7. Kandungan asam lemak hasil analisis menggunakan insturmen GCMS dengan menggunakan standart FAME (Fatty Acid Methyl Ester) terdeteksi 37 jenis senyawa asam lemak dengan turunan ester hasil gabungan dari semua sampel. Yang masing-masing sampel terdeteksi 20 jenis senyawa asam lemak. 8 jenis senyawa diantarax terdeteksi ada pada semua sampel baik itu sampel beras tanpa fermentasi, maupun sampel beras terfermentasi 3 jenis miselium jamur tiram *Pleurotus* spp.
8. Simulasi docking pada 5 senyawa dari profiling metabolit sekunder pada beras Pare Ambo' hitam terfermentasi menggunakan reseptor antioksidan, NADPH Oxidase (5O0x), dan reseptor antidiabetik Alpha Glucosidase (3w37). Pada reseptor antioksidan (5O0x) senyawa citric acid memiliki interaksi dengan nilai *binding affinity* yang mendekati nilai kontrol, yaitu -7.2 kcal/mol dari -8.5 kcal/mol untuk penambatan pada reseptor antioksidan, yang memiliki ikatan dengan residu asam amino Ser425, Asn572, Arg573, Glu574, dan Arg478. Reseptor alfa glucosidase (3w37), senyawa Citric acid juga memiliki nilai *binding affinity* lebih baik yaitu -8.2 kcal/mol dari -8.1 kcal/mol. Simulasi interaksinya lebih baik dibandingkan native ligand Acarbose. Ikatan hidrogen pada asam-asam amino jenis Asp357, Trp432, Asp568, His626, dan Arg552.

5.2 Saran

Saran untuk penelitian selanjutnya untuk dapat mengkaji potensi kandungan senyawa selain sebagai antioksidan dan antidiabetik. Dapat dilakukan penelitian lebih lanjut terkait analisis organoleptik terhadap beras Pare Ambo' hitam terfermentasi miselium jamur tiram jenis lainnya dan analisis komponen metabolit sekunder secara keseluruhan dibidang bioinformatika.

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LAMPIRAN

Lampiran 1. Hasil Analisis Proksimat.

ID ELSA BRIN	Nama Sampel	Berat kering %	Kadar Air %	Kadar Abu %	Protein Kasar %	Lemak kasar %	Serat kasar %
885-92662-5	TPJK	90.54	9.48	0.88	9.25	2.39	1.28
885-92662-6	JTP	90.98	9.46	0.60	9.37	2.10	1.65
885-92662-7	JTM	92.21	7.79	0.61	9.27	3.36	1.63
885-92662-8	JTC	92.80	7.20	0.88	9.67	3.36	1.67

Lampiran 2. Nilai % inhibisi uji Antioksidan Vitamin E

Konsentrasi	%INHIBISI
10	81.12
15	82.83
20	84.86
25	85.81
50	90.15

Lampiran 3. Nilai % inhibisi sampel uji Antioksidan (DPPH)

Perlakuan	% Inhibisi				
	100	200	300	400	500
Vitamin E	90.15				
TPJK	69.490	72.440	74.603	74.906	75.236
JTP	80.303	81.440	82.660	84.784	85.378
JTC	67.715	71.549	74.383	76.559	80.181
JTM	72.770	74.597	75.403	76.127	78.238
Perlakuan	STD. DEVIASI				
	100	200	300	400	500
VITAMIN E	0.110				
	0.001	0.000	0.001	0.000	0.000
	0.001	0.005	0.000	0.000	0.003
	0.016	0.001	0.000	0.001	0.000
	0.0008	0.0007	0.0004	0.0002	0.0013

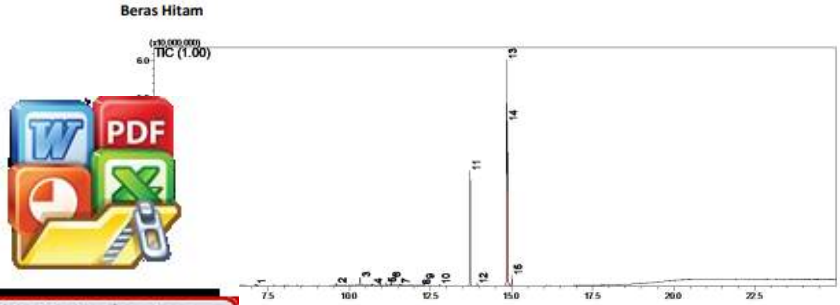
Lampiran 4. Nilai % inhibisi Uji antidiabetik Akarbosa

KONSENTRASI (PPM)	%INHIBISI
10	80.49909199
15	83.31204208
20	87.46940948
25	89.29175277
50	95.26207026

Lampiran 5. Nilai % inhibisi sampel uji Antidiabetik

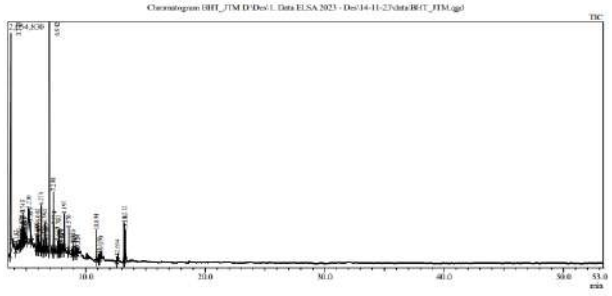
Perlakuan	% Inhibisi				
	100PPM	200PPM	300PPM	400PPM	500PPM
AKARBOSA	95.3				
TPJK	63.0	63.1	64.4	65.5	67.6
JTP	68.2	72.0	76.4	78.4	82.4
JTC	74.6	76.0	78.3	80.4	84.3
JTM	65.2	70.7	74.0	77.4	79.2
Perlakuan	Std Deviasi				
	100PPM	200PPM	300PPM	400PPM	500PPM
AKARBOSA	0.100				
TPJK	0.001	0.001	0.005	0.001	0.001
JTP	0.023	0.018	0.001	0.008	0.001
JTC	0.001	0.001	0.001	0.001	0.001
JTM	0.000	0.001	0.002	0.001	0.001

Lampiran 6. Profiling GCMS beras Pare Ambo' hitam tanpa fermentasi (TPJK)



Peaks	Ret. Time	Area	Height	Compound Name
1	7.109	1245151	382063	Butanedioic acid, hydroxy-, dimethyl ester
2	9.602	1041462	602037	3-Tetradecene, (Z)-
3	10.334	2455652	1941434	Citric acid, trimethyl ester
4	10.712	1030456	476986	Phenol, 3,5-bis(1,1-dimethylethyl)-
5	11.143	828051	651703	Methyl(methyl 3,4-di-O-methyl-.alpha.-D-mannopyranoside)uronate
6	11.285	1064692	660040	Cetene
7	11.558	465534	305313	Methyl(methyl 3,4-di-O-methyl-.alpha.-D-mannopyranoside)uronate
8	12.193	220848	158262	Carbonic acid, 2-ethoxyethyl neopentyl ester
9	12.317	959418	850749	Methyl tetradecanoate
10	12.792	434969	329510	5-Octadecene, (E)-
11	13.727	31044791	30072182	Hexadecanoic acid, methyl ester
12	13.946	692491	621678	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
13	14.854	69738026	59983250	Methyl 9-cis,11-trans-octadecadienoate
14	14.878	43116741	43367914	11-Octadecenoic acid, methyl ester
15	15.012	2885487	2686481	Methyl stearate

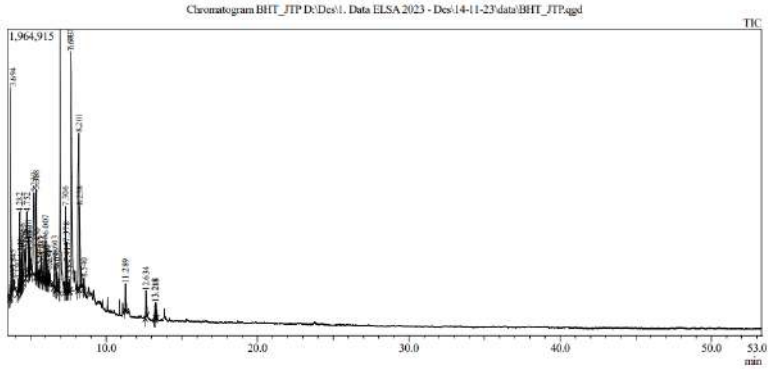
Lampiran 7. Kromatogram Profiling GCMS beras Pare Ambo' Hitam terfermentasi Jamur tiram Merah *Pleurotus djamor* (JTM)



Peak Report TIC

Peak#	R.Time	Area	Area%	Similarity	Base m/z	Name
1	3.733	6998465	25.30	96	45.05	L-Lactic acid
2	4.187	187225	0.68	88	43.05	1,2-Propanediol, 3-chloro-
3	4.459	109603	0.40	74	43.05	Pentanoic acid, 4-oxo-, methyl ester
4	4.620	589543	2.13	91	61.05	Glycerin
5	4.718	510465	1.85	73	72.10	L-Valine, methyl ester
6	4.745	637154	2.30	94	115.10	Butanedioic acid, dimethyl este
7	4.829	281673	1.02	68	98.10	1-Piperidinyl-2-trichlorophosphethane
8	4.867	176184	0.64	86	71.05	Pimethetrine
9	4.910	161372	0.58	81	91.10	Benzeneacetaldehyde
10	5.230	2301128	8.32	92	86.10	L-Leucine, methyl ester
11	5.385	507306	1.83	90	68.05	Levoglucosenone
12	5.900	548352	1.98	95	102.10	L-Aspartic acid, dimethyl ester
13	6.005	703670	2.54	66	117.10	Pentanoic acid, 3-hydroxy-, ethyl ester
14	6.098	251756	0.91	77	140.20	n-Octylsuccinic anhydride
15	6.120	203129	0.73	69	85.05	gamma.-Dodecalactone
16	6.275	439105	1.59	84	45.05	Ethanol, 2,2'-oxybis-
17	6.476	196886	0.71	79	45.05	Lactohydrazide
18	6.594	1053102	3.81	75	84.05	L-Glutamic acid, dimethyl este
19	6.680	143418	0.52	68	112.10	Quinolin-4-ol, decahydro-
20	6.713	122307	0.44	68	44.05	(S)-3-Aminobutyric acid, N-(n-propyloxycarbonyl)-, methyl ester
21	6.942	4133729	14.94	88	84.10	5-Benzyl-2-pyrrolidinone
22	7.290	1074924	3.78	96	88.10	L-Phenylalanine, methyl ester
23	7.374	473187	1.71	92	143.05	Citric acid, trimethyl ester
24	7.667	367879	1.33	89	60.05	beta.-D-Glucopyranose, 1,6-anhydro-
25	7.789	319860	1.15	93	131.05	Benzoic acid, 3-hydroxy-4-methoxy-, methyl ester
26	7.830	330634	0.92	63	86.15	Creatividine, 2-isopropyl-2-methyl-
27			0.46	55	164.10	1(2H)-Naphthalenone, 4-ethoxyoctahydro-8-methyl-
28			0.80	76	153.05	Vanillic acid
29			1.83	96	149.05	Diethyl Phthalate
30			1.29	74	170.10	Uracil, 1,3-dimethyl-6-hydrazino-
31			0.81	90	167.10	Benzenepropanoic acid, alpha.,4-dihydroxy-, methyl ester
32			0.33	72	72.10	2-(Propylamino)ethanol
33			0.78	88	70.10	3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione
34			0.61	89	107.10	L-Tyrosine, methyl ester
35			1.61	95	74.05	Hexadecanoic acid, methyl ester
36			0.59	64	84.10	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-
37			0.61	86	70.10	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-
38			0.41	65	128.15	4-Methoxy-2-methoxymethyl-pyrrolidine-1-carboxaldehyde
39			2.65	93	81.10	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
40			2.04	93	55.05	9-Octadecenoic acid, methyl ester, (E)-

