BIODIVERSITAS
Volume 26, Number 5, May 2025
Pages: 2278-2288

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260525

Traditional fermented anchovy rusip demonstrates antidiabetic effects through enzyme inhibition and metabolic improvement in diabetic rats

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Manuscript received: 30 January 2025. Revision accepted: 6 May 2025.

Abstract. *Kurnianto MA, Puspita AA, Winarti S, Munarko H, Aulia SS, Rini DM. 2025. Traditional fermented anchovy rusip demonstrates antidiabetic effects through enzyme inhibition and metabolic improvement in diabetic rats. Biodiversitas 26: 2278-2288.* Rusip, a traditional fermented anchovy, is a high-protein food with potential anti-diabetic properties. This study investigated the physicochemical properties and in vitro inhibitory activity of diabetes-related enzymes of rusip fermented at different time points (0, 7, 14, and 21 days). The optimal formulation was then subjected to further in vivo study with Streptozotocin-Nicotinamide (STZ-NA)-induced rats. The results revealed increased moisture, soluble protein, TVBN, and N-amino acid and decreased protein, fat, ash, carbohydrate, and pH of rusip during the 21-day fermentation time compared to the other fermentation stages. Glutamic acid and arginine, as well as Methyl-cis-10-pentadecenoate and Methyl-cis-4,7,10,13,16,19-docosahexaenoate, were the most dominant amino acids and fatty acids in rusip. The rusip showed α-glucosidase (53.8-59.8%) and α-amylase (40.3-46.7%) inhibitory activities at 20 mg/mL, with the lowest IC_{50} observed at day 14 of fermentation (30.52 mg/mL and 26.01 mg/mL). Further evaluation in STZ-NA-induced diabetes rats showed that rusip administration could reduce fasting blood glucose, HbA1c, and HOMA-IR and improve insulin levels, HOMA-B and QUICKI. Hepatoprotective (suppressing elevated AST, ALP, and ALT) and nephroprotective (decreasing urea and creatinine) effects were also observed. Rusip also improved lipid metabolism, reducing LDL, triglyceride, and cholesterol levels and returning HDL levels to normal, thereby reducing hypercholesterolemia. These findings suggest that rusip may mitigate diabetes-related metabolic disorders, highlighting its potential as a functional food for diabetes management.

Keywords: α-amylase inhibitor, α-glucosidase inhibitor, antidiabetic potential, fermented food, rusip

INTRODUCTION

Rusip, a traditional fermented fish product from Bangka Belitung, Indonesia, comprises anchovies, salts, and palm sugars. The mixture undergoes spontaneous fermentation under sealed conditions for 7-14 days (Susilowati et al. 2014; Koesoemawardani and Ali 2016; Kurnianto et al. 2023a). A study by Puspita et al. (2024) revealed that rusip possesses a high protein content (23.78-27.03%) and a low fat content (6.19-7.64%). Additionally, it exhibits a distinctive organoleptic profile, characterized by a thick texture, fishy aroma, light brown to dark ash color, and a sour taste. During the fermentation process of rusip, various bacteria, mainly Lactic Acid Bacteria (LAB), such as *Lactococcus* sp., *Leuconostoc* sp., and *Streptococcus* sp., undergo natural growth and produce a range of metabolites and enzymes, including proteolytic enzymes (Koesoemawardani and Yuliana 2013). Proteolytic enzymes facilitate the breakdown of the parent protein of anchovies into peptides and amino acids (Kurnianto et al. 2024).

Several studies have demonstrated that rusip possesses a range of functional activities that are beneficial to health, including antioxidant properties (Najafian and Babji 2018), angiotensin-converting enzyme inhibitor or antihypertensive (Rinto et al. 2021), and anti-cholesterol properties (Rinto et al. 2019). Our previous in-silico study also reported that rusip exhibits significant bioactive potential for preventing diabetes. This potential was attributed to its ability to inhibit Dipeptidyl Peptidase IV (DPP-IV) and α-glucosidase receptors, with an inhibition frequency of 47.2% (Kurnianto et al. 2023b). The diverse functional activities of rusip are likely due to Bioactive Peptides (BPs). BPs are short protein fragments consisting of 2-20 amino acids with low molecular weights (<6 kDa) that exhibit a variety of specific bioactivities (Tamam et al. 2018; Kurnianto et al. 2023a). Numerous studies have reported that BPs possess antioxidant, antithrombotic, antimicrobial, anti-cholesterol, immunomodulatory, anti-hypertensive, and anti-diabetic properties (Wang et al. 2015; Agrawal et al. 2016; Chalamaiah et al. 2019; Kurnianto et al. 2023a, 2023b).

Diabetes is a chronic metabolic disorder characterized by hyperglycemia, which is caused by an impairment in the secretion or effectiveness of insulin or a combination of both (American Diabetes Association 2009). Johnson et al. (2011) posited that several enzymes, including α-glucosidase, α-amylase, and DPP IV, regulate hyperglycemia in diabetes. The enzymes α-glucosidase and α-amylase are involved in the breakdown of carbohydrates into monosaccharides

within the digestive tract. Consequently, the inhibition of these enzymes is an effective method for reducing blood glucose levels. Conversely, DPP-IV inactivates the incretin hormone Glucagon-Like Peptide-1 (GLP-1) and gastric inhibitor peptides (Deng et al. 2018). BPs can prevent the onset of diabetes by inhibiting the activity of these three enzymes. Specifically, as α-glucosidase inhibitors, BPs block the absorption of carbohydrates, which reduces the sugar uptake that normally stimulates GLP-1 secretion, ultimately lowering blood sugar levels (Williams et al. 2020). By acting as α-amylase inhibitors, BPs decrease the hydrolysis of α-1,4-glycosidic bonds. This action slows carbohydrate digestion and delays glucose absorption, further contributing to reduced blood glucose levels (Ren et al. 2024). Concurrently, as DPP-IV inhibitors, BPs inactivate DPP-IV, thereby preventing the decomposition of GLP-1 (Elam et al. 2021).

Several fermented foods have been reported their activity in preventing diabetes, such as fermented soybean (Kwon et al. 2010), tempeh fermentation with *Lactobacillus plantarum* and *Rhizopus oligosporus* (Huang et al. 2018), fermented rice bran (Hu et al. 2023), fermented camel milk (Ayyash et al. 2020), and fermented fish sausage (Alkalbani et al. 2019). However, this activity has not been explored, especially for fermented fish. To date, the bioactivity of rusip has been the subject of a limited number of in vitro experiments, which have focused on their antioxidant, antihypertensive, and anti-cholesterol potential (Rinto et al. 2017; Najafian and Babji 2018; Rinto et al. 2021). The potential of rusip to prevent diabetes is yet to be evaluated through in vitro and in vivo studies. This study aimed to analyze and identify the physicochemical properties of rusip and the potential of rusip to inhibit the activity of α-amylase and α-glucosidase enzymes in vitro at different fermentation stages. The optimal formulation was further analyzed for administering Water-Soluble Extract (WSE) of rusip to improve the metabolism of Streptozotocin-Nicotinamide (STZ-NA)-induced rats.

MATERIALS AND METHODS

Preparation of rusip

Anchovies (150 g) were weighed, washed, and drained. 150 mg/g salt (Cap Kapal, Indonesia) was added and mixed. Then 100 mg/g palm sugar (Nira Murni, Indonesia) was added and mixed. The mixed ingredients were then kept in an incubator (Memmert, Germany) at 30°C for 0, 7, 14, and 21 days (Puspita et al. 2024).

Extraction of water-soluble fractions

The rusip sample and deionized water were mixed in a ratio of 1:10 and then homogenized for 30 minutes. The mixture was heated at 100°C for 10 minutes to inactivate the enzyme, then cooled to room temperature. The process was continued by filtering using cheesecloth and filter paper. The filtrate obtained was then re-filtered using a cellulose acetate membrane with a pore size of 0.22 µm, producing a Water-Soluble Extract (WSE) (Istiqamah et al. 2019; Amelia et al. 2024).

Physicochemical analysis

The proximate composition of rusip samples—including moisture, ash, protein, lipid, and carbohydrate content—was analyzed using the methods established by the AOAC (Desta et al. 2021). pH analysis was carried out based on the Kurnianto and Munarko (2022). N-amino content was analyzed using formol titration (Wikandari and Yuanita 2016). Soluble protein was analyzed using the Lowry method (Lowry et al. 1951; Sompinit et al. 2020). Total Volatile Base Nitrogen (TVBN) analysis was analyzed using the method by Yildiz et al. (2021).

In vitro α-amylase enzyme inhibition

The inhibitory activity of rusip against the α-amylase enzyme was evaluated following the methods of Apostolidis et al. (2007) and Wickramaratne et al. (2016). The Water-Soluble Extract (WSE) was centrifuged, and the supernatant was used to prepare working solutions (125-2000 µg/mL). Each solution (500 µL) was mixed with sodium phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/mL) and incubated at 25°C for 10 minutes. Starch solution (500 µL) was added, followed by further incubation. The reaction was stopped with 3,5-dinitrosalicylic acid, heated for 5 minutes, cooled, and the absorbance was measured at 540 nm using a spectrophotometer. The inhibitory activity was calculated as the percentage of inhibition:

$$\% \text{ Inhibition} = \left[\frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}}\right] \times 100\%$$

The sample and positive control concentrations were plotted against percent inhibition to generate a linear regression equation. The y-intercept represents the IC_{50}, indicating the WSE concentration required to inhibit 50% of α-amylase activity, while the slope reflects the WSE concentration evaluated.

