# 國立臺灣海洋大學食品科學系碩士班
# 專題討論書面報告

# 探討 *Laminaria japonica* 之發酵產物對抗氧化及抗發炎活性之影響


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## 摘要

本篇報告主要探討昆布 (*Laminaria japonica)* 萃取物及發酵產物之成分、抗氧化活性及抗發炎活性，並探討其對於腸道發炎現象是否具保護效果。Cui *et al*. (2016) 透過熱水及檸檬酸萃取法萃取昆布多醣 (LJPA-P)，並藉管柱層析分離純化出 LJPA-P1、P2、P3。結果顯示三種經純化之多醣的單醣組成、硫酸根基團、糖苷鍵、分子量和抗氧化活性均不相同。在所有樣品中，LJPA-P3 硫酸根基團和醣醛酸之含量最豐富，並表現出非常高的 ORAC 氧化自由基吸收能力和 ABTS 自由基清除活性。Lin *et al*. (2016) 研究昆布經枯草桿菌 (*Bacillus subtilis)* 發酵後，其抗氧化及抗發炎效果之變化。結果顯示，在含 5%（w/v）的昆布、2% 的額外氮源、起始 pH 為 6、37˚C、150 rpm 的條件下以 *Bacillus subtilis* 發酵 72 小時之產物具有最佳的抗炎作用。與 LPS 誘導組相比，NO 生成量下降至 27.6 % ± 5.2%。此外，發酵產物亦可抑制 LPS 誘導所造成之活性氧（ROS）、phosphorylated NF-κB 及 $PGE_2$ 的產生。腸道上皮屏障受損導致通透性增加時，易造成發炎反應，因此 Yang *et al*. (2019) 透過益生菌 (LGG、BB-12) 對昆布萃取物進行發酵，並分析萃取物及發酵物之抗發炎及對於腸道屏障系統的保護作用。結果顯示，昆布萃取物及發酵產物具有抑制 LPS 誘導的 NO 和 interleukin-6 (IL-6) 大量生成之功效、修復 Caco-2 細胞單層膜通透性增加現象及調控 tight junction 相關蛋白質 occludin 及 AMPK 之表現，進而達到保護腸道之效果。綜合上述，發酵可增強昆布多醣之抗氧化及抗炎活性，並可藉此開發出保護腸道之保健食品。

# 前言

潰瘍性結腸炎屬於發炎性腸道疾病的一種，其主要流行於歐洲及北美地區，亞洲地區病例相對較少。近年來由於飲食逐漸西化，亞洲地區流行率正逐年成長中。其發病原因尚不明瞭，可能原因為基因、環境壓力造成腸道黏膜屏障受損，使發炎物質進入組織中引起反覆的免疫反應所造成 (Wallace *et al*., 2014)。

Tight junction (TJ) 在腸道屏障系統中扮演重要角色，上皮細胞透過與 TJ 結合形成抵抗外界刺激的重要屏障。TJ 受損會導致屏障功能失調，導致各種病原體、抗原和內毒素可以輕易滲透到胃腸道的上皮內層，引起腸道異常炎症和發炎性腸道疾病 （IBD） 等慢性疾病 (Xavier & Podolsky, 2007)。Occludin 是 TJ 的組成蛋白之一，occludin 的缺乏會導致屏障通透性的增加，因此其對於 TJ 屏障的穩定性及完整性相當重要 (Kuo *et al*., 2019; Lee, 2015)。AMPK 是能量代謝的主要調節因子，具有一個催化 α-subunit，並有兩種異構體 α1 和 α2。 AMPKα1 主要在腸道上皮細胞中表達。AMPK 可藉由增加跨上皮電阻改善腸道屏障功能完整性 (Caplan *et al*., 2008; Sun *et al*., 2017)。

*L. japonica* 別名昆布、江白菜，屬於褐藻綱 (Phaeophyceae) 海帶目 (Laminariales) 海帶科 (Laminariaceae) 海帶屬 (Laminaria)，具有豐富營養價值，包含可溶性纖維（例如褐藻酸鹽和褐藻醣膠）以及脂溶性成分（例如褐藻素和海藻固醇）以及礦物質（例如鎂、碘、鈣、鐵和鋅）。其生理活應包含抗氧化 (Wang *et al*., 2008)、降血脂 (Huang *et al*., 2010)、抗菌 (Kim *et al*., 2013)、抗發炎 (Park *et al*., 2013)。

## 一、 昆布多醣之結構與抗氧化活性分析 (Cui *et al*., 2016)

昆布具有抗氧化特性，但其多醣的結構特徵與抗氧化活性之間的關係鮮少被探討，因此本篇利用改良萃取方法，並以管柱層析獲取純化樣品，最終分析樣品組成及抗氧化活性之差異。

使用樣品為昆布(青島)。將採摘獲得之昆布清洗、乾燥研磨成粉末，以 40 目之篩網過篩。以 120ºC 熱水 (1:30, w/v) 加熱 3 小時去除水溶性多糖，將其離心 (3000g, 15 min) 並乾燥 (40ºC) 後，將 100 g 乾燥產物與 3L 檸檬酸 (pH 2.0) 進行萃取。萃取產物以減壓濃縮方式濃縮至 600 L，與乙醇混合使乙醇濃度為 (80%, v/v) 後置於 4 ºC，隔日進行離心 (4000g, 20 min)，將離心產物以 Sevag reagent (chloroform:n-butyl alchol =4:1, v/v) 去除蛋白質得到粗萃取物 (LJPA-P)。最終以 DEAE-Sepharose fast flow column (2.5 x 20 cm) 及不同濃度梯度之 NaCl 分餾得到純化產物 (LJPA-P1~P3)。分析方面，對粗萃取物及純化產物進行成分組成、甲基化、紅外光譜、分子量及抗氧化能力分析。

粗萃取多醣之萃取率為 7.56％，純度為 67.37％ 。純化結果方面，以管柱層析得到主要產物之產物為 LJPA-P1~3，分別於 NaCl 濃度為 0.1、0.2、0.3 M 時析出，產率分別為 1.84％、3.42％ 及 0.93％，純度分別為 70.42%、61.65％ 和 54.94％，並且在純化過程會造成 18％ 耗損 (Figure 1)。成分組成方面，LJPA-P、LJPA-P1 及 LJPA-P2 主要單醣為 galactose、mannose 及 glucose。LJPA-P3 則只有 galactose 及 mannose。四種樣品中，僅有 LJPA-P 及 LJPA-P3 含有硫酸根團，並且 LJPA-P3 之含量為 LJPA-P 之三倍。醣醛酸組成方面，各樣品主要皆以 glucuronic acid 為主。有研究指出，硫酸根基團和醣醛酸可使多醣帶負電，使多醣具有更多的化學和生物活性 (Table 1)。紅外光譜儀可透過記錄分子吸收紅外光之後所呈的振動模式鑑別化合物和確定物質分子結構。3405－3423 和 1077 $cm^{-1}$ 處的信號表示 O－H 鍵的伸縮振動，表明多醣間存在強烈的分子間作用；2930－2933 $cm^{-1}$ 微弱信號表示 C－H 伸縮振動；1610 和 1420 $cm^{-1}$ 信號表示羧酸酯基團中不對稱的 C-O 和對稱的 C=O 伸縮振動；1298 – 1302 $cm^{-1}$ 微弱信號表示 C-H 彎曲振動；1235–1257 $cm^{-1}$ 表示 S=O 不對稱伸縮振動；1034±1 $cm^{-1}$ 表示醛醣醛酸中 C－O 與 C－O－SO 3 互相對稱伸縮振動；889-892 $cm^{-1}$ 信號表示 β- linked pyranose residue (Figure 2)。多醣分子大小方面以分子篩層析管柱進行分析，LJPA-

