highest (p<0.001) in the NC group and lowest (p<0.001) in the DM rats fed standard rations (PC) (**Figure 4C**). Among the tempe-treated groups, the non-germinated *bosok* tempe (DBNG) group had higher insulin levels than other tempe-fed groups, though not significantly different from the germinated *bosok* tempe (DBG) group. Conversely, the fresh non-germinated tempe (DFNG) group exhibited the lowest insulin levels among tempe-treated rats, though the difference was not statistically significant when compared to the fresh germinated tempe (DFG) and germinated *semangit* tempe (DTG) groups.

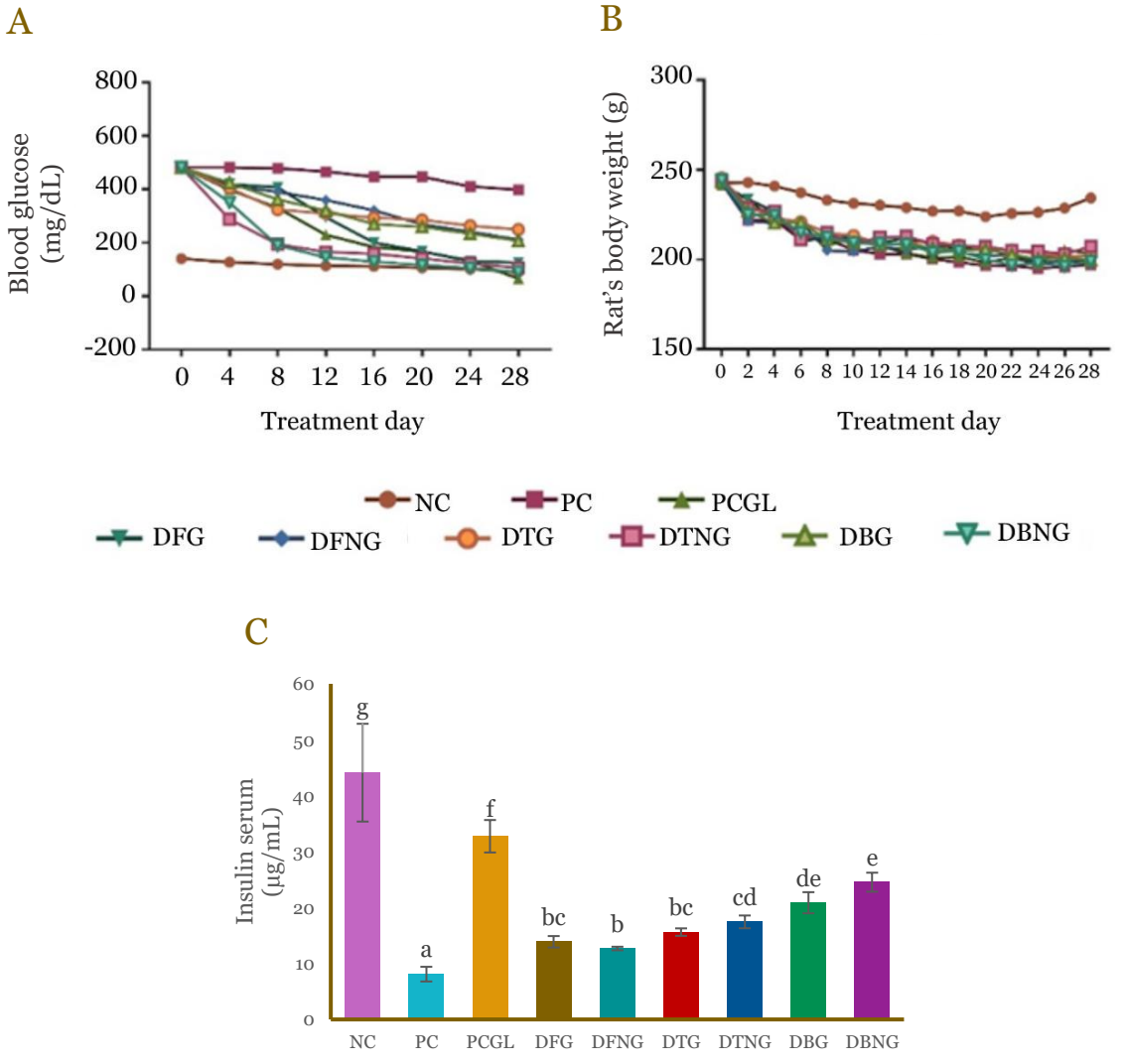

Figure 4. Effect of fresh, *semangit*, and *bosok* tempe against: (A) blood glucose level and (B) body weight (BW) during treatment, and (C) insulin serum after treatment. The difference in letters between samples indicates a significant difference at p<0.05 from other groups. DBG: diabetic rats + bosok germinated tempe; DBNG: diabetic rats + bosok non-germinated tempe; DFG: diabetic rats + fresh germinated tempe; DFNG: diabetic rats + fresh non-germinated tempe; DTG: diabetic rats + *semangit* germinated tempe; DTNG: diabetic rats + *semangit* non-germinated tempe; NC: negative control (normal rats + standard rations); PC: positive control (diabetic rats + standard ration); PCGL: positive control + gliclazide.

