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Comparative metabolomic analysis of Tempe from velvet bean, soybean, and their combination

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Abstract

Tempe, an Indonesian fermented soybean product, is a popular choice as a source of protein. Currently, Indonesia is very dependent on imported soybeans. Therefore, using local beans as raw material for tempe is necessary to reduce import dependence. Velvet beans (*Mucuna pruriens*) are legumes that have not been well utilized because they have many anti-nutritional compounds. Velvet beans have high carbohydrate and protein contents and other metabolites that can be utilized as antioxidant functional foods. Therefore, this research aims to analyzed the metabolites profile and explore the potential of velvet beans to partially replace soybeans in making tempe. Velvet bean seeds (VBS) and the tempe made from soybeans (SBT), velvet beans (VBT), and their combination (CSVT) were analyzed for metabolites profile by Gas Chromatography-Mass Spectrometry (GC-MS), and antioxidant activity by DPPH test (IC_{50}). Multivariate Principal Component Analysis (PCA) of the metabolomics study showed that all samples were separated based on Principal Component (PC) 1 with 58.3% variation caused by fermentation process. It was shown that organic acids and sugar derivatives were on the positive axis, while amino acids were on the negative axis. In addition, the samples were separated based on PC2 with a variation of 35.8%, which was caused by differences in its bioactive components. Allantoin and DOPA compounds were on the negative axis, while genistein and daidzein were on the positive axis. The lowest IC_{50} value for tempe was found in VBT (361 ppm), then followed by CSVT (2,216 ppm) and SBT (15,704 ppm). These results indicate that adding velvet beans to make tempe can increase its antioxidant capacity. CSVT had more diverse metabolites, which could increase their antioxidant activity. Furthermore, CSVT presents a promising avenue for local functional food diversification, particularly in supporting efforts to reduce the consumption of imported soybeans.

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1. Introduction

The adoption of a plant-based lifestyle has gained popularity among the public. Some studies have indicated that it can help reduce the risk of cardiovascular diseases, cancer,

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diabetes, and obesity (1,2). Moreover, plant-based diets can encourage the growth of gut microbiota (3). Soybean tempe is a popular plant in Indonesia and contains high level of protein (4). Soybean tempe also contains bioactive compounds, such as isoflavones that function as antioxidants (5). Therefore, tempe can be used as an alternative plant-based diet.

Some regions in Indonesia use velvet beans (*Mucuna pruriens*) to produce tempe. Velvet beans contain high levels of protein, carbohydrates, and fiber (6). Velvet bean is a legume that can grow on dry and infertile lands. It has a high nutritional content, is relatively inexpensive, and is grown locally in Indonesia. Hence, imports from other countries need not be imported. Like other legumes, Velvet beans generally contain anti-nutritional compounds, such as phytates, polyphenols, protease inhibitors, and aromatic amino acids (7). In addition, velvet beans have a high content of bioactive compounds, such as phenolic compounds and flavonoids which can be used as antioxidants (8). L-DOPA, phytates, tannins, oxalates, hydrogen cyanide, and trypsin inhibitors are commonly found in velvet beans (9). However, velvet bean tempe is only sometimes available and only needed in particular regions. Furthermore, few comprehensive studies have been conducted on tempe velvet bean.

Metabolomics based on gas chromatography-mass spectrometry (GC-MS) has recently been used for food analysis. This method can be used for volatile and non-volatile compounds, such as sugars, amino acids, and organic acids, owing to the development of various derivatization processes. In food science, metabolomic approaches can distinguish food samples by cultivar and authentication to prevent food fraud, characterize food sample profiles, and evaluate metabolite changes due to processing (10). Metabolomics using GC-MS is also commonly used for metabolite profiling of foods. One of which examined the metabolite profile of tempe. These studies examined the impact of various factors, such as the length of fermentation time (11), type of legumes and the location of tempe making (12), the origin of the raw material (13), the type of raw material, the environment when making tempe, and the type of starter used (14). Previous studies analyzing the metabolite profile of tempe from various legumes from Indonesia showed changes in amino acids caused by the fermentation process. In addition, there are also changes in amino acid composition in soybean tempe combinations with several different legumes (12).

However, metabolomic analysis to examine profile metabolites and the correlation with the antioxidant activity of velvet bean tempe, soybean tempe, or mixed legume tempe (velvet bean and soybean) is still limited. Therefore, the purpose of this study was to identify the distinguish of metabolite profiles of three types of tempe made from soybean, velvet bean, and soybean-velvet bean combination and to analyze how the metabolite correlation to antioxidant activity by Orthogonal Projections to Latent Structures (OPLS) method.

2. Materials and Methods

2.1. Materials

The materials used were soybean from Rumah Tempe Indonesia, velvet bean from PT Nagari Bumi Asri, tempe starter Raprima, and polypropylene plastic for packaging. GC-MS analysis utilized the following materials: ultrapure water (Genpure, Thermo Scientific, Osaka, Japan), ribitol (Fujifilm Wako Pure Chemical Industries, Ltd., Osaka, Japan), pure pyridine (Fujifilm Wako Pure Chemical Industries, Ltd., Osaka, Japan), GC-MS grade methanol (Kanto Chemical Co., Inc., Tokyo, Japan), GC-MS grade chloroform (Kishida Chemical Co., Ltd.), methoxyamine hydrochloride (Sigma Aldrich Japan), trifluoroacetamide (MSTFA) (GL

Sciences, Tokyo, Japan), and an alkene mix (C9-C40) (GL Sciences, Tokyo, Japan). Antioxidant analysis employed 1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid (Sigma-Aldrich).