In vitro α-glucosidase enzyme activity

The α-glucosidase activity of rusip was assessed using a modified method by Etsassala et al. (2020). A mixture of α-glucosidase enzyme (1 U/mL), phosphate buffer (100 mM, pH 6.8), and WSE samples was incubated at 37°C for 15 minutes. After incubation, the sample was added with 4-nitrophenyl-d-glucopyranoside (5 mM) and re-incubated for 20 minutes. The reaction was stopped with 0.1 M Na_2CO_3. Absorbance was measured at 405 nm, and the percentage of α-glucosidase inhibition was calculated:

$$\alpha\text{-glucosidase inhibitory activity (\%)} = 1 - \left(\frac{\text{Abs}_A}{\text{Abs}_B}\right) \times 100\%$$

Abs_A is the absorbance of the sample being tested, and Abs_B is the absorbance of the control (phosphate buffer). The IC_{50} value was subsequently calculated in a manner identical to that described in the previous section.

Amino acid and fatty acid analysis

The amino acid profile was analyzed using HPLC (Shimadzu CBM 20A). The sample was mixed with citrate phosphate buffer (pH 4.6) containing β-glucosidase (37

units), hydrolyzed at 37°C for 4 hours, and added ethanol. After centrifugation, the supernatant was concentrated. Amino acids were identified by retention time and spectra comparison with standards and quantified using peak area (Afifah et al. 2023). Fatty acid profiles were analyzed using GC-MS (Agilent 7890B). Samples were mixed with concentrated HCl, heated in a water bath to boiling for 3 hours, and cooled. The sample was extracted with diethyl and petroleum ether (1:1) and allowed to settle. The top layer of oil was taken and evaporated with N_2 gas. The oil was added to methanolic sodium and boron, heated at 60°C for 5-10, and extracted with saturated Heptane and NaCl. The top layer was removed and injected into GC. Fatty acids were identified using a flame ionization detector and recorded via chromatogram (Chiu and Kuo 2020; Fitriana et al. 2021).

Animals and experimental design

The study protocol was approved by the Animal Care and Use Committee (ACUC) of Airlangga University's Faculty of Veterinary Medicine (No. 3.KEH.119.08.2023). All rats were maintained following laboratory animal care guidelines. Male Wistar rats (n = 28), aged 3-4 months (250-300 g), were housed under controlled conditions (25°C, 12 h light/dark cycle, 35-60% humidity) and acclimatized for 7 days. They had access to a Comfeed AD II (Japfa Comfeed Indonesia, Indonesia) diet and distilled water during acclimatization. Rats were weighed before and after acclimatization, and healthy, similarly weighted rats were selected for the study.

After acclimatization, 28 rats were divided into 4 groups. To induce diabetes, 21 rats fasted for 12 hours, consumed a Comfeed AD II diet and received intraperitoneal injections of streptozotocin (STZ, 45 mg/kg body weight) and nicotinamide (NA, 110 mg/kg body weight) (Ardiana et al. 2018; Gad-Elkareem et al. 2019). They were given 5% glucose solution ad libitum to prevent hypoglycemia (Preetha et al. 2012). Diabetes was confirmed if fasting blood glucose levels exceeded 200 mg/dL after 72 hours (Banda et al. 2018; Gad-Elkareem et al. 2019). Rats with diabetes were then grouped into STZ-NA, DMG (STZ + Glibenclamide 0.45 mg/kg), and DMR (STZ + rusip 9 g/kg) groups, with via intragastrically rusip and Glibenclamide administered daily for 14 days. Six rats served as controls on a Comfeed AD II diet. Body weight and food intake were monitored daily.

Biochemical measurement

Fasting blood glucose level was determined using glucose oxidase-peroxidase aminoantipyrine (GOD-PAP, DiaSys, Indonesia) methods. HbA1c levels analysis was performed using the Epithod®616 HbA1c Test Kit (DxGen Corp, Republic of Korea). Plasma insulin levels were determined by an enzyme-linked immunosorbent assay kit (Mybiosource, USA) according to the manufacturer's instructions. Furthermore, insulin sensitivity levels were measured through three indices, including the Homeostasis Model Assessment (HOMA) index, both HOMA-IR and HOMA-B using calculations based on Khamchan et al. (2018) and de Fátima Haueisen Sander Diniz et al. (2020) while QUICKI is calculated based on logarithmic transformation according to Katz et al. (2000).

Analysis of lipid and liver profile

Lipid profiles for HDL and LDL levels were measured using the photometric cholesterol oxidase-p-aminophenazone method with DiaSys kits (Germany) following the manufacturer's instructions. Triglycerides and cholesterol were analyzed with Selectra Pro M (ELITech Clinical System SAS, French). Renal profile analyses, including urea and creatinine levels, were performed according to the Selectra Pro M analysis instructions (ELITech Clinical System SAS, French). Meanwhile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels were assessed using an optimized UV-test based on modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) methods with DiaSys kits (Germany), adhering to the manufacturer's protocol.

Statistical analysis

Quantitative data are presented as mean±standard deviation. One-Way Analysis of Variance (ANOVA) was used to compare differences between groups, while multiple comparisons were performed with the least significant difference (SPSS 25, USA). A $p<0.05$ was considered the minimum level of statistical significance.

RESULTS AND DISCUSSION

Physicochemical profile of rusip

During fermentation, the moisture content, N-amino acid, soluble protein, and TVB-N concentrations increased (Table 1 and Figures 1.A-1.C). These results are consistent with those of studies on fish sauce products fermented for 60 days, suanyu fermented for 6 weeks, and low-salt shrimp paste fermented for 12 weeks (Wang et al. 2017; Li et al. 2022; Takada et al. 2023). This trend is generally due to the synergistic effects of autolytic and microbial activities during fermentation (Faithong and Benjakul 2014; Peralta et al. 2021). More specifically, the metabolism of glucose to lactic acid by LAB and proteolysis might cause an increase in water content (Khositanon et al. 2021). The increase in soluble protein levels indicates substrate protein hydrolysis, which forms free amino acids and peptides (Wang et al. 2017). A high TVBN content also indicated the presence of proteolytic bacteria and the action of proteolytic enzymes in degrading protein and nitrogen (N)-containing compounds in the substrate (Lee et al. 2016; Bekhit et al. 2021).

In contrast, the ash, fat, protein, and carbohydrate contents showed a downward trend (Table 1). It may be due to the utilization of these components in the metabolism of LAB. García-Cano et al. (2019) and Islami et al. (2022) reported that the proteolytic and lipolytic activities of LAB degrade proteins and fats into amino acids, peptides, glycerol, and fatty acids. LAB breaks down carbohydrates into simple sugars for energy production (Yadav et al. 2010). Further sugar metabolism by LAB produces lactic, acetic, gluconic, and glucuronic acids, which accumulate and lower the pH

levels (Figure 1.D) (Wang et al. 2017; Zubaidah et al. 2020; Liu et al. 2023).

Inhibitory effects of rusip on α-amylase and α-glucosidase activities

The inhibitory effects of rusip on α-amylase and α-glucosidase activity have been observed to reduce postprandial hyperglycemia by slowing polysaccharide digestion (Sim et al. 2010; Date 2020). Our results demonstrated that a WSE of rusip showed increased enzyme inhibition at higher concentrations and longer fermentation durations (Figures 2 and 3). The highest inhibition occurred when rusip was fermented for 14 days, with an increase in α-amylase inhibition from 40.27% to 46.46% at 2000 µg/mL and α-glucosidase inhibition increasing from 56.06% to 59.80% at 1000 µg/mL. In addition, at this fermentation duration and concentration, the WSE of rusip showed the lowest IC_{50} values of 30.52 µg/mL for α-amylase and 26.01 µg/mL for α-glucosidase (Table 2). Similar trends have been reported for fish sausages fermented with *Enterococcus* spp., in which enzyme inhibition increased from 29.2 to 68.7% for amylase and 23.9 to 41.4% for glucosidase on the 14th day (Alkalbani et al. 2019).

The inhibitory ability of the WSE of rusip against α-amylase and α-glucosidase may be due to peptides released during protein hydrolysis in the fermentation process (Alkalbani et al. 2019). It is supported by increased N-amino acid, soluble protein, and TVBN levels in rusip, indicating proteolysis into peptides and amino acids (Chadong et al. 2015). Peptides inhibit glucose metabolism by interfering with enzymes via competitive inhibition involving hydrophobic interactions, hydrogen bonding, and van der Waals forces, thereby altering enzyme activity (Lu et al. 2023). Previous studies have shown α-glucosidase inhibition by GLLGY oligopeptides from fermented rice bran (Hu et al. 2023) and Ser-Thr-Tyr-Val peptides (Ibrahim et al. 2018). Additionally, peptides such as Cys-Ser-Ser-Val and Tyr-Ser-Phe-Arg from *Andrias davidianus* (Blanchard, 1871) demonstrate α-amylase and α-glucosidase inhibition with IC_{50} values of 2.86-13.76 µg/mL and 42.93-206 µg/mL, respectively (Ramadhan et al. 2017).