Effect of fresh, *semangit*, and *bosok* tempe on immunohistochemical staining of insulin pancreatic β-cells

The results of β-cell insulin staining using immunohistochemical techniques are presented in **Figure 5A**. The number and density of pancreatic β-cells were markedly reduced in diabetic rats compared to normal controls, as presented in **Figures 5B** and **5C**. Administration of gliclazide increased both the number (47.66 cells) and density (4.045 cells/1000 μm²) of β-cells, with the density not significantly different from that of normal rats (4.269 cells/1000 μm²). Feeding fresh, *semangit*, and *bosok* tempe flour, from both germinated and non-germinated soybeans, led to improvements in the Langerhans islet area, β-cell count, and β-cell density. Among the tempe treatments, *bosok* non-germinated tempe demonstrated the greatest effect, with the highest β-cell count (49.90 cells) and a β-cell density (3.891 cells/1000 μm²) in DM rats.

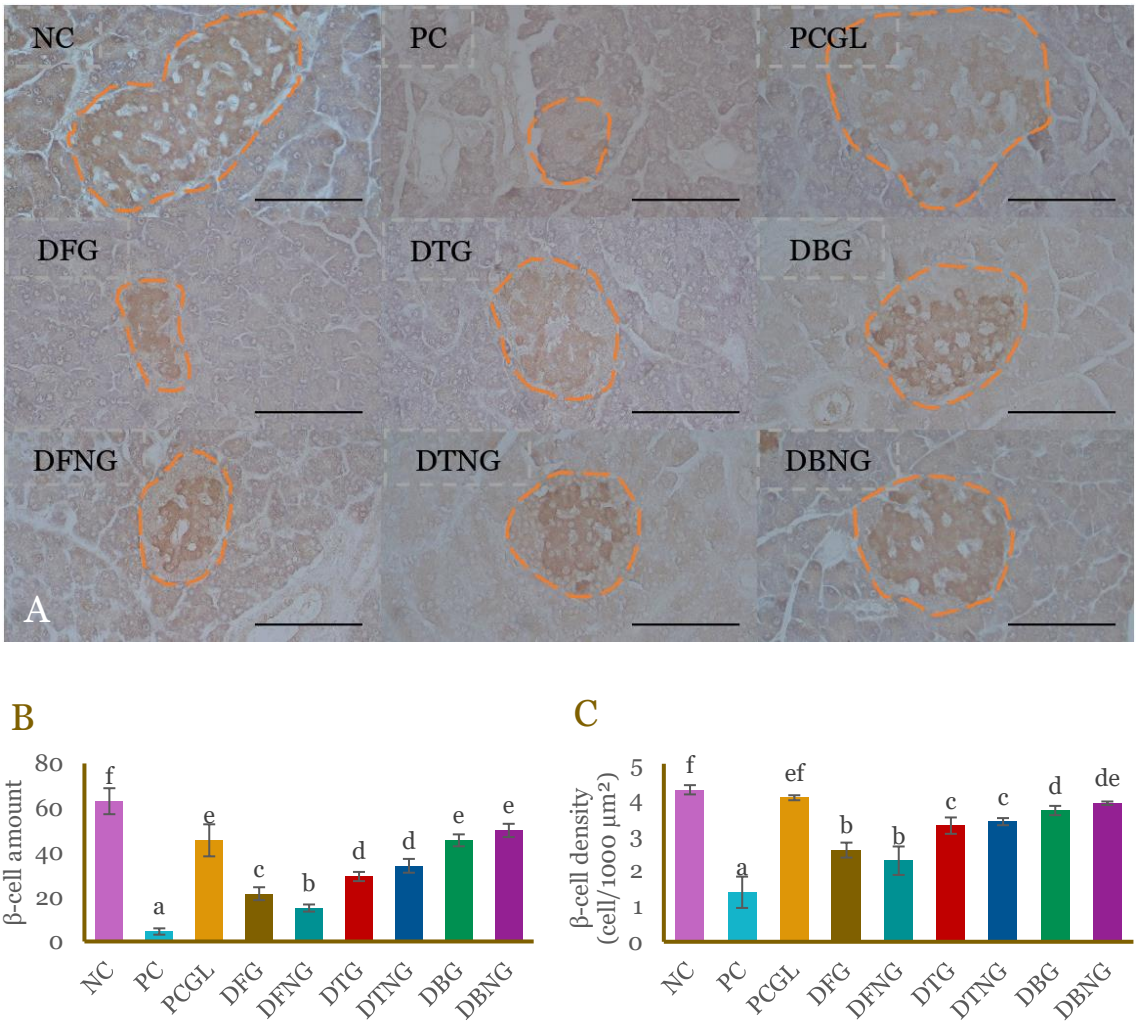

Figure 5. Pancreatic β-cell analysis using insulin immunohistochemical staining. (A) Representative photomicrographs of β-cells in the pancreatic islets of Langerhans across treatment groups (400× magnification, scale bar=100 μm). Brown staining indicates insulin-positive β-cells. (B) Quantification of β-cell number. (C) β-cell density in pancreatic tissue. The difference in letters between samples indicates a significant difference at $p<0.05$ from other groups. DBG: diabetic rats + *bosok* germinated tempe; DBNG: diabetic rats + *bosok* non-germinated tempe; DFG: diabetic rats + fresh germinated tempe; DFNG: diabetic rats + fresh non-germinated tempe; DTG: diabetic rats + *semangit* germinated tempe; DTNG: diabetic rats + *semangit* non-germinated tempe; NC: negative control (normal rats + standard rations); PC: positive control (diabetic rats + standard ration); PCGL: positive control + gliclazide.

Effect of fresh, *semangit*, and *bosok* tempe on glycogen content in liver and muscle tissues

The histological analysis of glycogen deposits in liver and muscle tissues is presented in **Figure 6**. High glycogen accumulation was indicated by intense magenta coloration, which was assessed both visually (**Figures 6A** and **6B**) and quantitatively (**Figures 6C** and **6D**). Diabetic rats exhibited low magenta intensity, reflecting depleted glycogen stores. Treatment with gliclazide increased glycogen deposition, as evidenced by the enhanced coloration in both liver and muscle tissues. Similarly, feeding fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans led to a notable increase in glycogen storage, as indicated by the more intense magenta staining (**Figures 6A** and **6B**).

Quantitative analysis confirmed that tempe supplementation significantly enhanced glycogen levels in the liver and muscle of DM rats compared to the positive control group (p<0.001). Among the treatments, *bosok* non-germinated tempe induced the highest glycogen accumulation, with significantly increased intensity values in both the liver (0.102 gv^{-1}) and muscle (0.009 gv^{-1}) (p<0.001) (**Figures 6C** and **6D**).