2.2. Production of Tempe

The soybean tempe production process starts with the sortation of soybean seeds, soaking for 2 hours, boiling for 10 minutes, soaking for 12 hours, dehulling, washing, draining, cooling, inoculation, packaging, and fermentation. For velvet bean tempe, it starts from selecting velvet bean seeds, first soaking for 48 hours, boiling for 60 minutes, second soaking for 48 hours, dehulling, washing, steaming for 15 minutes, draining, cooling, inoculation, packaging, and fermentation. For combination tempe, preparation was done according to the preparation of each tempe, and mixing was done at the stage before fermentation. The ratio of soybean and velvet bean was 61:39% (w/b), referring to a previous study on optimizing protein content and hardness of tempe produced (6).

2.3. Production of Tempe flour

Tempe flour was made by cutting tempe into blocks with a size of 2 cm x 1 cm, then dried using a freeze dryer (Operon FDB-5502, Korea) for 24 hours. Then, the dried tempe was pulverized using a miller (Panasonic MX-AC400, India) and sieved using an electromagnetic sieve shaker (BA200N, Spain), and 80-mesh sieve.

2.4. Extraction and Derivatization of Hydrophilic

Metabolite extraction and derivatization followed published extraction methods with some modifications (12). Into a 2mL microfuge tube was added 10 mg of each powdered tempe sample, 200 µL/mL ribitol as an internal standard, and 1 mL of a mixed solvent containing methanol, ultrapure water, and chloroform (5:2:2). After that, the mixture was lyophilized and freeze-dried. Next, 100 µL of methoxyamine in pyridine (20 mg methoxyamine/mL pyridine) was added. Furthermore, the mixture was then incubated in a shaker incubator (Eppendorf Ltd., Hamburg, Germany) at 30°C for 90 min for oximization. After oximization, silylation was carried out by adding 50 mL of MSTFA and incubating at 37°C for 30 min. The solution was transferred to a GC-MS vial for analysis at the final stage. In this experiment, three different biological samples from each sample were analyzed.

2.5. GC-MS Analysis

GC-MS analysis was performed using a GC-MS QP2010 Ultra (Shimadzu, Kyoto, Japan). The column used was an Inert-Cap 5 MS/NS (GL Sciences). System control and data acquisition were conducted using GC–MS solution software (Shimadzu). This method follows the method of previous publications (12). The Helium carrier gas used was set with a flow rate of 1.12 mL/min with a linear velocity of 39 cm/s. The derivatized samples were injected into the GC-MS in a 25:1 (v/v) split mode with an injection temperature of 230°C. The column temperature was held at 80°C for 2 minutes in the column, increased by 15°C/min until it reached 330°C, and then held for 6 minutes. The ion source and transfer line were at 200°C and 250°C, respectively. The ions were generated by electron ionization (EI) at 0.94 kV. Mass spectra were recorded at 6.67 scans per second over the mass range (m/z) 85-500. A mixture of standard alkenes (C_9-C_{40}) was initially prepared and injected to calculate the retention index (RI) as a tentative identification.

2.6. GC-MS Data Processing

Raw GC-MS data were converted to the AIA format using the GC-MS solution software package (Shimadzu) following a previously published method (12). The data were processed using MS-DIAL ver. 4.00 using GC/MS-5MP Library (Riken, Kanagawa, Japan) for peak alignment, filtering, and annotation. After each metabolite was annotated, the peak intensity was normalized to the peak intensity of ribitol as an internal standard. Then, metabolites were validated using internal library input via the MS Dial, GL-Sciences DB (InertCap 5MS-NP, Kovats RI MSP file) available online. Furthermore, the screening data for each annotated metabolite should show a relative standard deviation lower than 30% in the quality of the control samples. Afterwards, data visualization was performed using principal component analysis (PCA) and SIMCA ver. 18 (Umetrics, Umea, Sweden).

2.7. Antioxidant Activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay)

The dried sample weighed as much as 10 g, and 50 mL of 95% ethanol was extracted by vortexing in a closed tube. The tube containing the sample and solvent was then centrifuged at 4000 rpm for 5 minutes. A total of 1 mL of sample solution (supernatant) or standard solution was placed into a test tube, and then 7 mL of methanol was added (as a blank, the sample/standard was replaced with methanol so that the total methanol used was 8 mL). Then, 2 mL of DPPH solution was added (the final concentration of the DPPH solution was 0.2 mM) and vortexed thoroughly. The mixture was then incubated at room temperature for 30 minutes. The absorbance of the solution was measured at a wavelength/ λ 517 nm using a spectrophotometer (A=0 using distilled water). The antioxidant capacity was reported as the IC50 value, defined as the sample concentration inhibiting 50% of DPPH radicals, derived from the concentration-response curve (5).

3. Results and Discussion

3.1. Metabolite Changes in the Fermentation Process

Metabolites were analyzed using GC-MS from the aqueous extract of each sample, including velvet bean (VBS), soybean tempe (SBT), velvet bean tempe (VBT), and soybean-velvet bean combination tempe (61:39%, w/b) (CSVT). Forty-five metabolites were tentatively annotated (Table 1) from the four samples before and after fermentation by comparison with an internal library (RI and mass spectra). The obtained metabolites were classified into five major groups: amino acids and their derivatives, sugars and their derivatives, organic acids, isoflavones, and other compounds.

Based on the GC-MS analysis which was then visualized using Principal Component Analysis (PCA) for all samples, including velvet bean (VBS) and three types of tempe (SBT, VBT, and CSVT), a PCA score plot was obtained, which showed clear clustering between each sample based on principal components (PC) 1 and 2 (Figure 1A). Based on PC1, it could be seen that there is a separation of samples where the position of VBS was is on the far right, and the three types of tempe, namely VBT, CSVT, and SBT, were on the left, which was explained by a variance of 58.3%. In PC2, there was a grouping into 2: SBT and CSVT were on the positive axis, whereas VBT and VBS were on the negative axis, explained by a variance of 35.8%.

Table 1. List metabolite was annotated based on GC-MS analysis.