P1 和 LJPA-P2 平均分子量分別為 19.07 和 30.34 kDa，LJPA-P3 有兩個峰，平均分子量為 64.82 和 19.21 kDa (Figure 3)。抗氧化活性方面，在 ORAC 試驗中，LJPA-3 展現最佳抗氧化能力 （1247.22 μmol Trolox / g），其次為 LJPA-P （305.77 μmol Trolox / g）、LJPA-P1 （129.01 μmol Trolox / g）、LJPA-P2 （135.53 μmol Trolox / g）(Figure 4)。ABTS 試驗中，抗氧化活性之強度排列亦為如此 (Figure 5)，推測是由於醣醛酸及硫酸根具增強多醣抗氧化能力之作用 ∨ (Song *et al*., 2010; Sun-Waterhouse *et al*., 2007)。

## 二、 枯草桿菌發酵可增進昆布萃取物之抗發炎活性 (Lin *et al*., 2016)

昆布具有抗氧化及抗發炎活性，但多數萃取法是採用有機溶劑進行萃取，因此本篇選用 *Bacillus subtilis* 對昆布進行發酵，探討發酵過程對於昆布多醣之抗氧化及抗發炎活性的影響。

本篇昆布購自傳統市場 (產地為金門)。將昆布清洗、乾燥研磨成粉末 (1mm pore)。利用不同條件進行發酵後，測量其 pH、微生物生長曲線、NO 產生量試驗以找出適當的培養條件，發酵條件包含不同濃度之昆布、額外添加不同單醣、不同氮源、不同發酵溫度及不同之搖瓶速度。確認最佳發酵條件後，測量其產物之細胞毒性、細胞內活性氧 (ROS) 含量，以 western blot 分析發炎因子 NF-κB 及以 ELISA 分析前列腺素 E2($PAGE_2$)。發酵使用菌株為枯草桿菌 *Bacillus subtilis*，為革蘭氏陽性的好氧性細菌，普遍存在於土壤及植物體表，在人體及反芻動物腸胃道中亦可發現共生之 *B. subtilis*。

細胞實驗使用 RAW264.7 巨噬細胞。NO 抑制能力方面，以 LPS 誘導會導致 NO 生成量顯著上升，而給予 *L. japonica* 萃取物可有效減輕 LPS 誘導 NO 產生的現象，此外 *L. japonica* 在經 *B. subtilis* 發酵後，展現更佳的抑制 NO 的效果 (Figure 6)。推測是因為 *B. subtilis* 會釋放酵素將細胞壁分解，有利於抗發炎物質的釋放。以不同條件測試發酵之效果，可發現枯草桿菌皆在發酵 12 小時後

進入 stationary phase，並且 pH 達到發酵期間之最低值，推測是因昆布中 Mannitol 可作為枯草桿菌之能量來源並被代謝為酸性產物；12h 之後，pH 值上升，則是由於 Mannitol 被代謝完畢，改以氮源作為能量來源。最適合發酵條件為含量 5%（w / v）的昆布、2% 的額外氮源、起始 pH 為 6、37˚C、150 rpm，此條件之發酵產物具有最佳的抗炎作用。發炎抑制相關機制方面，活性氧被認為是發炎之第二信使。ROS 的過度表達會造成多種病理反應，並誘導促發炎細胞因子過度產生。昆布發酵產物具有抑制 LPS 刺激造成之 ROS 大量生成的效果，並且隨劑量提高，抑制現象呈現濃度依賴性 (Figure 12)。NF-κB 是發炎反應中關鍵轉錄因子，LPS 的刺激會造成 NF-κB 磷酸化進而誘導發炎相關物質生成釋放，例如: phospholipase A2 (PLA2)、 cyclooxygenase-2 (COX-2) 及 Prostaglandin E2 （$PGE_2$），昆布發酵產物可濃度依賴性的抑制 NF-κB 活化並抑制 $PGE_2$ 之生成 (Figure 13、14)。

## 三、 昆布發酵產物藉調控免疫反應及 tight junction 相關蛋白強化腸道屏障系統之功能性 (Yang *et al*., 2019)

腸道上皮細胞是作為腸道免疫防禦性最前線，其可耐受革蘭氏陰性菌等外部之刺激，保護組織免於受到破壞。昆布已被證實具有抗發炎及抗氧化之效果，但鮮少研究深入探討其對於腸道發炎之保護效果。此外，研究亦證明藉微生物發酵改善增強原材料之生物活性 (Kang *et al*., 2012; Lin *et al*., 2016; Martins *et al*., 2011)，因此本篇選用人類腸道共生菌株 *Lactobacillus rhamnosus GG* (LGG) 及 *Bifidobacterium animalis* subsp. *lactis* BB-12 (BB-12) 對昆布萃取物進行發酵，並藉人類腸道上皮細胞 Caco-2 模擬腸道屏障，研究昆布萃取物及發酵物分別對於腸道上皮屏障完整性和功能性具有何種效果。

本篇昆布購自傳統市場 (產地為濟州島)。將昆布清洗、乾燥研磨成粉末。以 200 mL double-distilled water ($ddH_2O$) 與 3 g 昆布乾燥粉末之比例，於常溫下進

行震盪萃取 24 小時，再以抽氣過濾去除雜質。此時，萃取物分為兩類：(1) 直接冷凍乾燥之產物 LJE (2) 分別添加 LGG、BB-12 或 LGG + BB-12 菌株，以 37 ºC 發酵 48 小時後才進行冷凍乾燥之產物 LJE-F1、LJE-F2 及 LJE-F3。化學分析方面，對樣品分析其 pH、總醣、還原醣及總酚含量。細胞實驗以 Caco-2 細胞進行實驗，進行實驗包含 MTT 細胞活力試驗、跨膜電阻試驗 transepithelium electrical resistance (TEER)、NO 生成試驗、ELISA 及 western blot。

成分組成變化方面，LJE-Fs 中 pH、總醣及還原醣之含量經過發酵皆顯著下降，但總酚之含量並為顯著差異 (Table 2)。根據研究，發酵過程除了透過合成方式提高食物營養成分外，也會造成生物利用度提高 (*Hubert et al., 2008; Katina et al*., 2007)，因此 LGG 及 BB-12 之發酵會導致 LJE-Fs 中總糖和還原醣等物質含量降低。細胞毒性方面，不同劑量之 LJE 或 LJE-Fs 對細胞存活率皆無顯著改變，最終選用細胞活性改變程度最小之劑量 (100 μg/ mL) 進行後續實驗 (Figure 16)。NO 釋放試驗方面，LJE 及 LJE-Fs 皆顯著改善 LPS 刺激造成 NO 大量生成之現象，但 LJE 及 LJE-Fs 間並無顯著差異 (Figure 17)。在細胞單層膜通透性保護效果方面，以 TEER 值判斷細胞單層膜的完整性，TEER 值愈低表示通透程度愈高，即細胞屏障受損愈嚴重。LJE 及 LJE-Fs 皆顯著抑制 LPS 刺激造成痛透性增加之現象，其中 LJE 與 LJE-F1 之改善效果更加優秀，其 TEER 值相較於未受刺激組無顯著差異 (Figure 18)。發炎相關細胞因子表現方面，LJE 及 LJE-F1 對於 TNF-α 皆具有顯著抑制效果，而 LJE-F2 及 LJE-F3 之 TNF-α 表現量雖然稍微降低，但皆未達到顯著差異。IL-6 表現量方面，LJE 與 LJE-Fs 皆能抑制其生成，但 LJE-Fs 展現更佳的抑制能力 (Table 3)。對於腸道屏障保護效果方面，選用 occludin 和 AMPK 兩種 TJ 相關蛋白進行評估。Occludin 蛋白表現量上，各樣品皆有顯著改善作用，但 LJE-F3 之效果相對其餘三者較差；AMPK 蛋白表現量上，LJE、LJE-F1 和 LJE-F3 顯著促進其磷酸化，而 LJE-F2 則否 (Figure 19)。以上結果表明 LJE 和 LJE-Fs 會差異性地調節 TJ 相關蛋白 (occludin 和 AMPK)