Tempe metabolite profile

Metabolite profiling of fresh, *semangit*, and *bosok* tempe, derived from both germinated and non-germinated soybeans, was performed using GC-MS, resulting in the identification of 154 compounds. Of these, 63 compounds were annotated using an in-house database. PCA was conducted to assess the effects of fermentation stage and soybean germination on metabolite composition. Four distinct clusters were observed (**Figure 7A**). The score plot showed that extended fermentation accounted for 33.5% of the total variance (PC1), separating the samples into four distinct groups. PC2 accounted for 22.4% of the variance and reflected changes associated with the transition from *semangit* to *bosok* tempe. These findings suggest that both fermentation duration and soybean germination may induce substantial changes in the metabolite profile of tempe.

The distribution of annotated metabolites across different tempe types is presented in the loading plot (**Figure 7B**). *Semangit* and *bosok* tempe exhibited a substantially higher number of dominant metabolites compared to fresh tempe, indicating that extended fermentation enhances the formation of simple compounds (**Figure 7B**). In contrast, fresh tempe contained only one dominant metabolite, highlighting the differences in metabolic profiles across fermentation stages.

The dominant metabolite composition for each PCA cluster is presented in **Table 2**. Fresh tempe was characterized by a single dominant metabolite, lysine. In contrast, *semangit* and *bosok* tempe exhibited a significantly greater number and variety of dominant metabolites. *Bosok* tempe produced from non-germinated soybeans showed the highest metabolite diversity, including amino acids, sugars, sugar alcohols, amines, and other compound classes. The extension of fermentation from *semangit* to *bosok* tempe resulted in notable changes in the dominant metabolite profiles of both germinated and non-germinated soybean tempe. A greater number of dominant metabolites was observed in *bosok* tempe derived from non-germinated soybeans compared to that from germinated soybeans.

The dominant metabolites identified in **Table 2** were further analyzed using OPLS-DA to determine their association with hypoglycemic activity. The analysis focused on key parameters, including the number of β-cells, insulin levels, and glycogen content in liver and muscle tissues. The validation results are summarized in **Table 3**. The model demonstrated strong predictive capability, with R^2X, R^2Y, and Q^2Y values exceeding 0.5, indicating a well-fitted model with high explanatory power for both the metabolite data (X variables) and biological outcomes (Y variables). Additionally, a CV-ANOVA value below 0.5 confirmed the statistical validity and reliability of the model. The lower R^2Y intercept value compared to the R^2Y value, as well as the lower Q^2Y intercept value compared to the Q^2Y value.