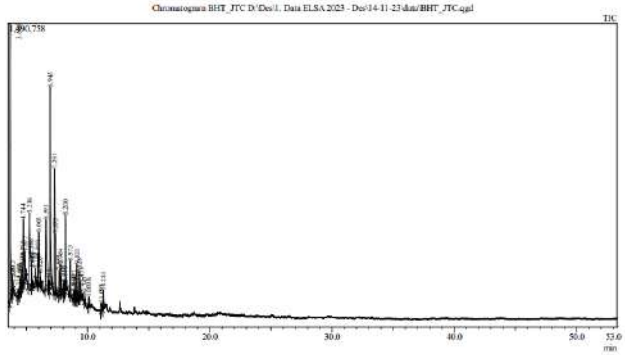
Lampiran 8. Kromatogram Profiling GCMS Beras Pare Ambo' Hitam terfermentasi Jamur tiram Putih *Pleurotus ostreatus* (JTP)



Peak Report TIC

Peak#	R.Time	Area	Area%	Similarity	Base m/z	Name
1	3.635	209628	0.44	93	43.05	Butanoic acid, 3-hydroxy-3-methyl-, methyl ester
2	3.694	3120808	6.58	98	45.05	L-Lactic acid
3	3.845	788273	1.66	82	88.05	Propanoic acid, 3-hydroxy-2-methyl-, methyl ester
4	4.191	198933	0.42	71	43.05	1-Di(tert-butyl)silyloxydodecane
5	4.282	700966	1.48	85	43.05	Acetic acid, diethyl-
6	4.340	324531	0.68	77	43.00	Acetic acid, diethyl-
7	4.419	288212	0.61	93	281.05	Cyclotetrasiloxane, octamethyl-
8	4.466	662156	1.40	93	43.05	Pentanoic acid, 4-oxo-, methyl ester
9	4.626	1166365	2.46	81	44.05	1,2,3,4-Butanetetrol, [S-(R*,R*)]-
10	4.752	2506796	5.28	78	72.10	Butanedioic acid, dimethyl ester
11	4.870	499935	1.05	89	71.05	Pantolactone
12	4.910	348184	0.73	90	91.10	Benzeneacetaldehyde
13	4.955	383183	0.81	86	43.05	Pentanoic acid, 4-oxo-
14	5.237	4306059	9.07	86	86.10	L-Leucine, methyl ester
15	5.388	1272979	2.68	94	98.05	Levoglucosenone
16	5.450	365310	0.77	91	73.05	Cyclopentasiloxane, decamethyl-
17	5.603	252227	0.53	71	43.05	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
18	5.737	417257	0.88	84	82.05	Dehydromevalonic lactone
19	5.779	295569	0.62	88	109.10	2-Furancarboxaldehyde, 5-(chloromethyl)-
20	5.925	723905	1.53	73	85.05	2-Methylpentyl isovalerate
21	6.007	1143493	2.41	69	69.05	2-Hydroxyisocaproic acid, pentafluoropropionate
22	6.080	276655	0.58	64	115.10	1,2-Cyclohexanediol, 1-(1-methylethyl)-, cis-
23	6.130	253794	0.53	85	85.05	4-Methoxycarbonyl-4-butanolide
24	6.234	230084	0.48	70	98.10	1,3-Cyclohexanediol, cis-
25	6.603	1011377	2.13	76	84.10	L-Glutamic acid, dimethyl ester
26	6.695	261016	0.55	65	112.15	1-Acetyl-3,3-pentamethylenediaziridine
27	6.760	475474	1.00	54	97.05	2-Methylpiperidine-1-thiocarboxylic acid 2-[1-[2-thiazolyl]ethylidene]hydrazide
28	6.947	5549843	11.69	97	84.05	L-Proline, 5-oxo-, methyl ester
29	7.306	2299915	4.85	93	88.05	L-Phenylalanine, methyl ester
30	7.378	500290	1.05	93	143.05	Citric acid, trimethyl ester
31	7.415	501770	1.06	55	60.00	cis-4-(Hydroxymethyl)cyclohexanecarboxylic acid
32	7.585	246596	0.52	59	43.05	Dimethyl 2-hydroxy-2-methylbutane-1,4-dioate
33	7.673	7786930	16.41	95	60.05	.beta.-D-Glucopyranose, 1,6-anhydro-
		02	8.50	72	149.05	Diethyl Phthalate
		15	5.17	93	73.05	1,6-Anhydro-.beta.-D-glucofuranose
		17	0.50	58	72.05	1-Methyl-3,6-diazahomoadamantan-9-one thiosemicarbazone
		74	0.87	84	70.05	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-
		32	0.98	66	128.10	4-Methoxy-2-methoxymethyl-pyrrolidine-1-carboxaldehyde
		63	0.52	93	67.05	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
		67	0.51	95	55.05	9-Octadecenoic acid, methyl ester, (E)-
		83	100.00			

Lampiran 9. Kromatogram Profiling GCMS beras Pare Ambo' hitam Terfermentasi Jamur Tiram Coklat *Pleurotus cystidiosus* (JTC)



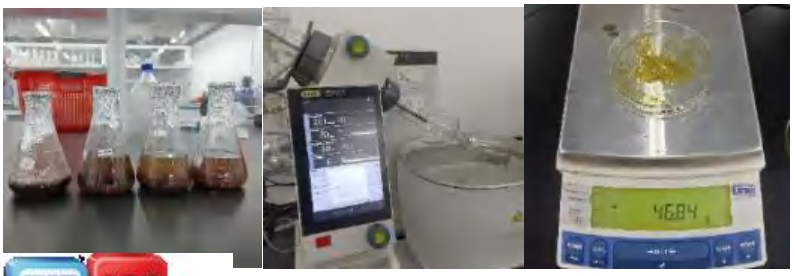
Peak Report TIC

Peak#	R.Time	Area	Area%	Similarity	Base m/z	Name
1	3.684	3819881	15.34	98	45.05	L-Lactic acid
2	3.813	213820	0.86	88	88.10	Propanoic acid, 3-hydroxy-2-methyl-, methyl ester
3	3.889	87536	0.35	81	88.10	Pentanoic acid, 3-hydroxy-2-methyl-, methyl ester
4	4.315	108684	0.44	87	57.10	Pentanoic acid, 4-methyl-2-oxo-, methyl ester
5	4.408	149671	0.60	86	281.05	Cyclotetrasiloxane, octamethyl-
6	4.460	351954	1.41	81	43.05	Pentanoic acid, 4-oxo-, methyl ester
7	4.610	512745	2.06	90	61.05	Glycerin
8	4.710	401854	1.61	75	72.10	2-Ethoxyethyl heptanoate
9	4.744	1047897	4.21	95	115.10	Butanedioic acid, dimethyl ester
10	4.877	559370	2.25	83	59.05	3,3-Dimethyldihydro-2,5-furandione
11	5.236	2727716	10.95	88	86.10	L-Leucine, methyl ester
12	5.386	484841	1.95	90	98.10	Levoglucosenone
13	5.445	209897	0.84	77	73.10	Cyclopentasiloxane, decamethyl-
14	5.707	64040	0.26	82	45.05	2-Butanone, 4-methoxy-
15	5.910	769101	3.09	93	102.10	L-Aspartic acid, dimethyl ester
16	6.003	1070844	4.30	67	69.05	Pentanoic acid, 3-hydroxy-, ethyl ester
17	6.120	154147	0.62	70	85.05	2,4:3,5-Dimethylene-l-iditol
18	6.593	2451915	9.85	92	84.05	L-Glutamic acid, dimethyl ester
19	6.845	116885	0.47	67	75.10	d-Galactose oxime
20	6.945	2486964	9.99	88	84.10	5-Benzyl-2-pyrrolidinone
21	7.291	2277341	9.14	97	88.05	L-Phenylalanine, methyl ester
22	7.375	542855	2.18	94	143.05	Citric acid, trimethyl ester
23	7.652	525367	2.11	92	60.00	.beta.-D-Glucopyranose, 1,6-anhydro-
24	7.764	241584	0.97	94	151.05	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester
25	7.812	130468	0.52	83	83.10	Nonanedioic acid, dimethyl ester
26	8.057	161479	0.65	71	168.05	Vanillic acid
27	8.140	172849	0.69	71	74.05	alpha.-Methyl-D-mannopyranoside
28	8.200	674823	2.71	96	149.05	Diethyl Phthalate
29	8.573	288873	1.16	72	170.10	Uracil, 1,3-dimethyl-6-hydrazino-
30	8.840	105477	0.42	75	137.10	Methyl 3-(4-hydroxy-3-methoxyphenyl)propanoate
31	8.923	201886	0.81	89	107.10	Benzenepropanoic acid, .alpha.,4-dihydroxy-, methyl ester
32	9.005	93497	0.38	74	72.10	(1-Ethyl-2-methylpropyl)methylamine
33	9.107	306648	1.23	88	101.10	1,3-Dioxolane, 4,5-dimethyl-2-pentadecyl-
		170374	0.68	89	70.05	3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione
		454280	1.82	93	107.10	L-Tyrosine, methyl ester
		118351	0.48	84	101.10	1,3-Dioxolane, 4,5-dimethyl-2-pentadecyl-
		94467	0.38	74	102.10	Octanedioic acid, 2-ethyl-, dimethyl ester
		93981	0.38	92	70.10	Cyclo(L-prolyl-L-valine)
		191285	0.77	84	70.10	Hexahydro-3-(1-methylpropyl)pyrrolo[1,2-a]pyrazine-1,4-dione
		268535	1.08	81	70.10	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-

Lampiran 10. Dokumentasi Foto kultivasi jamur tiram *Pleurotus* spp. media PDA & PDB