表現，達到增強腸上皮屏障功能之效用。

## 四、 結論

昆布可藉由抑制細胞內活性氧 ROS 的產生、NO 的生成及發炎相關細胞因子之表現進而減少發炎之程度，並可調節部分 tight junction 相關蛋白增強腸道屏障之功能，達到保護腸道免受外界刺激之作用。此外，透過發酵過程可增強昆布本來具有之抗氧化、抗發炎活性，因此發酵是一種具有前景性之產品製備方法。

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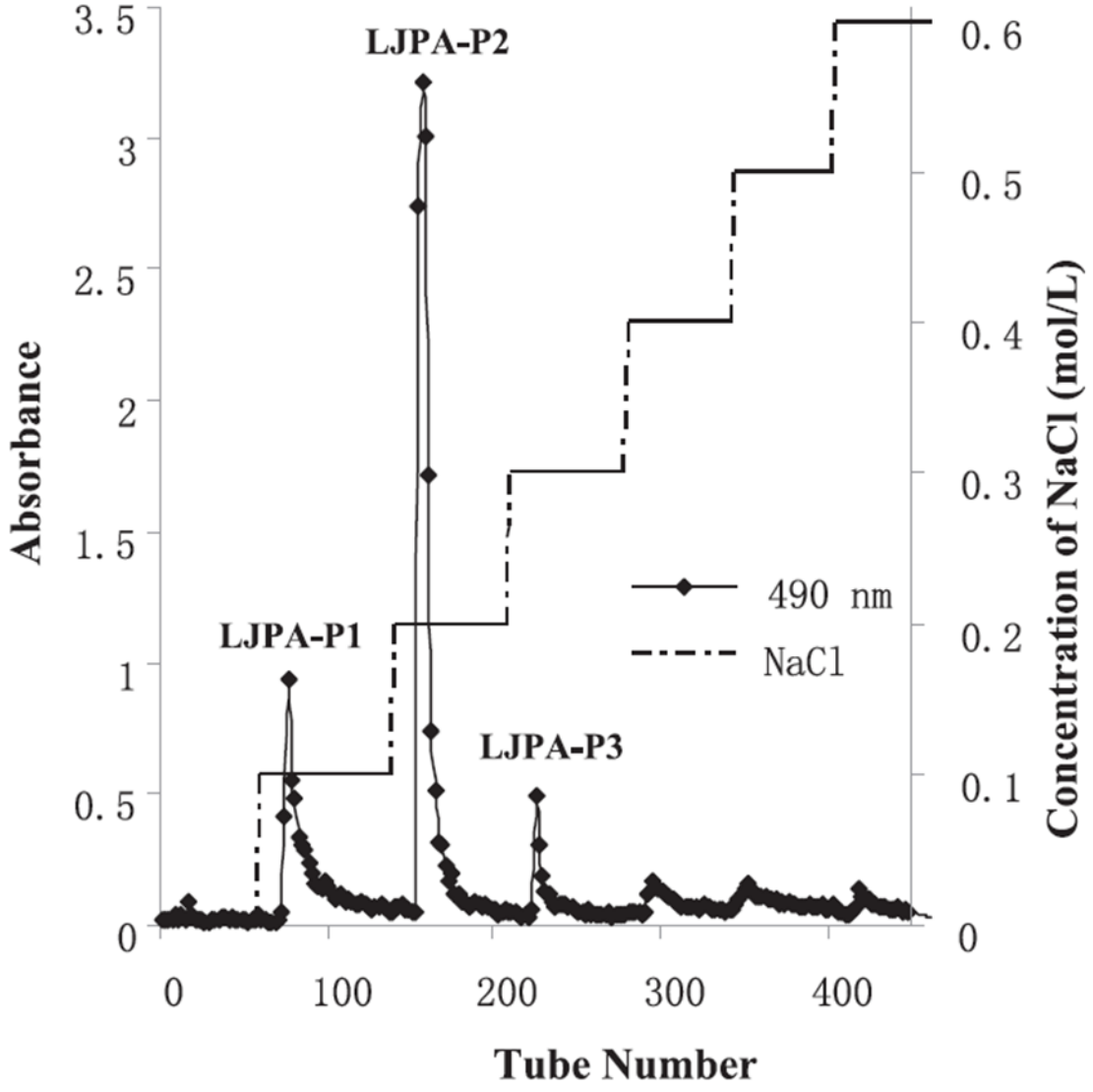


Figure 1. DEAE-Sepharose fast flow chromatogram of the crude polysaccharides from *Laminaria japonica* (LJPA-P) with distilled water, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 M NaCl elutions.

(Cui *et al*., 2016)