Metabolite Name	Class	Average Rt (min)	Quant mass
Alanine	amino	5.007	116.1
Malonic acid	organic	6.483	147.0733
Valine	amino	6.681	144.1461
Allantoic acid	organic	6.903	171.1115
2-Aminoethanol	others	7.407	174.12
Leucine	amino	7.491	158.1417
Phosphate	others	7.533	299.1
Glycerol	sugars	7.578	147.1
Isoleucine	amino	7.799	158.15
Proline	amino	7.814	142.1154
Glycine	amino	7.967	174.1267
Succinic acid (or anhydride)	organic	8.014	147.0867
Uracil	others	8.39	241.1125
Fumaric acid	organic	8.46	245.0857
Serine	amino	8.762	204.1458
Threonine	amino	9.134	218.1563
beta-Alanine	amino	9.584	248.1346
Malic acid	organic	10.434	147.09
Pyroglutamic acid	amino	10.766	156.1133
Aspartic acid	amino	10.822	232.14
4-Aminobutyric acid	amino	10.887	174.1179
4-Hydroxyphenethyl alcohol (Tyrosol)	others	11.34	179.1389
Glutamic acid	amino	11.998	246.1542
Phenylalanine	amino	12.042	218.1292
Asparagine	amino	12.593	116.1077
Arabitol	sugars	13.325	174.1125
Glutamine	amino	13.711	156.1083
Hypoxanthine	others	14	265.1125
Ornithine	amino	14.234	142.1125
Citric acid	organic	14.315	273.12
Isocitric acid	organic	14.354	245.0875
N-acetyl-alpha-D-glucosamine 1-phosphate	sugars	14.42	204.125
Fructose	sugars	14.986	103.0833
Allantoin	others	15.005	331.15
Galactose	sugars	15.182	147.0955
Histidine	amino	15.268	154.1167
Lysine	amino	15.289	156.15
Glucose	sugars	15.427	147.0909
Tyrosine	amino	15.451	218.1115
Mannitol	sugars	15.581	319.18
Sorbitol	sugars	15.666	147.0875
Galactitol	sugars	15.702	217.13

Metabolite Name	Class	Average Rt (min)	Quant mass
Galacturonic acid	sugars	15.714	333.18
Gluconic acid	sugars	16.26	147.0875
Xanthine	others	16.261	353.1542
Glucono-1,5-lactone	others	16.294	333.1429
Dopa	amino	17.055	218.1083
Inositol	sugars	17.104	305.1567
Sucrose	sugars	21.864	361.2
beta-Lactose	sugars	22.401	204.1214
Trehalose	sugars	22.631	361.2
Daidzein	isoflavones	23.542	398.1555
Genistein	isoflavones	23.748	471.1778
Raffinose	sugars	27.032	361.2

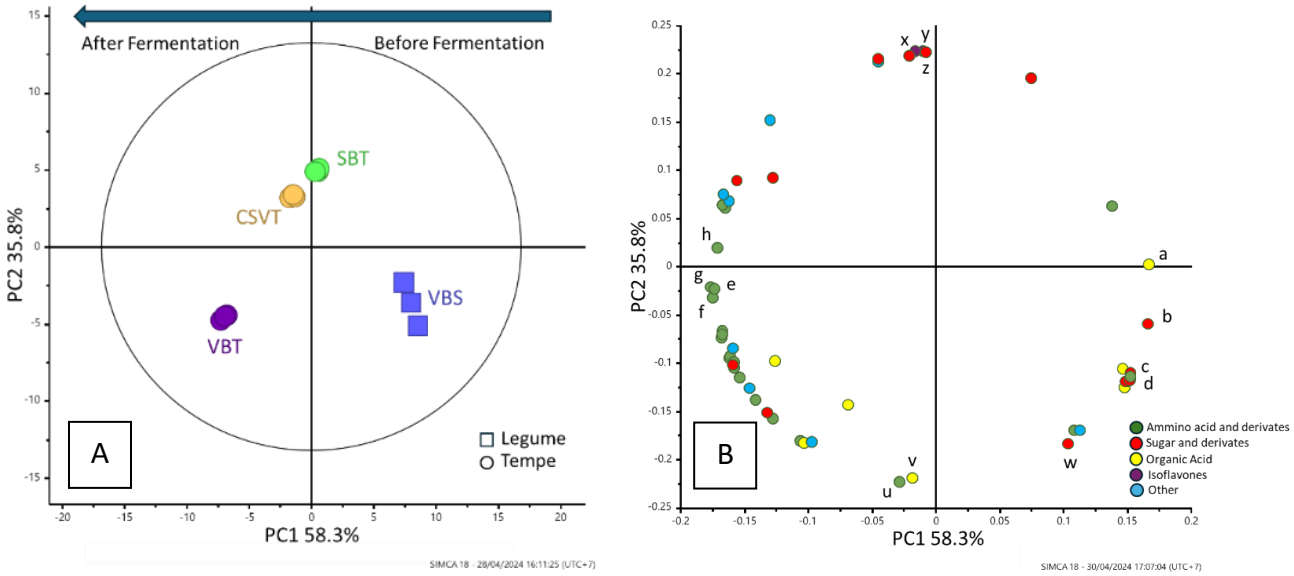

Figure 1. Principal component analysis (PCA) of legumes before and after fermentation produced based on GC-MS analysis. Velvet bean seed (VBS), Velvet bean tempe (VBT), Soybean Tempe (SBT), and Combination Soybean-Velvet bean tempe (CSVT): (A) score plot; (B) loading plot.