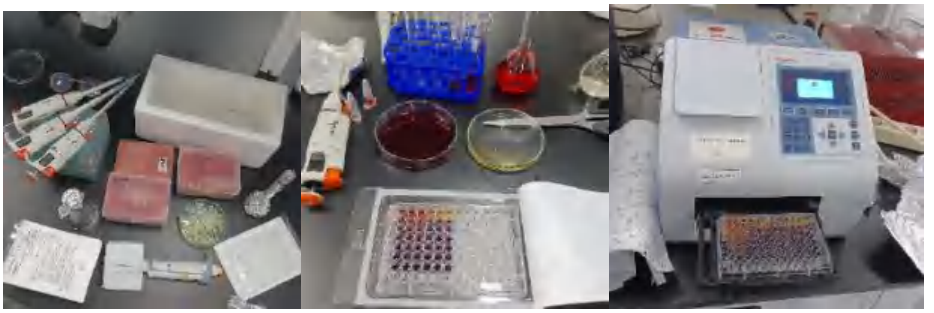
Lampiran 11. Dokumentasi Foto Sampel Beras Pare Ambo' Hitam

Lampiran 11. Dokumentasi Foto Sampel Analisis FAME GCMS



Lampiran 13. Dokumentasi Foto Sampel Analisis Profiling Metabolit HPLC

Lampiran 14. Dokumentasi Foto Uji Bioaktifitas Antioksidan dan Antidiabetik



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Bioactive metabolites, antioxidant, and antidiabetic activities of fermented local rice (*Pare ambo*) by *Pleurotus* spp.: *In-vitro* and *in-silico* approach

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ABSTRACT

Pare ambo, a local black rice variety popular in Tana Toraja (South Sulawesi), is recognized for its health benefits, especially for those managing diabetes or aiming to prevent obesity due to its antioxidant-rich composition. This study explored the impact of *Pleurotus* spp. Fermentation on *pare ambo*, examining its nutritional transformation. Proximate analysis indicated higher levels of crude protein (9.67 ± 0.73%), crude fat (3.36 ± 0.84%), and fiber (1.67 ± 0.33%) in *P. cystidiosus* fermentation compared to *P. ostreatus* and *P. djamor*. Antioxidant and antidiabetic activities significantly improved in all fermentations at 500 ppm concentration. *Pare ambo* fermented by all three *Pleurotus* species contained citric acid and trimethyl ester compounds, displaying superior antioxidant and antidiabetic activities, interacting with antioxidant receptor 5O0x (−7.2 kcal/mol) and antidiabetic receptor 3w37 (−8.2 kcal/mol) compared to the unfermented sample. Fatty acid profiling identified 32 compounds, predominantly FAMEs with ester groups. *Pleurotus cystidiosus* and *P. djamor* fermentations contributed 20 compounds, while *P. ostreatus* contributed 18 compounds. Prominent interactions were visualized between naphthalene, and decahydro-2,6-dimethyl, with 5O0x and 3w37. Anthocyanins were detected through HPLC separation in *P. cystidiosus* and *P. ostreatus* fermentation outputs. This study shows *pare ambo* as a

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[redacted]
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2024

promising functional food, abundant in natural antioxidants and antidiabetic compounds, highlighting the potential of *Pleurotus* spp. Fermentation to enhance the nutritional profile and bioactivity of indigenous rice varieties.

1. Introduction

Rice plays a pivotal role in providing daily caloric sustenance for over half of the global population. However, chronic malnutrition persists, affecting approximately 870 million people globally, with a significant portion residing in developing countries heavily reliant on rice for food security (). Adding to this challenge is the dual burden of malnutrition, contributing to the global rise of non-communicable diseases (). epidemiological investigations have revealed a correlation between reduced incidence of chronic diseases, including cancers and coronary heart disease, and the consumption of whole grain rice. This correlation is attributed to bioactive compounds present in rice (). Importantly, certain traditional landraces exhibit a higher accumulation of phytochemicals in rice grains compared to commercially cultivated varieties (). Pigmented rice, characterized by a red, black, or variable purple aleurone layer, stands out for its rich phytochemical content of high nutritional quality (). Despite the documentation of many cultured rice varieties in ancient texts and sporadic cultivation throughout Africa and Asia, their origins and dissemination remain largely shrouded in mystery (). Notably, black rice is considered a heirloom rice predating dynastic China, where it held distinguished status due to its rarity and served as a tribute (). The investigation into these diverse rice varieties holds promise for unveiling unique nutritional profiles and their potential implications for addressing prevailing global nutritional challenges.

Black rice is a nutritional powerhouse, containing a diverse array of beneficial components such as vitamin E, anthocyanin, phenolic acids, flavonoids, phytosterol, and antioxidants (Nacha et al., 2023;). These components contribute to its potential health benefits. Belonging to the family of polyphenols, anthocyanins constitute a group of naturally occurring pigments. These pigments are accountable for the vibrant red, purple, and blue hues found in various plants such as blackberries, blueberries, bilberries, grapes, strawberries, and black rice (). Recent evidence indicates that anthocyanins play a role in various biological effects, encompassing antioxidant activity, anti-hyperglycemia, anti-hyperlipidemia, anti-inflammation (;). A substantial quantity of cyanidin 3-glucoside (C3G) and peonidin 3-glucoside (P3G), the glycoside form of anthocyanins, was identified in this specific rice cultivar, as reported by (). Previous studies have detailed the diverse biological properties of black rice, encompassing antioxidant, anti-cancer, anti-hyperglycemic, anti-hyperlipidemic, and anti-inflammatory activities (;).

Pare ambo, a local black rice from Tana Toraja, has been identified as a rich source of metabolite compounds with the potential health benefits. This research focuses on understanding the dynamics in the profile of *pare ambo* and its potential bioactivity through fermentation, particularly using oyster mushrooms. Oyster mushrooms, for example *P. ostreatus*, could improve bioactive compounds and enhanced the nutritional content of rice (Nacha et al., 2023). Thus, choosing *P. ostreatus* as the fermentation agent aims to improve the nutritional quality and bioactivity of *pare ambo*. The research emphasizes mapping the metabolite profile during fermentation to provide a comprehensive overview of chemical transformations. Furthermore, the study explored potential bioactivity in-silico, offering predictive insights into antioxidant and antidiabetic activities of identified metabolite compounds. *In vitro* testing will validate and further investigate the underlying mechanisms of action.

2. Materials and methods

2.1. Fermentation of pare ambo rice with fungal mycelia

One hundred grams of finely milled *pare ambo* Toraja filler Flour, was placed in a sealed glass jar with Parchment paper. The jar was dried in an oven at 60 °C for 24 h, followed by a cooling period. White (*P. ostreatus* InaCC F150), red (*P. djamor* InaCC F135), and brown oyster mushroom (*P. cystidiosus* InaCC F100), cultivated in Potato Dextrose Broth (PDB), were inoculated into the substrate in a 6-mL volume (6:100 v/w). Batch fermentations were conducted at ambient temperature (25 °C) over a 3 day period. The fermented rice samples were then homogenized to a fine consistency ().

2.2. Proximate analysis of fermented pare ambo

The fermented *pare ambo* and the untreated rice sample, each finely ground and weighing 10 g, were placed into separate Ziplock plastic containers. Proximate composition was analyzed using the following tests: Crude Protein (CP) – IK.05 (Destructino Auto Analysis), Crude Fiber (CF) – IK.06 (Auto Fiber Analysis System), Crude Fat (CFat) – IK.07 (Auto Fat Extraction System), Moisture Content, and Ash Content in accordance with SNI 01-2891-1992 ().

2.3. 2,2-Diphenyl-1-picrylhydrazil (DPPH) antioxidant test

The fermented *pare ambo* samples and raw samples as a positive control at 1000 ppm were diluted into four concentrations using trations of 100 ppm, 50 ppm, 25 ppm, and 12.5 ppm. Vitamin E, serving as a positive control at 1000 ppm, concentrations, specifically, 20 ppm, 10 ppm, 5 ppm, and 2.5 ppm. A solution of DPPH (2,2-diphenyl-1-ed at a concentration of 100 ppm. For each diluted sample, 100 µL was withdrawn and mixed with 100 µL microplate, with each condition replicated three times. The microplate was then incubated for 30 min in

darkness. The samples were then analyzed using a spectrophotometer at a wavelength of 517 nm, and the mixture was shaken for 10 s. This process aimed to assess the antioxidant activity of the samples by measuring the reduction of DPPH, with lower absorbance indicating higher antioxidant capacity. The experiment was conducted to evaluate the potential antioxidant properties of the fermented rice samples and Vitamin E across different concentrations [illegible].

2.4. α-glucosidase inhibitory activity assay

The fermented *pare ambo* samples were prepared at a concentration of 1000 ppm using distilled water as a solvent. Acarbose served as the positive control. In a microplate, 20 μL of the sample was combined with 36 μL of phosphate buffer at pH 6.9 and 17 μL of p-Nitrophenyl-α-D-Glucoside (5 mM). The mixture was then incubated at 29 °C for 15 min. After the incubation period, 100 μL of Na_2CO_3 (200 mM) was added to the microplate. The analysis was carried out using a spectrophotometer at a wavelength of 400 nm, and the mixture was shaken for 10 s. This experimental procedure was designed to assess the alpha glucosidase inhibitory activity of the fermented rice samples at a concentration of 1000 ppm. The addition of Na_2CO_3 during the analysis facilitates the measurement of the released p-nitrophenol, and the reduction in absorbance at 400 nm is indicative of the inhibitory effect on alpha-glucosidase activity. The addition of acarbose as a positive control enables the comparison of the inhibitory potential of the fermented rice samples against a recognized standard [illegible].

2.5. Metabolite profiling of fermented pare ambo

The preparation of metabolite profiling of fermented pare ambo and controls involved the following steps: Twenty-five grams of finely ground rice sample were immersed in 75 mL of 1% HCl in methanol (v/v). The samples were subjected to agitation at 200 rpm, 26 °C for 10 h using a shaker in a dark room. The mixtures were filtered with an aspirator pump and Whatman Grade 1 filter paper. The extracts were evaporated using a vacuum set at 200-80 MBAR, a rotation speed of 60 rpm, a heating bath set at 35 °C, and a chiller set at 6 °C. The extracts were transferred to a microtube, concentrated for 30 min, and dissolved with 2% HCl in a 1:5 ratio. After filtration using a 0.45 μm syringe filter, the samples were subjected to GC-MS analysis. The analysis was conducted with the GC-MS-QP2010 Ultra (Shimadzu, Japan) program, using AOC-20i as the autosampler and Rxi-5MS (30 m × 0.25 mm i. d., 0.25 μm) as the column. Helium was used as the carrier gas, maintaining the column temperature at 60 °C, increasing at a rate of 10 °C per minute until reaching 240 °C. The injector and detector temperatures were set at 240 °C and 260 °C, respectively. Ionization was achieved through electron ionization at 70 eV, and the mass range is m/z 50–550. Compounds identified were compared with data from the National Institute of Standards and Technologies Mass Spectral Library (NIST) [illegible].

