



# Nutritional profiling and *in silico* analysis of pharmacological activities from local rice *Pulu Mandoti* fermented with *Pleurotus* spp.

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## Abstract

*Pulu Mandoti*, a local red rice (*Oryza sativa* L.) variety popular among Sulawesi residents, has gained recognition for its perceived health benefits, especially as a preferred dietary option for individuals with diabetes or those seeking to prevent obesity. Given the increasing consumption of mushrooms, particularly *Pleurotus* species, renowned for their nutritional and medicinal attributes, this study delves into the transformative effects of *Pleurotus* spp. fermentation on *Pulu Mandoti*, the indigenous rice variety. Proximate analysis disclosed elevated dry matter ( $91.99 \pm 0.61\%$ ), crude protein ( $8.55 \pm 0.15\%$ ), and crude fat ( $1.34 \pm 0.05\%$ ) in *Pleurotus cystidiosus* fermentation compared to *Pleurotus ostreatus* and *Pleurotus djamor*. Concurrently, antioxidant and antidiabetic activities were notably improved in all *Pleurotus* fermentations. *Pulu Mandoti* fermented with *P. cystidiosus* outperformed other treatments, aligning with molecular docking results pinpointing 11-Eicosenoic acid, methyl ester, and butylated hydroxytoluene as optimal interactors with antioxidant receptors 500x and 2CKJ. Butylated hydroxytoluene demonstrated interactions with the antidiabetic receptor 2QV4, along with 9-Octadecenoic acid, methyl ester. These compounds, previously unreported in *Pleurotus*, displayed promising attributes as antioxidants and antidiabetic agents. Furthermore, the investigation delved into the fatty acid profiles, emphasizing the diverse range of potential bioactive compounds in fermented *Pulu Mandoti*. The findings of this research present a potential functional food rich in natural antioxidants and antidiabetic compounds, highlighting the yet undiscovered capabilities of *Pleurotus* spp. fermentation in augmenting the nutritional composition and bioactivity of indigenous rice varieties, specifically *Pulu Mandoti*.

**Keywords** Antioxidant · Antidiabetic · Fatty acids · *In silico* · Oyster mushrooms

## Introduction

*Pulu Mandoti* is a variant of red glutinous rice characterized by a highly distinctive and strong aroma. It can only be cultivated in the village of Salukanan, making it an endemic crop in the Salukanan Village, Baraka Subdistrict, Enrekang Regency, South Sulawesi (Karim 2020). This variety exhibits a prolonged harvesting period of 170 days after sowing (DAS), with plant height ranging from 133 to 150 cm and 8–9 productive tillers. The grain morphology is characterized by plump, round grains with a straw-yellow color. *Pulu Mandoti* variety possesses a moderate amylose content of 20.16%. Rice with moderate amylose content is known for its soft texture without stickiness (Sahardi 2013). Locally known as fragrant *Pulu Mandoti*, this type of glutinous rice

is considered a local variety with high economic value, where the nutritional content plays a significant role. The protein content of *Pulu Mandoti* is known to be 8.89%, with glutelin being the most dominant protein fraction (Masniawati et al. 2013). Furthermore, it is highly probable that *Pulu Mandoti* possesses high antioxidant properties, as it produces a red pigment, leading the local community to refer to it as *pulu* for rice and *mandoti* for red.

Red rice can serve as an excellent alternative source of antioxidants. Ravichanthiran et al. (2018), in their review, concluded that red rice is associated with a broad spectrum of applications such as anti-diabetic, anti-cholesterol, cardio-protective, and antioxidant properties. Red rice is known for its red pigments containing antioxidant compounds believed to be beneficial for overall health (Arifin 2019). These varieties get their color from anthocyanins, which are recognized for their free radical scavenging and antioxidant capacities,

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along with other health benefits (Rathna et al. 2019). Antioxidants halt this chain reaction by scavenging free radical intermediates (Balitbangan 2015). The antioxidant activity plays a crucial role in preventing diabetic and reducing blood sugar levels by eliminating free radicals and preventing their excessive accumulation (Yan et al. 2016; Ghorbani 2017; Takagaki et al. 2019).

Hence, red rice emerges as a prospective dietary intervention for individuals with diabetes in forthcoming research. The diverse nutritional profile of red rice positions it as a highly promising functional food. Functional foods, recognized for imparting positive health benefits through their nutritional content, play a vital role in enhancing human health and reducing the risk of various diseases such as cardiovascular diseases, cancer, hyperlipidemia, osteoporosis, diabetes, and hypertension. In addition, they serve as agents for cardiovascular health, anti-obesity, anti-diabetic, anti-cancer, immune-boosting, management of chronic inflammatory disorders, and formulations for treating degenerative diseases, forming an integral part of regular diets (Morris et al. 2017; Reis et al. 2017; Raghavendra et al. 2018; Anusiya et al. 2021; Cateni et al. 2021; Kaur et al. 2021). Furthermore, by incorporating treatments involving edible mushrooms, our objective is to explore the fermentation of local *Pulu Mandoti* by three edible mushroom species, namely *Pleurotus ostreatus*, *Pleurotus cystidiosus*, and *Pleurotus djamor*.

Edible mushrooms have been utilized for their nutritional and medicinal attributes for millennia (Sun et al. 2020). Acknowledged as healthful foods, they boast low-fat content and elevated levels of protein, vitamins, and minerals (Atila et al. 2018), coupled with their distinctive flavor. The members of the *Pleurotus* genus (Jacq. Fr) P. Kumm constitute a diverse group of edible species with significant commercial importance (Zervakis et al. 2004; Selvakumar et al. 2008). As a source of nutraceuticals, oyster mushrooms are increasingly garnering attention for potential health benefits (Das et al. 2021). A recent study highlighted the potential use of steroids, fatty acids, and other compounds found in various *Pleurotus* species mushrooms as effective nutraceuticals (Illuri et al. 2022).

In obese human subjects, *Pleurotus eryngii* has been observed to regulate postprandial glycaemia by influencing glucose absorption (Kleftaki et al. 2022). As an initiative, this research holds significance as it endeavors to evaluate the nutritional value, antioxidant activity, and antidiabetic effects of treatments with three different *Pleurotus* species—*P. ostreatus*, *P. cystidiosus*, and *P. djamor* on the functional food, *Pulu Mandoti* local rice. This study contributes to understanding the health benefits of edible mushrooms, especially *Pleurotus* and to explore the relationship between antioxidant capabilities and antidiabetic effects of the resulting products. This finding may offer valuable insights into

the potential utilization of *Pleurotus* mushrooms as a natural and effective treatment for diabetic which may later be emerged as a promising functional food in the future.

## Methods

### Collection of *Pulu Mandoti*

Local rice (*Oryza sativa* L.), *Pulu Mandoti*, was retrieved from Salukanan Village, Baraka District, Enrekang Regency, South Sulawesi, Indonesia with a geographical coordinate: 3°22'56"S 119°53'51"E.

### Fermentation of *Pulu Mandoti*

One hundred grams of finely ground rice flour (based on dry weight) were placed into a jar and sealed with parchment paper. The substrate-filled jar was then oven-dried at 60 °C for 24 h and allowed to cool. White oyster mushrooms (*Pleurotus ostreatus* InaCC F10), red oyster mushrooms (*Pleurotus djamor* InaCC F135), and brown oyster mushrooms (*Pleurotus cystidiosus* InaCC F100), cultivated in Potato Dextrose Broth (PDB), were added to the substrate at a volume of 6 mL each. Optimal fermentation was carried out at  $\pm 25$  °C for 3 days. The fermented rice samples were subsequently blended to a smooth consistency.

### Proximate analysis

A 10-g sample of fermented *Pulu Mandoti* and an untreated *Pulu Mandoti* (control) were finely stored in ziplock plastic containers for proximate analysis, including crude protein (IK 05—destruction auto analysis), crude fiber (IK 06—auto fiber analysis system), crude fat (IK 07—auto fat extraction system), moisture content, and ash content with SNI 01-2891-1992 (Ranok et al. 2021; Supakot et al. 2022).

### Antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Fermented *Pulu Mandoti* samples and untreated raw samples (positive control) at 1000 ppm were diluted to four concentrations: 100, 50, 25, and 12.5 ppm with methanol. A 1000-ppm vitamin E solution (positive control) was diluted to four concentrations: 0, 10, 5, and 2.5 ppm. A DPPH solution was prepared at a concentration of 100 ppm. 100  $\mu$ L of the diluted samples were mixed with 100  $\mu$ L of the DPPH solution in a microplate, with each concentration tested in triplicate. After a 30-min incubation in the dark, the samples were analyzed using a spectrophotometer at 517 nm (Fidien et al. 2021).

### Antidiabetic assay (IC<sub>50</sub>) using $\alpha$ -glucosidase inhibitor method

Fermented *Pulu Mandoti* samples were prepared at a concentration of 1000 ppm using distilled water. Acarbose served as the positive control. 30  $\mu$ L of the sample was added to 36  $\mu$ L of pH 6.8 phosphate buffer, and 17  $\mu$ L of 4-Nitrophenyl- $\alpha$ -D-glucopyranoside (Sigma-Aldrich) was placed into a microplate. One-hundred  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> (200 mM) was added after a 15-min incubation at 39 °C. The analysis was conducted using a spectrophotometer at 400 nm.  $\alpha$ -Glucosidase inhibitory activity was expressed as a percentage of inhibition (Apostolidis et al. 2007).

### Fatty acids extraction

Fifty grams of finely ground rice were combined with a solvent mixture of chloroform and methanol (2:1) in a volume of 150 mL. The solvent mixture contained 0.01% Butylated Hydroxytoluene (BHT) and 0.75% KCl, added in a quantity of 10 mL. The solution was filtered using an aspirator pump and Whatman grade 1 filter paper. Fractionation of the sample was performed to separate the chloroform and methanol layers. The chloroform layer was separated by adding Na<sub>2</sub>SO<sub>4</sub> and filtered using an aspirator pump. Evaporation was carried out at a temperature of 35 °C. Lipids without a solvent were combined with 10 mL of chloroform and methanol (2:1) and rinsed with nitrogen. Two milliliters of the sample were mixed with 2 mL of NaOH + Methanol (1:1) and vortexed for 15 min. One milliliter of the solution was separated, and 1.5 mL of n-Hexane was added and vortexed. Three milliliters of Milli-Q water were added, and vortexed for 10 s. The upper layer of the sample was collected, filtered using a syringe (0.45  $\mu$ m) containing Na<sub>2</sub>SO<sub>4</sub>, and placed into an amber bottle and subjected to analysis by GC–MS. Fatty acids were identified based on the retention times of a SUPELCO 37 component FAME mixture (Sigma–Aldrich).