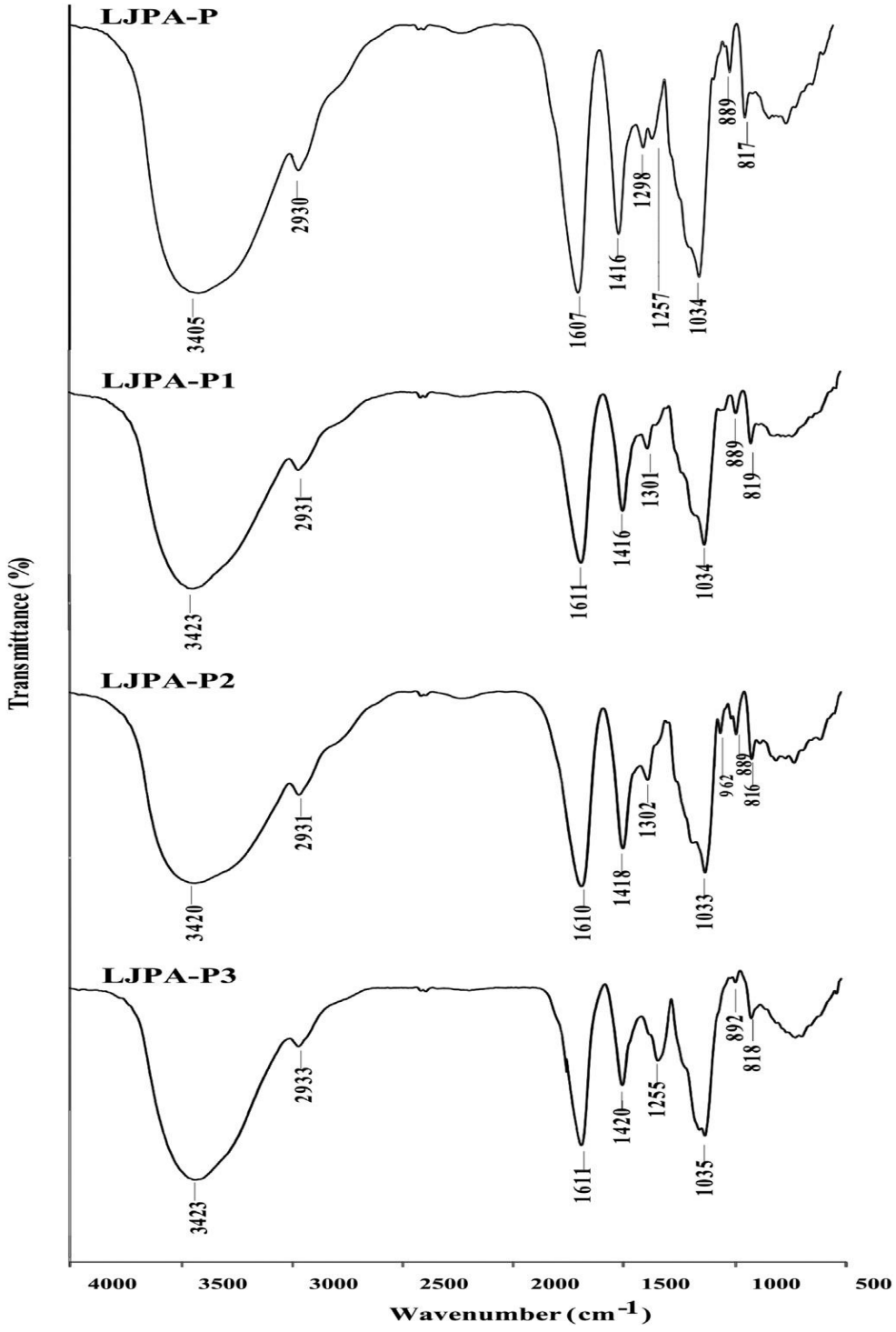


Figure 2. FT-IR spectra of LJPA-P, LJPA-P1, LJPA-P2 and LJPA-P3.

(Cui *et al*., 2016)

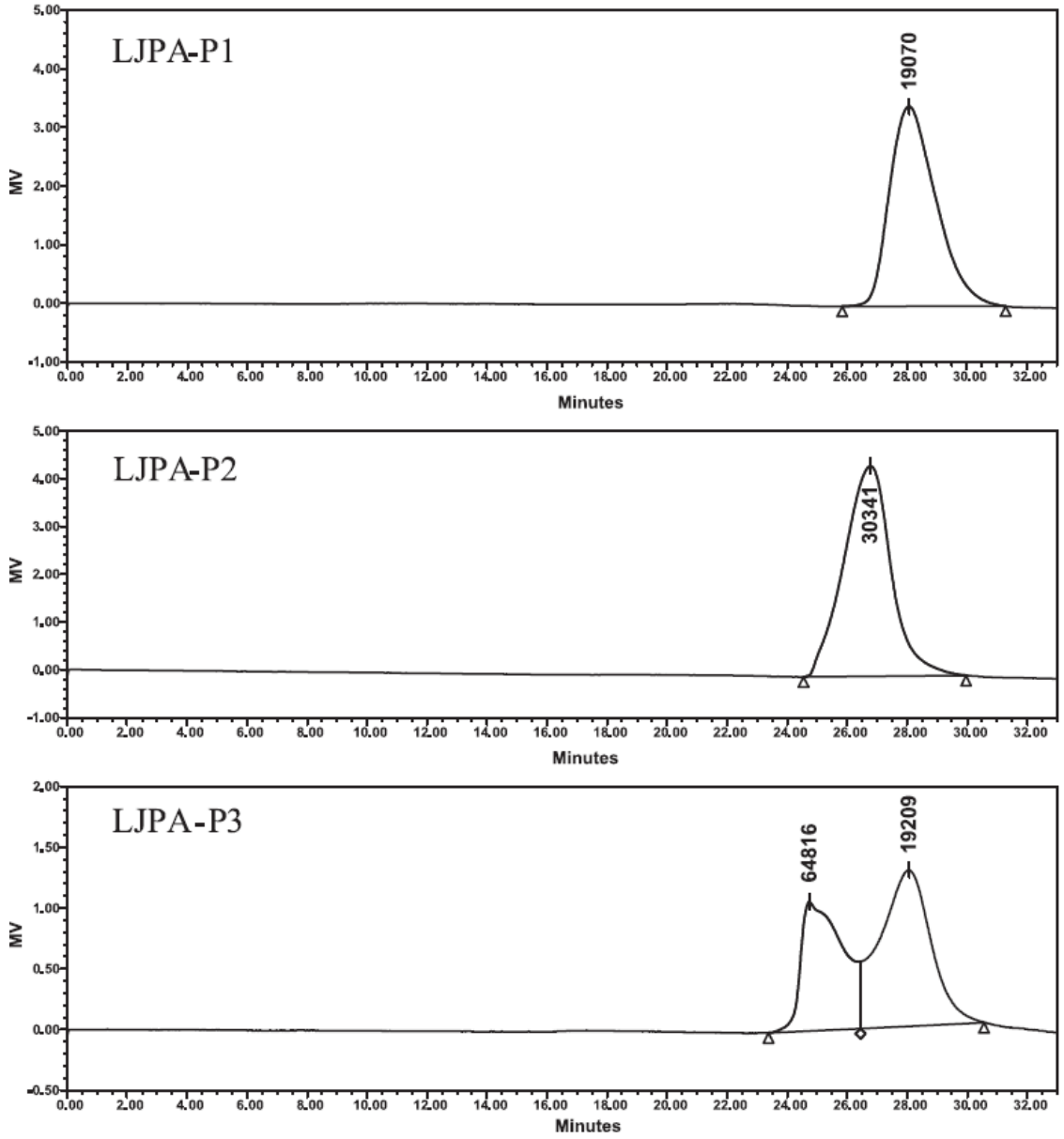


Figure 3. Molecular weights of LJPA-P1, LJPA-P2 and LJPA-P3.

(Cui *et al*., 2016)