The clustering of samples on PC1 occurred because of the differences in the metabolites in each sample. These results are similar to the findings of Prativi et al., who demonstrated that clustering of tempe samples can occur due to the accumulation of significantly different metabolites (11). This condition could be seen from the distribution of metabolites based on the PCA loading plot (Figure 1B), which showed that the metabolites of amino acid groups, such as serine, ornithine, glutamine, and threonine were collected on the negative axis of PC1on the left side. The metabolites of organic acid groups (malonic acid and isocitric acid) and sugars (mannitol and sucrose) were collected on the positive axis of PC1. This result indicates an increase in the relative intensity of amino acids and the formation of amino acids that were not found in the VBS sample/ velvet bean seeds.

The formation of new amino acid metabolites and the increase in relative intensity values of some amino acids were caused by proteolytic enzymatic reactions by *Rhizopus* spp—moulds, where long protein chains are broken down into amino acids (12). The fermentation process in the VBT tempe resulted in more amino acid accumulation than that in SBT and CSVT. Amino acids serine, ornithine, glutamine, and threonine were metabolites that significantly contributed to group separation and accumulated at the highest level in VBT but were low in velvet bean, thus placing the tempe group on the negative axis/ left side in PCA.

Meanwhile, the clustering of metabolites on the positive axis/ right side in PCA (Figure 1) was thought to be metabolites such as malonic acid, mannitol, galactitol, and DOPA, which significantly contributed to PCA clustering. This result was observed by comparing the relative intensities of each metabolite (Figure 2A). These metabolites had high intensity in velvet beans, but were minimal in tempe. This result is consistent with that of provious research (15,16), which showed a decrease in DOPA levels during the tempe-making process.

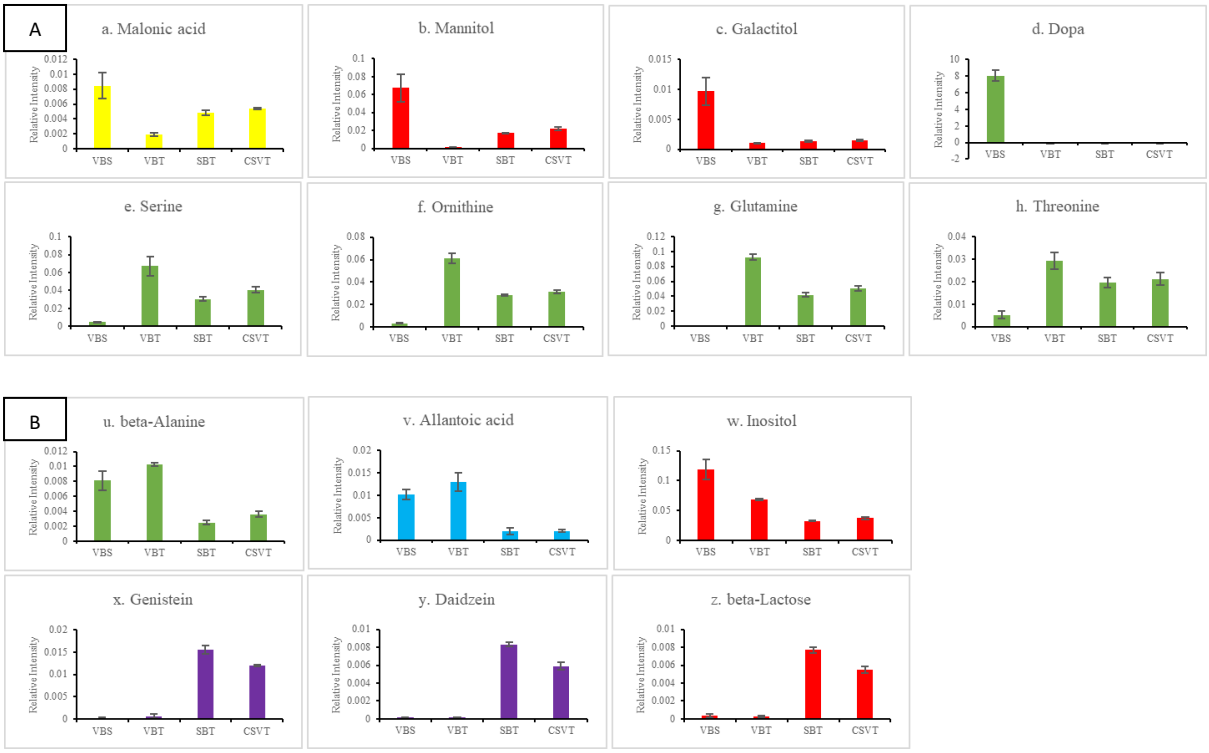

Figure 2. (A) Bar chart primary metabolites as discussed in the main text based on PC1, (B) Bar chart primary metabolites as discussed in the main text based on PC2.

The clustering of samples on PC2 occurred due to differences in the raw materials used to manufacture tempe, including soybean and velvet bean. SBT and CSVT were on the positive axis PC2 upper side because these raw materials were mainly soybean. VBT and VBS were in the negative axis PC2 lower side group because the raw material was velvet beans. This condition was also aligned with previous studies (6), where SBT and CSVT showed similarities in sensory acceptance and amino acid composition. When looking at the distribution of metabolites on the PCA loading plot (Figure1B) and bar chart (Figure 2B), it showed that genistein, daidzein, and beta-lactose were on the positive axis PC2, which indicated that these metabolites were found in the soybean-based tempe, including SBT and CSVT. According to

Astawan et al., soybean-based tempe have a relatively high content of the bioactive compounds daidzein and genistein as a result of the fermentation process, and these are isoflavones in their aglycone form (5). Meanwhile, beta-alanine, allantoic acid, and inositol significantly contributed to the separation of VBT and VBS, whose primary raw material was velvet bean (16).