### Metabolite profiling and fatty acid methy ester (FAME) identification by GC–MS

On the part of metabolite profiling, a total of 25 g of fermented *Pulu Mandoti* samples and raw samples were finely immersed in a solution of 75 mL of 1% HCl + 73 mL of methanol + 2 mL of 1% HCl. The samples were placed in a shaker at 200 rpm and incubated at 26 °C for 16 h in the dark. The suspension was filtered using an aspirator pump with Whatman No. 1 filter paper. Extraction and solvent separation were achieved through evaporation using a vacuum (200–80 MBAR), rotation at 60 rpm, a heating bath at 35 °C, and a chiller at 6 °C. The extract was then transferred into microtubes and concentrated for 30 min using a concentrator. The solution was dissolved in 2% HCl at a ratio of 1:5

(1 mL sample and 15 mL 2% HCl solution). The sample was filtered using a 0.45  $\mu$ m syringe and subjected to GC–MS analysis. The metabolite profiling and fatty acid identification were conducted using the GCMS-QP2010 Ultra (Shimadzu), equipped with AOC-20i as the autosampler. RTX-5MS (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) was used as a column, and helium was adjusted as the carrier gas. The column temperature was initially held at 55 °C and increased at a rate of 10 °C per minute to 250 °C. The injector and detector temperatures were set at 250 and 260 °C, respectively. Electron ionization at 70 eV generated ions, with a mass range of m/z 40–500. Compounds identified were compared with data from the National Institute of Standards and Technologies Mass Spectral Library (NIST).

### Molecular docking

Chemical compounds identified through GC–MS profiling were analyzed to determine potential candidates for antioxidants and antidiabetic agents. Several potentially relevant compounds were selected from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The compounds were stored in SDF file format, and these files were converted to PDB format using Open Babel 2.4.1. To prepare the target receptor proteins, three proteins were obtained from the RCSB PDB (<https://www.rcsb.org/>): 2CKJ and 500X for antioxidant testing and 2QV4 for antidiabetic testing. The downloaded receptor and ligand structures, converted to PDB format, underwent preparation using AutoDock Vina. The prepared receptor and ligand structures were saved in PDBQT format. Molecular docking was performed using AutoDock Vina through the Windows Command Prompt (CMD). The results of molecular docking were visualized using PLIP-tools and the Pymol program to identify amino acid residues involved in the binding interactions between the receptor and ligand.

### 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.

## Results

### Proximate nutritional evaluation

Table 1 presents the proximate composition of local fermented food, *Pulu Mandoti*, fermented with *Pleurotus* spp. for 3 days. The percentage of crude protein ( $8.55 \pm 0.15\%$ )

and crude fat ( $1.34 \pm 0.15\%$ ) in the fermentation treatment with *P. cystidiosus* was higher compared to other treatments and the control. These results correlated with the percentage of dry matter produced, with *P. cystidiosus* fermentation yielding  $91.99 \pm 0.61\%$  dry matter, although this relationship is not entirely linear between crude protein and dry matter in all treatments due to the different mushroom species involved. The increase in protein content could be attributed to the bioconversion of crude fiber and carbohydrates in the raw materials into mycelial protein or single-cell protein. This protein increment was likely facilitated by the presence of extracellular enzymes produced by *Pleurotus*, which inherently consist of proteins (Alemawor et al. 2009; Sherief et al. 2010).

Contrary to the achieved moisture content, the fermentation treatment by *P. cystidiosus* showed a lower value ( $8.01 \pm 0.33\%$ ) compared to other treatments and unfermented samples ( $12.38 \pm 1.03\%$ ). During the fermentation period, substrate hydrolysis occurred, leading to a reduction in the substrate's moisture content. The decrease in moisture content correlated with the fermentation duration, and it was later recognized that moisture concentration directly impact the shelf life of the final product, creating conditions supportive to microbial growth (Zewdie et al. 2008). The fermentation treatments with *P. cystidiosus* and *P. djamor* showed the lowest values for the percentage of fiber ( $0.78 \pm 0.04\%$ ), followed by fermentation with *P. ostreatus* ( $0.82 \pm 0.06\%$ ) and unfermented samples ( $0.84 \pm 0.08\%$ ). The reduction in fiber could be attributed to the metabolic capacity of fungi to hydrolyze and metabolize rice (substrate) as a carbon source to produce cell biomass (Madigan et al. 2002).

### Antioxidant activities

Inhibitory concentration ( $IC_{50}$ ) value of *Pulu Mandoti* fermented with *P. ostreatus*, *P. cystidiosus*, and *P. djamor* were 3.81, 1.12, and 4.50 ppm, respectively, which were similar to the unfermented sample ( $IC_{50} = 5.90$  ppm) as detailed in Table 2. Based on the results, it could be indicated that both *Pleurotus* fermentation and non-fermentation fell into the category of very strong antioxidants. Jun et al. (2003) stated that  $IC_{50}$  value below 50 ppm was categorized as very strong, followed by  $50 < IC_{50} < 100$  ppm (strong),  $100 < IC_{50} < 250$  ppm (moderate),  $250 < IC_{50} < 500$  ppm (weak), and  $IC_{50} > 500$  ppm (inactive). The DPPH radical scavenging activities of all fermented products were higher than control or unfermented samples (Fig. 2).

### $\alpha$ -Glucosidase inhibition activity

The  $\alpha$ -glucosidase inhibition results for the control (acarbose) and fermented *Pulu Mandoti* are depicted in Fig. 3.

Acarbose displayed a more potent  $\alpha$ -glucosidase inhibition activity compared to fermented products at 50 ppm. In the fermentation treatment with brown oyster mushrooms (*P. cystidiosus*), higher inhibition activity was observed at a concentration of 250 ppm compared to other fungal treatments and the control (unfermented).

### Metabolites profile of fermented products and unfermented samples

The peaks or ions of compounds present in fermented products and unfermented (control) sample, along with their relative abundances and elution times from the GC–MS analysis, are illustrated in Fig. 4. Out of the 173 detected compound types (data not shown), at least 57 identified compounds have antioxidant and antidiabetic activities, demonstrating both antioxidant and antidiabetic activities in *Pulu Mandoti*, whether fermented or unfermented (Table 3).

### Molecular docking of metabolites from fermented *Pulu Mandoti*

The results of molecular docking for several compounds derived from the GC–MS profiling of metabolites in fermented *Pulu Mandoti* with *Pleurotus* spp. revealed negative binding affinities in each docking experiment. Amino acid residue interactions were observed in certain compounds, overlapping with the native ligand of 5O0X, 2CKJ, and 2QV4 (Table 4). Figure 5 summarizes the 3D docking interactions of 5O0X, 2CKJ, and 2QV4 (native ligands) as antioxidant and antidiabetic receptors.

Several compounds resulting from the fermentation of mushrooms, when docked with the antioxidant receptors 5O0X and 2CKJ, exhibited negative binding affinities. For instance, the receptor 2CKJ interacting with octadecanoic acid from *P. cystidiosus* (PC) fermentation demonstrated a binding affinity of  $-4.0$  kcal/mol, with amino acid residues binding similarly to the native ligand, Lys422. The antioxidant receptor 5O0X, docked with hexanoic acid from *P. cystidiosus* (PC) fermentation, also displayed a negative binding affinity of  $-4.7$  kcal/mol, forming amino acid residue interactions in the Pro460 and Thr462 regions with the native ligand. Hexadecanoic acid from *P. djamor* (PD) fermentation and hexadecanoic acid from *P. ostreatus* (PO) fermentation exhibited negative binding affinities of  $-5.7$  kcal/mol and  $-5.5$  kcal/mol, respectively. These compounds also displayed interactions with amino acid residues in the regions of His459 and Pro460, analogous to the native ligand.

Compounds identified through GC–MS profiling were also interacted with antidiabetic receptors to identify potential compounds for use as antidiabetic candidates. Docking results for each compound with the administration of *Pleurotus* inoculants indicated that hexanoic acid and octadecanoic acid from



PC had binding affinities of  $-3.8$  kcal/mol. Docking results from PD fermentation with n-hexadecanoic acid showed a binding affinity of  $-4.1$  kcal/mol. Hexadecanoic acid from PO fermentation, when interacted with the antidiabetic receptor, exhibited a negative binding affinity of  $-3.8$ . Compounds from each mushroom fermentation treatment also formed several amino acid residue interactions in the antidiabetic receptor region. However, the negative binding affinity and amino acid residue interactions suggest that the profiled compounds from fermented rice with various mushrooms have potential antidiabetic activity.

### Effects of fermentation on the fatty acid composition of fermented *Pulu Mandoti*

In this study, the composition of compounds, particularly those belonging to the fatty acid group, in fermented *Pulo Mandoti* with *P. ostreatus*, *P. djamor*, and *P. cystidiosus* was observed. Major compounds exhibiting antioxidant and antidiabetic activities were identified (Fig. 6). The fatty acid content of lipids produced during the fermentation of *pulo mandoti* by three different species of *Pleurotus* was reported for the first time. GC-MS analysis revealed several fatty acid compounds with potential antioxidant and antidiabetic properties, with 21 compounds recorded after *P. ostreatus* fermentation, 19 compounds after *P. cystidiosus* fermentation, 17 compounds after *P. djamor* fermentation, and 15 compounds in raw *Pulu Mandoti* (Fig. 6). This data demonstrated that fermented products contained a higher number of fatty acid compounds compared to raw material.

### Molecular docking of fatty acid compounds from fermented *Pulu Mandoti*

The results of molecular docking for several fatty acid compounds identified through GC-MS profiling of fermented *pulo mandoti* with *Pleurotus* spp. indicated amino acid residues binding in regions analogous to the native ligands, serving as controls, namely 500X and 2CKJ (Fig. 7). Based on the binding affinity values, the best interaction between protein and ligand was observed with the receptor 500X from *P. cystidiosus* (PC) fermentation, where the compound 11-Eicosenoic acid, methyl ester exhibited a binding affinity of  $-6.4$  kcal/mol. Subsequently, the compounds 500X-PC, 500X-PO, 2CKJ-PO, 2CKJ-PC, 500X-PD, 2CKJ-PO, 2CKJ-PC, 500X-PO, and 500X-PD demonstrated respective free energy values of  $-6.3$ ,  $-6.2$ ,  $-5.7$ ,  $-5.2$ ,  $-4.8$ ,  $-4.7$ ,  $-4.6$ ,  $-4.6$ , and  $-4.5$  kcal/mol (Table 5).