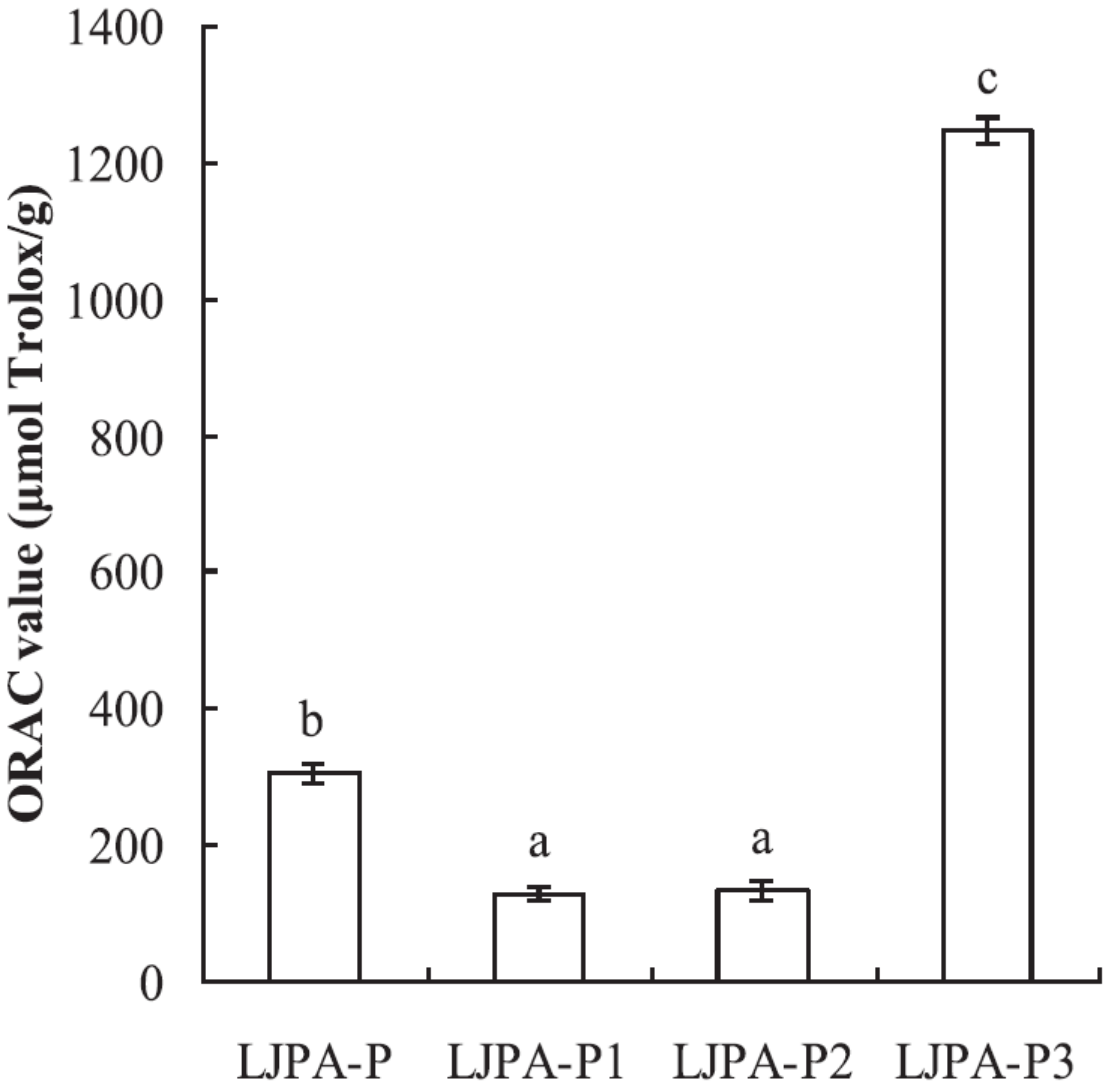


Figure 4. Oxygen radical absorbance capacity (ORAC) of LJPA-P, LJPA-P1, LJPA-P2 and LJPA-P3. Different letters indicate significant difference, $p < 0.05$.

(Cui *et al*., 2016)

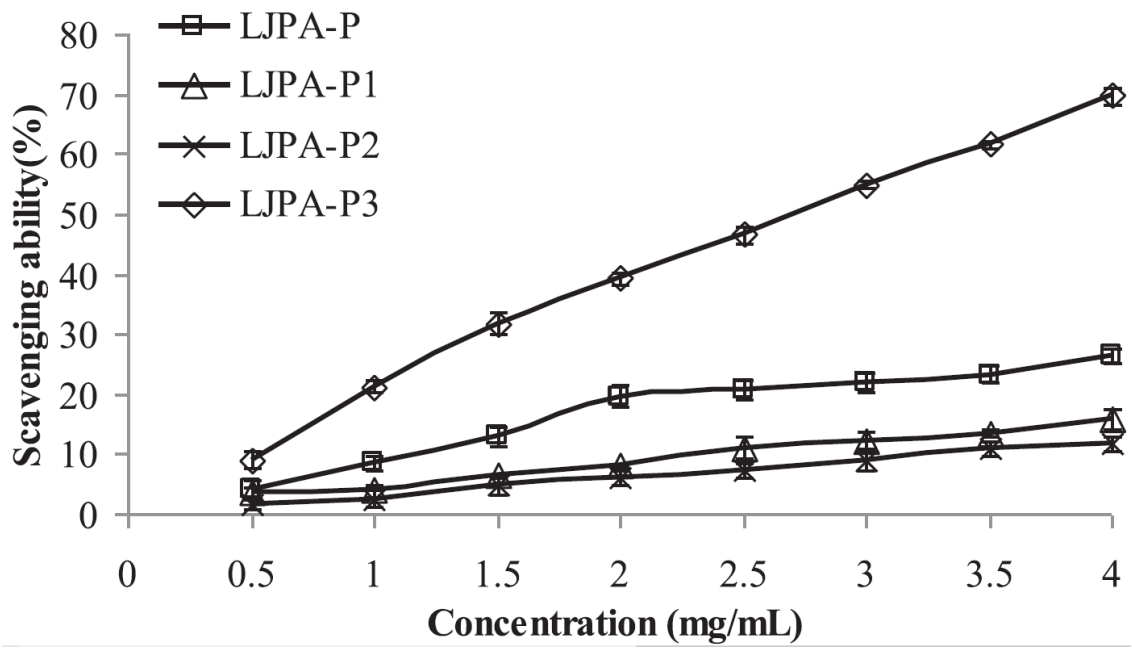


Figure 5. ABTS radical scavenging activity of LJPA-P, LJPA-P1, LJPA-P2 and LJPA-P3

(Cui *et al*., 2016)

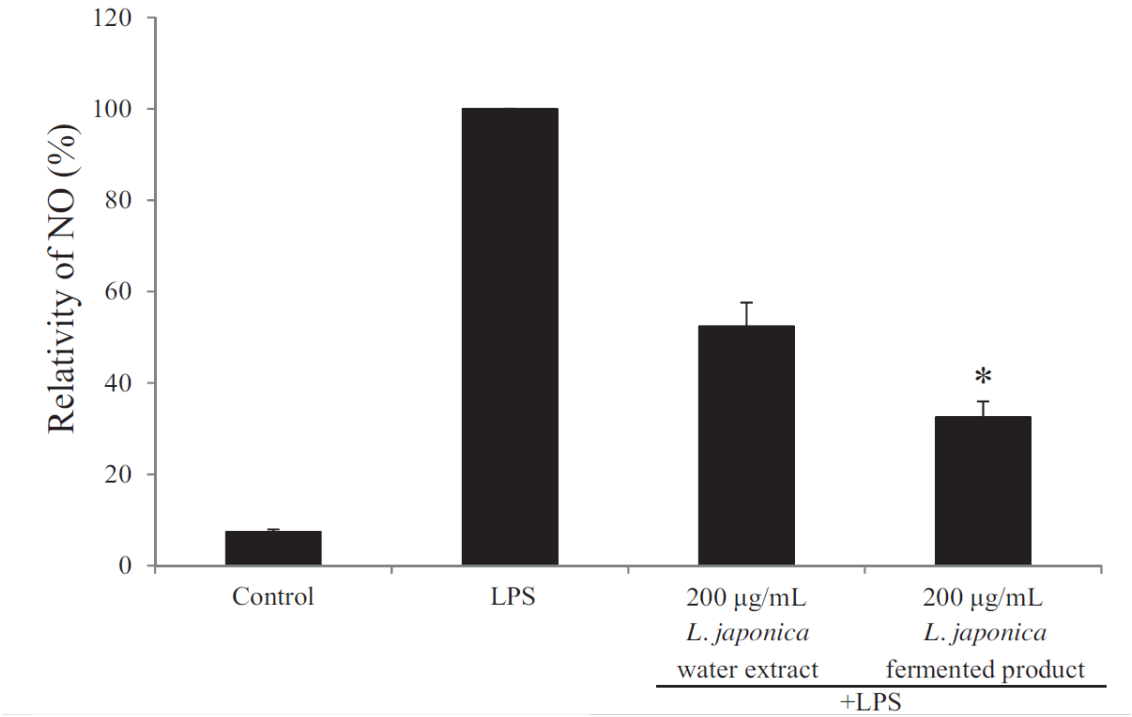


Figure 6. Effect of *L. japonica* fermented product and *L. japonica* water extract on nitric oxide (NO) production in LPS-induced RAW 264.7 cells. Data are expressed as mean ± SD (n = 3). *p < 0.05 compared to the *L. japonica* water extract.

