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Functional efficacy of tempeh oil microemulsion containing omega 3 for Alzheimer's protection

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Abstract

Alzheimer's disease (AD) is associated with ageing symptoms due to stress oxidative, neuroinflammation, acetylcholinesterase and butyrylcholinesterase activation, and Tau hyperphosphorylation. Tempeh has been recognized as one of the Indonesian traditional fermented foods made from soybean fermentation with a microbial consortium. Our previous study demonstrated that tempeh oil contains polyunsaturated fatty acids (PUFAs) and antioxidants for ageing prevention. Tempeh oil rich in PUFAs may be developed as a modern functional supplement for AD protection. This study aimed to extract tempeh oil, formulate a tempeh oil microemulsion supplement, and determine its functional efficacy towards Alzheimer's protection *via* AD-related enzymatic inhibition, antioxidant capability, and AD-related gene regulation in Schwann neuronal cell model *in vitro*. Tempeh oil microemulsion contained omega 3 and exerted acetylcholinesterase inhibitory and antioxidant activities. Tempeh oil microemulsion also had the capability of gene regulation related to AD in the lipopolysaccharide-induced Schwann neuronal cells model. These findings indicated that tempeh oil microemulsion effectively suppressed the gene expression of *BACE*, *APP*, and *TNF-α,* and increased the *NTrK* and *BDNF* gene expression in Schwann neuronal cells induced by lipopolysaccharide. Therefore, tempeh oil microemulsion containing omega 3 could be offered as an alternative modern functional food supplement for ageing protection in particular AD.

1. Introduction

During the ageing process, the human brain often experiences a decline in cognitive or intellectual function which causes a reduction in the functional capacity of the brain which generally occurs in 10% of people aged 65 years and over (van der Flier *et al*., 2018). The decline in brain function is triggered by the occurrence of neurodegenerative diseases such as Alzheimer's disease (AD) which is caused by the accumulation of beta-amyloid and Tau proteins that lead to plaque formation in brain tissue (Fabelo *et al.*, 2014). Tempeh is the indigenous traditional fermented food in Indonesia that is made from soybean fermentation with the microbial consortium. Tempeh consumption is effective in preventing several diseases like neurodegenerative disease, cholesterol, diabetes, diarrhoea, and others. Tempeh diet also increased acetylcholine production, reduced beta-amyloid production, and improved memory function in older people (Chan *et al*., 2018; Handajani *et al.,* 2020). Antioxidants in tempeh isoflavones have been reported to increase the activity of acetylcholine, an essential neurotransmitter involved in memory. Daidzein as one of the antioxidants in tempeh has a similar structure to oestrogen receptors that are useful for a pre-menopausal woman to prevent AD and delay the ageing neurotransmitters (Gohari and Akhlaghi, 2018). Our previous study showed that tempeh oil contained major polyunsaturated fatty acids (PUFAs) for ageing protection (Subali *et al.,* 2019). Another study also reported that docosahexaenoic acid (DHA) as one of omega 3 PUFAs had a protective effect on the change of amyloid-beta precursor protein (APP) cleavage in the amyloidogenic pathway that led to beta-amyloid accumulation and AD induction (Jicha and Markesbery, 2010). Thus, screening of omega 3 in tempeh is important, and the presence of omega 3 in tempeh might be expected to play a similar role to DHA in the prevention and protection of AD.

Microemulsion is a strategy to improve the solubility, stability and bioactivity of the product,

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especially the oil form. This strategy is suitable to protect the quality of tempe antioxidant compounds from damage due to food processing and food storage processes and to help improve the solubility of tempe oil (Iradhati and Jufri, 2017). Microemulsions are composed of water, oil, surfactants and co-surfactants, where the surfactants and co-surfactants play a role in dissolving and stabilising the oil by lowering the surface tension formed. The aggregates formed after the homogenization between surfactants and oil would be delivered easily in the blood. Hence, microemulsion formulation drug delivery is regarded as a powerful delivery system for the absorption of poorly soluble drugs like oil (Jadhav *et al*., 2006). This research aimed to formulate tempeh oil extract in microemulsion and test its efficacy for Alzheimer's protection *via* inhibiting acetylcholinesterase (AcHE) and butyrylcholinesterase (BcHe) activities and ameliorating gene expression related to AD in Schwann neuronal cell model *in vitro*.