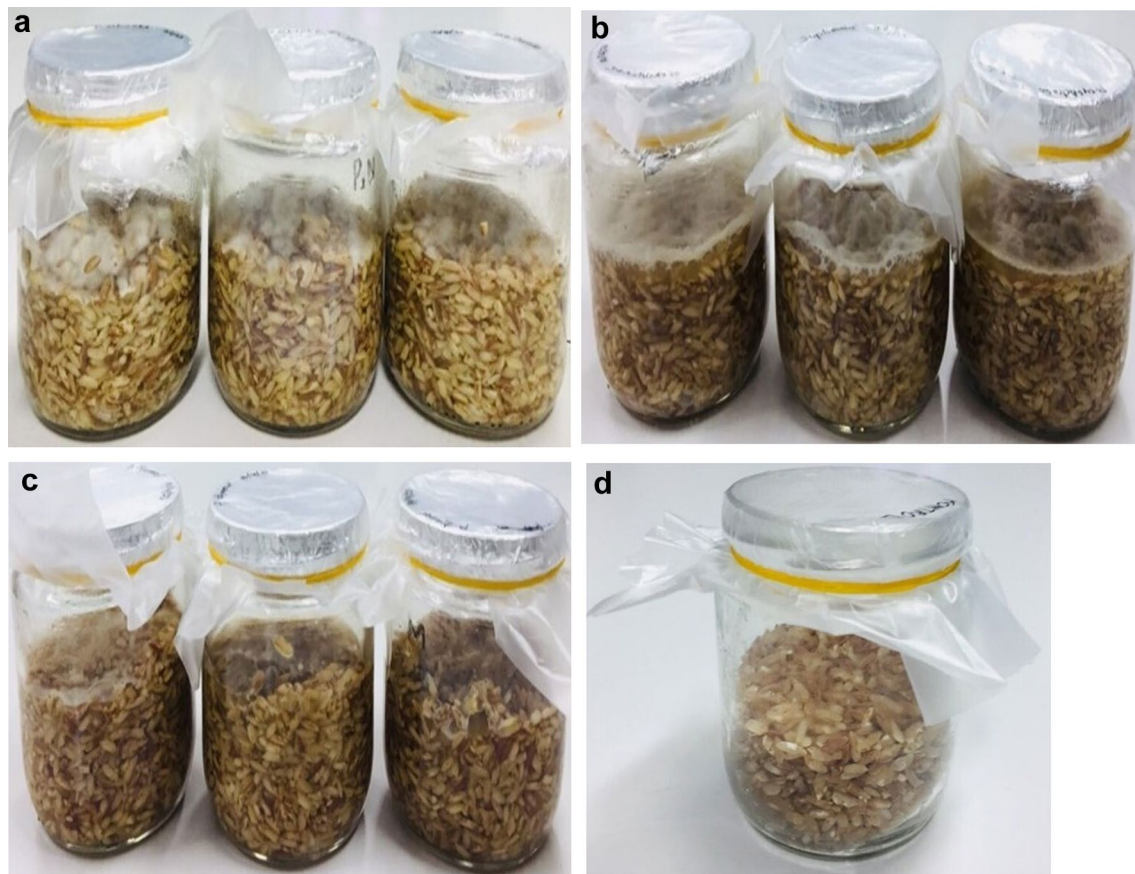
### Molecular docking of fatty acid compounds from fermented *Pulu Mandoti* as antioxidants and antidiabetic

Molecular docking for several fatty acid compounds of *Pulu Mandoti* fermented with *P. cystidiosus* revealed amino acid residues binding in regions analogous to the native ligands used as controls (500x, 2CKJ, and 2QV4) (Table 6). Based on the binding affinity values, the optimal interaction between protein and ligand is observed with the antioxidant receptor 500x and *P. cystidiosus* compounds i.e. 11-Eicosenoic acid, methyl ester, showing a binding affinity of  $-7.5$  kcal/mol. Notably, this interaction exhibits amino acid residue binding identical to the native ligand, specifically Thr484. Furthermore, the receptor 2CKJ binds with the compound butylated hydroxytoluene, presenting a binding affinity of  $-6.9$  kcal/mol, involving several amino acid residues that bind similarly to the native ligand, including Val259, Glu263, and Ile264. From the binding affinity and amino acid residue binding results, it can be inferred that Butylated Hydroxytoluene and 11-Eicosenoic acid, methyl ester hold potential as antioxidant candidates (Fig. 8).

Docking results using the antidiabetic receptor 2QV4 reveal binding between the protein and several compounds with potential as antidiabetic candidates. Negative binding affinity values and amino acid residues binding in the receptor region are observed for multiple test compounds. The compound 9,12-Octadecadienoic acid, methyl ester from the fermented rice with *P. cystidiosus* exhibits a binding affinity of  $-5.5$  kcal/mol, with amino acid residue binding at Gln63 identical to its native ligand. Negative binding affinity is demonstrated by the docking results between protein 2QV4 and the ligand 9-Octadecenoic acid, methyl ester ( $-5.0$  kcal/mol), with amino acid residue binding at Val107 similar to that of the native ligand. Butylated Hydroxytoluene, when docked with protein 2QV4, shows a relatively high binding affinity of  $-6.7$  kcal/mol, with several amino acid residue bindings not present in the native ligand. These docking results indicate that all three compounds exhibit antidiabetic activity, supported by negative binding affinity values and several amino acid residues binding in the same region as the native ligand.

### Discussion

For millennia, humans have incorporated mushrooms into their diets, but recent years have witnessed a substantial increase in their consumption, encompassing a diverse array of mushroom varieties. Mushrooms or edible fungi producing visible sporocarps stand as vital and beneficial foods, rich in vitamins and minerals, contributing to dietary health by being low in calories and fat, yet high in vegetable protein



**Fig. 1** Local rice *Pulu Mandoti* fermented by *P. ostreatus* (a), *P. cystidiosus* (b), *P. djamor* (c), and control (d)

**Table 1** Proximate composition of local rice, *Pulu Mandoti*, based on treatment of three *Pleurotus* inoculants

Treatment	Dry matter (%)	Moisture (%)	Total ash (%)	Crude protein (%)	Crude fat (%)	Fibre (%)
Fermented by <i>P. ostreatus</i>	90.03 ± 0.99	9.97 ± 0.64	0.30 ± 0.03	8.46 ± 0.42	1.27 ± 0.08	0.82 ± 0.06
Fermented by <i>P. cystidiosus</i>	91.99 ± 0.61	8.01 ± 0.33	0.29 ± 0.02	8.55 ± 0.15	1.34 ± 0.05	0.78 ± 0.04
Fermented by <i>P. djamor</i>	90.51 ± 0.93	9.49 ± 0.46	0.24 ± 0.02	8.24 ± 0.25	1.28 ± 0.07	0.78 ± 0.05
Control (raw)	87.62 ± 1.01	12.38 ± 1.03	0.37 ± 0.08	7.61 ± 0.69	0.57 ± 0.10	0.84 ± 0.08

**Table 2** IC<sub>50</sub> values of local rice *Pulu Mandoti* fermented with *Pleurotus* spp.

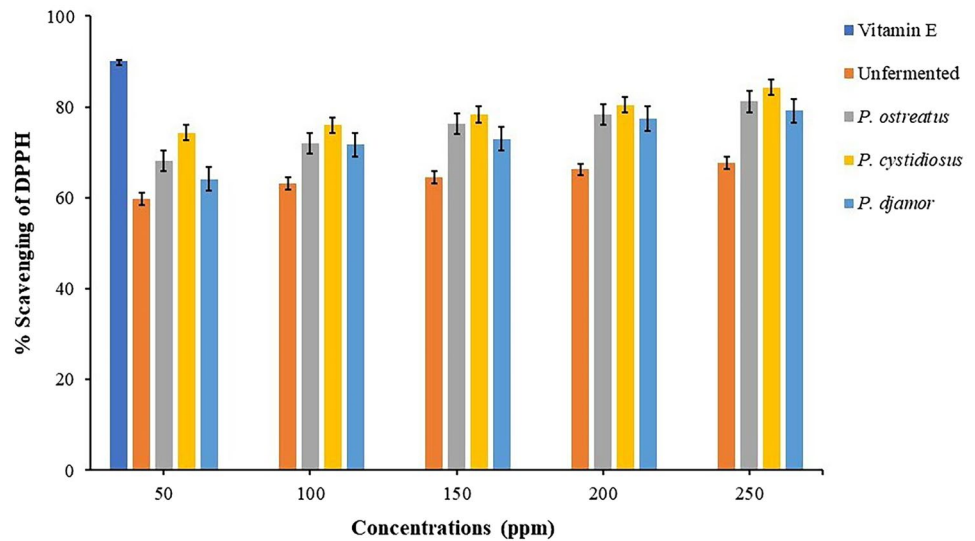
Treatment	IC <sub>50</sub> (ppm)	Category
Vitamin E	1.11	Very strong
Control	5.90	Very strong
<i>P. ostreatus</i>	3.81	Very strong
<i>P. cystidiosus</i>	1.12	Very strong
<i>P. djamor</i>	4.50	Very strong

(Krishna et al. 2023). Various medicinal benefits associated with mushrooms, particularly within the *Pleurotus* genus, emphasize their nutritional significance (Vega et al. 2022).

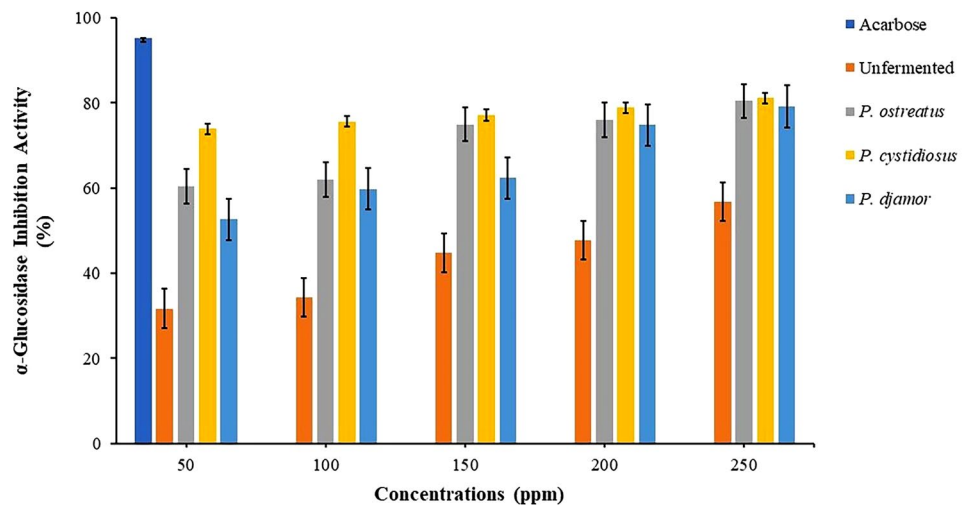
Nutritional analyses of *P. djamor* have indicated protein content ranging from 11.3 to 43.1%, total carbohydrates between 35.5 and 42.4%, fat levels from 0.1 to 4.6%, crude fiber spanning 7.3 to 12.2%, and ash content ranging from 6.2 to 8.3% (Vega and Franco 2013; Carrasco-González et al. 2017). Additionally, they serve as sources of essential vitamins, predominantly B-complex (B1, B2) and D, as well as minerals such as K, P, Mg, Ca, Na, Zn, and Fe (Salmones 2017). *Pleurotus cystidiosus* is noted for bisabolane-type sesquiterpenoids with antitumor properties (Zheng et al. 2015), while *P. ostreatus* contains proteoglycans with both antitumor and immunomodulating effects (Sarangi et al. 2006).