2.6. Fatty acid profile of fermented pare ambo

The fatty acids in fermented pare ambo samples and controls were analyzed qualitatively using GC-MS. Fifty grams of finely milled samples were added into a mixture of chloroform and methanol in a 2:1 ratio, with a total volume of 150 mL. The solvent mixture included 0.01% butylated hydroxytoluene (BHT). Then, 10 mL of 0.25% KCl solution was added, and the mixture was filtered using an aspirator pump and Whatman grade 1 filter paper. Fractionation was conducted to separate the chloroform and methanol layers. The chloroform layer was separated, Na_2SO_4 was added, followed by filtration using an aspirator pump. Evaporation occurred at 35 °C. Lipids without solvents were combined with 10 mL of chloroform and methanol in a 2:1 ratio, mixed with nitrogen, and 2 mL of the sample was taken. NaOH in methanol (2 mL, 1:1 ratio) was added, vortexed for 15 min, and 1 mL of the solution was then mixed with 1.5 mL of hexane and vortexed. Finally, 3 mL of milli-Q water was added and vortexed for 10 s. The upper layer of the sample was collected, filtered using a 0.45 mm syringe containing Na_2SO_4, and placed in an amber bottle for GC-MS analysis. GC-MS analysis was conducted using the GC-MS-QP2010 Ultra (Shimadzu, Japan) program with AOC-20i as the autosampler. The Rxi-5MS column (30 m × 0.25 mm i. d., 0.25 μm) was employed, and Helium served as the carrier gas. The column temperature was held at 55 °C, increasing by 10 °C/min and reaching 280 °C. The injector and detector temperatures were maintained at 250 °C and 220 °C, respectively. Ionization occurred at 70 eV, generating ions in the mass range of m/z 40–500. Compound identification was performed by comparing the obtained data with the National Institute of Standards and Technologies Mass Spectrum Library (NIST) [illegible].

2.7. Molecular docking analysis

This research employed molecular docking simulations to elucidate the binding pocket of ligands to the catalytic site of the receptor, utilizing the AutoDock Vina program [illegible]. Compounds identified through GC-MS profiling were analyzed to identify potential candidates for antioxidant and anti-diabetic targets. Several compounds with such potential were extracted from the [illegible]. The compounds were downloaded and stored in SDF file format, and these [illegible] to PDB formats using Open Babel 2.4.1. To prepare the target receptor proteins, three proteins 2QKJ and [illegible] proteins and 2QV4 as an antidiabetic test protein—were obtained from the RCSB PDB [illegible] tor and ligand files. Downloaded and converted to PDB format, underwent preparation using AutoDock r and ligand files were saved in PDBQT format, and the molecular docking process was executed through mpt (CMD) using AutoDock Vina. The results of the molecular docking were visualized using PLIP tools identify amino acid residues involved in the binding between the receptor and ligand. This comprehensive e potential molecular interactions and binding affinities, providing valuable insights into the antioxidant of the tested compounds [illegible].

2.8. Anthocyanin extraction and quantification

Extraction of anthocyanin was conducted based on the method described by [illegible]. Five grams of each sample were immersed in 15 mL of 1% HCl in methanol and sequentially extracted by shaking at 200 rpm for 16 h at room temperature in the dark. The solution was then filtered through Whatman grade 1 filter paper and evaporated using a rotary evaporator. The samples extracted with the rotary evaporator were dissolved in 2% HCl in methanol to achieve a final concentration of 1% mg/mL, and then filtered using a syringe equipped with a 0.45 μm nylon membrane filter. Extracts were analyzed using HPLC pressure gradient LC-2030C 3D (Shimadzu, Japan) following the method outlined by [illegible]. A linear gradient system was employed with mobile phase A (10% formic acid in DI water) and mobile phase B (methanol) as per HPLC standards. The gradient profile for standards and samples was set as follows: 5% solvent B for 1 min (minutes 0–1), 27% B for 14 min (minutes 1–15), holding at 27% B for 1 min, then increasing to 65% B for 1 min (minutes 16–18), and finally returning to 0% B for 5 min (minutes 18–23). Ultimately, the column was re-equilibrated for 7 min (minutes 23–30) using the initial conditions (0% B), with a flow rate of 300 μL/min at a temperature of 30 °C and an injection volume of 1 μL utilizing a C18 column. Peak reading was performed at 515 nm, and a sample injection volume of 25 μL was quantified using the reverse-phase HPLC method at 515 nm. A standard solution of cyanidin 3-glucoside was employed to generate the standard curve.

2.9. Statistical analysis

The data were subjected to analysis using Microsoft Excel 2021, employing the statistical formula for descriptive statistics to calculate 95% confidence intervals. All experiments were performed in triplicate, and the results are reported as mean values along with corresponding standard deviations.

3. Results

3.1. Proximate test in fermented pure ambo

The proximate analysis of fermented pure ambo involves a comprehensive assessment of its key nutritional components, considering dry matter, moisture, crude protein, crude fat, and fiber [illegible]. Samples were represented into following treatment codes: CO (pure ambo without fermentation/control), PO (fermented pure ambo by *Pleurotus ostreatus*), PD (fermented pure ambo by *Pleurotus djamor*) and PC (fermented pure ambo by *Pleurotus cystidiosus*).

Control group displayed the highest dry matter at 9.48 ± 1.02%, followed closely by fermentation with *P. ostreatus* (9.46 ± 0.68%), *P. djamor* (7.79 ± 0.91%), and the lowest in the *P. cystidiosus* treatment at 7.20 ± 0.66%. Notable differences in protein content were observed across treatments, with *P. cystidiosus* exhibiting the highest content at 9.67 ± 0.75%, followed by *P. ostreatus* (9.37 ± 0.55%), *P. djamor* (9.27 ± 0.91%), and the control (9.25 ± 0.98%). In terms of lipid content, the fermented samples by *P. cystidiosus* and *P. djamor* shared the same percentage (3.36 ± 0.84% and 3.36 ± 0.99%), while the control had 2.39 ± 1.16%, and *P. ostreatus* had 2.10 ± 0.54%. The highest moisture content was found in *P. cystidiosus* at 0.92 ± 0.65%, followed by the control (0.88 ± 0.98%), *P. djamor* (0.61 ± 0.87%), and *P. ostreatus* (0.60 ± 0.71%). Regarding fiber content, the *P. cystidiosus* treatment exhibited the highest fiber content at 1.67 ± 0.33%, followed by *P. ostreatus* (1.65 ± 0.29%), *P. djamor* (1.63 ± 0.46%), and the control (1.28 ± 0.93%). These findings collectively showing the diverse nutritional profiles resulting from different fermentation treatments.

3.2. Antioxidant and antidiabetic activities of fermented pure ambo

Significant variations in antioxidant activities were observed among the experimental treatments. The treatment *P. ostreatus* displayed the highest antioxidant activity with 85.37 ± 0.003% at 500 ppm, while the control sample exhibited the lowest antioxidant activity, with a percentage inhibition of 75.23 ± 0.003% at the same concentration [illegible]. In terms of antidiabetic activities, the treatment involving fermentation by *P. cystidiosus* demonstrated the highest percentage inhibition value at 84.07 ± 0.001% at 500 ppm, while the control displayed the lowest antidiabetic activity, recording a percentage inhibition value of 67.60 ± 0.003% at the same concentration [illegible]. The optimal IC_{50} value in the antioxidant assay was achieved in *P. djamor* treatment, measuring 2.43 ppm [illegible]. Conversely, the most favorable IC_{50} value in antidiabetic activity was documented in the *P. cystidiosus* treatment, totaling 3.11 ppm [illegible]. These results highlight the diverse and significant impacts of different treatments on both antioxidant and antidiabetic activities.

3.3. Compound profiling of fermented pure ambo using GC-MS

A total of 96 putatively identified compounds [illegible] were detected in three different mushroom treatments (A, B, C, D) and without any mushroom treatment. Out of the total 96 compounds, 72 were expressed at higher levels in the fermented samples with

[illegible] ambo (based on treatment in three Pleurotus species).

Dry matter (%)	Moisture (%)	Crude protein (%)	Crude fat (%)	Fiber (%)
9.48 ± 1.02	0.88 ± 0.71	9.25 ± 0.56	2.39 ± 0.54	1.28 ± 0.93
9.46 ± 0.68	0.60 ± 0.65	9.37 ± 0.55	2.10 ± 0.54	1.65 ± 0.29
7.79 ± 0.91	0.61 ± 0.87	9.27 ± 0.91	3.36 ± 0.99	1.63 ± 0.46
7.20 ± 0.66	0.92 ± 0.65	9.67 ± 0.75	3.36 ± 0.84	1.67 ± 0.33

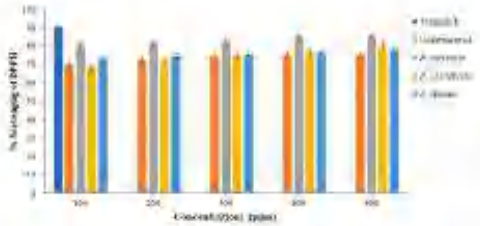

Fig. 1. DPPH free radical scavenging activity of local rice, *Pare ambo* fermented with different fungal inoculants.

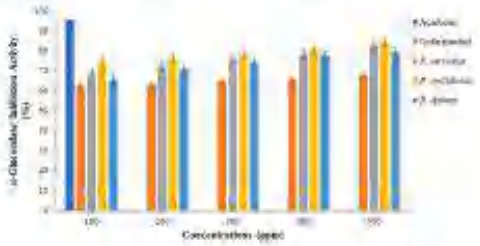

Fig. 2. α-Glucosidase inhibition assay of fermented *Pare ambo*.

Table 2
Inhibitory activity of local rice *Pare ambo* fermented with *Pleurotus* spp. On DPPH.

Treatment	IC_{50} (ppm)	Category
Vitamin E	1.11	Very strong
Unfermented	8.71	Very strong
P. ostreatus	5.93	Very strong
P. cystidiosus	3.60	Very strong
P. djamor	2.43	Very strong

Table 3
Inhibitory activity of local rice *Pare ambo* fermented with *Pleurotus* spp. On α-glucosidase.

Treatment	IC_{50} (ppm)	Category
Acarbose	1.05	Very strong
Unfermented	5.23	Very strong
P. ostreatus	3.42	Very strong
P. cystidiosus	3.11	Very strong
P. djamor	3.13	Very strong

mushrooms. The detected compounds predominantly include amino acids, peptides, esters, organic carboxylic acids, fatty esters, monosaccharides, and heterocyclic aromatic organics. Among the identified bioactive compounds, there is a possibility that they possess antioxidant and antidiabetic properties.