Amino acid and fatty acid profile of selected rusip formula

The most potent inhibition of α-amylase and α-glucosidase enzymes was found in rusip fermented for 14 days, which was further used for subsequent investigations. The amino acid and fatty acid profile of rusip fermented for 14 days were evaluated. The dominant amino acids in rusip were glutamic acid (1912.71 mg/100 g), arginine (1757.92 mg/100 g), and aspartic acid (1199.36 mg/100 g) (Table 3). Meanwhile, the fatty acids Methyl palmitoleate, Methyl cis-4,7,10,13,16,19-docosahexaenoate, and Methyl linolelaidate are high in rusip (Table 4). As raw materials, anchovies influence the dominance of amino or fatty acids. Mohanty et al. (2014) and Gencbay and Turhan (2016) stated that glutamic and aspartic acid are the dominant amino acids in anchovies (*S. commersonii* and *Engraulis encrasicholus*). Similar finding of glutamic acid dominance was reported in other fish fermentation products such as fish sauce (Puat et al. 2015), tuna by-product fish sauce (1.01% w/w) (Wenno and Loppies 2019), tuna fish sauce (Leiwakabessy et al. 2021), Zhayu (An et al. 2022) and fermented mackerel sausage (Afifah et al. 2023). Meanwhile, Kaya and Turan (2008) and Bayraklı (2023) reported that C22:6n3, C18:1n9c, C18:1n9t, and C16:0 are the dominant fatty acids in anchovies. Sayuti et al. (2022) also reported that C15:1(n-5) is the dominant fatty acid in smoked *Katsuwonus pelamis* (Linnaeus, 1758).

Table 1. Proximate content of rusip at different fermentation times

Proximate content	Fermentation time (Day)			
	0	7	14	21
Moisture	60.65±0.52^{d}	62.71±0.37^{c}	63.77±0.13^{b}	65.70±0.38^{a}
Ash	2.19±0.16^{a}	1.78±0.11^{b}	1.23±0.14^{c}	1.09±0.02^{c}
Protein	28.23±0.31^{a}	27.45±0.16^{a}	25.69±0.52^{b}	22.82±0.55^{c}
Fat	5.62±0.27^{a}	5.12±0.05^{ab}	4.53±0.30^{bc}	4.29±0.09^{c}
Carbohydrate	3.27±0.40	2.93±0.69	2.85±0.22	2.59±0.28

Note: Values without a common letter are significantly different (p<0.05) as determined by LSD

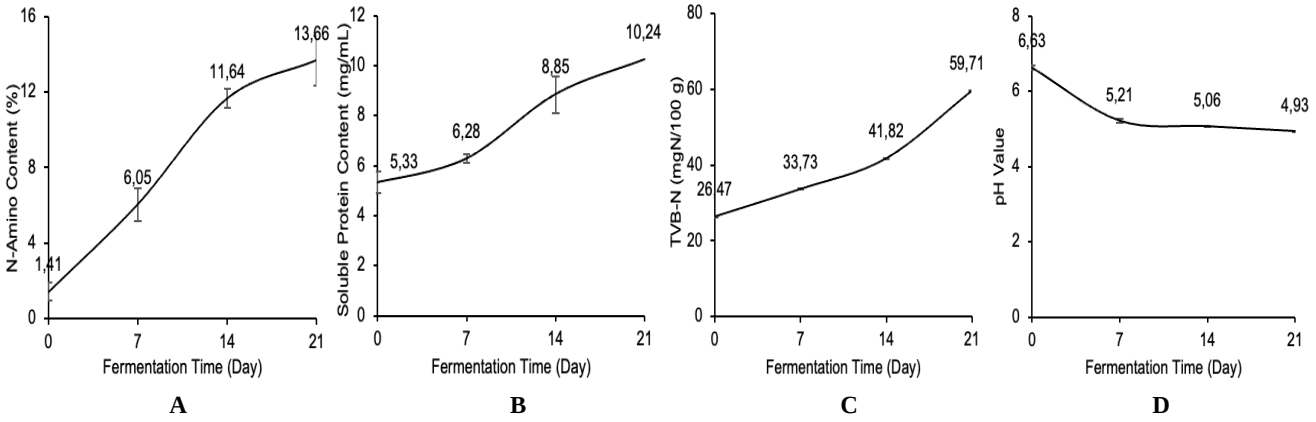

Figure 1. Chemical profile of fermented anchovy (rusip) during the fermentation period (0, 7, 14, and 21 days). A. N-Amino; B. Soluble Protein; C. TVBN; D. pH value

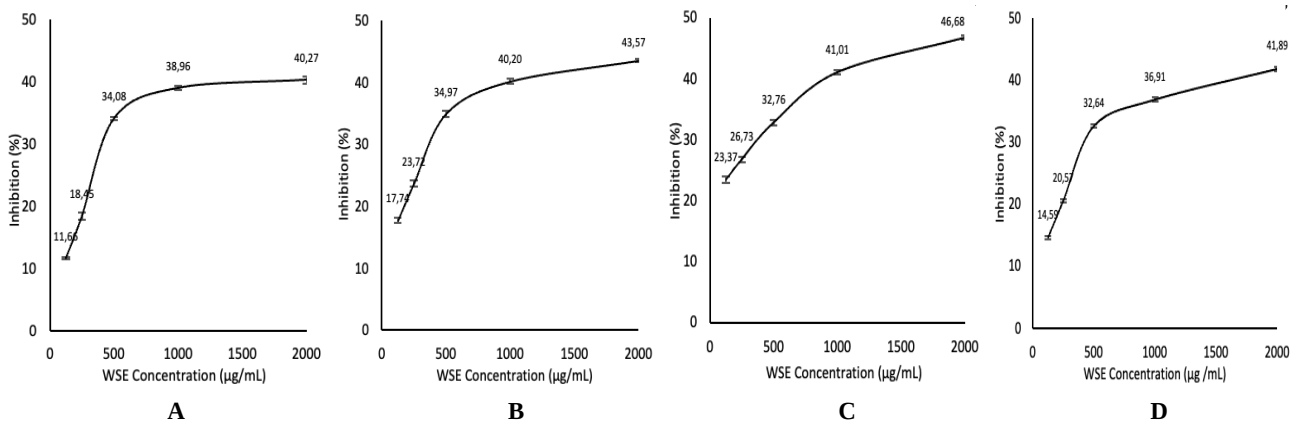

Figure 2. Inhibition of α-amylase by rusip at various fermentation times and different WSE of rusip concentration: A. Day 0; B. Day 7th; C. Day14th; D. Day 21st; WSE: Water-Soluble Extract

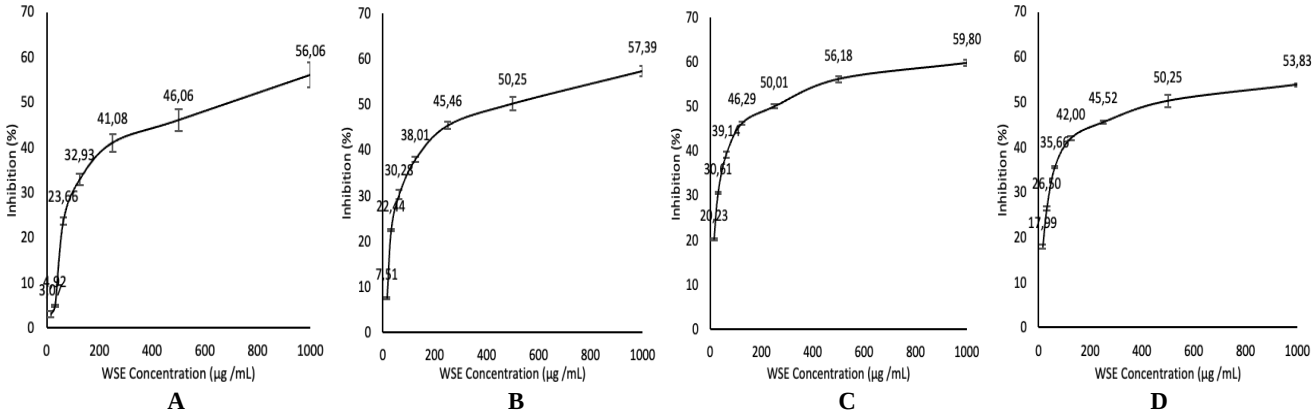

Figure 3. Inhibition of α-glucosidase by rusip at various fermentation times and WSE of rusip different concentrations. A. Day 0; B. Day 7th; C. Day 14th; D. Day 21st; WSE: Water-Soluble Extract

Table 2. IC_{50} value of rusip inhibiting α-amylase and α-glucosidase enzyme activity

Sample	IC_{50} value (µg/mL)	
	α-amylase	α-glucosidase
Acarbose*	17,43±1.45[a]	6.87±2.93[a]
Rusip 0 d	33.87±1.21[c]	50.90±2.96[c]
Rusip 7 d	31.07±1.89[b]	43.32±2.41[b]
Rusip 14 d	30.52±0.97[b]	26.01±1.31[b]
Rusip 21 d	37.73±1.85[d]	46.63±1.56[d]

Note: *Positive control; Values without a common letter are significantly different (p<0.05) as determined by LSD

The presence of glutamic acid, arginine, and aspartic acid in rusip contributes to α-amylase and α-glucosidase inhibitory activity, as these amino acids are associated with insulin regulation and secretion. Singh and Kaur (2016) and Ramadhan et al. (2018) highlighted that amino acids enhance insulin secretion from primary islet cells and β-cell lines. Furthermore, arginine is crucial in increasing insulin secretion through allosteric activation of metabolism or membrane depolarization (Salil et al. 2012). Additionally, van Loon et al. (2003) and Ramadhan et al. (2018) found that amino acids such as arginine, phenylalanine, leucine, and glutamic acid exhibit significant insulinotropic effects on pancreatic β-cells.