Motivated by the documented benefits of three *Pleurotus* species (*P. ostreatus*, *P. cystidiosus*, *P. djamor*) (Fig. 1),

**Fig. 2** DPPH free radical scavenging activity of local rice, *Pulu Mandoti* fermented with different fungal inoculants

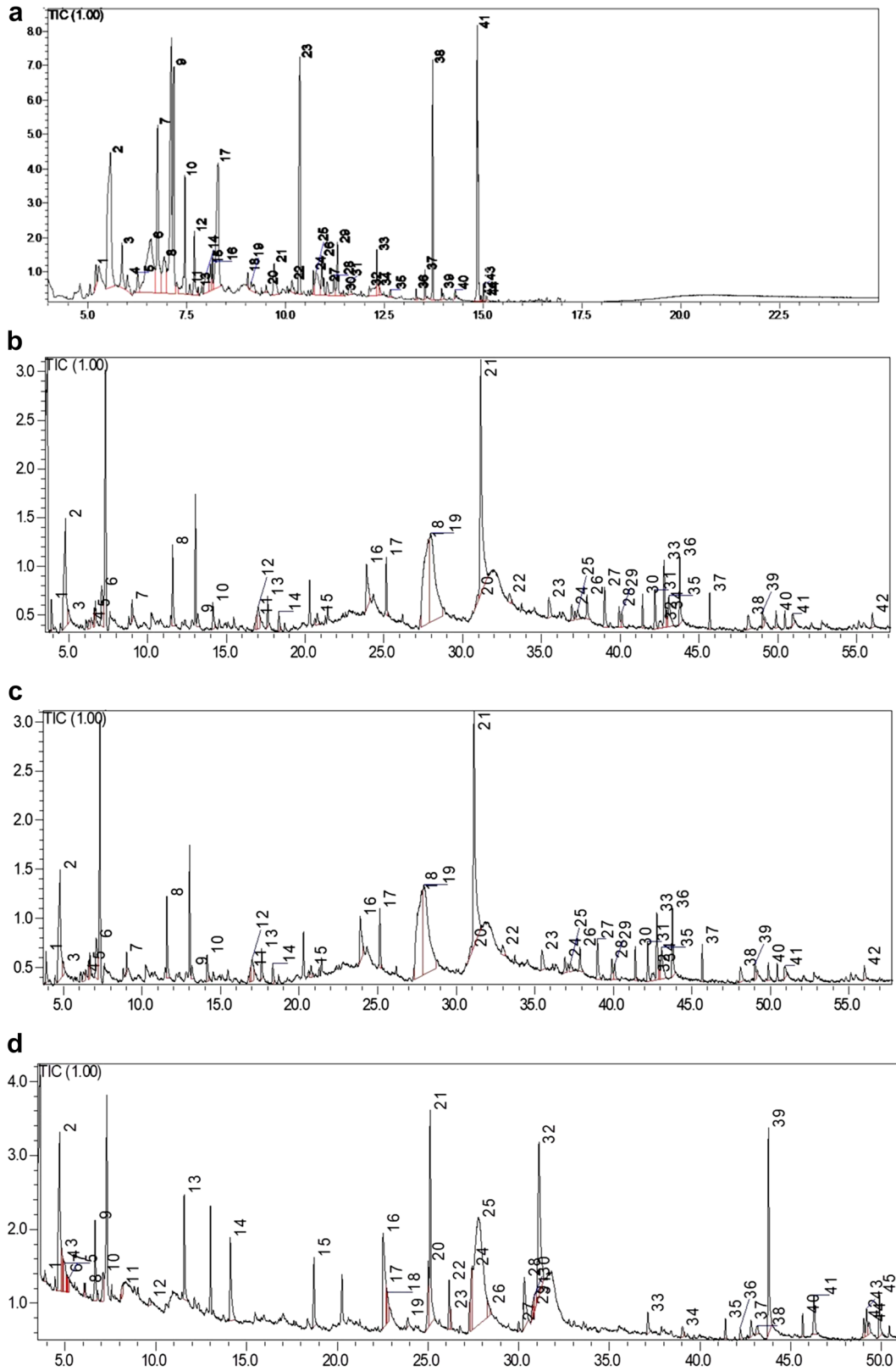


**Fig. 3**  $\alpha$ -Glucosidase inhibition assay of fermented *Pulu Mandoti* and acarbose (control) presented in percentages



we undertook the development of functional foods based on local commodity named as *Pulu Mandoti* or red rice fermented with those fungal inoculants. Proximate analysis revealed a notably high dry matter content in *P. cystidiosus* ( $91.99 \pm 0.61\%$ ), surpassing the other two mushroom treatments, with *P. ostreatus* showing the highest moisture content ( $9.97 \pm 0.64\%$ ). The increased moisture content could be attributed to the water absorbed by *Pulu Mandoti* during boiling before fortification. However, unfermented *Pulu Mandoti* exhibited higher moisture content ( $12.38 \pm 1.03\%$ ). Consistent with the previous findings, the moisture content of red rice varieties varies from 9.3 to 12.94%, correlating with the slightly reddish hue of *Pulu Mandoti* (Rathna et al. 2019). Furthermore, the fiber and ash content in the fermented products were lower compared to the unfermented counterpart.

The proximate analysis resulted in intriguing insights into the composition of *P. cystidiosus*, *P. ostreatus*, and unfermented *Pulu Mandoti* Rice. *P. cystidiosus* exhibited notably high dry matter content, surpassing the other two mushroom treatments, implying a relatively low moisture content. This suggests a significant proportion of non-water components like proteins, carbohydrates, and fats in *P. cystidiosus*. Conversely, *P. ostreatus* displayed the highest moisture content, likely influenced by species-specific composition. The moisture content in unfermented *Pulu Mandoti* Rice surpassed both mushrooms, potentially attributed to water absorption during boiling before fermentation, a common rice preparation practice. Consistent with literature, red rice varieties, including *Pulu Mandoti*, typically exhibit moisture content ranging from 9.3 to 12.94%. The slight reddish hue in *Pulu Mandoti* correlates with this moisture content, providing



**Fig. 4** GC chromatogram of methanol extract of *Pulu Mandoti*. **a** Control, Fermented by **b** *P. ostreatus*, **c** *P. cystidiosus*, **d** *P. djamor*



**Table 3** GC–MS profile of fermented and raw *Pulu Mandoi* extracts

Compound name	Similarity index (%)	Area	Chemical formula	Biological activity	Detection (√ = Present)			
					CO	PO	PC	PD
Carbamic acid, phenyl ester	86	2.47E+08	C8H7NO2	Antioxidant	√			
Dimethyl fumarate	89	32014097	C6H8O4	Antioxidant and antidiabetic	√			
1H-Imidazole-4-carboxylic acid, methyl ester	91	1.49E+08	C7H8N2O2	Antioxidant	√			
Butanedioic acid, monomethyl ester	93	66303702	C5H8O4	Antidiabetic	√			
Hepta-2,4-dienoic acid, methyl ester	79	69284981	C9H12O2	Antioxidant	√			
5-Hydroxymethylfurfural	81	1.65E+08	C6H6O3	Antioxidant and antidiabetic	√			
2-Methoxy-4-vinylphenol	94	6649240	C9H10O2	Antioxidant	√			
Methional	95	13630217	C4H8O2S	Antioxidant	√			
2-Pyridineacetic acid, hexahydro-	93	36126147	C7H13NO2	Antioxidant	√			
5-Oxotetrahydrofuran-2,3-dicarboxylic acid, dimethyl ester	94	11309207	C6H8O7	Antioxidant	√			
Benzenemethanol, .alpha.-1-pentynyl-	97	15112709	C11H12O	Antioxidant and antidiabetic	√			
2(3H)-Furanone, 5-ethylidihydro-5-methyl-	93	4693865	C7H10O2	Antioxidant	√			
Methyl tetradecanoate	84	23293560	C16H32O2	Anioxidant	√			
Pentadecanoic acid, 14-methyl-, methyl ester	95	87883193	C17H34O2	Antioxidant	√			
n-Hexadecanoic acid	94	7946714	C16H32O2	Antioxidant and antidiabetic	√	√	√	√
9,12-Octadecadienoic acid, methyl ester	87	1.62E+08	C19H34O2	Antioxidant and antidiabetic	√	√		
Sinapic acid methyl ester	83	2341934	C11H12O5	Antioxidant	√			
Methyl stearate	89	5750854	C19H38O2	Antioxidant and antidiabetic	√			
9,12-Octadecadienoic acid (Z,Z)-	95	3503598	C18H32O2	Antioxidant and antidiabetic	√	√		√
Butanoic acid, 2-methyl-, methyl ester	87	450368	C6H12O2	Antioxidant		√		
Benzeneacetaldehyde	93	712630	C8H8O	Anioxidant		√		
Phenol, 2,6-bis(1,1-dimethylethyl)-	85	452243	C14H22O	Antioxidant and antidiabetic		√		
4-Amino-5-cyclobutyl-1,2,4-triazole-3-thiol	89	384246	C6H10N4S	Antioxidant		√		
Diethyl phthalate	91	16413691	C12H14O4	Anioxidant		√	√	√
2(3H)-Furanone, 3-butyldihydro-	81	1112012	C8H12O2	Antioxidant		√		
(4-Hydroxy-2-mercapto-6-methyl-pyrimidin-5-yl)-acetic acid	85	701367	C9H10N2O3S	Antioxidant and antidiabetic		√		
Hexadecanoic acid, methyl ester	94	3484409	C17H34O2	Antioxidant and antidiabetic		√		
n-Hexadecanoic acid	89	4417074	C16H32O2	Antioxidant and antidiabetic	√	√	√	√
1,2-Diphenyltetramethyldisilane	83	762964	C14H20Si2	Antioxidant and antidiabetic		√	√	√
9,12-Octadecadienoic acid, methyl ester	88	6572662	C19H34O2	Antioxidant and antidiabetic	√	√		
9,12-Octadecadienoic acid (Z,Z)-	91	310172	C18H32O2	Antioxidant and antidiabetic	√	√		√
Nonanoic acid	87	10191480	C9H18O2	Anioxidant			√	
Benzeneacetaldehyde	82	769476	C8H8O	Anioxidant			√	
2-Furancarboxaldehyde, 5-(chloromethyl)-	89	1500344	C6H6Cl2O2	Anioxidant			√	
13-Docosenamide, (Z)-	83	1720851	C22H43NO	Anioxidant			√	
Hexanoic acid	91	20972325	C6H12O2	Antioxidant and antidiabetic			√	
Diethyl phthalate	86	21211743	C12H14O4	Antioxidant		√	√	√
2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	85	892008	C12H24N2O2	Anioxidant			√	
n-Hexadecanoic acid	91	3308126	C16H32O2	Antioxidant and antidiabetic		√	√	√
1,2-Diphenyltetramethyldisilane	87	1459520	C14H20Si2	Antioxidant and antidiabetic		√	√	√
Octadecanoic acid	93	899969	C18H36O2	Antioxidant and antidiabetic			√	
Chloromethyl 4-chlorodecanoate	88	763864	C11H20Cl2O2	Antioxidant				√
Levogluconone	83	5528467	C6H10O3	Antioxidant				√
L-Proline, 5-oxo-, methyl ester	91	11111966	C7H11NO3	Anioxidant				√
Piperidine, 2-(tetrahydro-2-furanyl)-	84	1459993	C9H15NO	Anioxidant				√
1,1-Cyclopentanediacetimide	87	4293935	C8H12N2O2	Anioxidant				√