(Lin *et al*., 2016)

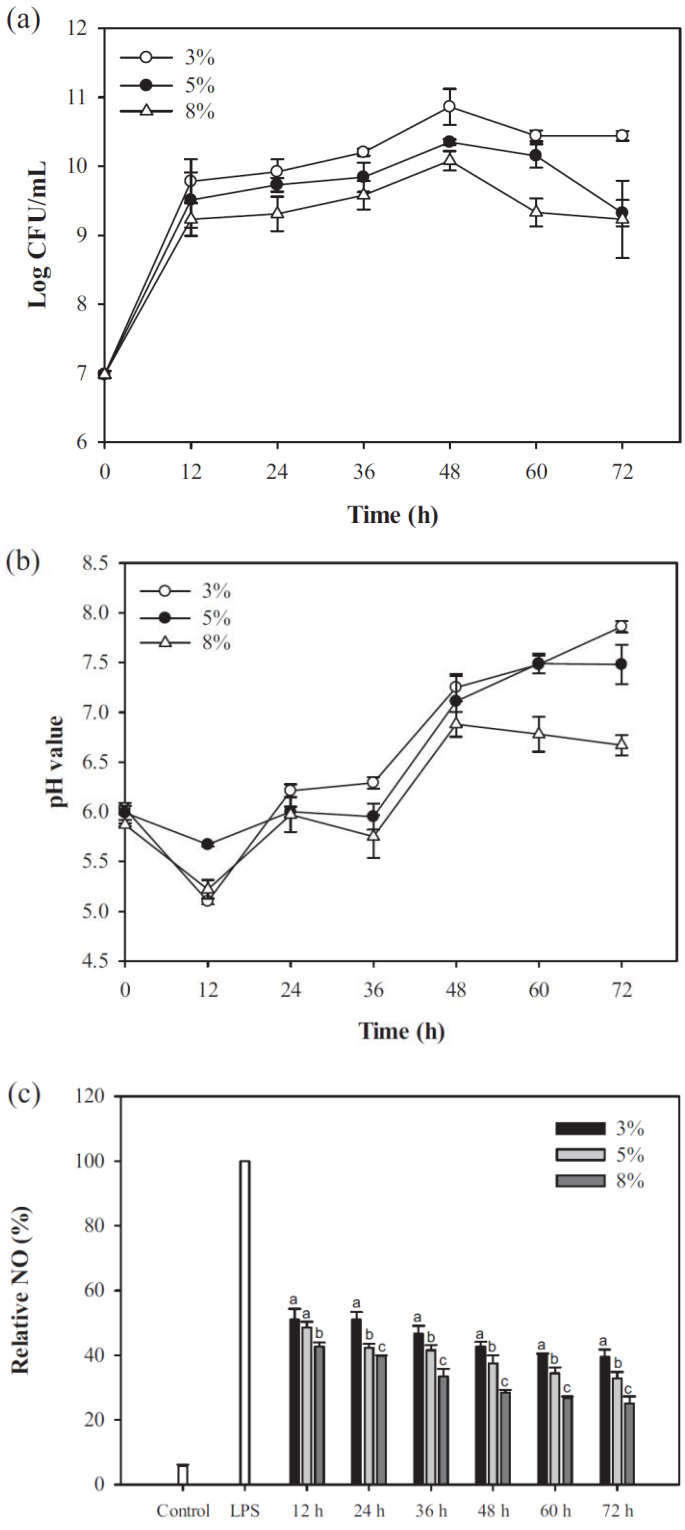


Figure 7. Effects of algae loadings on the (A) cell growth, (B) pH and (C) LPS induced NO production of the *L. japonica* fermentation. Data are expressed as mean ± SD (n = 3). Different letters at the top of the bars are significantly different ($p < 0.05$).

(Lin *et al*., 2016)

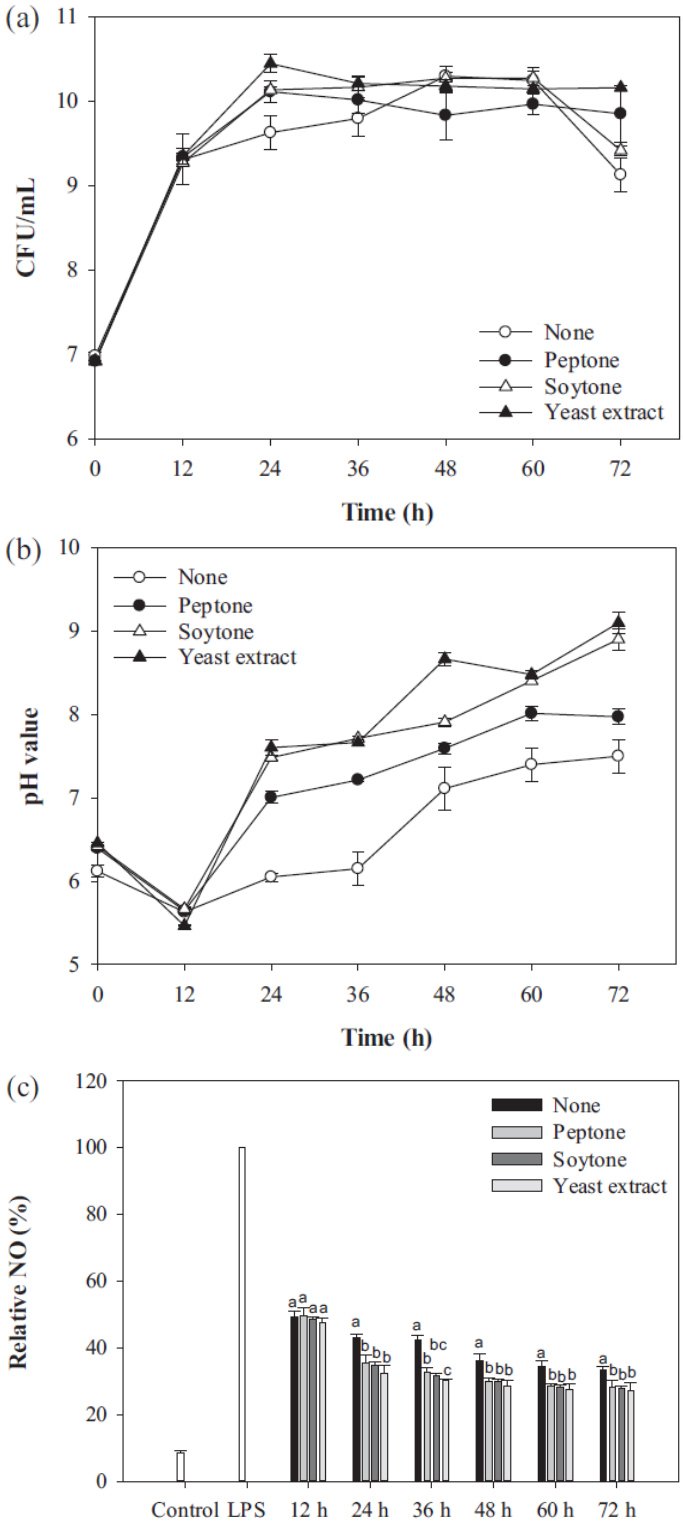


Figure 8. Effects of additional nitrogen source on the (A) cell growth, (B) pH and (C) LPS induced NO production of the *L. japonica* fermentation. Data are expressed as mean ± SD(n = 3). Different letters at the top of the bars are significantly different ($p < 0.05$).