Effects of STZ-NA-induced diabetes on fasting blood glucose, HbA1c, body weight, and insulin level

Fermented 14-day rusip with high diabetes-related enzyme inhibitory potential was further used in an in vivo study. STZ-NA induction in rats significantly increased fasting blood glucose and HbA1c levels and decreased insulin levels and body weight compared to control rats (Table 5). Increased fasting blood glucose levels are thought to be due to impaired sugar conversion, resulting in hyperglycemia (Rashid et al. 2019). This condition triggers the glycosylation of erythrocyte membranes and an increase in HbA1c levels, which is a significant factor in oxidative stress in diabetes (Wang et al. 2021). STZ-NA induction can also disrupt glucose homeostasis and decrease insulin levels. It triggers insulin deficiency, resulting in insulin catabolism, which can lead to weight loss (Rashid et al. 2019).

Table 3. Amino acid profile of rusip fermented for 14 days

Classification	Type of amino acids	Composition (mg/100 g)
Essential amino acids	L-Histidine (His)	251.58±4.62
	L-Isoleucine (Ile)	548.27±2.73
	L-Leucine (Leu)	953.37±2.75
	L-Phenylalanine (Phe)	528.08±6.61
	L-Threonine (Thr)	522.98±3.64
	L-Valin (Val)	677.01±0.28
	L-Lysine (Lys)	900.92±13.0
Non-essential amino acids	L-Alanine (Ala)	786.86±6.29
	L-Arginine (Arg)	1757.92±14.5
	L-Aspartic acid (Asp)	1199.36±10.1
	Glycine (Gly)	327.37±8.41
	L-Glutamic Acid (Glu)	1912.71±7.62
	L-Serin (Ser)	518.37±7.30
	L-Tyrosine (Tyr)	848.9±7.97

Table 4. Fatty acid profile of rusip fermented for 14 days

Types of fatty acids	Fatty acids	Composition (% relative)
Saturated fatty acids	C4:0	<0.1
	C6:0	<0.1
	C8:0	<0.1
	C10:0	<0.1
	C11:0	<0.1
	C12:0	0.523±0.04
	C13:0	<0.1
	C14:0	5.13±0.01
	C15:0	<0.1
	C16:0	<0.1
	C17:0	5.504±0.04
	C18:0	<0.1
	C20:0	0.859±0.05
	C21:0	<0.1
	C23:0	3.926±0.12
	C24:0	9.226±0.07
Unsaturated fatty acids	C14:1 (n-5)	<0.1
	C15:1 (n-5)	1.250±0.03
	C16:1 (n-7)	29.517±0.66
	C17:1 (n-7)	1.656±0.03
	C18:1 (n-9)	<0.1
	C18:1 (n-9)	9.150±0.05
	C18:2 (n-6)	9.428±0.06
	C18:2 (n-6)	<0.1
	C18:3 (n-6)	<0.1
	C20:1 (n-9)	<0.1
	C18:3 (n-3)	<0.1
	C20:2 (n-6)	0.522±0.02
	C22:0+C22:0+20:3 (n-6)	<0.1
	C20:3 (n-3)	<0.1
	C22:1 (n-9)	<0.1
	C20:4 (n-6)	<0.1
	C22:2 (n-6)	<0.1
	C20:5 (n-3)	<0.1
	C24:1 (n-9)	<0.1
	C22:6 (n-3)	23.305±0.08

In contrast, administering rusip and Glibenclamide (DMR and DMG groups) reduces blood glucose and HbA1c levels and increases insulin levels and body weight (Table 4). Similar results were shown in the administration of *F. olivieri* water fraction and pomegranate aril juice, which also significantly reduced fasting blood sugar and increased body weight and insulin levels compared to the negative control (rats with diabetes) (El-Beih et al. 2019; Rashid et al. 2019). Huang et al. (2018) also reported that feeding tempeh fermented with *L. plantarum* significantly reduces blood sugar and HbA1c. Based on the study results, the administration of rusip produced an anti-hyperglycemic effect, which might be due to the content of BPs formed during fermentation. These results confirm our previous in silico study that reported the potential of BPs from rusip as anti-diabetic (Kurnianto et al. 2023b).

The effect of rusip administration on insulin resistance and sensitivity was determined using HOMA-IR and QUICKI scores. The STZ-NA group showed significantly increased HOMA-IR scores and decreased QUICKI scores. Another parameter of β-cell secretory function and response seen through HOMA cell function (HOMA-B) also showed a significant decrease in the STZ-NA group (Figure 4). These results indicate that the rats experienced hyperglycemia and relative insulin deficiency and showed insulin resistance, which reflects the metabolic characteristics of type 2 diabetes mellitus (Nurdiana et al. 2017). It may be due to the upregulation of proinflammatory cytokines (TNF-α and IL-6), FFA, and adipose tissue expression levels of resistin, as well as the downregulation of adipose tissue PPARγ, GLUT4, adiponectin, and insulin receptor expression levels (Aziz et al. 2020).

In contrast, administration of rusip and glibenclamide in the DMR and DMG groups decreased the HOMA-IR score and increased QUICKI and HOMA-B scores, although not to normal levels (Figure 4). These results suggest a hypoglycemic effect of rusip, which is more attributed to its ability to improve insulin sensitivity rather than to increase insulin secretion (Azra 2022). Wang et al. (2024) also stated that the pathogenic factors contributing to hyperglycemia are closely related to the dysregulation of insulin-dependent pathways, including deficient insulin secretion and increased insulin resistance. However, further studies are needed to confirm these mechanisms. Importantly, our study showed that 14 days of treatment of rats with diabetes with 9 g/kg rusip lowered fasting blood glucose levels and normalized insulin levels. Thus, the administration of 9 g/kg rusip has higher anti-diabetic potentials than the administration of 0.45 mg/kg glibenclamide, an anti-diabetic drug that stimulates insulin release from pancreatic-β cells, in STZ-NA-induced diabetic rats (Furman 2007; Zhou et al. 2019).

Effects of STZ and NA-induced diabetes on liver enzyme, renal, and lipid profiles

The liver is the principal site of insulin action and the regulation of glucose homeostasis (Wang et al. 2024). High serum AST, ALP, and ALT levels indicate early liver damage, and they are correlated with an increased risk of diabetes (Chen et al. 2017; Karimabad et al. 2022). All three biomarkers were significantly higher in the STZ-NA group than in the control groups (Table 6). Azra (2022) reported that an increase in the levels of these three enzymes in liver cells indicated liver cell damage due to

oxidative stress. In contrast, the DMR and DMG groups showed decreased AST, ALT, and ALT levels. It suggests that rusip consumption has hepatoprotective effects, and even the administration of 9 g/kg rusip showed better hepatoprotective properties than the standard drug glibenclamide. This hepatoprotective effect may be due to the bioactive compounds of BPs that can maintain the hepatic redox status by enhancing enzymatic and non-enzymatic antioxidant defenses while reducing cellular damage, oxidative stress, and the presence of free radicals (Prathapan and Rajamohan 2011; Renjith et al. 2013). Our results are consistent with those of a previous study by Rashid et al. (2019) that showed decreased levels of AST, ALP, and ALT in the serum of rats with STZ-NA-induced diabetes treated with an extract of *Fagonia olivieri* DC. (Zygophyllaceae). Treatment with unfermented oat extract and *L. plantarum* fermented oat extract in rats with STZ-induced diabetes improved liver function by decreasing ALT, AST, and ALP levels (Algonaiman et al. 2022).

Table 5. Effect of STZ and NA-induced diabetes and rusip supplementation on body weight of rats, fasting blood glucose, and HbA1c

Group	Body weight (g)			Fasting blood glucose (mg/dL)		HbA1c (%)
	Initial body weight	Final body weight	Body weight gain	Initial	Final	
Control	187.42±1.90^{a}	207.42±2.64^{c}	20.00±1.00^{c}	68.2±1.39^{a}	69.44±1.78^{a}	3.86±0.20^{a}
STZ-NA	185.85±2.79^{a}	162.14±2.41^{a}	-23.71±2.14^{a}	271.42±2.70^{c}	272.58±3.02^{d}	6.78±0.75^{c}
DMG	189.14±2.41^{a}	190.85±2.91^{b}	1.71±0.95^{b}	268.01±3.26^{b}	125.32±3.15^{c}	4.63±0.15^{b}
DMR	186.14±3.84^{a}	190.85±3.44^{b}	4.71±1.38^{b}	268.05±3.69^{b}	97.8±4.51^{b}	4.24±0.20^{b}

Note: Control: No treatment; STZ-NA: Streptozotocin and Nicotinamide-induced diabetes rats; DMG: Streptozotocin and Nicotinamide-induced diabetes rats given Glibenclamide 0.45 mg/kg BW; DMR: Streptozotocin and Nicotinamide induced diabetes rats given rusip 9 g/kg BW; Value was mean±SD with n = 7. Values without a common letter are significantly different (p<0.05) as determined by LSD

Table 6. Effect of STZ and NA-induced diabetes and rusip supplementation on the liver profile of rats