2. Materials and methods

2.1 Sample preparation

Tempeh was made from soybeans based on the method of Hashim *et al*. (2018). Two hundred fifty grams of soybean was weighed, sorted, and soaked overnight at room temperature, followed by boiling for 30 mins. Boiled soybean was dried and the skin was peeled. The soybean was rewashed until clean and recoiled for 15 min, followed by drying at room temperature. Dried soybean was mixed with 0.25 g of yeast and coated with banana leaves. Fermentation occurred for 48 hrs. Tempeh was then cut into small pieces and dried using a freeze-dryer for 48 hrs. Dried tempeh was blended and filtered using a 100′ mesh filter to obtain tempeh powder.

2.2 Extraction of tempeh oil

Tempeh oil extraction was carried out according to the method of Subali *et al.* (2019) with slight modification. Approximately 1 g tempeh powder was macerated with 20 mL ethanol and incubated for 3 days at room temperature. The solution was centrifuged at 1000×*g* for 3 mins. The supernatant was separated and evaporated using a rotary evaporator to obtain tempeh oil extract (TOE).

2.3 Formulation of tempeh oil microemulsion

TOE was formulated into tempeh oil microemulsion (TOM) products based on the modified method of Iradhati and Jufri (2017). Approximately 1 mL of TOE for formulation 1 (TOM F1) and formulation 2 (TOM F2) were mixed with 2 mL of Tween 80 and stirred until homogenous. Then, 0.5 mL of ethanol and 0.1 mL of distilled water were added to each formula. For TOM F2, 1 mL of virgin coconut oil (VCO) was added and mixed until TOM was formed, homogenized, and stable.

2.4 Physicochemical characterization of tempeh oil microemulsion

TOM was further tested for its physicochemical characterization and stability according to the method of Iradhati and Jufri (2017). TOM was stored at room temperature and its physicochemical characteristics were observed on days 0, 7, 14, 2, and 28 with several parameters, including colour, smell, and form. For the stability test, TOM was centrifuged at 5600 × *g* for 60 min, followed by observation. TOM which has passed the physicochemical characterization test was packaged in capsule gel as a supplement product.

2.5 Analysis of polyunsaturated fatty acids content in tempeh oil microemulsion

TOM was analyzed by gas chromatography (GC) using a DB FastFAME capillary column and helium carrier gas (Shibahara *et al*., 2018). Volume injection was 0.1 µL and done with split injection mode, while the temperature was set at 240°C. For sample preparation, tempeh oil extract and TEM were added with 0.5 M KOH, isopropanol and hexane, and then centrifuged for 3 mins. The top layer (organic phase) was taken and transferred to a new tube. The hexane solvent was evaporated, and the methylation process was carried out. KOH solution was added to a tube containing lipid extract, mixed, and heated at 100°C, followed by cooling at room temperature. The solution was then added to 1.5 mL of 20% BF_3 in methanol. Natrium chloride and hexane were added and remixed. The solution formed 2 layers, and the top layer was transferred to a new tube containing anhydrous Na_2SO_4, and then incubated at room temperature. The solution was transferred to a vial and injected into the column.

2.6 Analysis of acetylcholinesterase and butyrylcholinesterase inhibitory activities

TOM was tested for its functional efficacy in inhibiting AChE and BChE activities (Ovais *et al.*, 2018). TOM was dissolved in ethanol at variation concentrations (12.5-500 µg/mL). The enzymatic reagents were prepared as follows: buffer A (50 mM Tris HCl pH 8), buffer B (50 mM Tris HCl pH 8 plus 0.1% BSA), buffer C (50 mM Tris HCl pH 8, 0.1 M NaCl, and 0.02 M $MgCl_2.6H_2O$), and buffer D (50 mM NaH_2PO_4-$Na_2H_2PO_4$ pH 7.6). AcHE and BcHE enzymes were diluted using buffer A. Next, 25 µL of TOM sample, 125 µL of DTNB 0.25 mM in buffer C, 50 µL of buffer B, and 25 µL of ATCI 2.5 mM in distilled water were added to a 96-well plate, mixed, and incubated at 30°C

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for 10 mins. Furthermore, 25 µL of AcHE and BcHE (0.22 U/ml) were added and mixed. Absorbance was measured at 412 nm every 30 s using a microplate reader. Galanthamine (Gal), a commercial drug for AD treatment, was used as a positive control (C+) and an enzymatic reagent was used as a negative control (C).