(Lin *et al*., 2016)

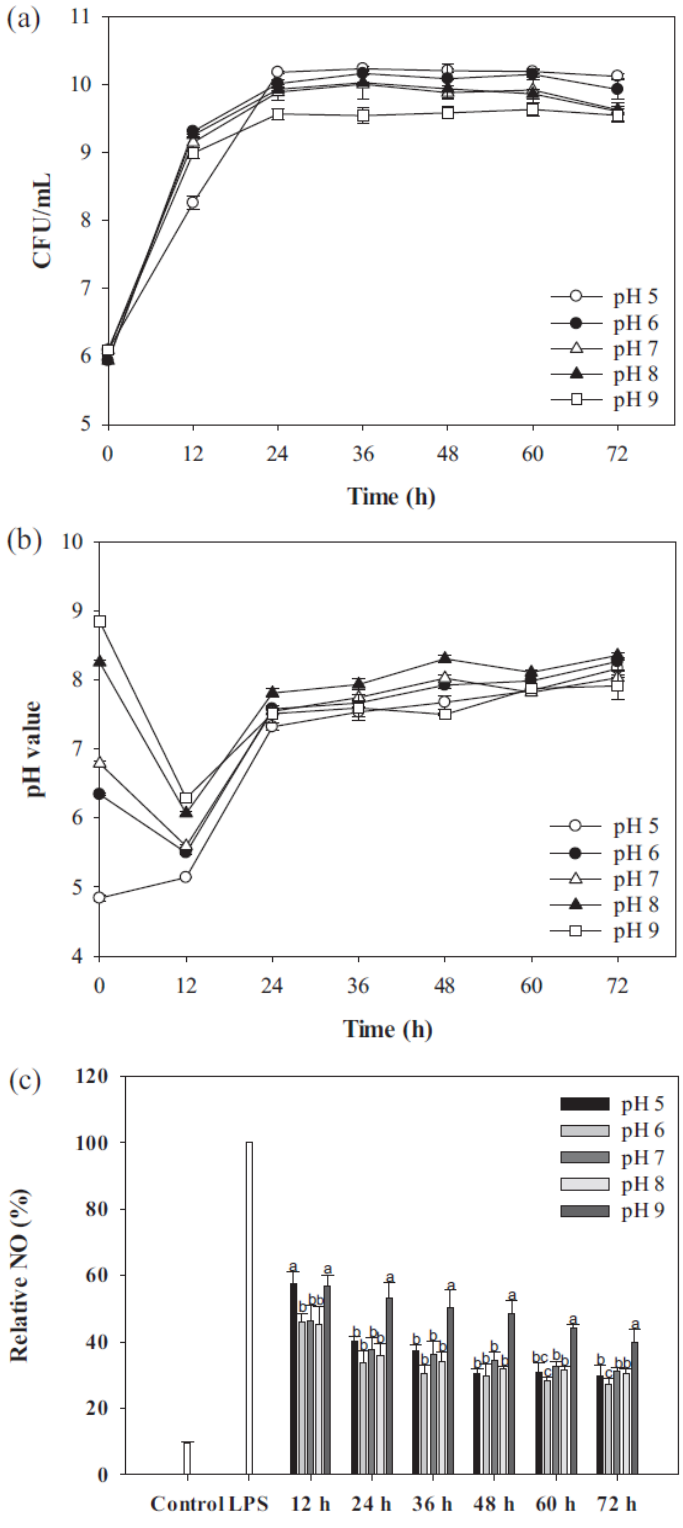


Figure 9. Effects of starting pH on the (A) cell growth, (B) pH and (C) LPS induced NO production of the *L. japonica* fermentation. Data are expressed as mean ± SD (n = 3).Different letters at the top of the bars are significantly different ($p < 0.05$).

(Lin *et al*., 2016)

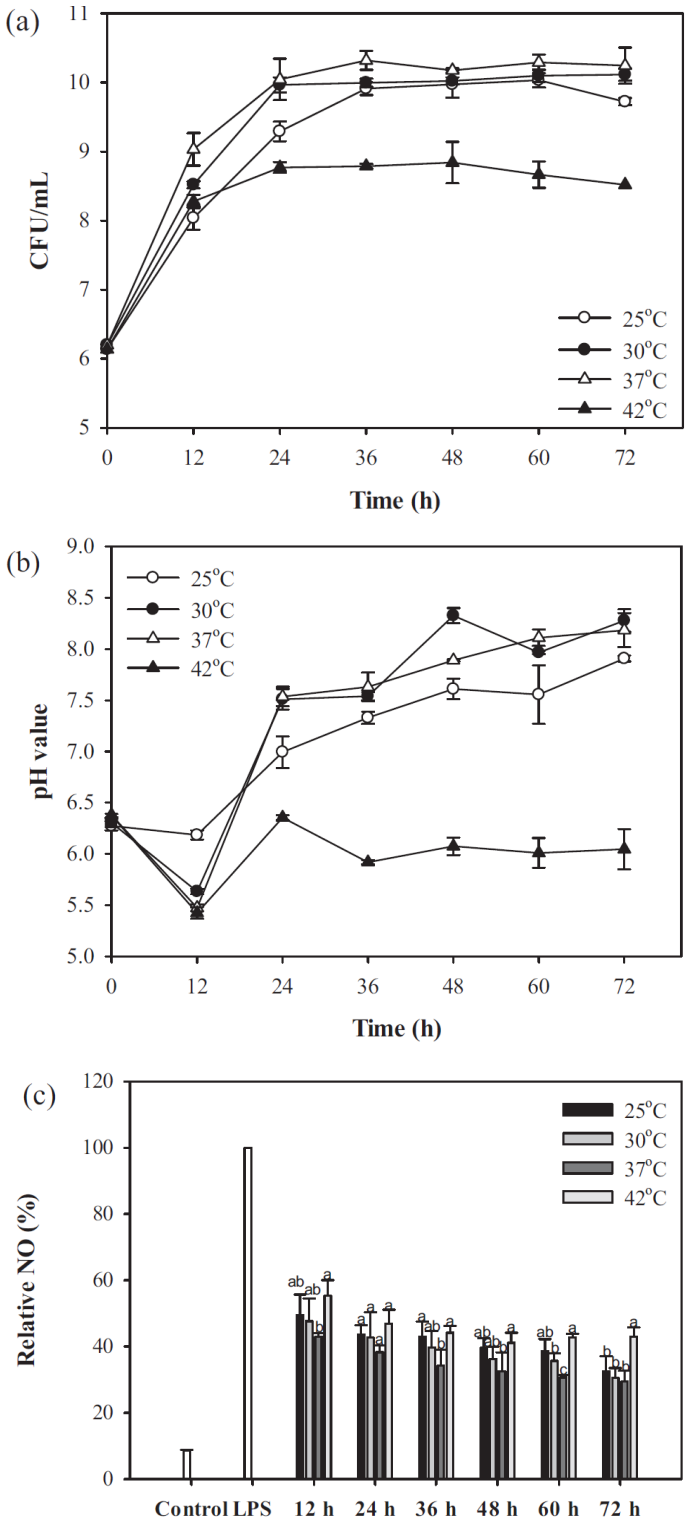


Figure 10. Effects of various temperatures on the (A) cell growth, (B) pH and (C) LPS induced NO production of the *L. japonica* fermentation. Data are expressed as mean ± SD(n = 3). Different letters at the top of the bars are significantly different ($p < 0.05$).