Group	Liver profile (U/L)			Lipid profile (mg/dL)				Renal profile (mg/dL)	
	AST	ALT	ALP	HDL	LDL	Triglyceride	Cholesterol	Urea	Creatinine
Control	19.14^{a}±0.62	39.11^{a}±0.55	38.79^{a}±1.24	50.09^{c}±2.21	16.45^{a}±2.57	64.27^{b}±3.65	56.52^{c}±4.12	40.14^{a}±2.54	0.32^{c}±0.02
STZ+NA	42.86^{d}±1.18	73.58^{c}±9.73	74.04^{d}±2.84	36.93^{a}±1.60	45.71^{c}±2.03	76.85^{a}±5.12	70.63^{a}±2.83	50.32^{a}±9.65	0.41^{a}±0.06
DMG	28.16^{c}±0.93	59.85^{b}±2.47	70.89^{c}±1.24	40.13^{b}±2.16	21.60^{b}±2.37	57.15^{c}±2.52	65.85^{b}±2.22	44.51^{a}±1.53	0.35^{b}±0.04
DMR	21.57^{b}±0.68	42.79^{a}±2.21	43.91^{b}±1.47	49.62^{c}±1.98	17.56^{a}±1.53	59.43^{c}±2.78	63.57^{b}±1.19	42.66^{a}±2.54	0.34^{b}±0.02

Note: Control: No treatment; STZ+NA: Streptozotocin and Nicotinamide-induced diabetes rats; DMG: Streptozotocin and Nicotinamide-induced diabetes rats given Glibenclamide 0.45 mg/kg BW; DMR: Streptozotocin and Nicotinamide induced diabetes rats given rusip 9 g/kg BW; Values without a common letter are significantly different (P<0.05) as determined by LSD; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein

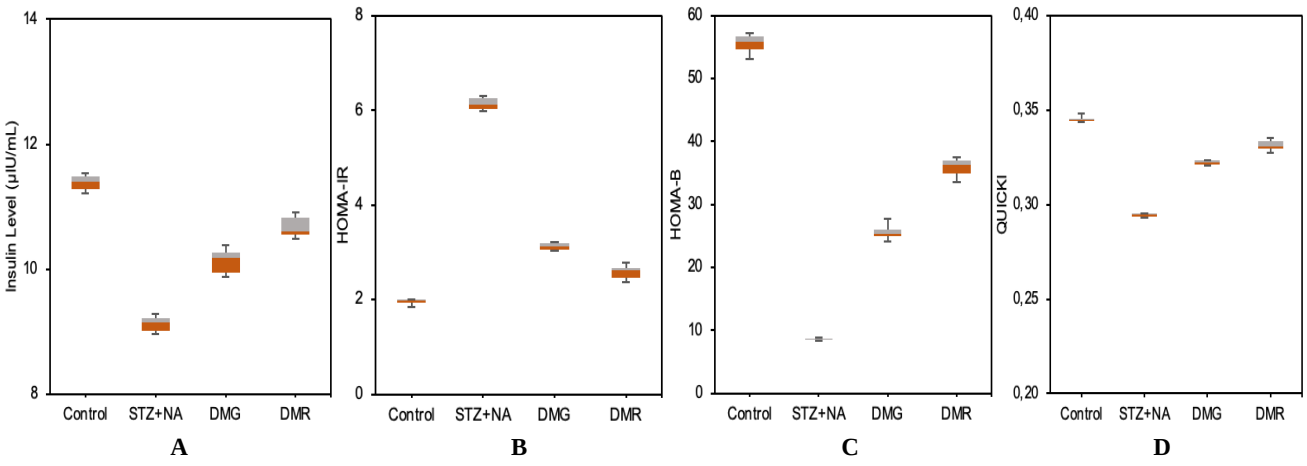

Figure 4. Effect of rusip and Glibenclamide administration on: A. Insulin production; and B, C, D. Sensitivity in Streptozotocin (STZ) and Nicotinamide (NA) induced diabetes rats. Control: No treatment; STZ+NA: Streptozotocin and Nicotinamide induced diabetes rats; DMG: Streptozotocin and Nicotinamide induced diabetes rats given Glibenclamide 0.45 mg/kg BW; DMR: Streptozotocin and Nicotinamide induced rats given rusip 9 g/kg BW; Data expressed as mean±SD

Analysis of urea and creatinine levels was performed to evaluate renal function. The result showed the STZ-NA group had significantly higher urea and creatinine levels than the control group (Table 6). Similar findings were reported by Gad-Elkareem et al. (2019) and Fagbohun et al. (2020), who showed increased urea and creatinine levels in rats with diabetes compared to normal rats. Urea and creatinine are end products of body metabolism that serve as markers of nephrotoxicity and are often used to diagnose renal damage (Khan and Sultana 2004). Elevated levels of these two markers reflect decreased glomerular filtration rate or impaired urinary excretion, indicating impaired renal function and metabolism (Pandya et al. 2016). However, DMR and DMG groups showed significantly reduced urea and creatinine levels. This decrease suggests that rusip may contain bioactive compounds with antioxidant and nephroprotective activities and the ability to lower serum urea levels in rats with diabetes (Rajasekaran and Kalaivani 2015; Sivamaruthi et al. 2018). The study of Rajasekaran and Kalaivani (2015) showed a similar trend, in which supplementation of fermented rice in STZ-induced diabetes rats showed a significant reduction in urea and creatinine levels. Administration of *L. fermentum* fermented skimmed milk also showed reduced urea levels in rats with diabetes (Kumari et al. 2024).

The administration of STZ-NA significantly altered lipid profile biomarkers in rats with diabetes, mainly by decreasing HDL levels and increasing LDL levels (Table 6). These results indicate dyslipidemia or a cholesterol imbalance, which is prevalent in type 2 diabetes mellitus (Vergès 2015). This increase in LDL levels was associated with decreased insulin levels due to the activation of HMGCoA-reductase, the enzyme responsible for synthesizing cholesterol-rich LDL. High LDL levels contribute to cholesterol deposition in the arteries and aorta, thereby increasing the risk of coronary heart disease in patients with diabetes (Rashid et al. 2019). A decrease in HDL levels indicates the inability to inhibit cholesterol deposition in the system, which increases the risk of atherosclerosis (Rashid et al. 2019). In the DMR and DMG groups, there was a decrease in LDL levels and an increase in HDL levels. These results indicate that administering rusip to rats with diabetes can alleviate the disruption of lipid metabolism, thereby reducing hypercholesterolemia. Consistent with other parameters, these results suggest that 9 g/kg body weight rusip is more effective than glibenclamide at modulating diabetic symptoms. Similar results were obtained after the consumption of soybean fermented with *Lactobacillus plantarum*, which resulted in a significant decrease in LDL levels to HbA1C but an increase in HDL levels in DM rats ($p<0.05$) (Huang et al. 2018). Huang et al. (2022) showed that fermented shrimp shells at 9 g/kg doses reduced hypercholesterolemia and hypertriglyceridemia in rats with diabetes.

In vitro and in vivo studies showed that rusip alleviates diabetes in rats. BPs were considered compounds that play a significant role in this activity. BPs exert anti-diabetic effects by enhancing insulin and AMPK signaling pathways, mimicking insulin to activate the insulin receptor and downstream pathways, including PI3K, protein kinase B, glycogen synthesis, and GLUT4-mediated glucose uptake. They also inhibit gluconeogenesis and stimulate glucose uptake via AMPK activation. Feedback inhibition of PI3K by mTOR complex 1 in obesity warrants further exploration (Li et al. 2018). Previous studies report that several fermented foods are known to have similar activities, such as fermented soybean (Kwon et al. 2010), fermented milk (Song et al. 2016), fermented oat (Algonaiman et al. 2022), Makgeolli (Korean rice wine) (Choi et al. 2014), Chouguiyu (Chinese traditional fermented fish) (Yang et al. 2022), and fermented rice bran (Hu et al. 2023). However, whether BPs or other water-soluble compounds in the rusip are responsible for this effect remains unclear, requiring further investigation.

In conclusion, this study demonstrated that rusip has anti-diabetic potential mainly through its ability to inhibit α-amylase and α-glucosidase enzymes. Rusip effectively regulates blood glucose, HbA1c levels and maintains insulin, HOMA-IR, HOMA-B, and QUICKI in STZ-NA-induced diabetes rats. In addition, rusip normalizes the liver marker enzymes associated with diabetes, such as AST, ALP, and ALT, and reduces other liver markers, such as urea and creatinine levels. Rusip also restores the abnormalities in HDL, LDL, triglyceride, and cholesterol levels in rats with diabetes, thereby protecting them from diabetes-associated tissue damage. Thus, our results suggest that the administration of rusip may serve as an alternative food for people with diabetes and its associated complications. However, the precise mechanisms underlying these effects remain to be elucidated and warrant further investigation.

REFERENCES

Aziz SMA, Ahmed OM, Abd El-Twab SM, Al-Muzafar HM, Amin KA, Abdel-Gabbar M. 2020. Antihyperglycemic effects and mode of actions of *Musa paradisiaca* leaf and fruit peel hydroethanolic extracts in nicotinamide/streptozotocin-induced diabetic rats. Evid Based Complement Alternat Med 2020: 9276343. DOI: 10.1155/2020/9276343.

Afifah DN, Sari IR, Prastifani NT, Fulyani F, Anjani G, Widyastuti N, Hastuti VN. 2023. Effect of fermentation time on nutrition content, physical properties, pH, amino acids, fatty acids composition and organoleptics on fermented mackerel sausage (*Rastrelliger kanagurta* Cuvier) characteristics. Intl J Food Stud 12: 57-70. DOI: 10.7455/ijfs/12.1.2023.a4.

Agrawal H, Joshi R, Gupta M. 2016. Isolation, purification and characterization of antioxidative peptide of pearl millet (*Pennisetum glaucum*) protein hydrolysate. Food Chem 204: 365-372. DOI: 10.1016/j.foodchem.2016.02.127

Algonaiman R, Alharbi HF, Barakat H. 2022. Antidiabetic and hypolipidemic efficiency of *Lactobacillus plantarum* fermented oat (*Avena sativa*) extract in streptozotocin-induced diabetes in rats. Fermentation 8 (6): 267. DOI: 10.3390/fermentation8060267.