3.2. Metabolite Profiles of Different Types of Tempe Legumes

The metabolite analysis was continued by focusing on three tempe types: SBT, VBT, and CSVT. PCA analysis indicated different clustering compared to the previous results. This result is shown in Figure 3. PCA score plot analysis showed clear clustering between each sample based on principal component (PC) 1 and 2. PC1 showed the clear separation of samples where the position of SBT and CSVT were on the right or positive axis, while VBT was on the left or negative axis PC1 with a 78.4% variance. Meanwhile, when looking at PC2, there was a grouping into two: SBT and VBT were below the negative axis, while CSVT was above or on the positive axis PC2 with an 11.1% variance (Figure 3).

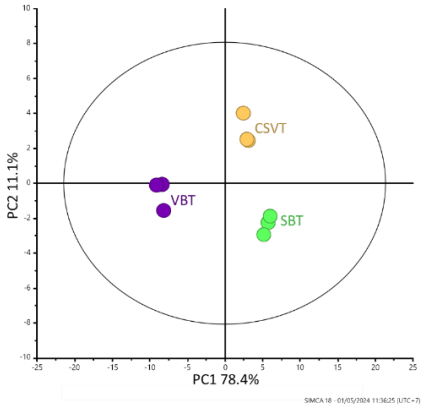

Figure 3. Principal component analysis (PCA) score plot results of legume tempe based on GC-MS analysis. Velvet bean tempe (VBT), Soybean Tempe (SBT), and Combination Soybean-Velvet bean tempe (CSVT).

The distribution of metabolites based on the PCA loading plot (Figure 4A) showed that amino acids were collected in the negative axis and isoflavones along the positive axis of PC1. Based on the results of the GC-MS analysis, it was found that the amino acids in VBT were significantly higher in the other samples. This condition can be seen from the heat maps for all metabolites in Figure 5. The difference in amino acid accumulation between VBT and SBT may depend on several factors. In addition to different raw materials, another factor is the activity of microorganisms during fermentation. This study found that even when using the same fungal strain for soybean tempe fermentation, different concentrations and compositions of amino acids can be produced (17). This could be because the fermentation process in tempe is also significantly influenced by the indigenous microflora present in each sample. Microorganisms, such as yeasts, lactic acid bacteria (LAB), and fungi, can interact during fermentation, potentially leading to diverse metabolites. The metabolites that contributed significantly to group separation were glycine, gluconic acid, and glucono-1,5-lactone, which could be seen from the high relative intensity compared to other amino acids. On the positive axis/right, cluster separation is caused by the metabolites galactose, genistein, and daidzein. These results were obtained by comparing the relative intensities of

each metabolite (Figure 4B). Isoflavones were found in SBT and CSVT was made from soybeans.

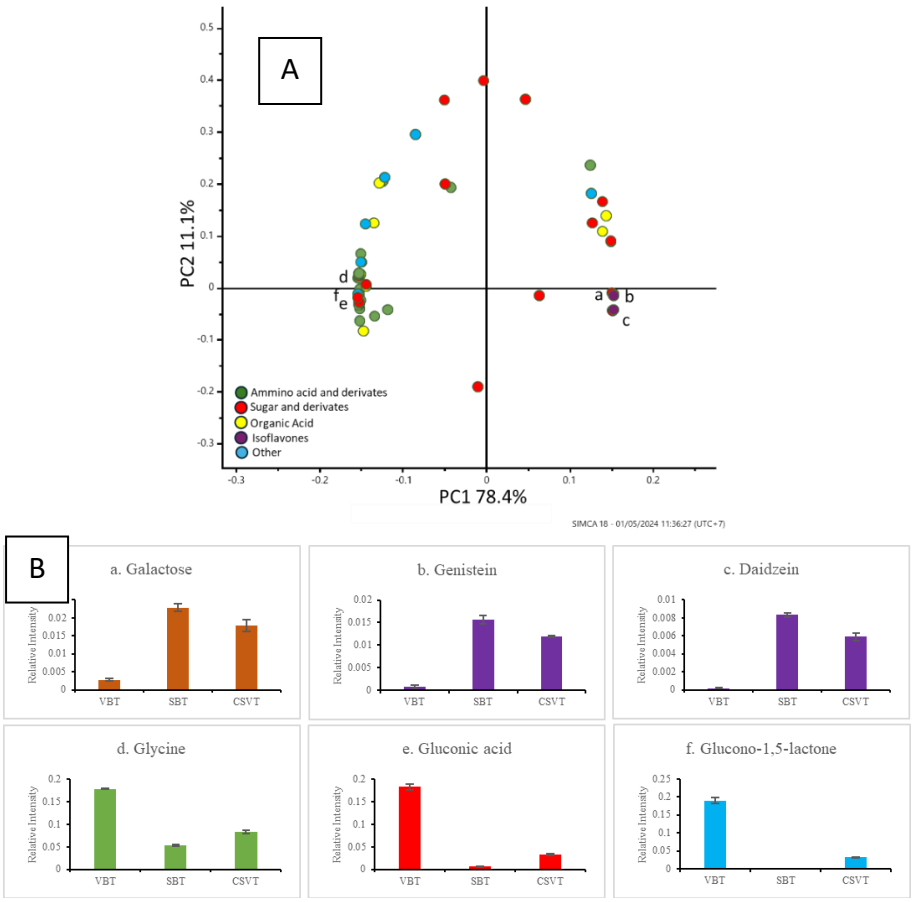

Figure 4. Principal component analysis (PCA) loading plot and bar chart VIP metabolite results of legume tempe based on GC-MS analysis. (A) Profile primary metabolites and grouping of metabolite types, (B) Bar chart primary metabolites as discussed in the main text.