2.7 Analysis of antioxidant activity

Antioxidant analysis of TOE and TOM was done using the ferric reducing antioxidant power (FRAP) method (Fernandes *et al*., 2016). FRAP reagent was prepared by mixing acetate buffer, $FeCl_3$, and 2,4,6-Tris (2-pyridyl)-S-triazine (TPTZ) in the ratio of 10:1:1. Acetate buffer was prepared by mixing acetic acid and sodium acetate, while 10 mM TPTZ was diluted in 40 mM HCl and 3mM $FeCl_3$ was made in 5 mM citric acid. All solutions for FRAP reagent were mixed and homogenized, followed by incubation at 37°C for 30 mins. For sample analysis, a 20 µL sample was mixed with 180 µL FRAP reagent, and then incubated at 37°C for 6 mins. The absorbance was measured at 595 nm and $FeSO_4.7H_2O$ was used as the standard solution.

2.8 Cell growth and treatment

Schwann neuronal cells (ATCC CRL3765) was used for the experimental Alzheimer's cell model and lipopolysaccharide (LPS) was employed to stimulate neuroinflammation in cells as one of Alzheimer's symptom (Panarsky *et al*., 2012). Cells were cultured in DMEM complex for 24 hrs until reached 80% of confluence. LPS from *Salmonella typhosa* at 1 µg/mL was used to induce neuroinflammation in cells. For treatment, cells were divided into five groups, including negative control (LPS untreated), positive control (LPS treated), TOE (50 and 100 mg/mL), and TOM F1 (50 and 100 mg/mL). After treatment, cells were incubated for 24 hrs, and the lysates were harvested for further analysis.

2.9 Quantitative PCR analysis

Total RNA was extracted using GENEzol reagent and quantified using Nanodrop. Total RNA was further analyzed for cDNA synthesis using SensiFAST™ cDNA Synthesis Kit (Bioline) prior to qPCR. Gene expression analysis was performed using qPCR with oligonucleotide primers specific for Alzheimer's genes, including brain-derived neurotrophic factor (*BDNF*), neurotrophic tyrosine receptor kinase 1 (*NTrk1*), beta-secretase (*BACE*), *APP*, and tumour necrosis factor-a (*TNF-a*) (Table 1). *Beta-actin* was used as the housekeeping gene. All oligonucleotide primers were designed using the Primer-BLAST program from the National Center for Biotechnology Information. The mixture reaction included 10 µl of SoFastÔ EvaGreen® Supermix, 1 µl of primer, 1 µl of template, and 8 µl of nuclease-free water. The qPCR reaction was carried out for 40 cycles. The conditions for the amplification reaction were 92°C for 5 s, annealing temperature for 30 s, and 72°C for 30 s.

2.10 Statistical analysis

Experiments were performed in three repetitions and data were presented as mean±standard deviation (SD). Significant differences between the control and sample treatment were statistically analyzed using paired *t*-tests. Gene expression was analyzed using one-way ANOVA with a significant difference of ($P < 0.05$).

3. Results and discussion

3.1 Tempeh oil microemulsion and its characteristics

Figure 1 shows TOE and TOM supplements. TOE had a very strong smell and yellow colour, while TOM effectively reduced the smell in TOE and maintained the colour stability. TOM was made in 2 formulas (TOM F1 and F2) with yields of 25 and 20% v/v, respectively. TOMs were packaged in gel capsule supplements for storage. Table 2 shows the physicochemical characteristic results of TOMs based on the observation

Table 1. Oligonucleotide primers for Alzheimer's markers.

Gene	Function	Sequences	Size (bp)
BDNF	Cholinergic system	F: GTGTGACCTGAGCAGTGGGCAAAGGA R: GAAGTGTACAAGTCCGCGTCCTTA	554
*NTrk*1	Cholinergic system	F: TTCAATGGCTCCGTGCTCAATG R: GGTCTCCAGATGTGCTGTTAGTGT	274
BACE	β-amyloid	F: GGATTATGGTGGCCTGAGCA R: CCAGGATGTTGAGCGTCTGT	667
APP	β-amiloid	F: AGCAGAAGGACAGACAGCAC R: AGTGGTCAGTCCTCGGTCAG	455
TNF-α	Inflammation	F: GGCAGGTCTACTTTGGAGTCATTG R: ACATTCGAGGCTCCAGTGAATTCGG	319
B-actin	Internal control	F: TGGAATCCTGTGGCATCCATGAAAC R: TAAAACGCAGCTCAGTAACAGTCCG	439