Alkalbani NS, Turner MS, Ayyash MM. 2019. Isolation, identification, and potential probiotic characterization of isolated lactic acid bacteria and in vitro investigation of the cytotoxicity, antioxidant, and antidiabetic activities in fermented sausage. Microb Cell Fact 18 (1): 188. DOI: 10.1186/s12934-019-1239-1.

Amelia V, Nurhamzah LY, Lioe HN, Sitanggang AB, Adawiyah DR, Kusumaningrum HD. 2024. Characterization and peptide identification of umami fractions from rusip-A traditional fermented anchovy product. J Food Sci 89 (12): 8326-8341. DOI: 10.1111/1750-3841.17532.

American Diabetes Association. 2009. Diagnosis and classification of diabetes mellitus. Diabetes Care 32: S62-S67. DOI: 10.2337/dc09-S062.

An Y, Cai X, Cong L, Hu Y, Liu R, Xiong S, Hu X. 2022. Quality improvement of Zhayu, a fermented fish product in China: Effects of inoculated fermentation with three kinds of lactic acid bacteria. Foods 11 (18): 2756. DOI: 10.3390/foods11182756.

Apostolidis E, Kwon Y-I, Shetty K. 2007. Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov Food Sci Emerg Technol 8 (1): 46-54. DOI: 10.1016/j.ifset.2006.06.001.

Ardiana L, Sauriasari R, Elya B. 2018. Antidiabetic activity studies of white tea (*Camellia sinensis* (L.) O. Kuntze) ethanolic extracts in streptozotocin-nicotinamide induced diabetic rats. Pharmacogn J 10 (1): 186-189. DOI: 10.5530/pj.2018.1.31.

Ayyash M, Olaimat A, Al-Nabulsi A, Liu S-Q. 2020. Bioactive properties of novel probiotic *Lactococcus lactis* fermented camel sausages: Cytotoxicity, angiotensin converting enzyme inhibition, antioxidant capacity, and antidiabetic activity. Food Sci Anim Resour 40 (2): 155-171. DOI: 10.5851/kosfa.2020.e1.

Azra JM. 2022. Efektivitas Serbuk Minuman Kelapa (*Cocos nucifera*) Muda dalam Memperbaiki Metabolisme pada Tikus Diabetes yang Diinduksi oleh Streptozotocin. [Dissertation]. IPB University, Bogor. [Indonesian]

Banda M, Nyirenda J, Muzandu K, Sijumbila G, Mudenda S. 2018. Antihyperglycemic and antihyperlipidemic effects of aqueous extracts of *Lannea edulis* in alloxan-induced diabetic rats. Front Pharmacol 9: 1099. DOI: 10.3389/fphar.2018.01099.

Bayraklı B. 2023. A study on fatty acid composition and quality indicators of anchovy (*Engraulis encrasicolus*) oils from different factories. Mar Sci Technol Bull 12 (4): 522-529. DOI: 10.33714/masteb.1356285.

Bekhit AE-DA, Holman BWB, Giteru SG, Hopkins DL. 2021. Total Volatile Basic Nitrogen (TVB-N) and its role in meat spoilage: A review. Trends Food Sci Technol 109: 280-302. DOI: 10.1016/j.tifs.2021.01.006.

Chadong K, Yunchalard S, Piyatheerawong W. 2015. Physicochemical characteristics and protein degradation during fermentation of Plaa-som, A traditional fermented fish product of North-Eastern Thailand. Indian J Tradit Knowl 14 (2): 220-225.

Chalamaiah M, Keskin, US, Hong H, Wu J. 2019. Regulatory requirements of bioactive peptides (protein hydrolysates) from food proteins. J Func Foods 58: 123-129. DOI: 10.1016/j.jff.2019.04.050.

Chen SC-C, Tsai SP, Jhao J-Y, Jiang W-K, Tsao CK, Chang L-Y. 2017. Liver fat, hepatic enzymes, alkaline phosphatase and the risk of incident type 2 diabetes: A prospective study of 132,377 adults. Sci Rep 7 (1): 4649. DOI: 10.1038/s41598-017-04631-7.

Chiu H-H, Kuo C-H. 2020. Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. J Food Drug Anal 28 (1): 60-73. DOI: 10.1016/j.jfda.2019.10.003.

Choi J-S, Seo HJ, Lee YR, Kwon S-J, Moon SH, Park S-M, Sohn JH. 2014. Characteristics and in vitro anti-diabetic properties of the Korean rice wine, makgeolli fermented with *Laminaria japonica*. Prev Nutr Food Sci 19 (2): 98-107. DOI: 10.3746/pnf.2014.19.2.098.

Date K. 2020. Regulatory functions of α-amylase in the small intestine other than starch digestion: α-glucosidase activity, glucose absorption, cell proliferation, and differentiation. In: Takada A (eds). New Insights into Metabolic Syndrome. IntechOpen, London. DOI: 10.5772/intechopen.92660.

de Fátima Haueisen Sander Diniz M, Beleigoli AMR, Schmidt MI, Duncan BB, Ribeiro ALP, Vidigal PG, Benseñor IM, Lotufo PA, Santos IS, Griep RH, Barreto SM. 2020. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and metabolic syndrome at baseline of a multicentric Brazilian cohort: ELSA-Brasil study. Cad Saude Publica 36 (8): e00072120. DOI: 10.1590/0102-311X00072120.

Deng Y, Gruppen H, Wierenga PA. 2018. Comparison of protein hydrolysis catalyzed by bovine, porcine, and human trypsins. J Agric Food Chem 66 (16): 4219-4232. DOI: 10.1021/acs.jafc.8b00679.

Desta DT, Kelikay GN, Zekwos M, Eshete M, Reda HH, Alemayehu FR, Zula AT. 2021. Influence of fermentation time on proximate composition and microbial loads of Enset, (*Ensete ventricosum*), sampled from two different agroecological districts. Food Sci Nutr 9 (10): 5641-5647. DOI: 10.1002/fsn3.2527.

Elam E, Feng J, Lv Y-M, Ni Z-J, Sun P, Thakur K, Zhang J-G, Ma Y-L, Wei Z-J. 2021. Recent advances on bioactive food derived anti-diabetic hydrolysates and peptides from natural resources. J Funct Foods 86: 104674. DOI: 10.1016/J.JFF.2021.104674.

El-Beih NM, Ramadan G, El-Husseiny EA, Hussein AM. 2019. Effects of pomegranate aril juice and its punicalagin on some key regulators of insulin resistance and oxidative liver injury in streptozotocin-nicotinamide type 2 diabetic rats. Mol Biol Rep 46 (4): 3701-3711. DOI: 10.1007/s11033-019-04813-8.

Etsassala NGER, Badmus JA, Marnewick JL, Iwuoha EI, Nchu F, Hussein AA. 2020. Alpha-glucosidase and alpha-amylase inhibitory activities, molecular docking, and antioxidant capacities of *Salvia aurita* constituents. Antioxidants 9: 1149. DOI: 10.3390/antiox9111149.

Fagbohun OF, Awoniran PO, Babalola OO, Agboola FK, Msagati TAM. 2020. Changes in the biochemical, hematological and histopathological parameters in STZ-Induced diabetic rats and the ameliorative effect of *Kigelia africana* fruit extract. Heliyon 6 (5): e03989. DOI: 10.1016/j.heliyon.2020.e03989.

Faithong N, Benjakul S. 2014. Changes in antioxidant activities and physicochemical properties of Kapi, a fermented shrimp paste, during fermentation. J Food Sci Technol 51 (10): 2463-2471. DOI: 10.1007/s13197-012-0762-4.

Fitriana LY, Maharani N, Anjani G, Afifah DN. 2021. Nutrient content and fatty acid profile of fermented shrimp (*Litopenaeus vannamei*) sausage. Food Res 5 (S3): 76-84. DOI: 10.26656/fr.2017.5(S3).002.

Furman BL. 2007. Glibenclamide. In: Enna SJ, Bylund DB (eds). xPharm: The Comprehensive Pharmacology Reference. Elsevier, New York.

Gad-Elkareem MAM, Abdelgadir EH, Badawy OM, Kadri A. 2019. Potential antidiabetic effect of ethanolic and aqueous-ethanolic extracts of *Ricinus communis* leaves on streptozotocin-induced diabetes in rats. PeerJ 7: e6441. DOI: 10.7717/peerj.6441.

García-Cano I, Rocha-Mendoza D, Ortega-Anaya J, Wang K, Kosmerl E, Jiménez-Flores R. 2019. Lactic acid bacteria isolated from dairy products as potential producers of lipolytic, proteolytic and antibacterial proteins. Appl Microbiol Biotechnol 103 (13): 5243-5257. DOI: 10.1007/s00253-019-09844-6.

Gencbay G, Turhan S. 2016. Proximate composition and nutritional profile of the black sea anchovy (*Engraulis encrasicholus*) whole fish, fillets, and by-products. J Aquat Food Prod Technol 25 (6): 864-874. DOI: 10.1080/10498850.2014.945199.

Hu J, Lai X, Wu X, Wang H, Weng N, Lu J, Lyu M, Wang S. 2023. Isolation of a novel anti-diabetic α-glucosidase oligo-peptide inhibitor from fermented rice bran. Foods 12: 183. DOI: 10.3390/foods12010183.

Huang C-H, Lin C-H, Huang H-H, Tsai G-J. 2022. Development of fermented shrimp shell product with hypoglycemic and hypolipidemic effects on diabetic rats. Metabolites 12 (8): 695. DOI: 10.3390/metabo12080695.