The metabolite profile of each sample was observed using the heat map shown in Figure 5. The red colour in the figure indicated a positive correlation, indicating that the metabolite was prominently abundant in the sample. Based on the relative intensity value, it is known that amino acids such as glutamic acid, alanine, threonine, serine, and valine are abundant in VBT. Lysine and histidine are amino acids in SBT (7). Furthermore, SBT contains genistein and daidzein isoflavones, which are antioxidant compounds (6). Bioactive components such as GABA (4-aminobutyric acid), were also visualized on the heatmap, showing a decreasing intensity due to fermentation. Therefore, VBS, which accumulated the highest amount of GABA, then blended with soybean, which also had a considerably high GABA intensity, serves as a solution to increase the GABA concentration in the CSVT tempe, as depicted in the graph. The metabolites in CSVT reflect the composition of its raw materials, a combination of soybean and velvet bean These results align with the research by Rahmawati et al., which demonstrated that the metabolomic profiling of tempe made with mixed beans can be a strategy to enhance metabolites (amino acid or bioactive compound) that are generally low in a single type of legume, thus complementing the metabolite composition in mixed-legume tempe (12).

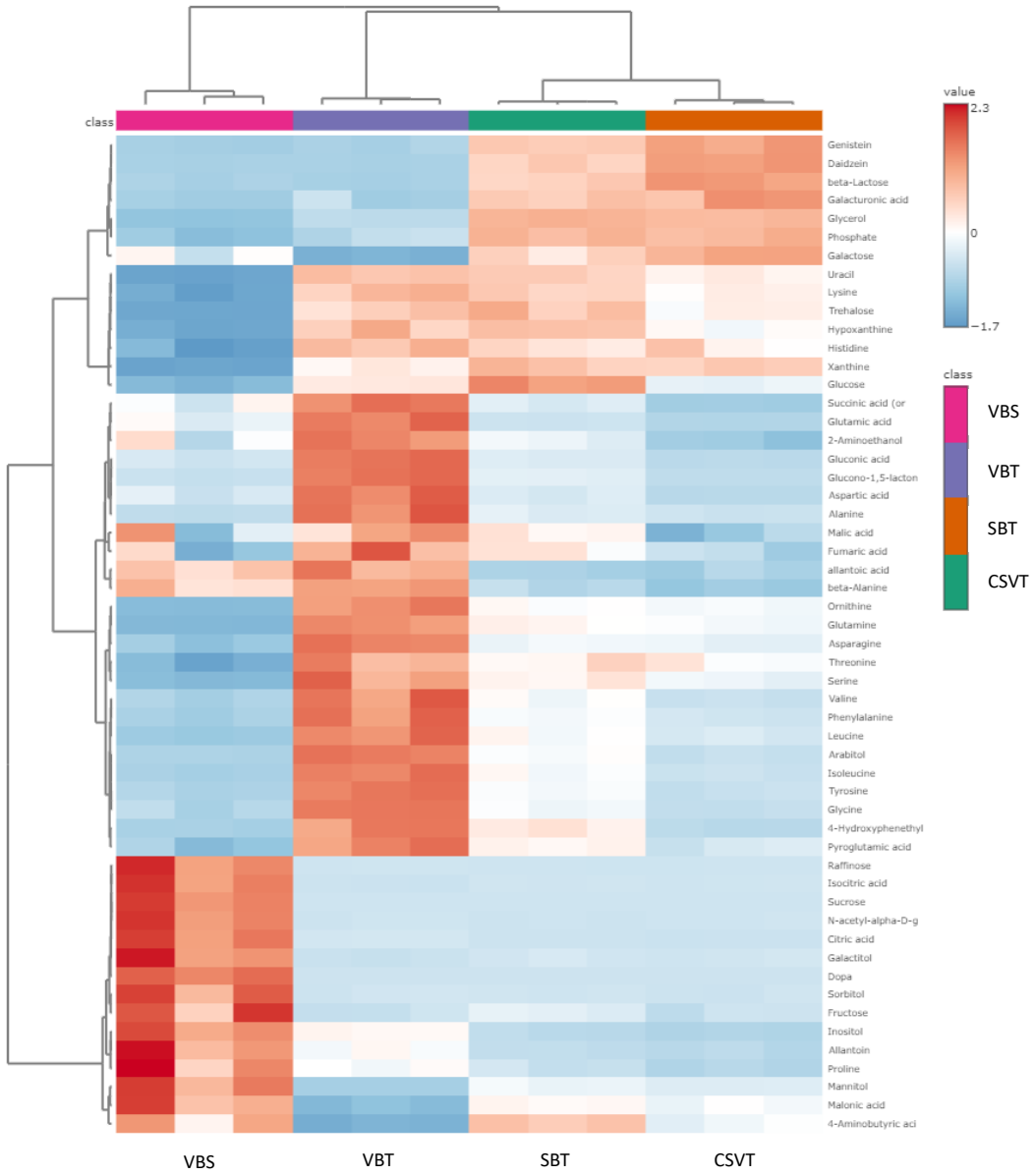

Figure 5. Heatmaps profile primary metabolites for all samples. Velvet bean seed (VBS), Velvet bean tempe (VBT), Soybean Tempe (SBT), and Combination Soybean-Velvet bean tempe (CSVT).

3.3. Antioxidant Activity of Various Tempe

The antioxidant activity of tempe flour was analyzed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The antioxidant activity of the food ingredients was measured using the DPPH free radical inhibition method with the IC_{50} parameter. The IC_{50} value is the effective concentration required to inactivate 50% of free radicals, which mean that the lower the IC_{50} value, the higher the antioxidant activity (18). Foods with high antioxidant activity can be beneficial for preventing metabolic diseases, including obesity, as suggested Astawan et al. (19). The different types of legumes used in the production of tempe (Figure 6) significantly affected the IC_{50} value ($p<0.05$). The lowest IC_{50} value was observed for VBT 361 ppm. This value indicated that the antioxidant activity of velvet bean tempe flour was significantly higher than that of other tempe flours. Compared to the study by Astawan et al. (2023), VBT still exhibited a lower IC50 value than tempe flour made from germinated and non-

germinated soybeans, ranging from 2487 to 2642 ppm (5). Figure 6 also shows that increasing the amount of soybean decreased the antioxidant activity (increasing the IC_{50} value).