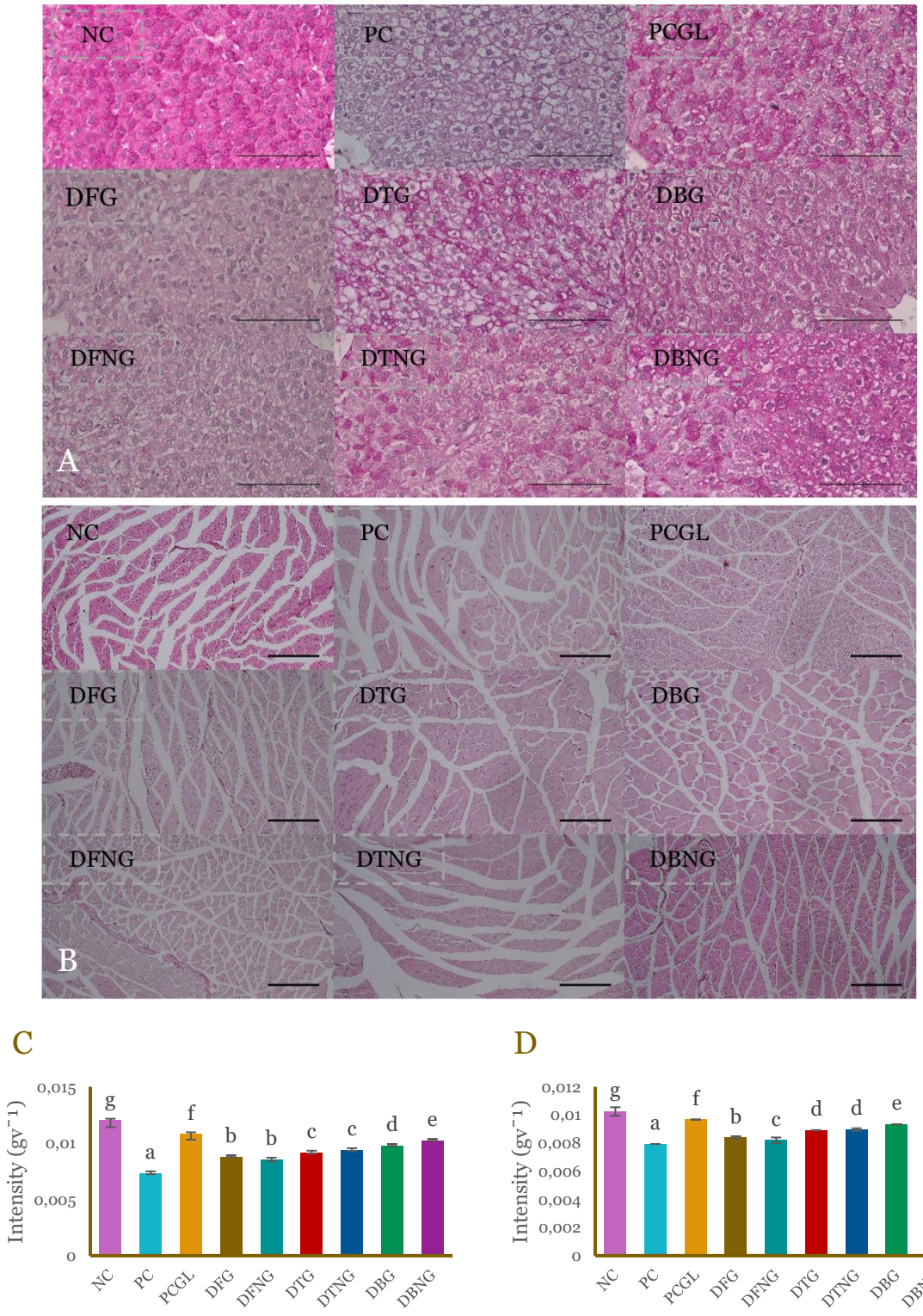

Figure 6. Histological analysis of liver and muscle tissues using Periodic Acid-Schiff (PAS) staining. (A) Photomicrograph of liver glycogen deposition in rats following treatment (400× magnification, scale bar=100 µm). (B) Photomicrograph of muscle glycogen deposition in rats following treatment (100× magnification, scale bar=100 µm). The magenta coloration indicates glycogen accumulation. (C) Quantitative analysis of glycogen intensity in liver tissue. (D) Quantitative analysis of glycogen intensity in muscle tissue. The difference in letters between samples indicates a significant difference at $p<0.05$ from other groups. DBG: diabetic rats + *bosok* germinated tempe; DBNG: diabetic rats + *bosok* non-germinated tempe; DFG: diabetic rats + fresh germinated tempe; DFNG: diabetic rats + fresh non-germinated tempe; DTG: diabetic rats + *semangit* germinated tempe; DTNG: diabetic rats + *semangit* non-germinated tempe; NC: negative control (normal rats + standard rations); PC: positive control (diabetic rats + standard ration); PCGL: positive control + gliclazide.