**Table 3** (continued)

Compound name	Similarity index (%)	Area	Chemical formula	Biological activity	Detection (√ = Present)			
					CO	PO	PC	PD
2,4-Pentadienamide, N,N-diethyl-	93	14454875	C10H17N2	Anioxidant			√	√
D-Allose	89	45670233	C6H12O6	Antioxidant	√			√
Bicyclo[2.2.1]heptan-2-one, 1-ethenyl-7,7-dimethyl-	84	626038	C10H16O	Anioxidant				√
Diethylphthalate	91	5811361	C12H14O4	Anioxidant		√	√	√
Heptanoic acid, butyl ester	88	671143	C11H22O2	Anioxidant				√
Diethyl phthalate	90	18836948	C12H14O4	Anioxidant		√	√	√
Tetradecanoic acid	93	1454407	C14H28O2	Antioxidant				√
cis-10-Heptadecenoic acid	95	790970	C17H32O2	Antioxidant				√
n-Hexadecanoic acid	94	12893738	C16H32O2	Antioxidant and antidiabetic	√	√	√	√
1,2-Diphenyltetramethyldisilane	96	1212393	C14H18Si2	Antioxidant and antidiabetic		√	√	√
9,12-Octadecadienoic acid (Z,Z)-	93	1124085	C18H32O2	Antioxidant and antidiabetic	√	√		√

CO control, PO *Pleurotus ostreatus*, PC *Pleurotus cystidiosus*, PD *Pleurotus djamor*

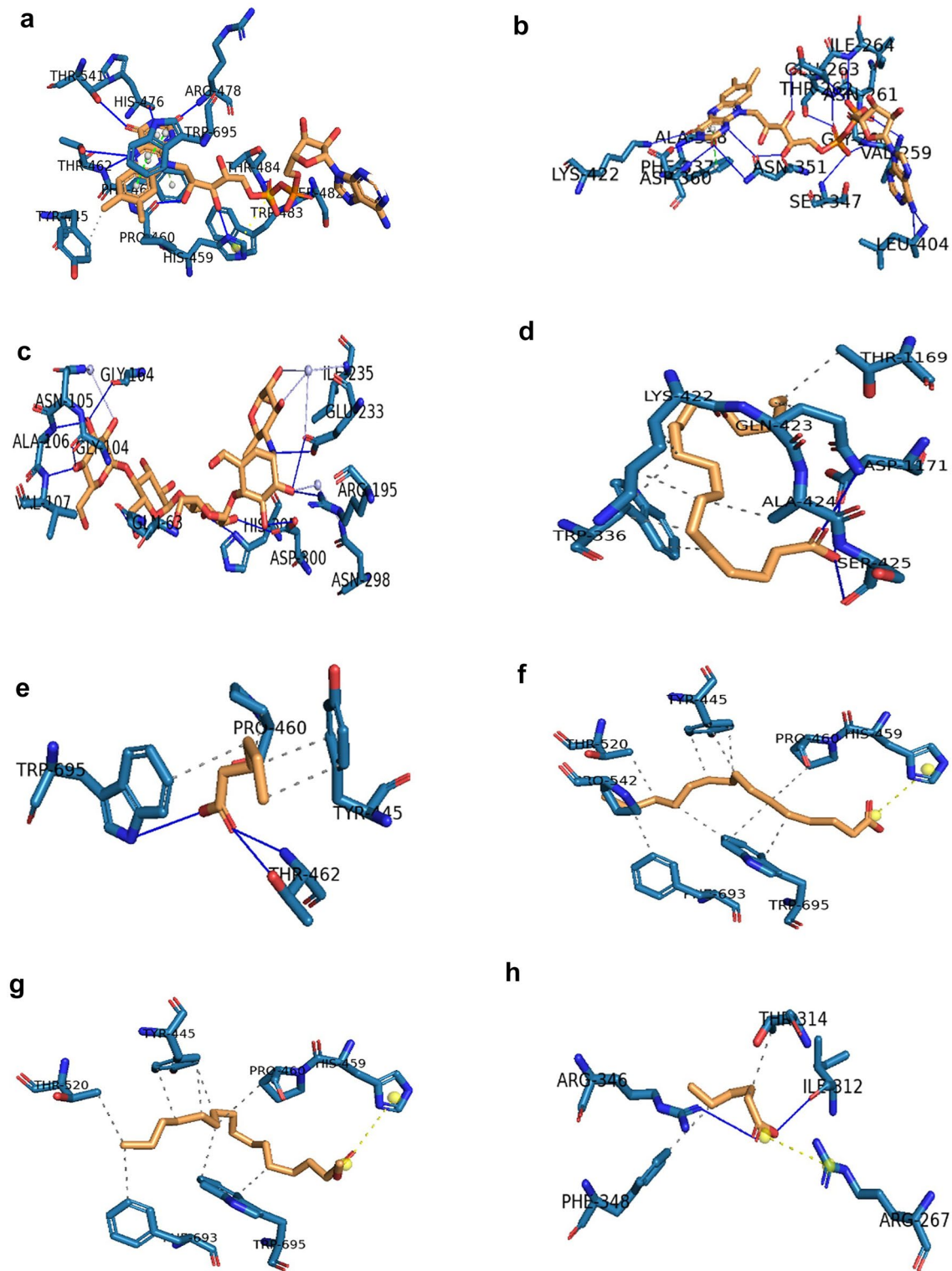
**Table 4** Receptor-ligand interaction among selected compounds of fermented and unfermented *Pulu Mandoti* with their respective binding affinity and bond types

Receptor	Treatment(s)	Compound	Binding affinity (kcal/mol)	H bond and hydrophobic interactions
2CKJ	Control	Native ligand	-9.4	Val259, Gly260, Asn261, Thr262, Glu263, Ile264, Ser347, Asn351, Asp360, Leu404, Lys422
500X	Control	Native ligand	-8.6	His459, Pro460, Thr462, His476, Arg478, Ser482, Trp483, Thr484, Thr541
2QV4	Control	Native ligand	-9.0	Gln63, Gly104, Asn105, Ala106, Val107, Gly164, Arg195, Glu233, Asp300, His305
2CKJ	PC	Octadecanoic acid	-4.0	Trp336, Lys422, Gln423, Ala424, Ser425, Thr1169, Asp1171
500X	PC	Hexanoic acid	-4.7	Tyr445, Pro460, Thr462, Trp695
500X	PD	n-Hexadecanoic acid	-5.7	Tyr445, His459, Pro460, Thr520, Pro542, Phe693, Trp695
500X	PO	Hexadecanoic acid	-5.5	Tyr445, His459, Pro460, Thr520, Phe693, Trp695
2QV4	PC	Hexanoic acid	-3.8	Ile312, Thr314, Arg346, Phe348
	PC	Octadecanoic acid	-3.8	Thr11, Phe335, Asp402, Gly403
	PD	n-Hexadecanoic acid	-4.1	Asn279, Glu282, Gly283, Pro332, Phe406
	PO	Hexadecanoic acid	-3.8	Ala310, Arg346, Phe348

context to observed values. Moreover, fermented products (mushrooms and rice) demonstrated lower fiber and ash content compared to the unfermented counterpart. This reduction during fermentation may stem from the breakdown of cell wall components and mineral loss. Changes in proximate composition underscore fermentation's impact on the nutritional profile, suggesting potential alterations in dietary attributes and bioactive components of the studied mushrooms and rice (Gupta et al. 2013; Gogavekar et al. 2014; Fernandes et al. 2015; Huyen et al. 2019; Omarini et al. 2019; Melanouri et al. 2022; Nacha et al. 2023).

Fermentation products by *P. cystidiosus* had the highest protein content among the three treatments ( $8.55 \pm 0.15\%$ ), while unfermented (control) one had the lowest

( $7.61 \pm 0.69\%$ ), as protein amount was directly influenced by the inherent nature of mushroom. This result is in accordance with the findings of Bao et al. (2013), who observed an increase in the protein content of rice fermented with *Pleurotus*. This observed protein variation is linked to the inherent characteristics of *P. cystidiosus*, suggesting that this specific *Pleurotus* species contributes to a higher protein yield during the fermentation of *Pulu Mandoti* Rice. This finding aligns with previous research showing an increase in protein content in fermented rice with *Pleurotus*. In our investigation, the crude lipid content in fermented *P. cystidiosus* was significantly higher ( $1.34 \pm 0.05\%$ ) compared to the unfermented counterpart. The lipid composition of edible mushrooms predominantly comprises unsaturated



**Fig. 5** 3D interactions of 500X (a), 2CKJ (b) and 2QV4 (c) (native ligands) 2CKJ-PC (Octadecanoic acid) (d), 500X-PC (Hexanoic acid) (e), 500X-PD (n-Hexadecanoic acid) (f), 500X-PO (Hexade-

canoic acid) (g), 2QV4-PC (Hexanoic acid) (h), 2QV4-PC (Octadecanoic acid) (i), 2QV4-PD (n-Hexadecanoic acid) (j) and 2QV4-PO (Hexadecanoic acid) (k) with amino acid residues

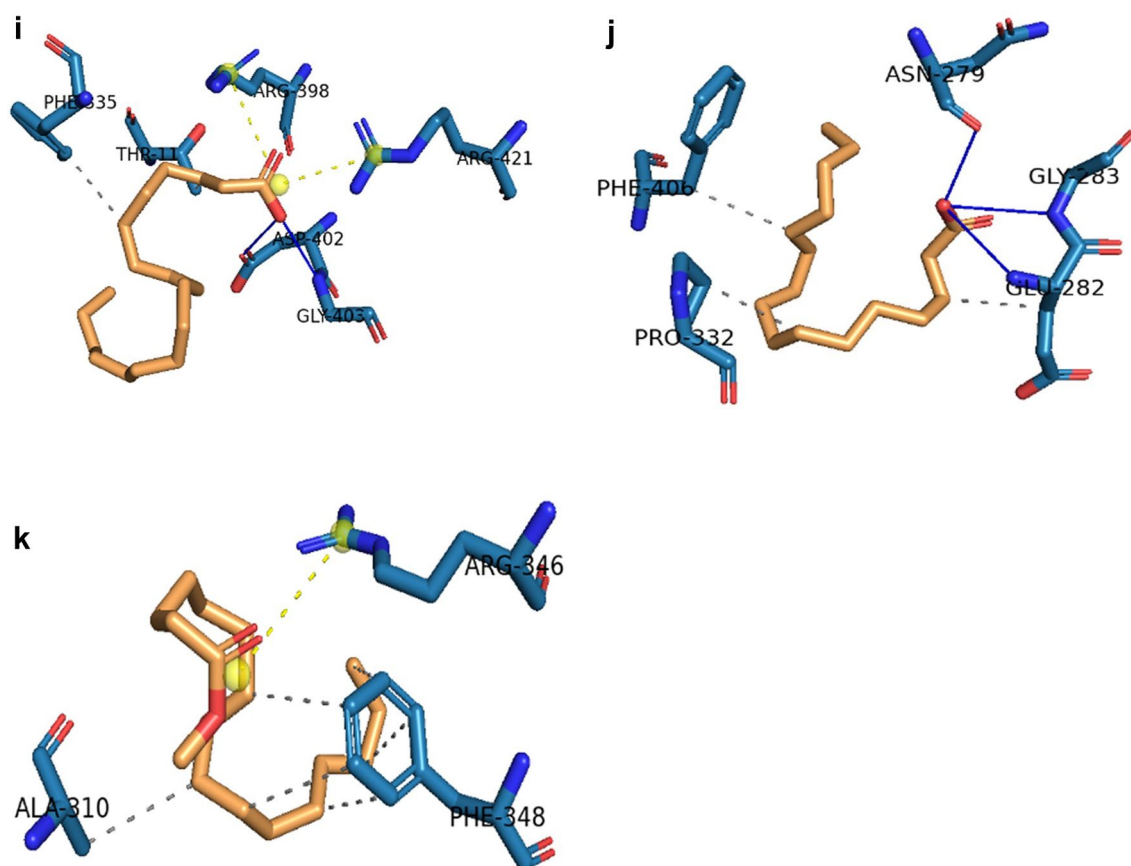


Fig. 5 (continued)