The IC_{50} value for VBT was higher than that for CSVT, and the IC_{50} value for CSVT was higher than that for SBT. These results were thought to be due to the abundance of bioactive compounds that function as antioxidants, such as 4-Hydroxyphenethyl alcohol (tyrosol) in velvet beans (20). Velvet beans are commonly incorporated into the human diet as supplements to promote healthy living. Velvet beans have a high contents of phenolics, flavonoids, and proanthocyanidins (21). Some studies have shown that velvet beans contain L-3,4-dihydroxyphenylalanine (L-DOPA), lectin, isoflavanones, and some alkaloids, which are responsible for the enormous bioactivity of its crude extracts (22). On the other hand, the soybean tempe contained high amino acid content; and isoflavone antioxidant compounds, such as daidzein and genistein (4).

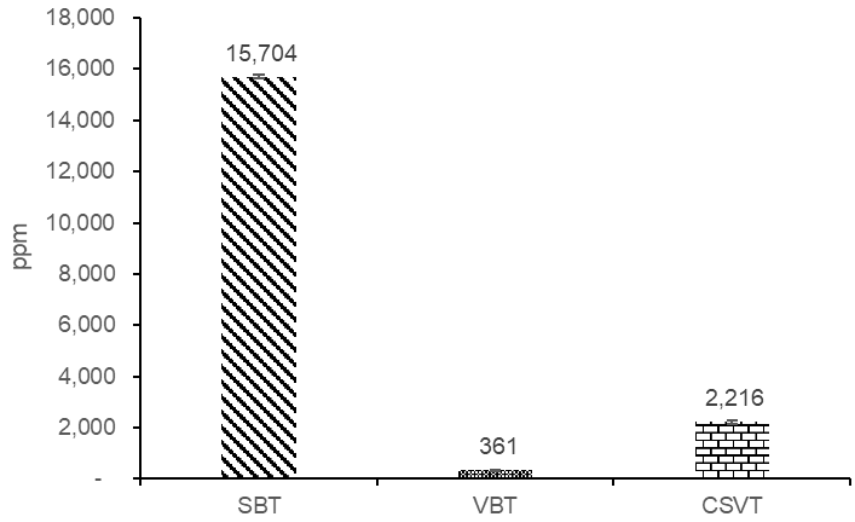

Figure 6. The IC_{50} value of various tempe flour. SBT (soybean tempe), VBT (velvet bean tempe), and CSVT (combine soybean and velvet bean tempe).

The tempe combination of soybeans and velvet beans was made from a mixture of two types of beans, and the compound content varied more than that of a single bean. Increasing the number and type of metabolites in the combination tempe increased antioxidant activity (Figure 6). This condition can be seen in the metabolite contents shown in Table 1. The combination tempe made from soybeans and velvet beans contained genistein and daidzein, which came from soybeans, and 4-Hydroxyphenethyl alcohol (tyrosol) from velvet beans. These three compounds are known to have antioxidant activities (5,20).

3.4. Orthogonal Projection to Latent Structures of Various Tempe

Orthogonal projection of latent structures (OPLS) regression analysis is commonly used to predict the value of the Y-variable (response variable) using the X-variable (explanatory variable). In addition, OPLS regression was conducted to identify metabolites that were highly influenced by differences in the response variable (22). To analyze the contribution of metabolites responsible for antioxidant activity, OPLS regression analysis was conducted with antioxidant activity as the response variable and annotated metabolites of tempe as the explanatory variable. The linearity (represented by the R^2 value) and predictability (shown by

the Q2 value) of the model were utilized to evaluate the quality of the model. A good model has an R^2 value larger than 0.6 and a Q^2 value greater than 0.5 (23).

The constructed OPLS regression model is shown in Figure 7, where the R^2 and Q^2 values of the model showed values of 0.9733 and 0.937, indicating that the model accurately represented these relationships. The important variables in the projection (VIP) in the OPLS regression analysis are indicated by statistically significant metabolites. Metabolites considered essential to the model had a VIP score > 1 (24) (Table 2).

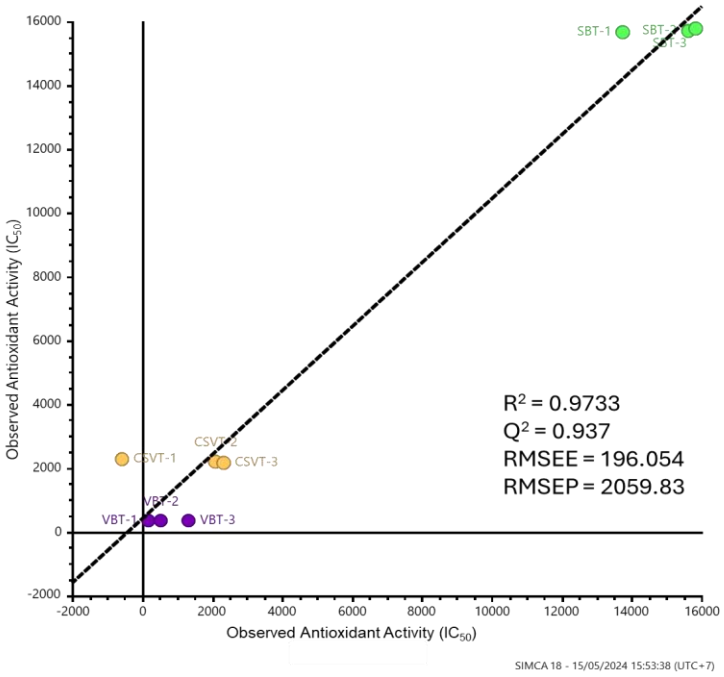

Figure 7. Orthogonal projection to latent structures (OPLS) regression results from the various tempe flour. SBT (soybean tempe), VBT (velvet bean tempe), and CSVT (combine soybean and velvet bean tempe).