3.4. Molecular docking of selected metabolites in fermented pare ambo

The results of molecular docking for various compounds identified through GC-MS profiling of metabolites in fermented *pare ambo* with *Pleurotus* spp. Indicated negative binding affinities in each docking experiment. Interactions with amino acid residues were observed in specific compounds, aligning with the native ligands of antioxidants and antidiabetics, namely 5O0X (NADPH Oxidase) and 3w37 (alpha-glucosidase) (Table 5).

[illegible]ry of the 3D docking interactions with the NADPH Oxidase receptor (5O0x) serving as the receptor to as-
idant capabilities and alpha-glucosidase (3w37) as the antidiabetic receptor (native ligands).

-ligand interaction through molecular docking

inity data for binding interactions on the receptor protein 5O0x as a test for antioxidants, obtained from
ions sequentially, where the compound citric acid, trimethyl ester, exhibits a value of −7.2 kcal/mol, fol-
2,3-dihydro-3,5-dihydroxy-6-methyl- (−5.6 kcal/mol), pentanoic acid, 3-hydroxy-2-methyl-, methyl ester,

Tabel 4
Chemical compounds detected using GC-MS in fermented *Pare ambo* and control sample.

No	Compound	Area	Formula	MW (g/mol)	Detection (√ = Present)			
					CO	PC	PD	PO
1	Butanedionic Acid, hydroxy, dimethyl ester	1245151	$C_6H_8O_5$	118.09 g/mol	✓	✓	✓	✓
2	3-tetradecene, (Z)-	1041462	$C_{14}H_{28}$	196.37 g/mol	✓			
3	Citric acid, trimethyl ester	2455652	$C_9H_{14}O_7$	192.12 g/mol	✓	✓	✓	✓
4	Phenol, 3,5-bis(1,1-dimethylethyl)-	1030456	$C_{14}H_{22}O$	220.35 g/mol	✓			
5	Methyl (methyl 3,4-di-o-methyl- α -d-Mannopyranoside) uronate	828051	$C_{10}H_{18}O_7$	250.25 g/mol	✓			
6	Cetene	1064692	$C_{16}H_{32}$	224.42 g/mol	✓			
8	Carbonic acid, 2-ethoxyethyl neopentyl ester	220848	$C_{10}H_{20}O$	244.3703 g/mol	✓			
9	Methyl tetradecanoate	959418	$C_{15}H_{30}O_2$	242.40 g/mol	✓			
10	5-octadecene, (E)-	434969	$C_{18}H_{36}$	252.5 g/mol	✓			
11	Hexadecanoic acid, methyl ester	31044791	$C_{17}H_{34}O_2$	270.45 g/mol	✓		✓	
12	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4hydroxy-, methyl ester	692491	$C_{18}H_{28}O_3$	294.77 g/mol	✓	✓	✓	
13	Methyl 9-cis, 11-trans-octadecadienoate	69738026	$C_{19}H_{34}O_2$	294.5 g/mol	✓			
15	11-octadecenoic acid, methyl ester	43116741	$C_{19}H_{36}O_2$	296.5 g/mol	✓			
16	Methyl stearate	2885487	$C_{19}H_{38}O_2$	298.5 g/mol	✓			
17	l-Lactic acid	3819881	$C_3H_6O_3$	90.08 g/mol		✓	✓	✓
18	Propanoic acid, 3-hydroxy-2-methyl-, methyl ester	213820	$C_5H_{10}O_3$	118.13 g/mol		✓	✓	
19	Pentanoic acid, 3-hydroxy-2-methyl-, methyl ester	87536	$C_7H_{14}O_3$	146.18 g/mol		✓		
20	Pentanoic acid, 4-methyl-2-oxo-, methyl ester	108684	$C_7H_{12}O_3$	144.17 g/mol		✓		
21	Cyclotetrasiloxane, octamethyl-	149671	$C_8H_{24}O_4Si_4$	296.61 g/mol		✓		✓
22	Glycerin	512745	$C_3H_8O_3$	92.09 g/mol		✓	✓	
23	2-Ethoxyethyl heptanoate	401854	$C_{11}H_{22}O_3$	202.29 g/mol		✓		
24	3,3-Dimethyldihydro-2,5-furandione	559370	$C_6H_8O_3$	128.13 g/mol		✓		
25	l-Leucine, methyl ester	2727716	$C_6H_{13}NO_2$	131.17 g/mol		✓	✓	✓
26	Levoglucosenone	484841	$C_6H_6O_3$	126.11 g/mol		✓	✓	✓
27	Cyclopentasiloxane, decamethyl-	209897	$C_{10}H_{30}O_5Si_5$	370.77 g/mol		✓		✓
28	2-Butanone, 4-methoxy-	64040	$C_5H_{10}O_2$	102.13 g/mol		✓		
29	l-Aspartic acid, dimethyl ester	769101	$C_6H_{11}NO_4$	133.1 g/mol		✓	✓	
30	2,4:3,5-Dimethylene-l-idinol	154147	$C_8H_{14}O_6$	206.19 g/mol		✓		
31	l-Glutamic acid, dimethyl ester	2451915	$C_7H_{13}NO_4$	175.18 g/mol		✓	✓	✓
32	d-Galactose oxime	116885	$C_6H_{13}NO_6$	195.17 g/mol		✓		
33	5-Benzyl-2-pyrrolidinone	2486964	$C_{11}H_{13}NO$	175.23 g/mol		✓	✓	
34	l-Phenylalanine, methyl ester	2277341	$C_{10}H_{13}NO_2$	179.22 g/mol		✓	✓	✓
35	β-d-Glucopyranose, 1,6-anhydro-	525367	$C_6H_{10}O_5$	162.14 g/mol		✓	✓	✓
36	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	241584	$C_9H_{10}O_4$	182.17 g/mol		✓	✓	
37	Nonanedioic acid, dimethyl ester	130468	$C_{11}H_{20}O_4$	216.27 g/mol		✓		
38	Vanillic acid	161479	$C_8H_8O_4$	168.15 g/mol		✓	✓	
39	α.-Methyl-d-mannopyranoside	172849	$C_7H_{14}O_6$	194.18 g/mol		✓		
40	Diethyl Phthalate	674823	$C_{12}H_{14}O_4$	222.24 g/mol		✓	✓	✓
41	Uracil, 1,3-dimethyl-6-hydrazino-	288873	$C_6H_{10}N_4O_2$	170.17 g/mol		✓	✓	
42	Methyl 3-(4-hydroxy-3-methoxyphenyl)propanoate	105477	$C_{11}H_{14}O_4$	210.23 g/mol		✓		
43	(1-Ethyl-2-methylpropyl)methylamine	93497	$C_7H_{17}N$	115.22 g/mol		✓		
44	1,3-Dioxolane, 4,5-dimethyl-2-pentadecyl-	306648	$C_{20}H_{40}O_2$	312.5 g/mol		✓		
45	3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo [1,2-a]pyrazine-1,4-dione	170374	$C_8H_{12}N_2O_2$	168.19 g/mol		✓	✓	
46	l-Tyrosine, methyl ester	454280	$C_{10}H_{13}NO_3$	195.21 g/mol		✓	✓	
47	Octanedioic acid, 2-ethyl-, dimethyl ester	94467	$C_{12}H_{22}O_4$	230.3 g/mol		✓		
48	Cyclo (L-prolyl-l-valine)	93981	$C_{10}H_{16}N_2O_2$	196.2 g/mol		✓		
49	Hexahydro-3-(1-methylpropyl)pyrrolo [1,2-a]pyrazine-1,4-dione	191285	$C_{10}H_{16}N_2O_2$	196.25 g/mol		✓		
50	Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	268535	$C_{10}H_{16}N_2O_2$	196.25 g/mol		✓	✓	✓
51	1,2-Propanediol, 3-chloro-	187225	$C_3H_7ClO_2$	110.54 g/mol			✓	
52	l-Valine, methyl ester	510465	$C_6H_{13}NO_2$	131.17 g/mol			✓	
53	1-Piperidynyl-2-triethylsilylethane	281673	$C_{12}H_{25}NSi$	227.46 g/mol			✓	
54	Pantolactone	176184	$C_6H_{10}O_3$	130.139 g/mol			✓	✓
55	Benzeneacetaldehyde	161372	C_8H_8O	120.15 g/mol			✓	✓
56	n-Octylsuccinic anhydride	251756	$C_{12}H_{20}O_3$	212.28 g/mol			✓	
57	Y.-Dodecalactone	203129	$C_{12}H_{22}O_2$	198.3 g/mol			✓	
		439105	$C_6H_{12}O_3$	120.15 g/mol			✓	
		198886	$C_4H_8N_2O_2$	104.11 g/mol			✓	
		143148	$C_8H_{17}NO$	155.24 g/mol			✓	
	V-(n-propyloxycarbonyl)-, methyl ester	122307	$C_6H_9NO_2$	103.12 g/mol			✓	
	l-methyl-	255604	$C_7H_{15}NO$	129.199 g/mol			✓	
	thoxyoctahydro-8-methyl-	127770	$C_{12}H_{22}O_2$	210.31 g/mol			✓	

(continued on next page)

Table 4 (continued)

No	Compound	Area	Formula	MW (g/mol)	Detection (√) – Present			
					CO	PC	PO	PU
64	2-(Propylamino)ethanol	89416	$C_5H_{13}NO$	103.16 g/mol			✓	
65	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	164426	$C_{14}H_{26}N_2O_2$	226.32 g/mol			✓	
66	4-Methoxy-2-methoxymethyl-pyrrolidine-1-carbonaldehyde	113219	$C_8H_{15}NO_3$	173.21 g/mol			✓	✓
67	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	733121	$C_{19}H_{34}O_2$	294.5 g/mol			✓	✓
68	Butanoic acid, 3-hydroxy-2-methyl-, methyl ester	209428	$C_6H_{12}O_3$	132.16 g/mol				✓
69	1-Di (tert-butyl) silyloxydodecane	198935	$C_{26}H_{58}OSi_2$	434.8 g/mol				✓
70	Acetic acid, dimer	709088	$C_4H_8O_4$	116.11 g/mol				✓
71	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	1166368	$C_4H_{10}O_4$	122.12 g/mol				✓
72	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	282227	$C_6H_8O_4$	144.12 g/mol				✓
73	Dehydroascorbic acid dimer	417257	$C_6H_6O_5$	162.13 g/mol				✓
74	2-Furancarboxaldehyde, 5-(chloromethyl)-	295389	$C_5H_3ClO_2$	144.55 g/mol				✓
75	2-Methylpentyl nonanoate	722905	$C_{15}H_{30}O_2$	186.29 g/mol				✓
76	2-Hydroxyoctadecanoic acid, pentafluoropropionate	1143493	$C_{23}H_{37}F_5O_4$	488.53 g/mol				✓
77	1,2-Cyclohexanediol, 1-(1-methylethyl)-, (S)-	276855	$C_9H_{18}O_2$	158.24 g/mol				✓
78	4-Methoxycarbonyl-4-butanolide	253794	$C_6H_8O_4$	144.12 g/mol				✓
79	1,3-Cyclohexanediol, cis-	259084	$C_6H_{12}O_2$	116.16 g/mol				✓
80	1-Acetyl-3,3-pentamethylenetriazirine	261016	$C_8H_{14}N_2O$	152.21 g/mol				✓
81	2-Methylpentadec-1-ylsuccinic acid 2-[1-(2-hydroxy)-ethyl]ethyl ester	475474	$C_{21}H_{40}O_5$	282.4 g/mol				✓
82	L-Proline, 5-oxo-, methyl ester	5549843	$C_6H_9NO_3$	143.14 g/mol				✓
83	cis-4-(Hydroxymethyl)cyclohexaneacetic acid	591770	$C_9H_{16}O_3$	172.22 g/mol				✓
84	Dimethyl 3-hydroxy-2-methylpentane-1,4-dioate	246906	$C_7H_{12}O_5$	176.17 g/mol				✓
85	1,6-Anhydro-beta-d-glucofuranose	3452815	$C_6H_{10}O_5$	162.14 g/mol				✓
86	1-Methyl-3,5-diazabicyclo[3.3.1]nonane-2,4-dithione-7-carboxamide	237417	$C_{10}H_{15}N_3S_2$	241.37 g/mol				✓

Note: CO = Control, PO = *Pleurotus sarcoides*, PC = *Pleurotus cystidiosus*, PU = *Pleurotus tuber*.