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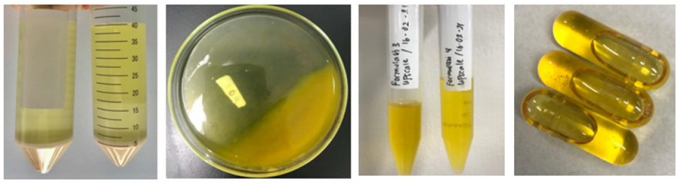
Table 2. Physicochemical characterization of tempeh oil microemulsion.

TOM	Parameters	Days				
		0	7	14	21	28
F1	Colour	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent
	Smell	Oil Aromatic	Oil Aromatic	Oil Aromatic	Oil Aromatic	Stronger Oil Aromatic
	Form	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation
F2	Colour	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent	Yellow Transparent
	Smell	Oil Aromatic	Oil Aromatic	Oil Aromatic	Oil Aromatic	Oil Aromatic
	Form	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation

TOM: tempeh oil microemulsion, F1: formula 1, F2: formula 2.

on days 0, 7, 14, 21 and 28. There was no phase separation in microemulsion products (TOM F1 and F2) nor change in colour and smell, indicating that these TOM products were stable. In terms of PUFA detection, GC data demonstrated that TOM F2 contained higher omega 3 PUFA compared to TOM F1 (Table 3). In addition, both TOMs also had omega 6 PUFA.



(a) (b) (c) (d)

Figure 1. Extraction of tempeh oil and formulation of tempeh oil microemulsion. Note: (a), tempeh supernatant; (b), tempeh oil extract; (c), tempeh oil microemulsion; and (d) tempeh oil microemulsion in gel capsule supplement.

Table 3. PUFA content in tempeh oil microemulsion.

TOM	Omega 3 (mg/mL)	Omega 6 (mg/mL)
F1	3.97±0.05	35.10±0.15
F2	14.96±0.33	11.00±0.00

TOM: tempeh oil microemulsion, F1: formula 1, F2: formula 2.

In this study, tempeh was made by conducting soybean fermentation using a microorganism consortium, followed by tempeh powder production and tempeh oil extraction. TOE was obtained with a high yield (~70%). Fermentation of tempeh has two stages, including the first fermentation by bacterial activity that happens during soaking of the soybean and the second fermentation by moulds when inoculated. During the fermentation, yeasts also support interactions between microorganisms, form the texture, and biosynthesis of tempeh flavour.

For the supplement product, TOE was formulated in microemulsion supplement products (TOM) (Figure 1). There were 2 formulas of TOM, including TOM F1 (TOE with Tween 80 and ethanol) and TOM F2 (TEM F1 with VCO addition). Our results demonstrated that both TOM F1 and F2 had good physicochemical characteristics in terms of homogeneity, colour, and smell (Table 2). There was no phase separation in TOMs, indicating that the products were consistently homogenous and stable. Also, there was no change in colour and smell in TOMs after observation for 28 days.

The microemulsion is a dispersion system between oil and water which is stabilized by surfactant and cosurfactant or without cosurfactant. Characteristics of microemulsion are thermodynamically stable, isotropic systems, composed of water, oil, and surfactant, the small size of droplets (d < 100 nm), low viscosity and high solubility due to low interfacial tension (Iradhati and Jufri, 2017). Microemulsion is one of the drug delivery systems that can increase the bioavailability of a drug or product that is difficult to dissolve in water. The use of surfactants in microemulsion formulation can help to increase the penetration rate of drug transport to the intestinal walls. Microemulsion can be applied in several types of drug delivery, including oral delivery (Jadhav *et al*., 2006). This technique is appropriate for the formulation of TOMs as a food supplement candidate for the protection of AD.