The results showed that many metabolites had coefficient values greater than 1, but based on the top 10, uracil, lysine, malic acid, 4-hydroxyphenethyl alcohol (Tyrosol), trehalose, hypoxanthine, 2-aminoethanol, serine, pyroglutamic acid, and daidzein were positively correlated with antioxidant activity. This result is shown in Figure 8, where the relative intensity values of the metabolites were in line with the increase in antioxidant activity. As shown in the graph (Figure 8), the eight identified relative intensity VIP metabolites consistently demonstrated that the VBT sample, which exhibited the highest antioxidant activity, had a consistently higher intensity than the other two samples, with the exception of trehalose and daidzein. Conversely, the SBT sample, which possessed the lowest antioxidant activity, also displayed the lowest relative intensity of VIP metabolite values among the three samples, with the sole exception of daidzein. Therefore, a positive correlation between antioxidant activity and the relative intensity of VIP metabolites was clearly illustrated. Some metabolites such as 4-Hydroxyphenethyl alcohol (tyrosol) and daidzein have been reported to act as antioxidants (5,11,25).

Table 2. Antioxidant activity highly influenced the Variable Important in Projection (VIP) Metabolite list.

Metabolite Name	Coefficient
Uracil	1.24779
Lysine	1.17869
Malic acid	1.17535
4-Hydroxyphenethyl alcohol (Tyrosol)	1.17398
Trehalose	1.16223
Hypoxanthine	1.14211
2-Aminoethanol	1.12008
Serine	1.10955
Pyroglutamic acid	1.10495
Daidzein	1.10378
Arabitol	1.10289
Genistein	1.09595
Fumaric acid	1.09376
beta-Lactose	1.09211
Galactose	1.08255

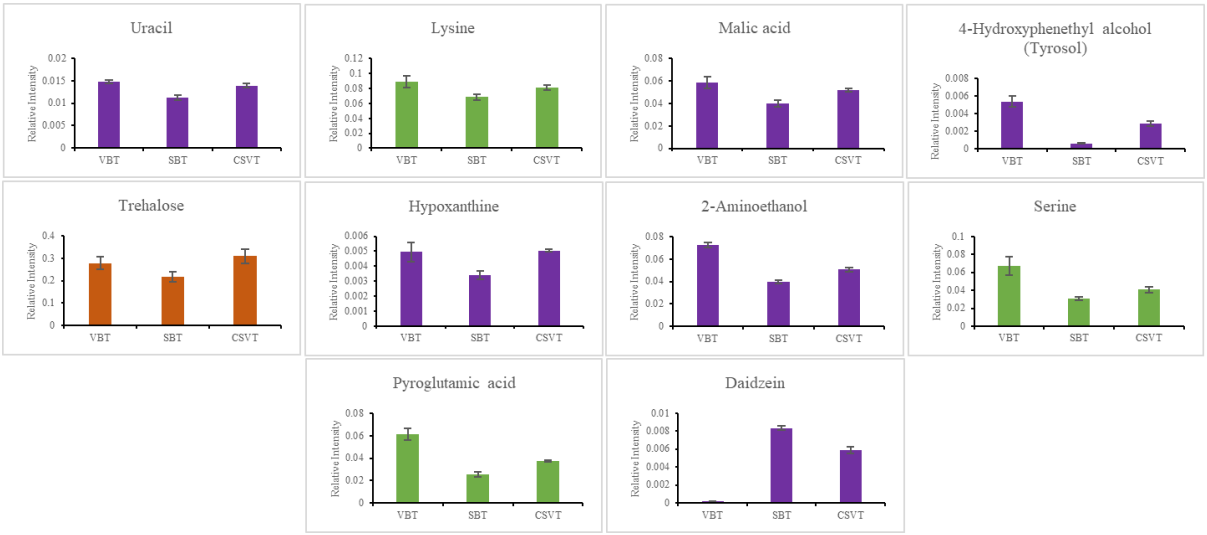

Figure 8. Top 10 VIP Metabolites from the various tempe flour. SBT (soybean tempe), VBT (velvet bean tempe), and CSVT (combine soybean and velvet bean tempe).

4. Conclusions

Metabolomic analysis revealed distinct metabolite profiles and abundances across the soybean tempe, velvet bean tempe, and their combination. The fermentation process greatly influenced changes in metabolite profiles, including the amounts of amino acids, sugars, and organic acids. The difference in profile made the tempe combination of soybean and velvet richer than tempes derived from one type of legume (soybean or velvet bean). Metabolites such as amino acids and organic acids were more abundant in the combined tempe than in soybean tempe alone. Similarly, the bioactive components daidzein and genistein in combined tempe exhibited a better intensity profile than that of tempe made solely from velvet beans.

Velvet bean tempe demonstrated the highest antioxidant activity among the tested samples, a characteristic that is likely attributed to its unique metabolite profile. Specifically, VIP metabolites such as uracil, lysine, malic acid, 4-hydroxyphenethyl alcohol (tyrosol), trehalose, hypoxanthine, 2-aminoethanol, serine, pyroglutamic acid, and daidzein likely contributed to the observed antioxidant capacity of the tempe samples. The high antioxidant activity of velvet bean tempe suggests its potential as a functional food ingredient with enhanced health benefits.

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Author Contributions

M.A. and T.W. conceived and designed the experiments, and wrote the paper; R.P.R. performed the experiments, analyzed the data,interpreted the data and wrote the paper; R.I. performed the experiments; R.E.S, S.P.P, and A.A.T supported the writing of the paper.

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Data Availability Statement

Invalid.

Conflicts of Interest

No conflict of interest

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