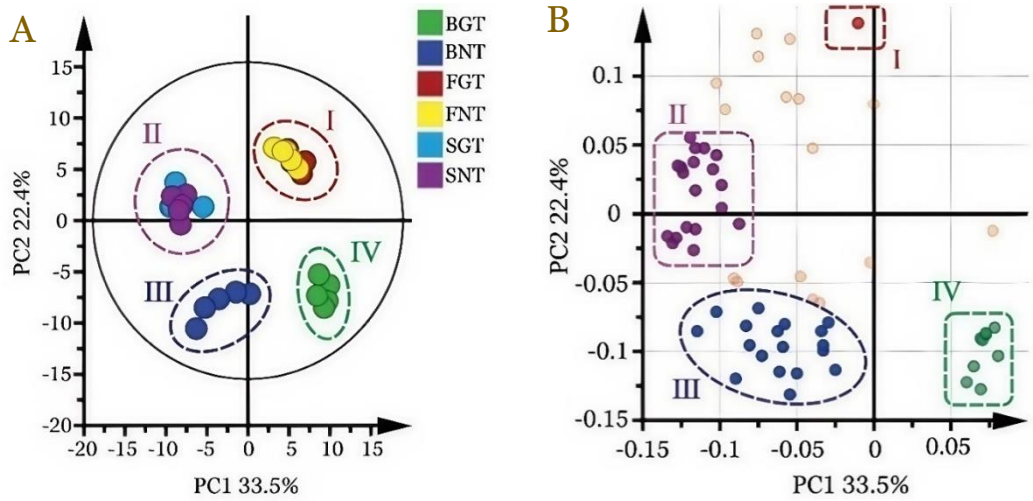

Figure 7. Metabolite profiling of tempe samples (fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans) based on data from gas chromatography-mass spectrometry (GC-MS) analysis using principal component analysis (PCA). (A) Score plot of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans based on metabolite profile. Clusters 1 (fresh tempe from germinated and non-germinated soybeans) and 4 (*bosok* tempe from germinated soybeans) were located on the positive side of PC1, while clusters 2 (*semangit* tempe from germinated and non-germinated soybeans) and 3 (*bosok* tempe from non-germinated soybeans) appeared on the negative side. These findings indicate that fermentation duration and soybean germination significantly alter the metabolite profile of tempe. (B) Loading plots of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans based on metabolite profiles. *Semangit* and *bosok* tempe contained significantly more dominant metabolites than fresh tempe, suggesting that longer fermentation promotes the production of simpler compounds. BGT: *bosok* germinated tempe; BNT: *bosok* non-germinated tempe; FGT: fresh germinated tempe; FNT: fresh non-germinated tempe; SGT: *semangit* germinated tempe; SNT: *semangit* non-germinated tempe.

Table 2. The composition of dominant metabolites in each cluster in the principal component analysis (PCA) model

Cluster I (FGT and FNT)	Cluster II (SGT and SNT)	Cluster III (BNT)	Cluster IV (BGT)
Lysine[a]	Glutamic acid[a]	β-alanine[a]	Phenylalanine[a]
	Glycine[a]	β-lactose[b]	Isoleucine[a]
	Alanine[a]	Trehalose[b]	Leucine[a]
	Proline[a]	2-aminoadipatic acid[c]	Valine[a]
	Aspartic acid[a]	2-hidroxyglutaric acid[c]	Linoleic acid[c]
	Asparagine[a]	Alantoic acid[c]	Oleic acid[c]
	Glucose[b]	3-phosphoglycerate acid[c]	Pantothenic acid[c]
	Galactose[b]	Succinic acid[c]	1,3-propandiamine[e]
	Pyroglutamic acid[c]	Citric acid[c]	Cadaverine[e]
	Malonic acid[c]	Nicotinic acid[c]	
	Gliseric acid[c]	Oxalic acid[c]	
	Fumaric acid[c]	3-Hydroxy-3-methyl glutaric acid[c]	
	3-hydroxyanthranilate[c]	Mannitol[d]	
	Malic acid[c]	Meso-erythritol[d]	
	saccharic acid[c]	Xylitol[d]	
	2-Aminoetanol[e]	Timin[e]	
	Putrescine[e]	Glukono-1,5-lakton[f]	
	Xanthine[e]	Daidzein[f]	
	Phosphate[f]		

BGT: *bosok* germinated tempe; BNT: *bosok* non-germinated tempe; FGT: fresh germinated tempe; FNT: fresh non-germinated tempe; SGT: *semangit* germinated tempe; SNT: *semangit* non-germinated tempe
The value of contributed metabolites is above 0.85
[a]Amino acids
[b]Organic acids
[c]Sugars
[d]Alcoholic sugars
[e]Amine
[f]Others

Table 3. Orthogonal projection to latent structure–discriminant analysis (OPLS-DA) model validation between tempe metabolites and blood glucose reduction parameters

Parameter	R^2X	R^2Y	Q^2Y	CV-Anova	R^2Y intercept	Q^2Y intercept
Increase in the number of β-cell	0.535	0.936	0.907	1.59E-12	0.389	-0.469
Increase in insulin Level	0.523	0.912	0.849	6.11E-10	0.378	-0.467
Increase in liver glycogen	0.546	0.902	0.861	2.27E-10	0.395	-0.456
Increase in muscle glycogen	0.551	0.922	0.883	2.65E-11	0.377	-0.476