Table 5
Binding affinity value of ligands or detected metabolites from fermented *Pyrr-ambo* and interacting amino acids among target proteins.

Compounds	Receptor	Amino acid residue	Binding affinity (kcal/mol)	Note
Citric acid, trimethyl ester	5O0x	Ser425, Asn572, Arg573, Glu574, Arg478	−7.2	All fermented treatments
Citric acid, trimethyl ester	3w37	Asp357, Trp432, Asp568, His626, Arg552	−8.2	All fermented treatments
Pentanoic acid, 3-hydroxy-2-methyl-, methyl ester	3w37	Trp329, Ile358, Trp432, Phe601, Arg552, His626	−5.7	PC
Pentanoic acid, 3-hydroxy-2-methyl-, methyl ester	5O0x	Phe461, Trp695, Thr462, Thr543	−4.9	PC
L-Valine, methyl ester	3w37	Trp329, Phe601, Asp357, Arg552, Asp568, His626	−5.4	PO
L-Valine, methyl ester	5O0x	Phe461, Ile538, Thr462, His476, Thr543	−4.9	PO
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	5O0x	Thr462, Thr543, Phe461	−5.6	PO
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	3w37	Trp329, Trp432, Arg552, Asp568, His626	−6.3	PO
Carbonic acid, 2-ethoxyethyl tetradecyl ester	3w37	Thr227, Asn241, Asp245,	−4.0	CO
Carbonic acid, 2-ethoxyethyl tetradecyl ester	5O0x	Phe697, Glu691	−3.4	CO

Note: CO = Control, PO = *Pleurotus sarcoides*, PC = *Pleurotus cystidiosus*, PU = *Pleurotus tuber*.

and L-valine, methyl ester (−4.9 kcal/mol). Meanwhile, the interaction with the receptor protein 3w37 as a test for antidiabetics, the best simulation interaction is also with the compound citric acid, trimethyl ester (−8.2 kcal/mol). This compound is present in all fermentations of the three *Pleurotus* mushroom species. It is followed by the compounds 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (−6.3 kcal/mol), pentanoic acid, 3-hydroxy-2-methyl-, methyl ester (−5.7 kcal/mol), and L-Valine, methyl ester (−5.4 kcal/mol). The docking simulations on both NADPH oxidase receptor (5O0x) and alpha-glucosidase receptor (3w37) show the best results with the compound citric acid, trimethyl ester, forming hydrogen bonds with different amino acids on each receptor. When binding to the 5O0x receptor, it forms hydrogen bonds with amino acids Ser425, Asn572, Arg573, Glu574, Arg478, and Asp357. While binding to the 2w37 receptor, it forms hydrogen bonds with Trp432, Asp568, His626, and Arg552. This compound, previously identified in *pyrr-ambo* extract (Leonti et al., 2021), has not been reported in rice fermented by *Pleurotus* before.

in fermented pure-ambo

al agent was aimed to improve the nutritional component of raw substrate of pre-conditioned food into , the extraction of total fatty acid content from the fermented samples, converted to FAMEs, were identi-

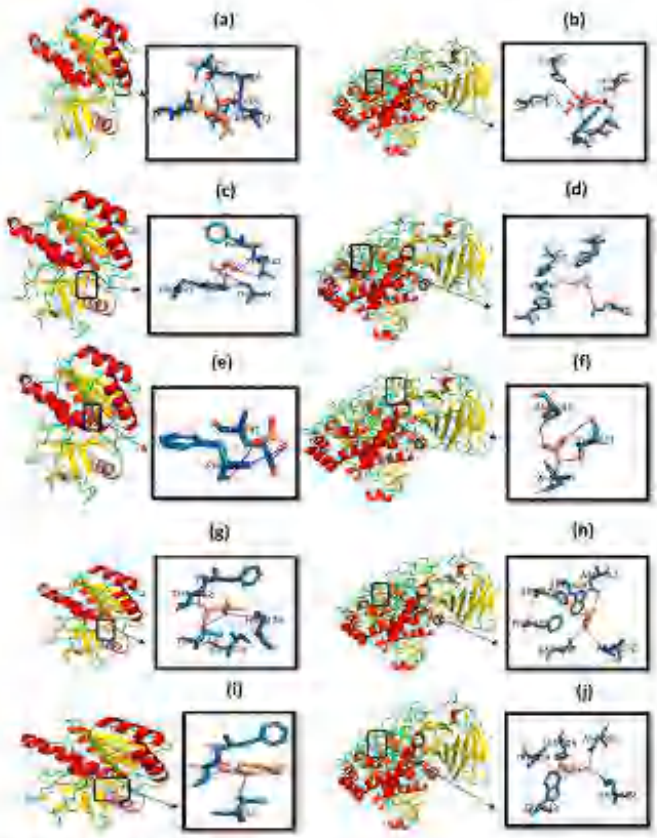

Fig. 3. 3D docking interactions of 500x and 3w37 (native ligands) selected metabolites from fermented and unfermented rice bran oils as antioxidants and antidiabetics. (a) Citric acid + 500x; (b) citric acid +3w37, (c) Pentanoic acid +500x, (d) Pentanoic acid 3w37; (e) Carbonic acid + 500x, (f) Carbonic acid +3w37, (g) L-valine +500x, (h) L-valine +3w37, (i) 4H-pyran +500x, (j) 4H-pyran +3w37.

fied and profiled through GC MS analysis. A total of 37 fatty acid compounds were successfully observed [illegible], with 32 compounds belonging to the ester group, and two ketones, one each for ether and methyl ester groups. The control, PC and PO treatments exhibited 20 FAMEs, while the PO treatment had 18 FAMEs.

3.7. Molecular docking of selected metabolites from pure amla as bioactive compounds

Table 7 presents the binding affinity values for interaction with the receptor protein 500x as a test for antioxidants, obtained from the best sequential simulation interactions. The compounds include naphthalene, decahydro-2,6-dimethyl- (−7.2 kcal/mol), furan, 2,5-dihydro-2,2-dimethyl-5-(1-methylethenyl)-3-(1-methylethyl)- (E) (−6.2 kcal/mol), cyclohexanol, 2-methyl-5-(1-methylethyl)- (−6.0 kcal/mol), decanoic acid, 10-(2-hexylcyclopropyl) (−5.7 kcal/mol), and cis-Vaccenic acid (−5.5 kcal/mol). The interaction with the receptor protein 3w37 as a test for antidiabetics in the best simulation interaction also involves naphthalene, decahydro-2,6-dimethyl-, with a binding affinity of −6.3 kcal/mol, followed by cyclohexanol, 2-methyl-5-(1-methylethyl)- (−6.1 kcal/mol), cis-Vaccenic acid (−5.8 kcal/mol), and decanoic acid, 10-(2-hexylcyclopropyl) (−5.3 kcal/mol). The best results in docking simulations involving NADPH oxidase receptor (500x) and alpha-glucosidase receptor (3w37) are demonstrated by the decahydro-2,6-dimethyl- (Fig. 7). This compound can form hydrogen bonds with different amino acids on binding to the 500x receptor, it forms hydrogen bonds with amino acids Phe461, Val460, Thr484, Ile538. While binding to the 3w37 receptor, it forms hydrogen bonds with Trp432, Phe476, Phe601, and Trp329. Overview of the 3D docking interactions with the NADPH Oxidase receptor (500x) serving as the receptor to antioxidant capabilities and alpha-glucosidase (3w37) as the antidiabetic receptor (native ligands).

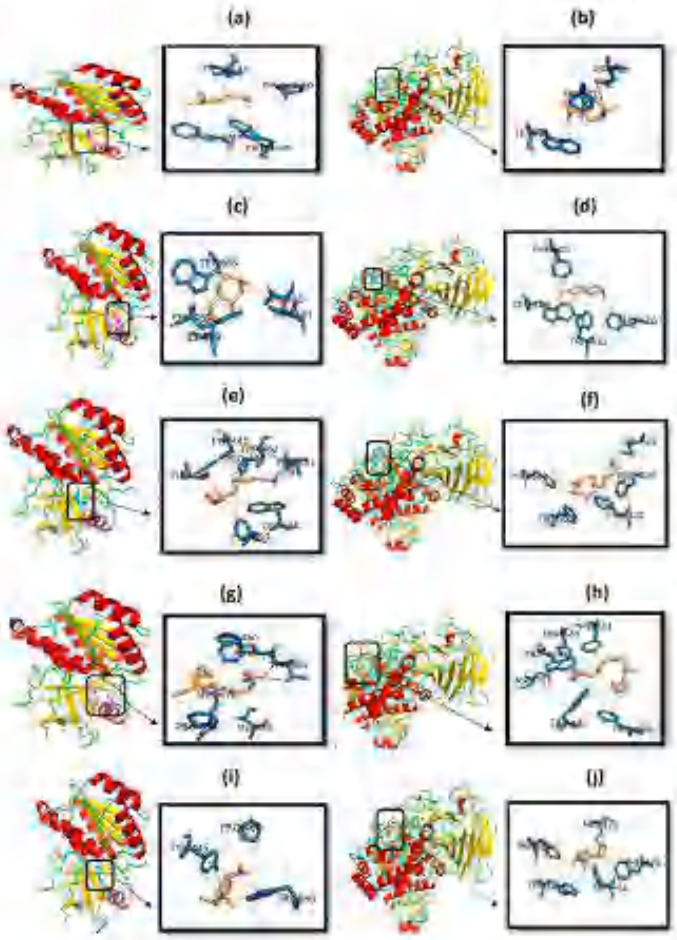

Fig. 4. 3D docking interactions of 5OOx and 3w37 (native ligands) of fatty acid compounds from fermented and unfermented rice Pari seeds as antioxidant and antidiabetic. (a) Cyclohexanol +5OOx, (b) Cyclohexanol +3w37, (c) Naphthalene +5OOx, (d) Naphthalene +3w37, (e) Cis vaccenic acid +5OOx, (f) Cis vaccenic acid+3w37, (g) Decanoic acid +5OOx, (h) Decanoic acid+3w37, (i) Furan +5OOx, (j) Furan+3w37.