After formulation and characterization, TOM products were further determined for their PUFA composition using GC analysis. TOMs contained omega 3 and omega 6 (Table 3), whereas TOM F2 exerted higher omega 3 content compared to that of the F2 one. It seems that the VCO addition in the TOM F2 formula also contributed to the increased level of omega 3. The ageing brain is prone to the development of neurodegenerative diseases such as Alzheimer's disease (AD). Consumption of omega-3 is important for neurocognitive development and normal brain function, where the cognitive function of the elderly increases and slows the risk of AD (Ward *et al*., 2012). Omega 3 is a

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polyunsaturated long chain that can be found in foods and can not be synthesized by the body. Omega 3 consists of alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and DHA. EPA and DHA have the ability to reduce brain cell damage caused by beta-amyloid plaque and inhibit the formation of Tau protein. Omega 3 also prevents neuronal apoptosis by inhibiting caspase 3 activity and regulates BDNF synthesis where BDNF plays a role in synaptic development, synaptic plasticity, and cognitive function (Dyall, 2015).

3.2 Effect of tempeh oil microemulsion on inhibiting AcHE and BcHE activities

Figure 2a demonstrated that TOMs exerted a higher moderate inhibitory effect at 30-50% towards AChE activity than that of the TOE. At 100 mg/mL, TOM F2 inhibited AChE activity up to 50%. Compared to the Gal standard, TOMs had a lower inhibition against AChE. Moreover, both TOE and TOMs also had a slight

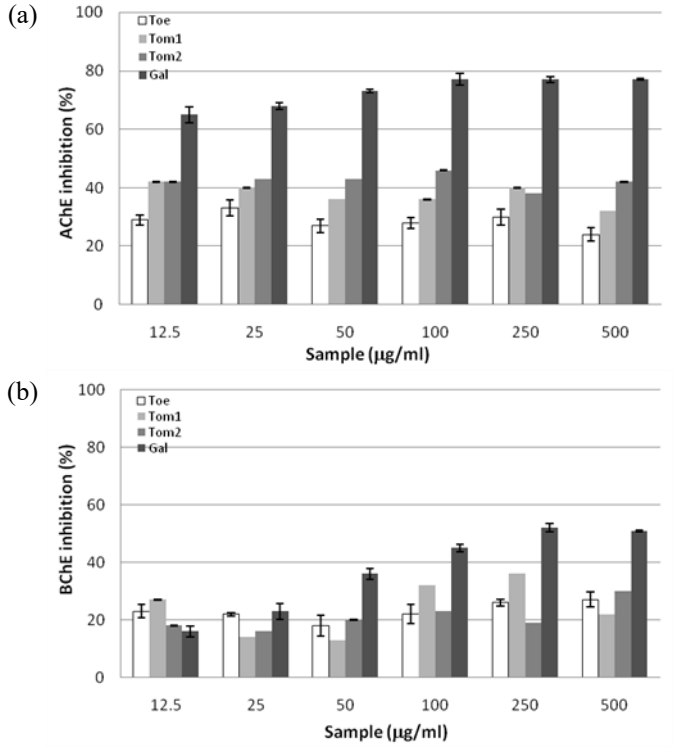

Figure 2. Effect of tempeh oil extract and tempeh oil microemulsion on inhibiting acetylcholinesterase and butyrylcholinesterase activities. Toe: tempeh oil extract, Tom1: tempeh oil microemulsion formula 1, Tom2: tempeh oil microemulsion formula 2, Gal: galanthamine standard.

inhibition (15-30%) on BChE activity as well as Gal standard (Figure 2b).

TOMs were further tested for their functional efficacy to inhibit AChE and BcHE enzymes as well as TOE. Our data showed that TOMs exerted higher potency for inhibiting AChE than TOE one (Figure 2a). Microemulsion formula for TOE increased its functional efficacy for enzyme inhibitory mechanism. Microemulsion has lipophilic and hydrophobic compounds. AChE has 3 domain active sites, including choline-binding site, acyl binding pocket, and peripheral anionic site. The acyl binding pocket is a hydrophobic site in which hydrophobic compounds in microemulsion (TOMs) have more binding sites compared to that of oil extract one (TOE) (Chaiyana *et al.,* 2010).

Interestingly, for BChE results, our findings demonstrated that both TOE and TOMS had lower efficacy in inhibiting BChE as well as Gal standard (Figure 2b). It is assumed that the ability of TOMs on AChE inhibition is correlated with the use of ATCI substrates that is specific for degradation by AChE. Based on these results, TOMs could be offered as AChE inhibitor candidates for AD protection. In this study, Gal is used as a drug standard (positive control) that has acted as an AChE inhibitor that directly inhibits both AChE and BChE activities. Gal is a reversible inhibitor type, and the binding of Gal to AChE will slow down the catabolism of Ach leading to the increased level of Ach in the synaptic cleft (Handajani *et al*., 2020).