Discussion

This study evaluated the characteristics (proximate composition, color, texture, and pH) and metabolite profiles of three tempe variants—fresh, *semangit*, and *bosok*—as well as their effects on BW, blood glucose levels, serum insulin, pancreatic β-cell count, and glycogen content in liver and muscle tissues in a diabetic rat model. The proximate compositions of fresh, *semangit*, and *bosok* tempe from germinated soybeans were not significantly different, except for moisture content. Color analysis showed a decrease in lightness of *semangit* and *bosok* tempe, indicating reduced brightness compared to fresh tempe. A previous study reported that the decline in lightness with prolonged fermentation is due to moisture loss, which increases solid concentration and results in a darker appearance [18]. The results for red-green axis, yellow-blue axis, and hue angle values suggest that extended fermentation in non-germinated soybean tempe tends to increase redness, while in germinated soybean tempe it tends to reduce yellowness. The increase in red-green axis value, as suggested by a previous study [19], may be due to the spontaneous growth of *Citrobacter freundii* during soybean soaking. The chroma values show that color saturation decreases in germinated soybean tempe but increases in non-germinated soybean tempe with longer fermentation time.

The hardness and slicebility of tempe increase during the addition of fermentation time. Tempe made from germinated soybeans has a lower slicebility. No previous studies have reported the texture profile of fermented tempe for up to seven days of fermentation. The higher hardness and slicing power of bosok tempe compared to fresh tempe were caused by a decrease in water content. The texture of tempe is greatly influenced by the moisture content. The loss of water in the food matrix makes the matrix structure harder [20].

Bosok tempe has the highest pH, followed by *semangit* tempe. This suggests that the addition of fermentation time can decrease the total organic acids, leading to an increase in pH. The fermentation process can further increase organic acids in tempe, especially lactic acid [21]. The use of lactic acid by microbes during fermentation is positively correlated with the production of alkaline compounds. At the same time, the process of protein and amino acid metabolism produces ammonia [22], which causes the increasing pH of tempe during further fermentation.

The results of this study indicated that the administration of fresh, *semangit*, and *bosok* tempe from germinated and non-germinated soybeans effectively reduced hyperglycemic symptoms in DM rats. This was evidenced by decreased blood glucose levels and an increase in the number of β-cells in the pancreas. Improvements in the pancreatic function were accompanied by increased insulin secretion, which enhances glucose metabolism in the body. The elevated glycogen deposits in the liver and muscles of DM rats consuming tempe suggest that increased insulin secretion positively impacts glucose absorption in cells. Enhanced glucose absorption in cells can fulfill their energy requirements, prompting the body to activate energy reserve formation mechanisms, such as glycogenesis, leading to increased glycogen deposits [23].

The results of this study reinforced the idea and previous reports that tempe has hypoglycemic activity since tempe was able to lower blood glucose levels in DM rats [24-27]. In addition, tempe was able to increase insulin levels in the blood of DM rats [25]. *Bosok* tempe from non-germinated soybeans have the highest hypoglycemic activity compared to other types of tempe. The extended fermentation time showed a significant increase in hypoglycemic activity, while the soybean germination did not show a significant increase. This result aligned with our previous study, which shoswed the high concentration of daidzein, genistein, and total phenolic compounds in tempe *bosok* made from non-germinated soybeans [28].

The distribution of annotated metabolites in tempe is presented in the loading plot (**Figure 7B**). Metabolites are widely distributed in the *semangit* and *bosok* tempe clusters. The

dominant metabolites responsible for the sample grouping were classified into four clusters based on their contribution values exceeding 0.85. The number of dominant metabolites in *semangit* and *bosok* tempe is higher than that found in fresh tempe (**Table 2**). Further fermentation processes lead to the production of dominant metabolites of the organic acid group, amino acids, sugars, and sugar alcohols [29]. This increase can be attributed to the ongoing hydrolysis process during advanced fermentation, resulting in the formation of simpler compounds.

Previous research has shown that the fermentation process produces more metabolites in tempe, which is dominated by amino acids, organic acids, sugars, and other simple compounds [17,30]. Extended fermentation times promote ongoing hydrolysis, resulting in the production of more simple compounds [31-33]. Additionally, vitamins in tempe such as nicotinic acid and pantothenic acid, along with fatty acids like oleic acid and linoleic acid fatty acids, have been reported to increase during fermentation [18,20]. Similar results were observed in this study.

The further fermentation process from *semangit* tempe to *bosok* tempe results in varying dominant metabolites in non-germinated soybean tempe compared to germinated soybean tempe. Notably, *bosok* tempe made from non-germinated soybeans exhibits more dominant metabolites than that made from germinated soybeans. The precise reasons for this disparity remain unclear. It is hypothesized that differences in the hydrolysis mechanism between germinated and non-germinated soybean tempe may arise after the fermentation period of five days.

Bosok tempe from non-germinated soybeans has the highest hypoglycemic effect compared to other types of tempe. Extending the fermentation time led to a significant increase in hypoglycemic activity, while soybean germination did not show a significant increase. This result aligns with the abundance of metabolites found in non-germinated *bosok* tempe due to extended fermentation (**Figure 7B** and **Table 2**). This further supports the hypothesis that certain metabolites in non-germinated *bosok* tempe contribute to its hypoglycemic activity.