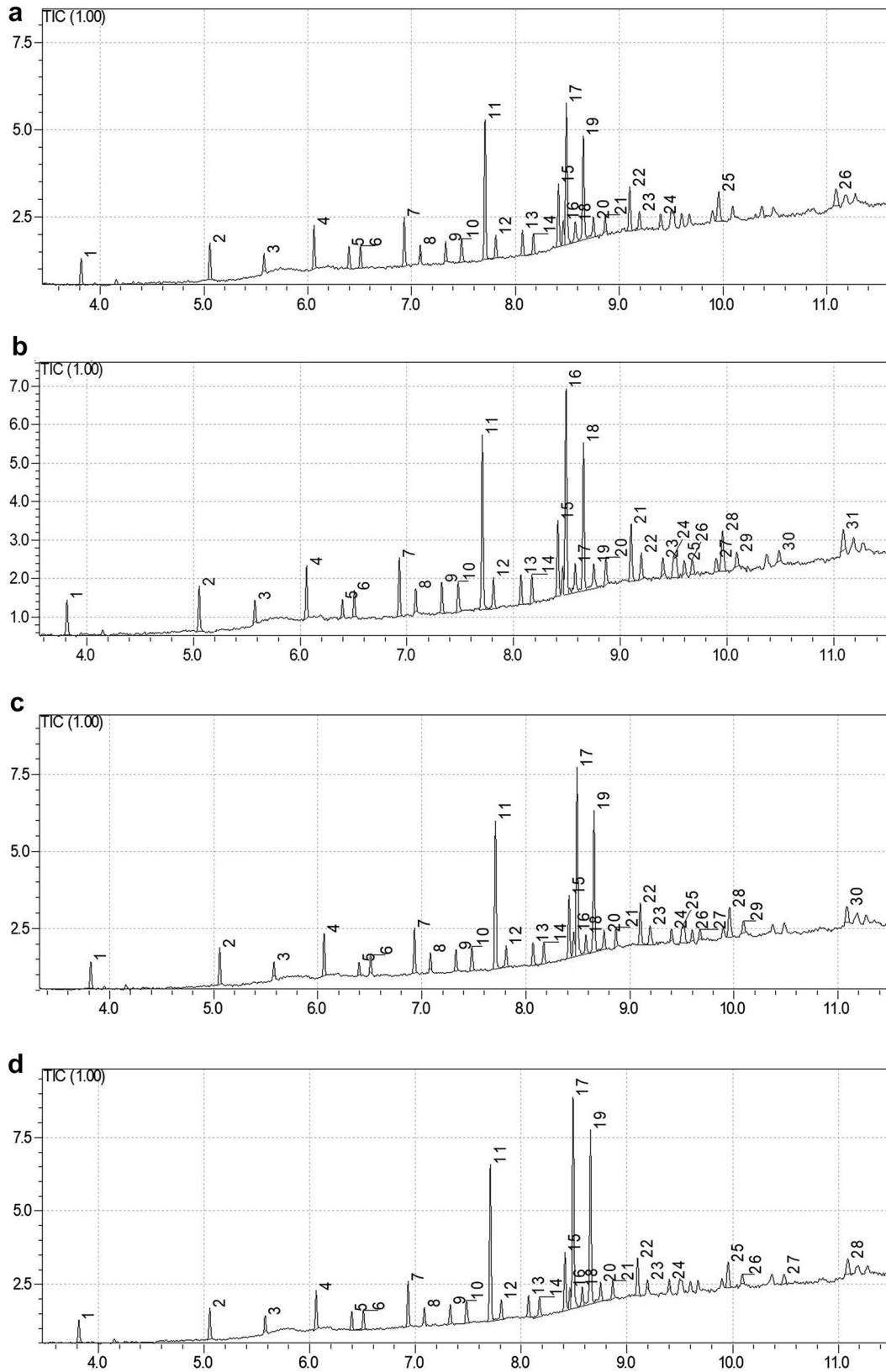
fatty acids, which are considered less detrimental to health compared to the saturated fatty acids present in animal fats (Sahoo et al. 2022). The slight increase in crude fat content percentage, in comparison to the control, is attributed to the inherently low fat content in *Pleurotus* spp. (Gupta et al. 2013; Koutrotsios et al. 2014; Fernandes et al. 2015; Ng et al. 2017). Various lipid classes constitute the fat fraction of mushrooms, including free fatty acids, mono-, di-, and triglycerides, sterols, sterol esters, and phospholipids (Lavelli et al. 2018). As a result, our findings provide detailed fatty acid data for each *Pleurotus* treatment subjected to fortification. The detailed fatty acid data for each *Pleurotus* fermentation treatment enhances our understanding of the lipid composition, highlighting potential health benefits associated with increased protein and unsaturated fatty acid content in fermented *Pulu Mandoti* Rice products (Gupta et al. 2013; Gogavekar et al. 2014; Lavelli et al. 2018; Omarini et al. 2019; Postemsky et al. 2019; Nacha et al. 2023).

Antioxidant activity was observed in each *Pleurotus* treatment, evaluated based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results indicate higher values

in *P. cystidiosus*, exhibiting an inhibition percentage of  $84.27 \pm 0.001\%$  at a concentration of 250 ppm and an  $IC_{50}$  value of 1.12 ppm, compared to fermentation treatments with *P. ostreatus* ( $81.16 \pm 0.001\%$ ) and *P. djamora* ( $79.19\% \pm 0.001\%$ ), having  $IC_{50}$  values of 3.81 ppm and 4.50 ppm, respectively. This observation could be attributed to *P. cystidiosus* having the highest total phenolic and flavonoid contents, contributing to its relatively robust antioxidant activity. Hoa et al. (2017) reported that *P. cystidiosus* exhibits highly efficient scavenging ability against DPPH free radicals due to its rich total phenolic content.

The evaluation of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay reveals distinctive variations in antioxidant potential among *Pleurotus* treatments. *Pleurotus cystidiosus* demonstrated the highest antioxidant activity, with a substantial inhibition percentage of  $84.27 \pm 0.001\%$  at a concentration of 250 ppm and an impressively low  $IC_{50}$  value of 1.12 ppm. This suggests a potent ability of *P. cystidiosus* to scavenge DPPH free radicals, highlighting its effectiveness against oxidative stress. The superior antioxidant performance aligns with





**Fig. 6** GC chromatograms showing fatty acid composition of **a** unfermented and fermented *Pulu Mandoti* by **b** *P. ostreatus*, **c** *P. cystidiosus*, **d** *P. djamor*

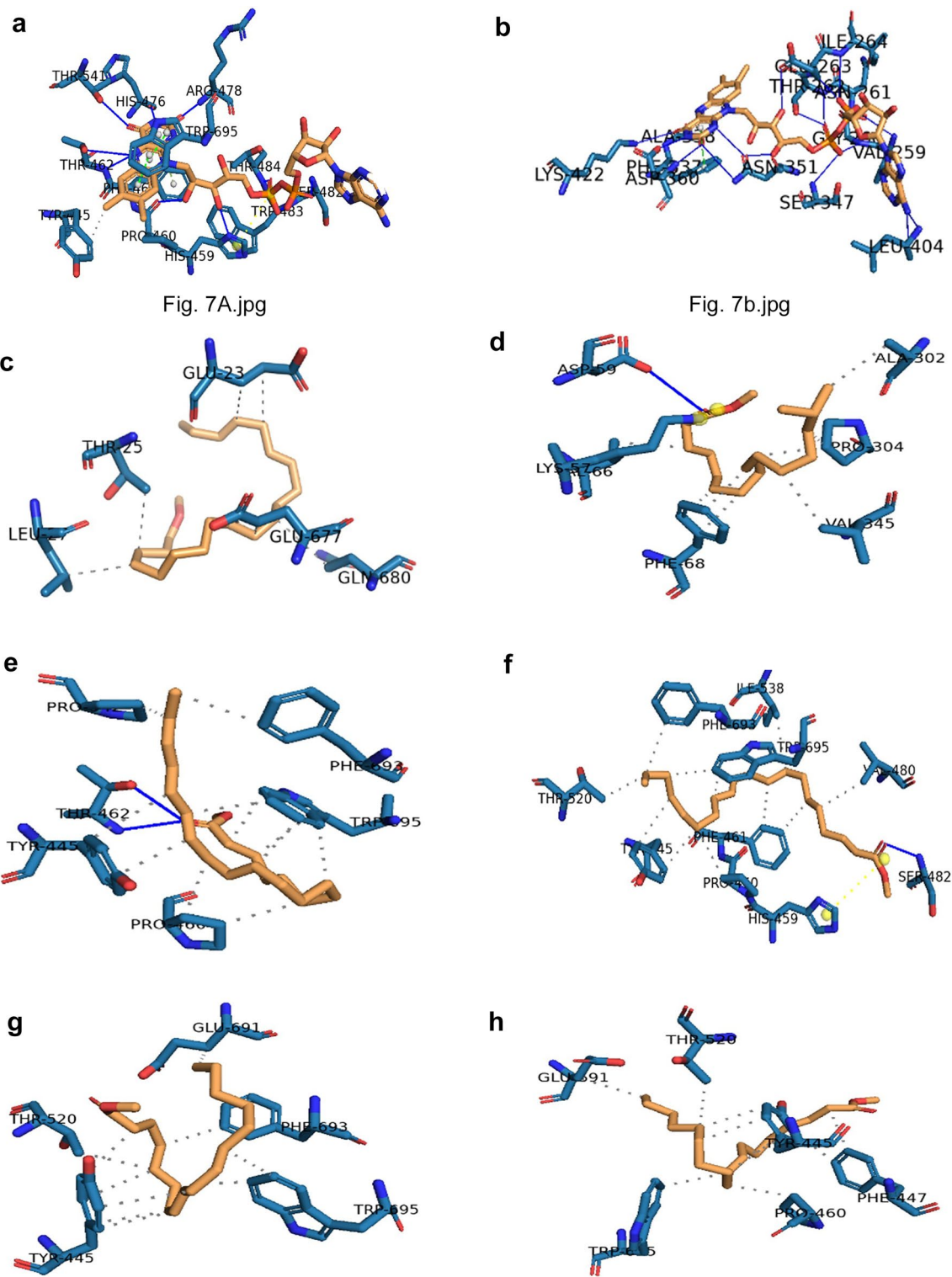


Fig. 7A.jpg

Fig. 7b.jpg

**Fig. 7** 3D interactions of 500x (a) and 2CKJ (b) (native ligands) PC (11,14,17-Eicosatrienoic acid, methyl ester) (c), PC (Hexadecanoic acid, 15-methyl-, methyl ester) (d), PC (11,14,17-Eicosatrienoic acid, methyl ester) (e), PC (11-Eicosenoic acid, methyl ester) (f), PD (5,8,11-Heptadecatrienoic acid, methyl ester) (g), PD (8-Octa-