AD is also known to be influenced by the role of the cholinergic neurons. The deficit of cholinergic function is associated with the degradation of learning capacity and memory. In Alzheimer's pathology, the increasing hydrolysis of acetylcholine (Ach) by AChE and BChE results in a significant decrease in Ach concentration. Ach has a role as a neurotransmitter and is synthesized by a choline acetyltransferase (ChAT) enzyme. Thus, AChE and/or BChE inhibitor agents can be used to treat AD by targeting them.

3.3 Antioxidant activity of tempeh oil microemulsion

Antioxidant activity was determined by FRAP assay based on OD value for free radical scavenging action. Both TOE and TOM exerted antioxidant capacity in the dose-dependent manner (Figure 3). Interestingly, TOE showed a higher antioxidant effect compared to that of TOM.

It has been known that antioxidant capacity is also associated with a therapeutic strategy for ageing-related diseases including AD. In a previous study, tempeh isoflavones have been claimed to have a protective effect against beta-amyloid 42 mediated impairment of memory and learning (Glazier and Bowman, 2001). Genistein in tempeh isoflavones is used as a substitute for estrogen and a relatively selective estrogen receptor of ERβ. Genistein also has a structural similarity with estrogen and presents in areas of the brain associated with memory and learning. In elderly women, it is noted that the amount of ERβ is reduced (Uddin and Kabir, 2019). The binding genistein to ERβ plays a role in

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ameliorating the memory deficit due to AD, especially in elderly women. Oxidative stress is known as one of the factors in AD pathogenesis. The generation of reactive oxygen species (ROS) in the brain can be increased due to the beta-amyloid accumulation that results in oxidative stress. Fascinatingly, genistein exerts an antioxidant effect against beta-amyloid accumulation and reduces ROS production (Soni *et al.,* 2014). Genistein and daidzein have been reported to inhibit apoptosis in the cells and reduce caspase 3 up-regulation (Uddin and Kabir, 2019).

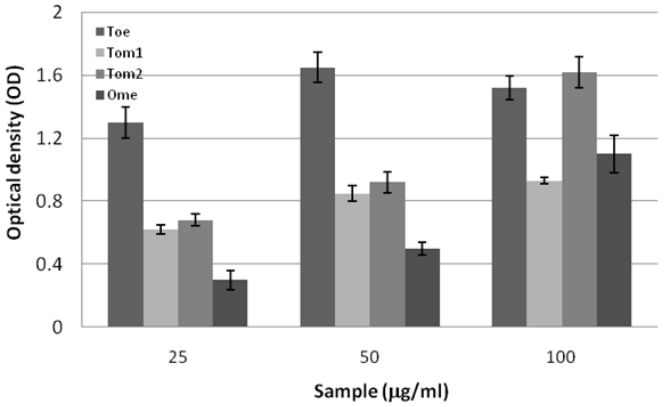

Figure 3. Antioxidant activity of tempeh oil extract and tempeh oil microemulsion. Toe: tempeh oil extract, Tom1: tempeh oil microemulsion formula 1, Tom2: tempeh oil microemulsion formula 2, Ome: commercial omega 3.

3.4 Effect of tempeh oil microemulsion on regulating gene expression related to AD in LPS-induced Schwann neuronal cells

TOM was tested against 5 gene markers related to AD in Schwann neuronal cells induced by LPS (Figure 4). TOM at a low concentration (50 mg/mL) affected the expression of down-regulatory genes, such as *BACE, APP,* and *TNF-α* in LPS-induced Schwann cells (Figures 4a, 4b, and 4c). TOM was found to slightly increase the up-regulation of *NTrK* and *BDNF* genes compared to that of TOE (Figures 4d and 4e). Both TOEs and TOMs showed similar actions on the regulation of genes related to AD in LPS-induced Schwann cells.