Huang Y-C, Wu B-H, Chu Y-L, Chang W-C, Wu M-C. 2018. Effects of tempeh fermentation with *Lactobacillus plantarum* and *Rhizopus oligosporus* on streptozotocin-induced type ii diabetes mellitus in rats. Nutrients 10 (9): 1143. DOI: 10.3390/nu10091143.

Ibrahim MA, Bester MJ, Neitz AWH, Gaspar ARM. 2018. Structural properties of bioactive peptides with α-glucosidase inhibitory activity. Chem Biol Drug Des 91 (2): 370-379. DOI: 10.1111/cbdd.13105.

Islami P, Rianingsih L, Sumardianto. 2022. Pengaruh penambahan gula terhadap lemak pada terasi udang rebon (*Acetes* sp.) dengan lama fermentasi yang berbeda. Jurnal Ilmu dan Teknologi Perikanan 4 (1): 24-30. DOI: 10.14710/jitpi.2022.12873. [Indonesian]

Istiqamah A, Lioe HN, Adawiyah DR. 2019. Umami compounds present in low molecular umami fractions of asam sunti-A fermented fruit of *Averrhoa bilimbi* L. Food Chem 270: 338-343. DOI: 10.1016/j.foodchem.2018.06.131.

Johnson MH, Lucius A, Meyer T, de Mejia EG. 2011. Cultivar evaluation and effect of fermentation on antioxidant capacity and in vitro inhibition of α-amylase and α-glucosidase by highbush blueberry (*Vaccinium corombosum*). J Agric Food Chem 59 (16): 8923-8930. DOI: 10.1021/jf201720z.

Karimabad MN, Khalili P, Ayoobi F, Esmaeili-Nadimi A, La Vecchia C, Jamali Z. 2022. Serum liver enzymes and diabetes from the Rafsanjan cohort study. BMC Endocr Disord 22: 127. DOI: 10.1186/s12902-022-01042-2.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. 2000. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 85: 2402-2410. DOI: 10.1210/jcem.85.7.6661.

Kaya Y, Turan H. 2008. Fatty acids composition of Anchovy (*Engraulis encrasicolus* L. 1758) oil produced in Sinop-Turkey. J FisheriesSciences.com 2 (5): 693-697. DOI: 10.3153/jfscom.2008036.

Khamchan A, Paseephol T, Hanchang W. 2018. Protective effect of wax apple (*Syzygium samarangense* (Blume) Merr. & L.M. Perry) against streptozotocin-induced pancreatic ß-cell damage in diabetic rats. Biomed Pharmacother 108: 634-645. DOI: 10.1016/j.biopha.2018.09.072.

Khan N, Sultana S. 2004. Abrogation of potassium bromate-induced renal oxidative stress and subsequent cell proliferation response by soy isoflavones in Wistar rats. Toxicology 201 (1-3): 173-184. DOI: 10.1016/j.tox.2004.04.012.

Khositanon P, Panya N, Roytrakul S, Krobthong S, Chanroj S, Choksawangkarn W. 2021. Effects of fermentation periods on antioxidant and angiotensin I-converting enzyme inhibitory activities of peptides from fish sauce by-products. LWT 135: 110122. DOI: 10.1016/j.lwt.2020.110122.

Koesoemawardani D, Ali M. 2016. Rusip with alginate addition as seasoning. Jurnal Pengolahan Hasil Perikanan Indonesia 19 (3): 277. DOI: 10.17844/jphpi.v19i3.14549. [Indonesian]

Koesoemawardani D, Yuliana N. 2013. Karakter rusip dengan penambahan kultur kering: *Streptococcus* sp. Jurnal Sains dan Teknologi Indonesia 11 (3): 205-211. DOI: 10.29122/jsti.v11i3.834. [Indonesian]

Kumari VBC, Huligere S, Jayanthi MK, Goh KW, Desai SM, Kalabharthi HL, Ramu R. 2024. Characterization of *Lactobacillus* spp. as probiotic and antidiabetic potential isolated from Boza, traditional fermented beverage in Turkey. Intl J Microbiol 2024: 2148676. DOI: 10.1155/2024/2148676.

Kurnianto MA, Defri I, Syahbanu F, Aulia SS. 2024. Fish-derived bioactive peptide: Bioactivity potency, structural characteristics, and conventional and bioinformatics approaches for identification. Future Foods 9: 100386. DOI: 10.1016/j.fufo.2024.100386.

Kurnianto MA, Munarko H. 2022. Pengaruh penambahan kultur starter dan metabolit *Lactobacillus casei* terhadap mutu mikrobiologi sosis fermentasi ikan patin (*Pangasius* sp.). Jurnal Kelautan dan Perikanan Terapan 5 (1): 27-37. DOI: 10.15578/jkpt.v5i1.10970. [Indonesian]

Kurnianto MA, Syahbanu F, Defri I, Sanjaya YA. 2023a. Prospects of bioinformatics approach for exploring and mapping potential bioactive peptide of rusip (the traditional Indonesian fermented anchovy): A review. Adv Food Sci Sustain Agric Agroind Eng 6 (2): 116-133. DOI: 10.21776/ub.afssaae.2023.006.02.3.

Kurnianto MA, Syahbanu F, Hamidatun H, Sanjaya YA. 2023b. Prediction and mapping of potential bioactive peptides from traditional fermented anchovy (rusip) using bioinformatics approaches. Squalen Bull Mar Fish Postharvest Biotechnol 18 (2): 93-105. DOI: 10.15578/squalen.725.

Kwon DY, Daily 3rd JW, Kim HJ, Park S. 2010. Antidiabetic effects of fermented soybean products on type 2 diabetes. Nutr Res 30 (1): 1-13. DOI: 10.1016/j.nutres.2009.11.004.

Lee Y-C, Kung H-F, Huang C-Y, Huang T-C, Tsai Y-H. 2016. Reduction of histamine and biogenic amines during salted fish fermentation by *Bacillus polymyxa* as a starter culture. J Food Drug Anal 24 (1): 157-163. DOI: 10.1016/j.jfda.2015.02.002.

Leiwakabessy J, Sormin RBD, Mailoa MN. 2021. Amino acid profile of tuna (*Thunnus albacores*) fish sauce fermented by salt and papain enzyme. IOP Conf Ser: Earth Environ Sci 777: 012027. DOI: 10.1088/1755-1315/777/1/012027.

Li S, Liu L, He G, Wu J. 2018. Molecular targets and mechanisms of bioactive peptides against metabolic syndromes. Food Funct 9 (1): 42-52. DOI: 10.1039/C7FO01323J.

Li W, Mi S, Liu X, Sang Y, Sun J. 2022. Variations in the physicochemical properties and bacterial community composition during fermentation of low-salt shrimp paste. Food Res Intl 154: 111034. DOI: 10.1016/j.foodres.2022.111034.

Liu H, Yang S, Liu J, Lu J, Wu D. 2023. Effect of salt concentration on Chinese soy sauce fermentation and characteristics. Food Biosci 53: 102825. DOI: 10.1016/j.fbio.2023.102825.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. J Biol Chem 193 (1): 265-275. DOI: 10.1016/S0021-9258(19)52451-6.

Lu H, Xie T, Wu Q, Hu Z, Luo Y, Luo F. 2023. Alpha-glucosidase inhibitory peptides: Sources, preparations, identifications, and action mechanisms. Nutrients 15 (19): 4267. DOI: 10.3390/nu15194267.

Mohanty B, Mahanty A, Ganguly S et al. 2014. Amino acid compositions of 27 food fishes and their importance in clinical nutrition. J Amino Acids 2014: 269797. DOI: 10.1155/2014/269797.

Najafian L, Babji AS. 2018. Fractionation and identification of novel antioxidant peptides from fermented fish (*pekasam*). J Food Measur Charac 12: 2174-2183. DOI: 10.1007/s11694-018-9833-1.

Nurdiana S, Goh YM, Ahmad H, Dom SM, Azmi NS, Zin NSNM, Ebrahimi M. 2017. Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with *Ficus deltoidea* and vitexin. BMC Complement Alternat Med 17: 290. DOI: 10.1186/s12906-017-1762-8.

Pandya D, Nagrajappa AK, Ravi KS. 2016. Assessment and correlation of urea and creatinine levels in saliva and serum of patients with chronic kidney disease, diabetes and hypertension-A research study. J Clin Diagn Res 10: ZC58-ZC62. DOI: 10.7860/JCDR/2016/20294.8651.

Peralta EM, Endoma Jr. LF, Sefil AS, Palmos GN. 2021. Chemical and antioxidant activity changes in Philippine green mussel (*Perna viridis*) during fermentation at varying salt concentrations. Philipp J Sci 150 (6B): 1711-1722. DOI: 10.56899/150.6B.09.

Prathapan A, Rajamohan T. 2011. Antioxidant and antithrombotic activity of tender coconut water in experimental myocardial infarction. J Food Biochem 35: 1501-1507. DOI: 10.1111/J.1745-4514.2010.00471.X.

Preetha PP, Devi VG, Rajamohan T. 2012. Hypoglycemic and antioxidant potential of coconut water in experimental diabetes. Food Funct 3 (7): 753-757. DOI: 10.1039/C2FO30066D.

Puat SNA, Huda N, Abdullah WNW, Al-Karkhi AFM, Ardiansyah. 2015. Chemical composition and protein quality of fish sauces (*Kecap Ikan* and Nampla). Asia Pac J Sustain Agric Food Energy 3 (2): 2-9.