The identification of tempe metabolites associated with hypoglycemic activity was carried out by parameters of cell-β count, insulin levels, as well as liver and muscle glycogen. The results of tempe metabolite analysis related to hypoglycemic activity are presented in **Table 6**. Metabolite identification was conducted by considering the variable importance in projection (VIP) value, Y-related value, and findings from previous research. The metabolites identified as significantly contributing to the number of β-cells, insulin levels, liver glycogen, and muscle glycogen were those with VIP values above 0.5 and positive Y-related values, as supported by earlier studies.

The identification results revealed that there were ten tempe metabolites associated with hypoglycemic activity, namely daidzein, genistein, trehalose, xylitol, mannitol, alanine, leucine, phenylalanine, isoleucine, and 2-aminoadipic acid (**Table 6**). These metabolites operate through four mechanisms of lowering blood glucose. Daidzein, genistein, xylitol, and trehalose are able to increase the number of pancreatic β-cells. An increase in the number of pancreatic β-cells can elevate insulin levels, which is further supported by the insulinotropic amino acids leucine, isoleucine, phenylalanine, and alanine, along with 2-aminoadipic acid. Additionally, the increase in glycogen deposits in the liver and muscles, driven by the fulfillment of glucose needs, is influenced by daidzein, genistein, mannitol, and xylitol.

The results of the OPLS analysis showed that the metabolites with the most significant hypoglycemic effects were found in *bosok* tempe from non-germinated soybeans. This is aligned with our previous research, which demonstrated that *bosok* tempe from non-germinated soybean has the potential to act as a hypoglycemic agent, marked by the highest levels of hypoglycemic compounds and antioxidants compared to other tempe, such as daidzein, genistein, phenolic compounds, and insulinotropic free amino acids. *Bosok* tempe from non-germinated soybeans also contains low molecular weight peptides, indicating the potential for an increase in bioactive peptides that have hypoglycemic effects [28]. The extended fermentation time of tempe was found to increase hypoglycemic compounds, while soybean germination did not affect the level of these compounds significantly.

Conclusion

Extending the fermentation duration of tempe was found to enhance the production of hypoglycemic and antioxidant compounds, as evidenced by elevated serum insulin levels, increased numbers of pancreatic β cells, and greater glycogen accumulation in the liver and muscles of diabetic rats. Among the dietary interventions, *bosok* tempe demonstrated the most pronounced effects in improving these parameters compared to fresh or *semangit* tempe. This enhanced bioactivity was attributed to the broader range of metabolites associated with DM regulation that accumulated during prolonged fermentation. However, the type of soybean—germinated or non-germinated—did not significantly affect metabolite enrichment in tempe. These findings suggest that extended fermentation may be optimized to enhance the functional properties of tempe, particularly for applications in diabetes management. Further investigation is warranted to elucidate the mechanisms of action and to assess the clinical relevance of the identified bioactive compounds.

Ethics approval

This research procedure has received permission from the IPB Animal Ethics Commission with number 281/KEH/SKE/I/2025.

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Conflict of interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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References

1. Abdurrasyid Z, Astawan M, Lioe HN, Wresdiyati T. Physicochemical and antioxidant properties of germinated soybean tempe after two days additional fermentation time. Biointerface Res Appl Chem 2023;13(3):238-252.
2. Astawan M, Cahyani AP, Wresdiyati T. Antioxidant activity and isoflavone content of overripe Indonesian tempe. Food Res 2023;7 Suppl 1:42-50.
3. Yoshari RM, Astawan M, Prangdimurti E, Wresdiyati T. The production process of tempe protein isolate from germinated soybeans and its potential as an antidiabetic. Food Res 2023;7 Suppl 1:71-79.