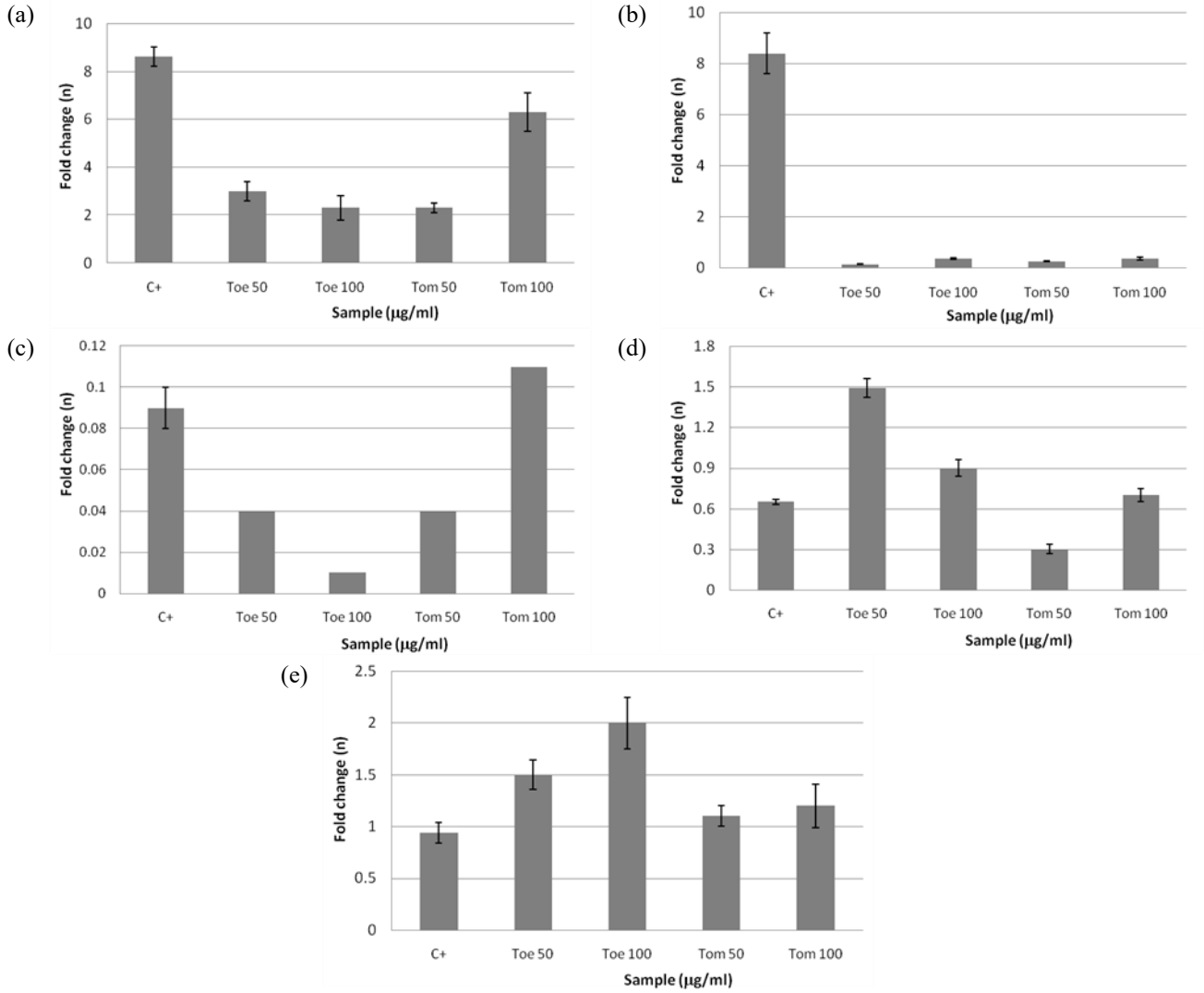

Figure 4. Effect of tempeh oil extract and tempeh oil microemulsion on inhibiting gene expression related to Alzheimer's disease, including (a) *BACE*, (b) *APP*, (c) *TNF-α*, (d) *NTRK1*, and (e) *BDNF* in lipopolysaccharide-induced Schwann neuronal cells. acetylcholinesterase and butyrylcholinesterase activities. Data is statistically significantly different at $P < 0.05$ to control. C+: positive control (LPS treated), TOE: tempeh oil extract, TOM: tempeh oil microemulsion formula 1, Gal: galanthamine standard.