3.8. Analysis of anthocyanin compounds (cyanidin-3-glucoside)

The detection of cyanidin-3-glucoside compounds in the four Toraja black rice samples was conducted, and the chromatogram results of the HPLC analysis are depicted in Fig. 5 with variations in retention time detection. Table 6 shown the concentration values in each treatment of *Pleurotus*. The unfermented treatment, detected cyanidin-3-glucoside compound at a retention time of 29.394 min, with an area detection of 15.852 at the peak spectrum absorption height of 821. The detected concentration of the compound was 0.528 (Fig. 5A), for the sample fermented by *P. djamor*, detected cyanidin-3-glucoside at a retention time of 29.301, with an area of 20,643 mAU, indicating a compound concentration of 0.688 (Fig. 5B). The sample fermented by *P. ostreatus*, detected the compound with the highest area among the three samples, measuring 186.435, and a peak absorption height of tected concentration of the compound at 6.209 (Fig. 5C). The *P. cystidiosus* sample, was detected at a with an area of 185,167 and a peak absorption height of 8102, indicating a detected concentration of 0).

Table 6
Fatty acid profile in the fruit of Fruit Acid Methyl Ester (FAMEs) from fermented rice grain.

No	Compounds	Area	Formula	MW	Chemical	Biological activity	Detection (√ = Present)			
							CO	PC	PO	PO
1	Furan, 2,5-dihydro-2,2-dimethyl-5-(1-methylethenyl)-3-(3-methylbutyl)-	1424804	$C_{12}H_{20}O$	180.29 g/mol	Ether	–	✓	✓	✓	✓
2	Methyl tetradecanoate	7171441	$C_{15}H_{30}O_2$	242.4 g/mol	Ester		✓	✓	✓	✓
3	9-Hexadecenoic acid, methyl ester, (Z)-	1523383	$C_{17}H_{32}O_2$	268.4 g/mol	Methyl ester	Antidiabetic	✓	✓	–	–
4	Tetradecanoic acid, 12-methyl-, methyl ester	23932216	$C_{16}H_{32}O_2$	256.42 g/mol	Ester	–	✓	–	–	✓
5	Pentadecanoic acid	2716820	$C_{15}H_{30}O_2$	242.4 g/mol	Ester	–	✓	–	–	✓
6	E,E-4,13-Nonadecatriene	4672404	$C_{19}H_{34}$	262.5 g/mol	Ester	–	✓	–	–	–
7	9-Octadecenoic acid, methyl ester, (E)-	5269765	$C_{19}H_{36}O_2$	296.5 g/mol	Ester	–	✓	✓	✓	✓
8	Phytol	5995625	$C_{20}H_{40}O$	296.5 g/mol	Ester	Antioxidant	✓	✓	✓	–
9	Methyl stearate	5081263	$C_{19}H_{38}O_2$	298.5 g/mol	Ester		✓	✓	✓	✓
10	2-Chloroethyl linolenate	3820980	$C_{20}H_{33}ClO_2$	342.9 g/mol	Ester		✓	–	–	–
11	Decanoic acid, 10-(2-hexylcyclopropyl)	5903298	$C_{19}H_{36}O_2$	296.5 g/mol	Ester	–	✓	–	–	–
12	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl	5155198	$C_{24}H_{44}O_2$	364.5 g/mol	Ester	–	✓	✓	✓	–
13	2,2-2,13-Octadecadien-1-ol	5420173	$C_{18}H_{34}O$	266.5 g/mol	Ester	–	✓	✓	✓	✓
14	9-Octadecenethioic acid, 12-hydroxy-, S-butyl ester	7074978	$C_{22}H_{42}O_2S$	370.6 g/mol	Ester		✓	✓	✓	–
15	Octanoic acid, 3-oxo-, methyl ester, cis-	5056837	$C_{19}H_{36}O_3$	312.5 g/mol	Ester	Antioxidant	✓	✓	✓	✓
16	cis-11-Eicosenoic acid, methyl ester	14215864	$C_{21}H_{42}O_2$	324.5 g/mol	Ester	Antioxidant	✓	–	–	–
17	2-Furancarboxylic acid, 5-hexyltetrahydro-, methyl ester	5267547	$C_{12}H_{20}O_3$	212.3 g/mol	Ester	Antioxidant & Antidiabetic	✓	–	✓	✓
18	Methyl 18-methylnonadecanoate	12811096	$C_{21}H_{42}O_2$	326.6 g/mol	Ester	Antioxidant	✓	✓	✓	✓
19	PGH1, methyl ester	4854494	$C_{21}H_{34}O_5$	366.5 g/mol	Ester	–	✓	–	–	–
20	7-Tetradecenal, (E)-	4003143	$C_{14}H_{26}O$	210.36 g/mol	Ester	Antioxidant	✓	✓	✓	✓
21	Butylated Hydroxytoluene	5167815	$C_{15}H_{24}O$	220.35 g/mol	Ester	Antioxidant	–	✓	✓	–
22	Octadecanoic acid, 2-hydroxy-2-octadecyl-, methyl ester, (2R*,3R*)-	37750-57-6	$C_{37}H_{74}O_3$	319.9 g/mol	Ester		–	✓	✓	–
23	cis-Vaccenic acid	6061282	$C_{18}H_{34}O_2$	282.5 g/mol	Ester	Antidiabetic	–	✓	–	–
24	Bicosanoic acid, octadecyl ester	39289206	$C_{38}H_{76}O_2$	341.4 g/mol	Ester	–	–	✓	–	–
25	9-Hydroxy-dodecanoic acid, methyl ester	2754063	$C_{13}H_{26}O_3$	230.34 g/mol	Ester	Antioxidant	–	✓	–	–
26	14-Octadecadien-1-ol, acetate, (Z,Z)-	16183264	$C_{20}H_{36}O_2$	324.5 g/mol	Ester	Antioxidant	–	✓	✓	✓
27	2-Butyl-3,5-methyl-5-(2-methylprop-2-enyl) cyclohexanone	22784475	$C_{15}H_{26}O$	222.37 g/mol	Ketone	–	–	✓	–	–
28	Eicosanoic acid, methyl ester	7124505	$C_{21}H_{42}O_2$	354.6 g/mol	Ester		–	✓	✓	–
29	Cyclohexanone, 2-(1-methyl-2-nitroethyl)-	79635826	$C_9H_{15}NO_3$	185.22 g/mol	Ketone		–	–	✓	✓
30	Naphthalene, decahydro-2,6-dimethyl-	2020095	$C_{12}H_{24}$	168.3 g/mol	Ester		–	–	✓	
31	Undecanoic acid, 9,8-dimethyl-10-oxo-, methyl ester	3026661	$C_{14}H_{26}O_3$	242.35 g/mol	Ester	–	–	–	✓	✓
32	Heneicosane-1,2-diol	28740328	$C_{21}H_{44}O_2$	258.44 g/mol	Ester	–	–	–	✓	
33	Eicosadienoic acid, methyl ester	18862393	$C_{21}H_{38}O_2$	298.5 g/mol	Ester	Antioxidant & Antidiabetic	–	–	–	✓
34	Octadecanoic acid	4434186	$C_{18}H_{36}O_2$	284.5 g/mol	Ester	–	–	–	–	✓
35	Tricyclo-[20.8.0.0.(7,16)]triacontane, (1(22),7(16)-diepoxy-	4859062	$C_{30}H_{54}O_2$	444.7 g/mol	Ester	–	–	–	–	✓
36	Cyclohexanol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.beta.)-	4748093	$C_{10}H_{20}O$	156.26 g/mol	Ester		–	–	–	✓

Note: CO = Control, PO = *Pleurotus ostreatus*, PC = *Pleurotus cystidiosus*, PO = *Pleurotus djamor*.

4. Discussion

Comprehensive and targeted metabolite profiling of local riceberry or pure *ambo* cultivar (*Oryza sativa* L.) fermented by three *Pleurotus* species has been accommodated in our study. Fermentation treatments involving the edible mushroom, *Pleurotus* have been shown to increase crude fat and protein content, particularly when fermenting rice into *pure ambo* using *Pleurotus cystidiosus* (protein 9.67 ± 0.73%, fat 3.36 ± 0.84%). [illegible] reported similar findings, indicating that the bioconversion using *Pleurotus* mushrooms could elevate crude protein levels. This increase may be attributed to the loss of carbon as CO_2 during fermentation, leading to a relatively higher nitrogen content ([illegible]). Differences in protein content may also be associated with an increase in fungal biomass. However, in contrast to typical patterns, this study observed an inverse relationship between fungal dry matter content. Fermentation of rice by *P. cystidiosus* exhibited higher protein content but the (7.20 ± 0.66%). [illegible] also reported similar results, where rice koji fermented by *As*- gh protein content (9.5 g/100 g) but low dry matter content (45.1 g/100 g). We assume that fluctuating ation may lead to mycelium growth instability in *Pleurotus* species, even when fungal species, temperature, strained.

Table 7
Receptor-ligand interaction of fatty acid compounds from fermented and unfermented rice *Pleurotus* co-ocations with 3W37 and 5006 receptors.

Compounds	Receptor	Binding affinity (kcal/mol)	Amino acid residue	[illegible]
Furan, 2,5-dihydro-3,4-dimethyl-5-(1-[illegible])-3-(1-methylethyl)-	3W37	−5.9	[illegible]	All fermented samples
Furan, 2,5-dihydro-3,4-dimethyl-5-(1-[illegible])-3-(1-methylethyl)- (E)-	[illegible]	−6.2	[illegible]	All fermented samples
[illegible]	3W37	−5.1	[illegible]	[illegible]
[illegible]	5006	−6.0	[illegible]	[illegible]
Naphthalene, decahydro-2,6-dimethyl-	3W37	−6.3	[illegible]	[illegible]
Naphthalene, decahydro-2,6-dimethyl-	5006	−7.2	[illegible]	[illegible]
cis-Vaccenic acid	3W37	−5.8	[illegible]	[illegible]
cis-Vaccenic acid	[illegible]	−5.5	[illegible]	[illegible]
[illegible]	3W37	−5.3	[illegible]	Unfermented (control)
[illegible]	5006	−5.1	[illegible]	Unfermented (control)

Crude fat was higher in the *Pleurotus cystidiosus* fermentation treatment than in the *P. ostreatus* fermentation (2.10 ± 0.54%) which was contrary to a report by [illegible] on *P. ostreatus* extract, which showed a higher percentage of the element (3.05%). This difference could be influenced by the substrate composition for mushroom growth. Even within the same species, variations in the growth substrate can impact the metabolic cycle of edible mushrooms in producing macromolecular products such as fat. Edible mushrooms, such as *P. ostreatus*, thriving in natural habitats, may accumulate a comprehensive combination of internal and external growth factors in contrast to those cultivated on specific substrates like pare ambo. The concentration of crude fat, regardless of being low or high, does not exert a significant impact on the nutritional quality, providing a distinct advantage. The fat content in edible mushrooms primarily consists of unsaturated fatty acids, which pose fewer health risks compared to the saturated fatty acids found in animal fats ([illegible]).