decenoic acid, methyl ester) (h), PO (9,12,15-Octadecatrienoic acid, methyl ester) (i), PO (Methyl eicosa-5,8,11,14,17-pentaenoate) (j), PO (9,12,15-Octadecatrienoic acid, methyl ester) (k) and PO (Methyl eicosa-5,8,11,14,17-pentaenoate) (l) with amino acid residues of. Numbering of a chemical compound according to the order in

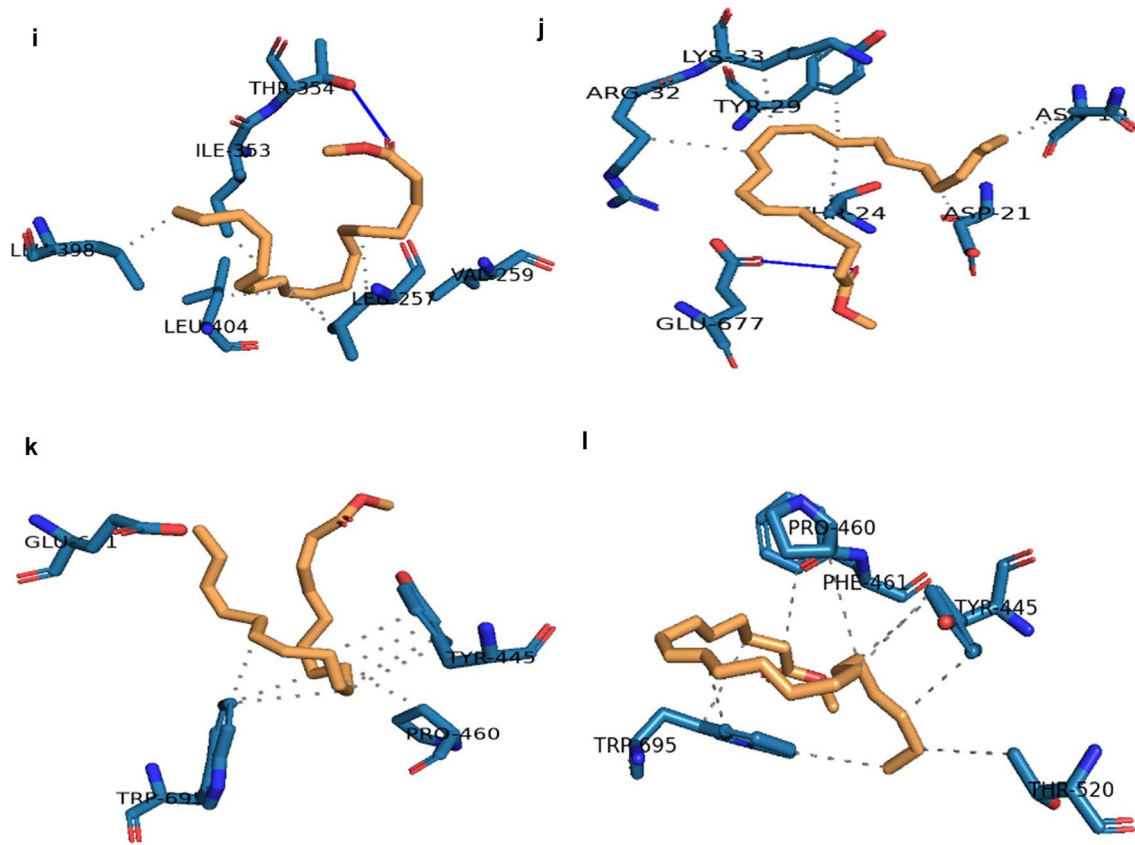


Fig. 7 (continued)

the notably high total phenolic and flavonoid contents in *P. cystidiosus*, establishing a clear correlation between these bioactive compounds and enhanced antioxidant activity.

In contrast, *P. ostreatus* and *P. djamor*, while still showing significant antioxidant activity with inhibition percentages of  $81.16 \pm 0.001\%$  and  $79.19 \pm 0.001\%$ , respectively, exhibited relatively higher  $IC_{50}$  values of 3.81 and 4.50 ppm. These values indicate that higher concentrations of extracts from *P. ostreatus* and *P. djamor* are needed for 50% inhibition of DPPH radicals compared to *P. cystidiosus*. The data highlights distinct antioxidant capacities among *Pleurotus* species, with *P. cystidiosus* standing out as particularly efficient in neutralizing free radicals. These findings offer valuable insights into the potential health benefits linked to consuming *Pulu Mandoti* Rice fermented with various *Pleurotus* species, emphasizing the importance of selecting mushrooms with high phenolic and flavonoid contents for enhanced antioxidant intake (Huyen et al. 2019; Omarini et al. 2019; Postemsky et al. 2019; Nacha et al. 2023).

In normal cellular metabolism, the human body continually produces reactive oxygen species (ROS) and free radicals. Endogenous enzymatic and non-enzymatic defense mechanisms typically eliminate these radicals and species. However, under certain conditions such as smoking, air

pollution, drug use, inflammation, and irradiation, endogenous antioxidant systems can become overactive, leading to oxidative stress and various conditions, including atherosclerosis, aging, and cancer (Khanday et al. 2019). The consumption of dietary antioxidants is inversely correlated with the risk of developing oxidative stress-related diseases. The identification of antioxidants in functional food sources, including mushrooms, is becoming an increasingly significant area of interest.

The  $\alpha$ -glucosidase inhibitory activity of fermented *Pulu Mandoti* Rice and its raw material, with a specific focus on the influence of different *Pleurotus* species on this bioactivity were investigated. Results revealed that the fermentation involving *P. cystidiosus* demonstrated the highest  $\alpha$ -glucosidase inhibition activity, reaching an impressive 81.11%, surpassing inhibition levels observed in treatments with other *Pleurotus* species. This indicates that *P. cystidiosus* possesses distinctive properties significantly contributing to the enhanced  $\alpha$ -glucosidase inhibitory potential of the fermented rice. The substantial inhibitory effect on  $\alpha$ -glucosidase is particularly relevant for managing blood glucose levels, highlighting the potential of *Pulu Mandoti* Rice fermented with *P. cystidiosus* as a functional food product with antidiabetic properties.

**Table 5** Receptor-ligand interaction of fatty acid compounds from fermented and unfermented *Pulu Mandoti* as antioxidants with their respective binding affinity and bond types

Receptor	Treatment	Compound	Binding affinity (kcal/mol)	H bond and hydrophobic interactions
2CKJ (Antioxidant)	Control	Native ligand	-9.4	Val259, Gly260, Asn261, Thr262, Glu263, Ile264, Ser347, Asn351, Asp360, Leu404, Lys422
500X (Antioxidant)	Control	Native ligand	-8.6	His459, Pro460, Thr462, His476, Arg478, Ser482, Trp483, Thr484, Thr541
2CKJ	PC	1s1,14,17-Eicosatrienoic acid, methyl ester	-5.2	Glu23, Thr25, Leu27, Glu677, Gln680
2CKJ	PC	Hexadecanoic acid, 15-methyl-, methyl ester	-4.6	Asp59, Val66, Phe680, Ala302, Pro304, Val345
500X	PC	11,14,17-Eicosatrienoic acid, methyl ester	-6.3	Tyr445, Pro460, Thr462, Pro542, Phe693, Trp695
500X	PC	11-Eicosenoic acid, methyl ester	-6.4	Tyr445, Pro460, Phe461, Val480, Ser482, Thr520, Ile538, Phe693, Trp695
500X	PD	5,8,11-Heptadecatrienoic acid, methyl ester	-4.8	Tyr445, Thr520, Glu691, Phe693, Trp695
500X	PD	8-Octadecenoic acid, methyl ester	-4.5	Tyr445, Phe447, Pro460, Thr520, Glu691, Trp695
2CKJ	PO	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	-5.7	Leu257, Val259, Ile353, Thr354, Leu398, Leu404
2CKJ	PO	Methyl eicosa-5,8,11,14,17-pentaenoate	-4.7	Asn19, Asp21, Thr24, Tyr29, Arg32, Lys33, Glu677
500X	PO	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	-4.6	Tyr445, Pro460, Glu691, Trp695
500X	PO	Methyl eicosa-5,8,11,14,17-pentaenoate	-6.2	Tyr445, Pro460, Phe461, Thr520, Trp695