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It is known that the early onset of AD is caused by mutations in the *APP* gene (Weggen and Beher, 2012). *APP* gene mutations cause abnormalities in APP metabolism and beta-amyloid deposition in the brain. In the normal brain, cleavage of APP protein is induced by alpha-secretase in the non-amyloidogenic pathway, while in pathological conditions including AD, APP is cleaved by BACE in the amylodoigenic pathway. BACE is an enzyme that regulates the accumulation of beta-amyloid. Therefore, the use of BACE as a marker target for AD is ideal. The decreased level of BACE is associated with lowering the risk of AD development (Li *et al*., 2012). In line with our data (Figure 4a), *BACE* gene expression was significantly attenuated by treatment of TOE and TOM in LPS-induced Schwann cells. It seems that TOM and TOE containing omega 3 exerted the potential ability to suppress the expression of the *BACE* gene in LPS-induced Schwann neuronal cells.

The decrease of APP expression can prevent the formation of aggregates of beta-amyloid causes AD (Basun *et al.,* 2008). Beta-amyloid is toxic to neurons, causes apoptosis and cell death, and disturbs the homeostatic balance of calcium ions (Li *et al.*, 2012). DHA omega 3 has been reported to act as a neuroprotective agent by reducing beta-amyloid toxicity (Lim *et al*., 2005). As shown in Figure 4b, TOE and TOM significantly ameliorated the gene expression of *APP* compared to that of control in LPS-induced Schwann neuronal cells. In terms of dosage, both TOE and TOM treatments were found to be more effective at lower concentrations (50 µg/mL) on down-regulating *APP* genes compared to the positive control. Statistically, there was no significant difference between *APP* gene levels in TOE and TOM ($P>0.05$).

Experimental and clinical evidence has demonstrated that in the Alzheimer's brain, there is an increased synthesis of pro-inflammatory cytokines, such as TNF-α. This cytokine also participates in the development of chronic inflammation when not counterbalanced by anti-inflammatory cytokines, and stimulates the expression of APP and BACE that induces the formation of senile plaque (Chang *et al*., 2017). Our data expressed that *TNF-α* gene expression was quite low in Schwann neuronal cells induced by LPS (Figure 4c). It is due to the non-selective capability of LPS as a stimuli agent for inducing pro-inflammatory cytokines at various cell types including Schwann neuronal cells. Treatments of TOE and TOM effectively suppressed *TNF-α* gene expression, but not in TOM at high concentrations (100 µg/ml). It seems that the functional efficacy does not correlate with the dose-dependent pattern.

Furthermore, we also tested the efficacy of TOE and TOM for generating gene expression of *NTRK1* and *BDNF* in LPS-induced Schwann cells (Figures 4d and 4e). TOE had a significant up-regulatory effect on *NTRK* and *BDNF* gene expression in cells compared to TOMs. Interestingly, TOE exerted a dose-dependent pattern on the up-regulation of *BDNF* gene expression in LPS-induced Schwann cells. High levels of these *NTRK* and *BDNF* gene expressions can lower the risk of AD. The NTRK gene is located on chromosome 1 and codes three tyrosine receptor kinase receptors (TrkA, TrkB, and TrkC) for specific mechanisms of activation and regulation signalling in the brain (Chen *et al*., 2008).

In AD pathology, there is a significant down-regulation of TrkA, TrkB, and TrkC expression in individual cholinergic nucleus basalis neurons. Activation of the BDNF/TrkB pathway in cells causes dephosphorylation of Tau, while inactivation of TrkB decreases the dephosphorylation of Tau (Tanila, 2017). Moreover, the reduction of BDNF-TrkB signalling expression has a crucial role in AD pathology which is indicated by the formation of Aβ and neurofibrillary tangles composed of hyperphosphorylated Tau protein. These findings suggest that *NTRK* and *BDNF* are potential gene markers for the development of AD.

4. Conclusion

Tempeh oil extract has been successfully formulated into microemulsion supplement products containing omega 3. These tempeh microemulsion products had the capability to protect against Alzheimer's disease through several mechanisms like inhibiting acetylcholine esterase activity, exerting antioxidant activity, and regulating several genes related to Alzheimer's disease in cell models *in vitro*. Tempeh oil microemulsion effectively down-regulated the gene expression of *BACE*, *APP,* and *TNF-α* and up-regulated the *NTrK* and *BDNF* gene expression in Schwann neuronal cells induced by lipopolysaccharide. In conclusion, tempeh oil microemulsion containing omega 3 could be offered as an alternative modern functional food supplement for ageing protection in particular Alzheimer's disease.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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