4. Rachmawati NA, Astawan M, Wresdiyati T. Haematological and biochemical serum profiles of experimental rats fed with GMO and non-GMO soybean. J Gizi Pangan 2021;16(28):167-176.
5. Abdurrasyid Z, Astawan M, Lioe HN, Wresdiyati T. Evaluation of hypoglycaemic potency in tempe with soybean germination process and extended fermentation time. Food Res 2023;7 Suppl 1:217-227.
6. Astawan M. Pengaruh konsumsi tempe dari kedelai germinasi dan non-germinasi terhadap profil darah tikus diabetes. J Pangan 2019;28(2):135-144.
7. Astawan M, Rahmawati IS, Cahyani AP, *et al*. Comparison between the potential of tempe flour made from germinated and nongerminated soybeans in preventing diabetes mellitus. HAYATI J Biosci 2020;27(1):17-23.
8. Astawan M, Wresdiyati T, Yoshari RM, *et al*. The physicochemical properties of tempe protein isolated from germinated and non-germinated soybeans. J Nutr Sci Vitaminol 2020;66:S215-S221.
9. Kanetro B. Amino acid profile of soybean (*Glicine max*) sprout protein for determining insulin stimulation amino acids. Int Food Res J 2018;25(6):2497-2502.
10. Kadar AD, Astawan M, Putri SP, Fukusaki E. Metabolomics based study of the effect of raw materials to the end product of tempe—an indonesian fermented soybean. Metabolites 2020;10(9):367.
11. Puspitasari A, Astawan M, Wresdiyati T. The effect of soybeans germination on proximate composition and isoflavones bioactive components of fresh and semangit tempe. Pangan 2020;1(29):35-44.
12. Association of Official Analytical Chemistry. Official methods of analysis of the Association of Official Analytical Chemists. Washington: AOAC International; 1995.
13. Astawan M, Wresdiyati T, Purnomo EH, Purwanto A. Equivalence test between the physicochemical properties of transgenic and non-transgenic soy flour. J Nutr Sci Vitaminol 2020;66:S286-S294.
14. Association of Official Analytical Chemistry. Official methods of analysis of the Association of Official Analytical Chemists. Washington: AOAC International; 2019.
15. Buchwalow IB. Immuno-histochemistry basics and methods. New York: Springer New York LLC; 2010.
16. Wresdiyati T, Iskandar DC, Sa'diah S, Astawan M. In vitro and in vivo hypoglycemic activity test of Indonesian *Cajanus cajan* leaves and *Zingiber officinale* extracts. Malaysian J Med Heal Sci 2020;16(13):13-14.
17. Rahmawati D, Astawan M, Putri SP, Fukusaki E. Gas chromatography-mass spectrometry-based metabolite profiling and sensory profile of Indonesian fermented food (tempe) from various legumes. J Biosci Bioeng 2021;132(5):487-495.
18. Murata K, Ikehata H, Miyamoto T. Studies on the nutritional value of tempeh. J Food Sci 2006;32:580-586.
19. Muzdalifah D, Athaillah ZA, Nugrahani W, Devi AF. Colour and pH changes of tempe during extended fermentation. AIP Conf Proc 2017;1803(1):020036.
20. Bisping B, Hering L, Baumann U, *et al*. Tempe fermentation: Some aspects of formation of gamma-linolenic acid, proteases and vitamins. Biotechnol Adv 1993;11(3):481-493.
21. Astawan M, Damayanti AF, Wresdiyati T, *et al*. Potential of soybean-velvet bean combination tempe in improving cognitive function. Narra J 2024;4(3):e1365.
22. Sitanggang AB, Lesmana M, Budijanto S. Membrane-based preparative methods and bioactivities mapping of tempe-based peptides. Food Chem 2020;329:127193.
23. Govindarajan S, Babu SN, Vijayalakshmi MA, *et al*. *Aloe vera* carbohydrates regulate glucose metabolism through improved glycogen synthesis and downregulation of hepatic gluconeogenesis in diabetic rats. J Ethnopharmacol 2021;281:114556.
24. Bintanah S, Kusuma HS. Pengaruh pemberian bekatul dan tepung tempe terhadap profil gula darah pada tikus yang diberi alloxan. J Pangan Gizi 2010;01(02).
25. Huang YC, Wu BH, Chu YL, Chang W. Effects of tempeh fermentation with *Lactobacillus plantarum* and *Rhizopus oligosporus* on streptozotocin-induced type II diabetes mellitus in rats. Nutrients 2018;10(9):1143.
26. Masdar H, Satriyasumatri T, Hakiki M, *et al*. Histological apperarance of diabetes-rat pancreas administrated by soybean compared to tempeh. AIP Conf Proc 2019;2108(1):020012.
27. Yoshari RM, Aini AN, Prangdimurti E, *et al*. Pengaruh konsumsi tempe dari kedelai germinasi dan non-germinasi terhadap profil darah tikus diabetes. J Pangan 2019;28(2):135-144.
28. Abdurrasyid Z, Astawan M, Lioe HN, Wresdiyati T. Evaluation of hypoglycaemic potency in tempe with soybean germination process and extended fermentation time. Food Res 2023;7(1):217-227.
29. Nurkolis F, Taslim NA, Lee D, *et al*. Mechanism of action of isoflavone derived from soy-based tempeh as an antioxidant and breast cancer inhibitor via potential upregulation of miR-7-5p: A multimodal analysis integrating pharmacoinformatics and cellular studies. Antioxidants 2024;13(6):632.

30. Ahnan-winarno AD, Xiao H, *et al*. Tempeh : A semicentennial review on its health benefits, fermentation, safety, processing, sustainability, and affordability. Compr Rev Food Sci Food Saf 2021;21(2):1717-1767.
31. Baumann U, Bisping B. Proteolysis during tempe fermentation. Food Microbiol 1995;12:39-47.
32. Chang CT, Hsu CK, Chou ST, *et al*. Effect of fermentation time on the antioxidant activities of tempeh prepared from fermented soybean using *Rhizopus oligosporus*. Int J Food Sci Technol 2009;44(4):799-806.
33. Rizal S, Kustyawati ME, Murhadi, Hasanudin U. The growth of yeast and fungi, the formation of β-glucan, and the antibacterial activities during soybean fermentation in producing tempeh. Int J Food Sci 2021;2021:6676042.