Puspita AA, Winarti S, Kurnianto MA. 2024. Profil fisikokimia, mikrobiologi dan organoleptik rusip ikan teri (*Stolephorus* sp.) dengan lama fermentasi berbeda. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 19 (1): 51-60. DOI: 10.15578/jpbkp.v19i1.1011. [Indonesian]

Rajasekaran A, Kalaivani M. 2015. Protective effect of Monascus fermented rice against STZ-induced diabetic oxidative stress in kidney of rats. J Food Sci Technol 52 (3): 1434-1443. DOI: 10.1007/s13197-013-1191-8.

Ramadhan AH, Nawas T, Zhang X, Pembe WM, Xia W, Xu Y. 2017. Purification and identification of a novel antidiabetic peptide from Chinese giant salamander (*Andrias davidianus*) protein hydrolysate against α-amylase and α-glucosidase. Intl J Food Prop 20: S3360-S3372. DOI: 10.1080/10942912.2017.1354885.

Rashid U, Khan MR, Sajid M. 2019. Antioxidant, anti-inflammatory and hypoglycemic effects of *Fagonia olivieri* DC on STZ-nicotinamide induced diabetic rats-In vivo and in vitro study. J Ethnopharmacol 242: 112038. DOI: 10.1016/j.jep.2019.112038.

Ren J, Dai J, Chen Y, Wang Z, Sha R, Mao J. 2024. Physiochemical characterization and ameliorative effect of rice resistant starch modified by heat-stable α-amylase and glucoamylase on the gut microbial community in T2DM mice. Food Funct 15 (10): 5596-5612. DOI: 10.1039/D3FO05456J.

Renjith RS, Chikku AM, Rajamohan T. 2013. Cytoprotective, antihyperglycemic and phytochemical properties of *Cocos nucifera* (L.) inflorescence. Asian Pac J Trop Med 6 (10): 804-810. DOI: 10.1016/S1995-7645(13)60142-X.

Rinto R, Nopianti R, Herpandi, Oktaviani S. 2017. Fractionation of anticholesterol bioactive compounds from Bekasam (Indonesian fermented fish product). Pertanika J Trop Agric Sci 40 (3): 399-406.

Rinto R, Lestari SD, Putri NA. 2019. Aktivitas antioksidan dan antikolesterol ekstrak rusip. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 14 (1): 1. DOI: 10.15578/jpbkp.v14i1.568. [Indonesian]

Rinto R, Widiastuti I, Samudera BT. 2021. Novel antihypertension bioactive compounds from rusip. IOP Conf Ser: Earth Environ Sci 810: 012002. DOI: 10.1088/1755-1315/810/1/012002.

Salil G, Nevin KG, Rajamohan T. 2012. Arginine-rich coconut kernel diet influences nitric oxide synthase activity in alloxan diabetic rats. J Sci Food Agric 92 (9): 1903-1908. DOI: 10.1002/jsfa.5558.

Sayuti M, Salampessy RBS, Asriani, Nurbani SZ, Saidin. 2022. Chemical and hedonic characteristics of smoked *Katsuwonus pelamis* (Fufu fish) from Sorong, West Papua, Indonesia. Biodiversitas 23 (3): 1707-1713. DOI: 10.13057/biodiv/d230363.

Sim L, Jayakanthan K, Mohan S, Nasi R, Johnston BD, Pinto BM, Rose DR. 2010. New glucosidase inhibitors from an ayurvedic herbal treatment for type 2 diabetes: Structures and inhibition of human intestinal maltase-glucoamylase with compounds from *Salacia reticulata*. Biochemistry 49 (3): 443-451. DOI: 10.1021/bi9016457.

Singh B, Kaur A. 2016. Antidiabetic potential of a peptide isolated from an endophytic *Aspergillus awamori*. J Appl Microbiol 120 (2): 301-311. DOI: 10.1111/jam.12998.

Sivamaruthi BS, Kesika P, Prasanth MI, Chaiyasut C. 2018. A mini review on antidiabetic properties of fermented foods. Nutrients 10 (12): 1973. DOI: 10.3390/nu10121973.

Sompinit K, Lersiripong S, Reamtong O, Pattarayingsakul W, Patikarnmonthon N, Panbangred W. 2020. In vitro study on novel bioactive peptides with antioxidant and antihypertensive properties from edible rhizomes. LWT 134: 110227. DOI: 10.1016/j.lwt.2020.110227.

Song R, Yang P, Wei R, Ruan G. 2016. Antioxidative, antibacterial, and food functional properties of the half-fin anchovy hydrolysates-glucose conjugates formed via Maillard reaction. Molecules 21 (6): 795. DOI: 10.3390/molecules21060795.

Takada K, Ikezawa K, Maeda N, Sato T, Fukui Y, Takano T, Funatsu Y. 2023. Effects of different fermentation starter cultures on the quality of fish sauce prepared from deep-sea smelt. Food Sci Technol Res 29 (4): 289-300. DOI: 10.3136/fstr.FSTR-D-23-00021.

Tamam B, Syah D, Lioe H, Suhartono M, Kusuma W. 2018. Beberapa penciri berbasis sekuens untuk mengenali sifat fungsional peptida bioaktif: Studi eksplorasi. Jurnal Teknologi dan Industri Pangan 29 (1): 1-9. DOI: 10.6066/jtip.2018.29.1.1. [Indonesian]

van Loon LJC, Kruijshoop M, Menheere PPCA, Wagenmakers AJM, Saris WHM, Keizer HA. 2003. Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes. Diabetes Care 26 (3): 625-630. DOI: 10.2337/diacare.26.3.625.

Vergès B. 2015. Pathophysiology of diabetic dyslipidaemia: Where are we?. Diabetologia 58: 886-899. DOI: 10.1007/s00125-015-3525-8.

Wang J, Li C, Xue J, Yang J, Zhang Q, Zhang H, Chen Y. 2015. Fermentation characteristics and angiotensin I-converting enzyme-inhibitory activity of *Lactobacillus helveticus* isolate H9 in cow milk, soy milk, and mare milk. J Dairy Sci 98 (6): 3655-3664. DOI: 10.3168/jds.2015-9336.

Wang C, Luo D, Zheng L, Zhao M. 2024. Anti-diabetic mechanism and potential bioactive peptides of casein hydrolysates in STZ/HFD-induced diabetic rats. J Sci Food Agric 104 (5): 2947-2958. DOI: 10.1002/jsfa.13187.

Wang W, Xia W, Gao P, Xu Y, Jiang Q. 2017. Proteolysis during fermentation of Suanyu as a traditional fermented fish product of China. Intl J Food Prop 20 (Sup 1): S166-S176. DOI: 10.1080/10942912.2017.1293089.

Wang Y, Yang P, Yan Z, Liu Z, Ma Q, Zhang Z, Wang Y, Su Y. 2021. The relationship between erythrocytes and diabetes mellitus. J Diabetes Res 2021: 6656062. DOI: 10.1155/2021/6656062.

Wenno MR, Loppies CRM. 2019. Physico-chemical characteristics and amino acid profile of fermented sauce made from tuna loin by-product. IOP Conf Ser: Earth Environ Sci 370: 012006. DOI: 10.1088/1755-1315/370/1/012006.

Wickramaratne MN, Punchihewa JC, Wickramaratne DBM. 2016. In-vitro alpha amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. BMC Complement Alternat Med 16 (1): 466. DOI: 10.1186/s12906-016-1452-y.

Wikandari PR, Yuanita L. 2016. The effect of degradation of proteolytic enzyme on angiotensin converting enzyme inhibitor activity of Bekasam with *Lactobacillus plantarum* B1765. Agritech 36 (2): 170-175. DOI: 10.22146/agritech.12861. [Indonesian]

Williams L, Alshehri A, Robichaud B, Cudmore A, Gagnon J. 2020. The role of the bacterial muramyl dipeptide in the regulation of GLP-1 and glycemia. Intl J Mol Sci 21 (15): 5252. DOI: 10.3390/ijms21155252.

Yadav M, Jain S, Tomar R, Prasad GBKS, Yadav H. 2010. Medicinal and biological potential of pumpkin: An updated review. Nutr Res Rev 23 (2): 184-190. DOI: 10.1017/S0954422410000107.

Yang D, Li C, Li L, Wang Y, Chen S, Zhao Y, Hu X, Rong H. 2022. Discovery and functional mechanism of novel dipeptidyl peptidase IV inhibitory peptides from Chinese traditional fermented fish (Chouguiyu). Curr Res Food Sci 5: 1676-1684. DOI: 10.1016/j.crfs.2022.09.025.

Yildiz E, Sumnu G, Kahyaoglu LN. 2021. Monitoring freshness of chicken breast by using natural halochromic curcumin loaded chitosan/PEO nanofibers as an intelligent package. Intl J Biol Macromol 170: 437-446. DOI: 10.1016/j.ijbiomac.2020.12.160.

Zhou J, Kang X, Luo Y, Yuan Y, Wu Y, Wang M, Liu D. 2019. Glibenclamide-induced autophagy inhibits its insulin secretion-improving function in β cells. Intl J Endocrinol 2019: 1265175. DOI: 10.1155/2019/1265175.

Zubaidah E, Arum MS, Widyaningsih TD, Rahayu AP. 2020. Sauerkraut with the addition of *Lactobacillus casei*: Effects of salt and sugar concentrations on fermentation and antioxidant activity. Curr Nutr Food Sci 16: 1265-1269. DOI: 10.2174/1573401316666200217112642.