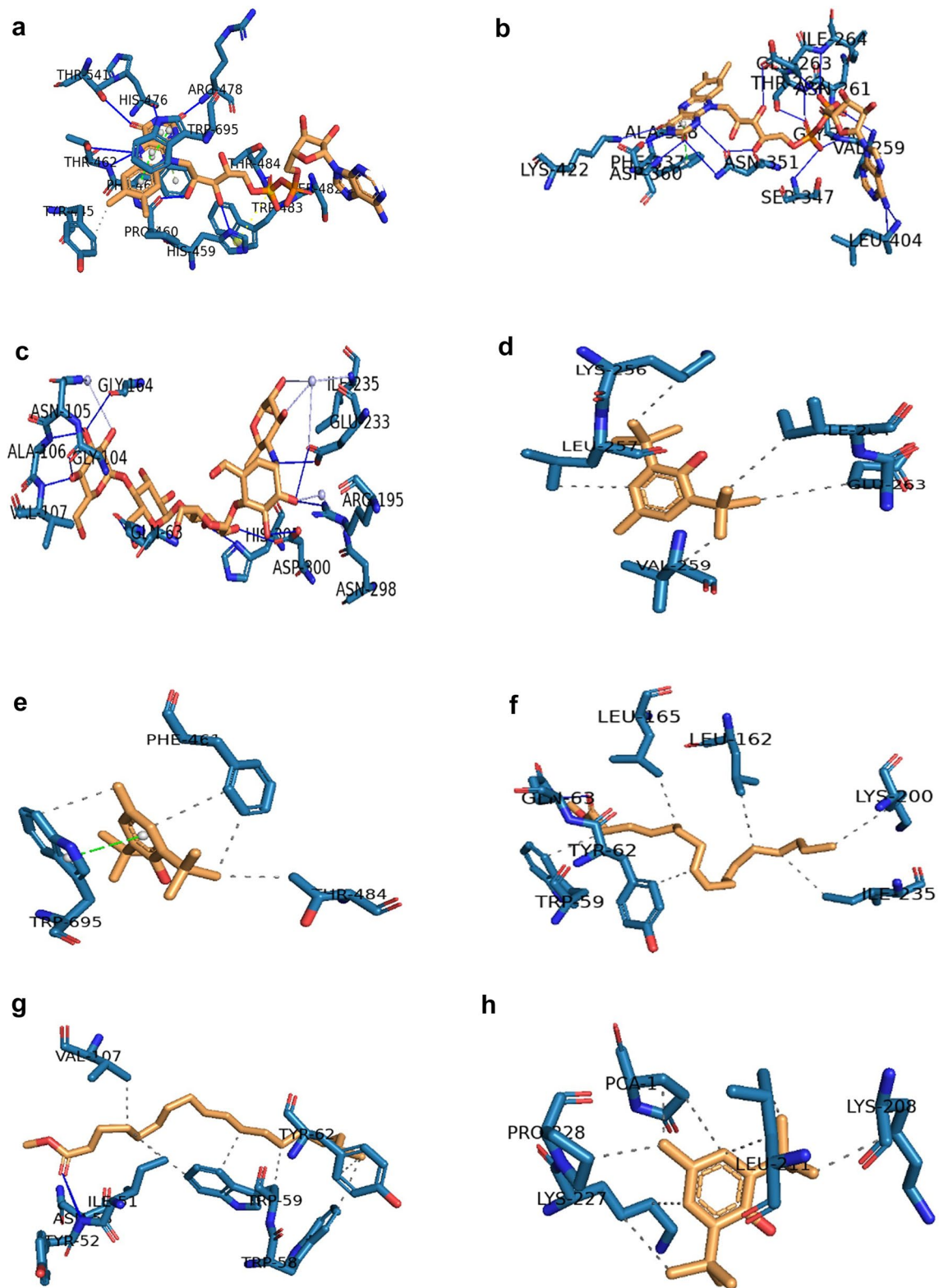
**Table 6** Receptor-ligand interaction of fatty acid compounds from fermented and unfermented *Pulu Mandoti* as antioxidants and antidiabetic with their respective binding affinity and bond types

Receptor	Treatment	Compound	Binding affinity (kcal/mol)	H bond and hydrophobic interactions
2CKJ (Antioxidant)	Control	Native ligand	-9.4	Val259, Gly260, Asn261, Thr262, Glu263, Ile264, Ser347, Asn351, Asp360, Leu404, Lys422
500X (Antioxidant)	Control	Native ligand	-8.6	His459, Pro460, Thr462, His476, Arg478, Ser482, Trp483, Thr484, Thr541
2QV4 (Antidiabetic)	Control	Native ligand	-9.0	Gln63, Gly104, Asn105, Ala106, Val107, Gly164, Arg195, Glu233, Asp300, His305
2CKJ	PC	Butylated hydroxytoluene	-6.9	Lys256, Leu257, Val259, Glu263, Ile264
500X	PC	11-Eicosenoic acid, methyl ester	-7.5	Phe461, Thr484, Trp695
2QV4	PC	9,12-Octadecadienoic acid, methyl ester	-5.5	Trp59, Tyr62, Leu162, Gln63, Leu165, Lys200, Ile235
2QV4	PC	9-Octadecenoic acid, methyl ester	-5.0	Ile51, Tyr52, Asn53, Trp58, Trp59, Tyr62, Val107
2QV4	PC	Butylated hydroxytoluene	-6.7	Pca1, Lys208, Leu211, Lys227, Pro228

Moreover,  $\alpha$ -glucosidase inhibition activity was observed in treatments with *P. ostreatus* and *P. djamor*, albeit at varying levels, suggesting that multiple *Pleurotus* species contribute to the antidiabetic effects of fermented *Pulu Mandoti*. This broadens the scope of potential mushroom varieties for developing functional food products targeting glycemic control. The findings signify the potential of fermented products as functional foods with implications

for diabetes management (Huyen et al. 2019; Omarini et al. 2019; Postemsky et al. 2019; Nacha et al. 2023). These align with previous research by Bello et al. (2017), suggesting that certain *Pleurotus* species, including *P. ostreatus*, could serve as alternative therapeutic options for type-2 diabetes treatment, providing a natural and dietary approach to mitigate reliance on conventional medications. The results highlight the differential  $\alpha$ -glucosidase inhibitory activities of various





**Fig. 8** 3D interactions of 500x (a), 2CKJ (b) and 2QV4 (c) (native ligands) PC (Butylated Hydroxytoluene) (d), PC (11-Eicosenoic acid, methyl ester) (e), PC (9,12-Octadecadienoic acid, methyl ester)

(f), PC (9-Octadecenoic acid, methyl ester) (g), and PC (Butylated Hydroxytoluene) (h) with amino acid residues

*Pleurotus* species in the context of fermented *Pulu Mandoti* Rice. The study not only added the potential health benefits of *P. cystidiosus* in enhancing antidiabetic properties but also expands the repertoire of *Pleurotus* species that may contribute to the development of functional foods relevant to blood sugar regulation and type-2 diabetes management (Huyen et al. 2019; Omarini et al. 2019; Nacha et al. 2023).

Fatty acid compounds were profiled from each treatment to highlight the uniqueness of fermented products from *pulo mandoti*. Fermentation treatments revealed a more varied profile of fatty acid compounds with potential antioxidant and antidiabetic properties compared to the control. Subsequently, selected antioxidant and antidiabetic compounds were further analyzed through a molecular docking approach. The analysis initiated by examining fatty acid compounds in the fermentation treatments involving three *Pleurotus* species. The findings reveal that these compounds interact with amino acid residue regions possessing receptors identical to their native ligands. Specifically, certain amino acids formed bonds with the 2CKJ receptor in *P. ostreatus* concerning the compound 9,12,15-octadecatrienoic acid, methyl ester. Notably, this compound, not previously documented in *Pleurotus* species, has been reported in the seed oil of *Opuntia ficus-indica*. Belonging to the esters category, it exhibits antioxidant, anti-inflammatory, antibacterial, antifungal, and anticancer properties (Alqurashi et al. 2022). Amino acid residues on the 500x receptor indicated binding with compounds in each treatment, namely, *P. cystidiosus* fermentation (compounds 11,14,17-eicosatrienoic acid, methyl ester, and 11-eicosenoic acid, methyl ester). The compound 11,14,17-eicosatrienoic acid, methyl ester has been reported in *P. ostreatus* (Das et al. 2023). Yang et al. (2018) reported that 11-eicosenoic acid, methyl ester was successfully extracted from *Pleurotus ferulae* and demonstrated antioxidant and antitumor activities. The *P. djamor* fermentation treatment yielded the compound 8-octadecenoic acid, methyl ester, and the *P. ostreatus* fermentation treatment yielded the compounds 9,12,15-octadecatrienoic acid, methyl ester, and methyl eicosa-5,8,11,14,17-pentae-noate. Octadecenoic acid, methyl ester has been reported in the edible wild mushroom, *Phlebopus beniensis*, showing antioxidant activity and other biological bioactivities (Campi et al. 2023). Docking results indicate that fatty acid profiling compounds have the potential to be candidates for antioxidants based on their negative binding affinity and amino acid residue interactions in the receptor regions, consistent with several test compounds.

The molecular docking analysis of fatty acid profiles concentrated on compounds generated from fermenting *Pulu Mandoti* using *P. cystidiosus*, considering its potential as a functional food with natural antioxidant and antidiabetic properties. The analysis revealed negative binding affinity values and identified interactions between receptor proteins

(antioxidant: 500X and 2CKJ, and antidiabetic: 2QV4) and potential compounds acting as ligands through amino acid residues. The results indicated that compounds such as 11-Eicosenoic acid, methyl ester, and butylated hydroxytoluene exhibited optimal interactions with the antioxidant receptors 500x and 2CKJ. Furthermore, butylated hydroxytoluene demonstrated interactions with the antidiabetic receptor 2QV4 alongside the compound 9-Octadecenoic acid, methyl ester. Butylated hydroxytoluene is a lipid compound from the primary volatile group found in *Pleurotus* (Zhao et al. 2023). Fatty acids naturally exist in a mixed form of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). Although the lipid content in mushrooms is relatively small, essential fatty acids constitute a significant portion of the lipid composition in various instances (Pedneault et al. 2006). The lipid composition of dehydrated mushrooms ranges from 1.75 to 15.5% per 100 g (Hong et al. 1988). Fatty acids play a crucial role in cellular functions, aiding in muscle contraction and overall metabolic regulation at the cellular, tissue, and organism levels. Polyunsaturated fatty acids contribute to various regulatory activities (Das et al. 2023).

## Conclusions

This study successfully reports the nutritional profile, antioxidant and antidiabetic activities, and molecular docking simulations of potential compounds in a local rice variety, *Pulu Mandoti* from Sulawesi after fermentation with *P. ostreatus*, *P. djamor*, and *P. cystidiosus*. Proximate analysis of fermented *pulo mandoti* with *P. cystidiosus* revealed higher percentages of dry matter ( $91.99 \pm 0.61\%$ ), crude protein ( $8.55 \pm 0.15\%$ ), and crude fat ( $1.34 \pm 0.05\%$ ) compared to fermentations with *P. ostreatus* and *P. djamor*. The antioxidant and antidiabetic activities in all fermentation treatments with *P. ostreatus*, *P. djamor*, and *P. cystidiosus* showed higher inhibition values than the unfermented or raw sample. Fermented *Pulu Mandoti* with *P. cystidiosus* exhibited superior inhibitory effects in antioxidant and antidiabetic tests compared to *P. ostreatus* and *P. djamor*. The molecular docking analysis of the fatty acid profiles in the fermentation *pulo mandoti* with *P. cystidiosus* identified compounds such as 11-Eicosenoic acid, methyl ester, and butylated hydroxytoluene, exhibiting optimal interactions with antioxidant receptors 500x and 2CKJ. Furthermore, butylated hydroxytoluene demonstrated interactions with the antidiabetic receptor 2QV4 alongside the compound 9-Octadecenoic acid, methyl ester. Local rice, *Pulu Mandoti*, fermented with *P. cystidiosus* holds significant potential as a functional food due to its diverse array of natural antioxidant and antidiabetic compounds.

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**Data availability** The authors declare data transparency.

## Declarations

**Conflicts of interest** The authors declare no conflict of interest.

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