A functional food with improved antioxidant and antidiabetic qualities relies heavily on the presence of metabolite pool with certain bioactivities. Within this symbiotic relationship, fungal mycelium particularly from oyster mushrooms may serve as catalysts for breaking down complex compounds in *pare ambo*, leading to the liberation of any bioactive metabolites. Our treatments exhibited significant antioxidant activities, aligning with reports that specific rice cultivars possess antioxidant properties ([illegible]). [illegible] also reported that *P. ostreatus* can enhance antioxidant capacity by 70% on day 2 of fermentation compared to the control of lime-cooked maize wastewater or *Nejayote*. Antioxidant activity may be influenced by various factors, including enzymatic degradation and oxidation of phenolic compounds ([illegible]). Among the array of enzymes, fungi produce laccases, oxidase enzymes that act on phenols and similar substrates. *Pleurotus* spp. have been documented to produce laccases ([illegible]). The study by [illegible] and [illegible] highlights the substantial increase in phenolic content and antioxidant activity of ginger and chia seeds through solid state fermentation (SSF) by *Trichoderma* spp. In ginger, SSF by *Trichoderma viride* resulted in enhanced phenolic content and potent antioxidant activity, along with significant antibacterial effects against human pathogenic bacteria. Similarly, SSF of chia seeds by *Trichoderma reesei* led to a remarkable increase in phenolic content and antioxidant activity, with new phenolic compounds identified. The activities of fungal phenolic-associated enzymes strongly correlated with the phenolic content of the fermented products, suggesting the potential of SSF by *Trichoderma* spp. to enrich botanicals with enhanced health-promoting properties. Extracts from fermented *pare ambo* with enhanced ability to prevent oxidation could be a promising ingredient for designing various functional foods and for specific use as nutraceuticals.

In addition, the fermentation extracts also displayed potential antidiabetic activities. Although there are no reports suggesting that *pare ambo* potentially possesses antidiabetic activity, the contribution of *Pleurotus* mycelium growth is evident. The multidirectional health promoting and therapeutic effects of mushrooms of the *Pleurotus* genus result from the presence of secondary metabolites, which have been isolated from both oyster mushroom fruiting bodies and mycelia ([illegible]). Bioactive substances exhibit immunostimulatory, anti-neoplastic, anti-diabetic, anti-atherosclerotic, anti-inflammatory, hepatoprotective, and antioxidative properties ([illegible]).

The aromatic compound profile in fermented *pare ambo* was elucidated through GC-MS analysis, a robust analytical methodology for identifying and quantifying volatile and semi-volatile compounds. The fermentation process generated a spectrum of aromatic compounds, including esters, aldehydes, ketones, and phenolic compounds, each significantly influencing the sensory attributes of the fermented product. Esters contributed to fruity and floral notes, while aldehydes and ketones imparted nutty and buttery aromas. Phenolic compounds, derived from the fermentation of phenolic-rich substrates, introduced nuanced spicy or smoky characteristics. The precise profiling of these aromatic compounds via GC-MS facilitated a comprehensive understanding of the complex flavor profile [illegible] shedding light on the intricate microbial and biochemical processes underlying their formation during the [illegible] analytical approach contributes to a broader comprehension of the organoleptic properties and chemical [illegible] ed products. The identified metabolites in fermented *pare ambo*, exhibiting antioxidant and antidiabetic [illegible] lude: butanedioic acid, hydroxy, dimethyl ester; citric acid, trimethyl ester; phenol, 3,5-bis(1,1-[illegible] id, 2-ethoxyethyl neopentyl ester; hexadecanoic acid, methyl ester; benzenepropanoic acid, 3,5-bis(1,1-

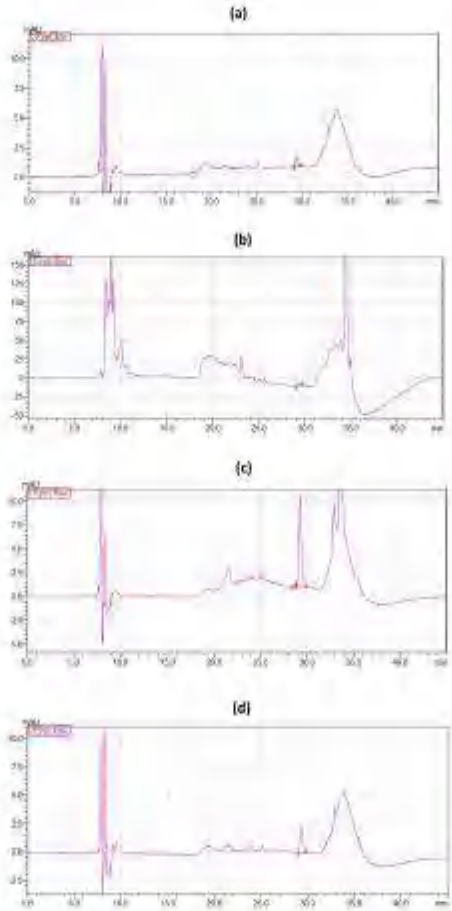

Fig. 5. Chromatogram showing anthocyanin compound through HPLC analysis (a) Unfermented (control), (b) Fermented sample by *P. djamor* (PD), (c) Fermented sample by *P. ostreatus* (PO), (d) Fermented sample by *P. cystidiosus* (PC).

Tabel 8
Retention time of detected Cyanidin-3-glycoside of fermented and unfermented *Pare ombo*.

Treatment(s)	Retention time (min)	Area	Peak absorbance (mAU)	Concentrations (µg/ml)
Unfermented (control)	29.394	15852	821	0.528
PO	29.243	186,435	9453	6.209
PD	29.301	20,643	1145	0.688
PC	28.482	185,167	8102	6.167

Note: PO = *Pleurotus ostreatus*, PC = *Pleurotus cystidiosus*, PD = *Pleurotus djamor*.

dimethylethyl)-4hydroxy-, methyl ester; vanillic acid; pantolactone; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-. Our findings are also supported by Sánchez-García et al., (2023), who described that vanillic acid in fermented dried lentils and quinoa by ntioxidant properties. On the other hand, lentil extract fermented with *Aspergillus oryzae* LBA01 and *As*so contains vanillic acid, showed antioxidant and antidiabetic activities (Magro et al., 2018). The com imethyl ester as an antioxidant was reported in spontaneously fermented cocoa beans (Septiani et al., ne was identified in fermented soybeans by *Trichoderma reesei* F.417 (Ramadan et al., 2014). The com ihydro-3,5-dihydroxy-6-methyl was successfully identified in fermented tea flower petals by *Saccha* et al., 2021), and another fermentation product containing this compound is kombucha (Khan et al.,

dant properties. The compound naphthalene, decahydro-2,6-dimethyl-, has been previously isolated from the crude extract of *Trichoderma asperellum* ([illegible]), root extract of *Althaea officinalis* ([illegible]), and crude extract of *Bacillus licheniformis* ([illegible]).

Intensive research has focused on identifying flavonoids in mushrooms, which constitute a substantial group of phenolic compounds. Flavonoids, encompassing flavonols, flavones, flavanones, isoflavones, flavanols, and anthocyanins, share a structural origin from the parent substance flavone, consisting of two benzene rings (A and B) combined with a pyran one (C) ([illegible]). The composition of anthocyanins becomes crucial due to its association with antioxidant activity post-treatment with *Pleurotus* mushroom fermentation. [illegible] demonstrated that fungi could produce anthocyanins, a metabolite often thought to exist only in natural plants. Fermented rice with *P. djamor* exhibited a higher concentration of anthocyanins (6.20) compared to treatments using two other *Pleurotus* mushroom species. This antioxidant property has been proven to be influenced by the diversity of compounds present ([illegible] 2014), including the quantity of anthocyanins in rice fermentation products by *Pleurotus* mushrooms. Although the initial GC-MS analysis of the control (unfermented) revealed profiles of antioxidant metabolite compounds and FAMEs, the anthocyanin concentration was only 0.528. The analysis of anthocyanin concentrations correlated with the highest antioxidant activity in the PD treatment, with an inhibition percentage value of 86.37% at a concentration of 500 ppm. In contrast, the lowest inhibition percentage (75.25%) at the same concentration was recorded in the control. Many previous studies have indicated that anthocyanins exhibit potent antioxidant activity ([illegible]). Anthocyanins are glycoside derivatives, and their reduction may be related to the role of glycosidase, which further reduces antioxidant activity. Glycoside precursors were identified, and their reduction is inseparable from the role of glycosidases. *Pleurotus ostreatus* can produce glycosidase (enzyme) to decompose glycosides, releasing aromatic substances and enhancing the nutritional quality of *pare ambo*. Compared with the control group, the DPPH free radical-scavenging power and ferric reducing antioxidant power showed less loss of antioxidant activity.

5. Conclusion

In general, it can be concluded from this study that the supplementation of mycelium from the three *Pleurotus* species can naturally enhance nutritional values towards improvement compared to the non-supplemented counterpart. The fungi improved the quality of raw material, *pare ambo*, as indicated by a superior profile of antioxidant and antidiabetic activities compared to non-fermented sample. This is further supported by the validation of molecular docking, metabolite profiles, and fatty acid methyl esters (FAMEs), which have the potential to serve as robust compounds with antioxidant and antidiabetic properties. There is a tendency for anthocyanin concentrations to increase when treated with the three *Pleurotus* mushrooms compared to untreated samples. The resulting fermented sample of local rice, *pare ambo* could be utilized in rice-based products as multifunctional modified functional foods.

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CRediT authorship contribution statement

Muthia Lestari: Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Apon Zaenal Mustopa:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization. **Andi Masniawati:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fatimah Fatimah:** Validation, Methodology, Investigation, Data curation. **Herman Irawan:** Validation, Methodology, Investigation. **Des Saputro Wibowo:** Visualization, Validation, Methodology, Formal analysis, Data curation. **Jendri Mamangkey:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Data curation, Conceptualization. **Baso Manguntungi:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nur Rohmah:** Validation, Investigation, Data curation. **Adrian Hartanto:** Writing – review & editing, Visualization, Validation, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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