(Lin *et al*., 2016)

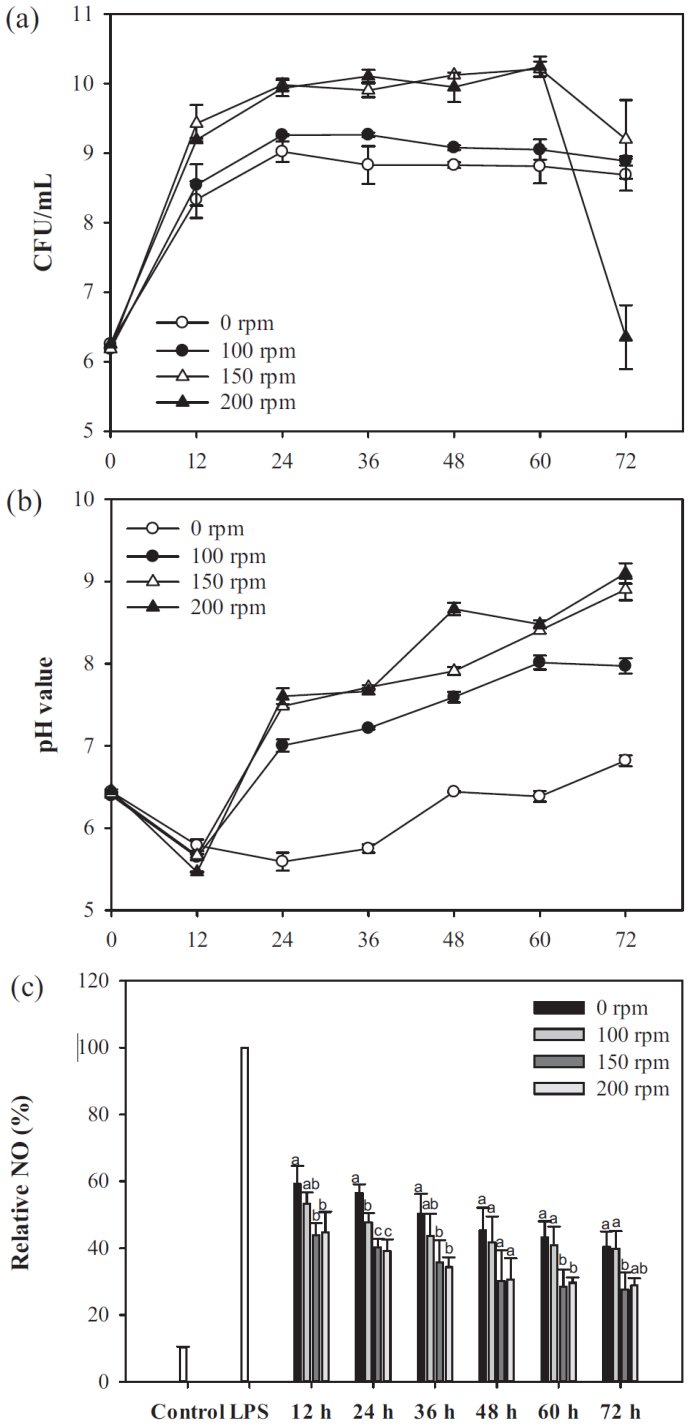


Figure 11. Effects of various agitation on the (A) cell growth, (B) pH and (C) LPS induced NO production of the *L. japonica* fermentation. Data are expressed as mean ± SD (n = 3). Different letters at the top of the bars are significantly different ($p < 0.05$).

(Lin *et al*., 2016)

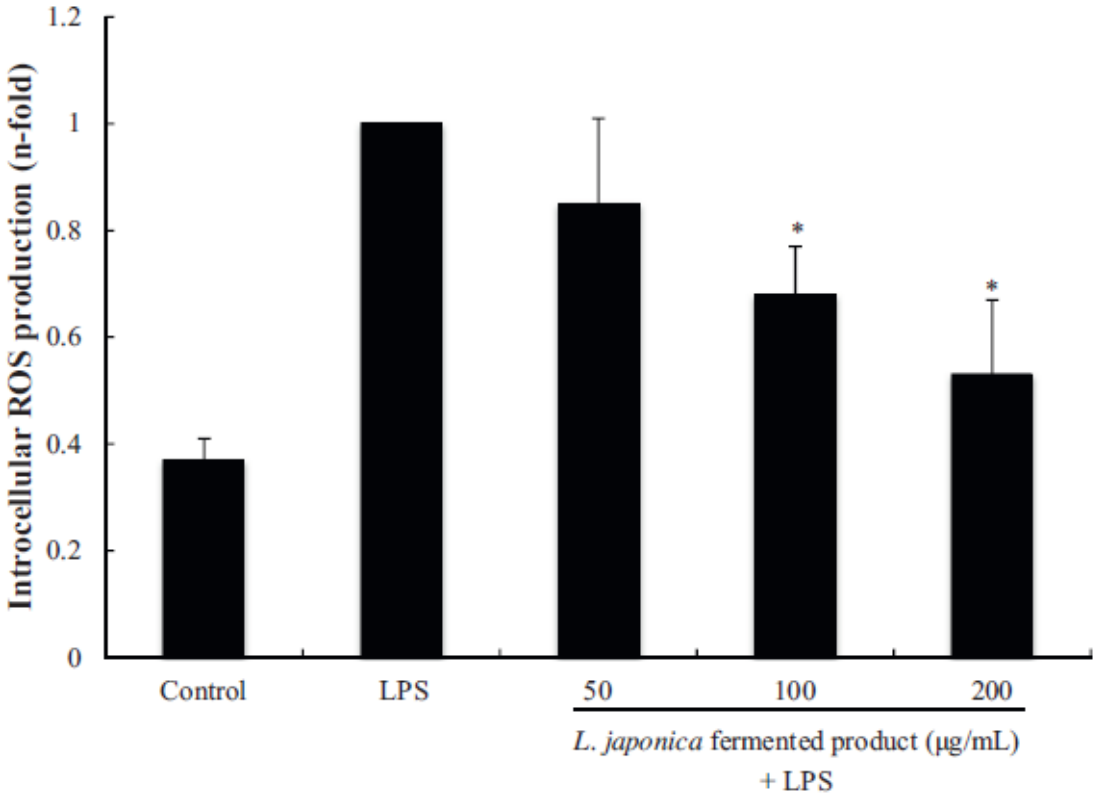


Figure 12. Effect of *L. japonica* fermented product on intracellular ROS level in LPS-induced RAW 264.7 cells. Data are expressed as mean ± SD (n = 3). *p < 0.05compared to the LPS.

(Lin *et al*., 2016)

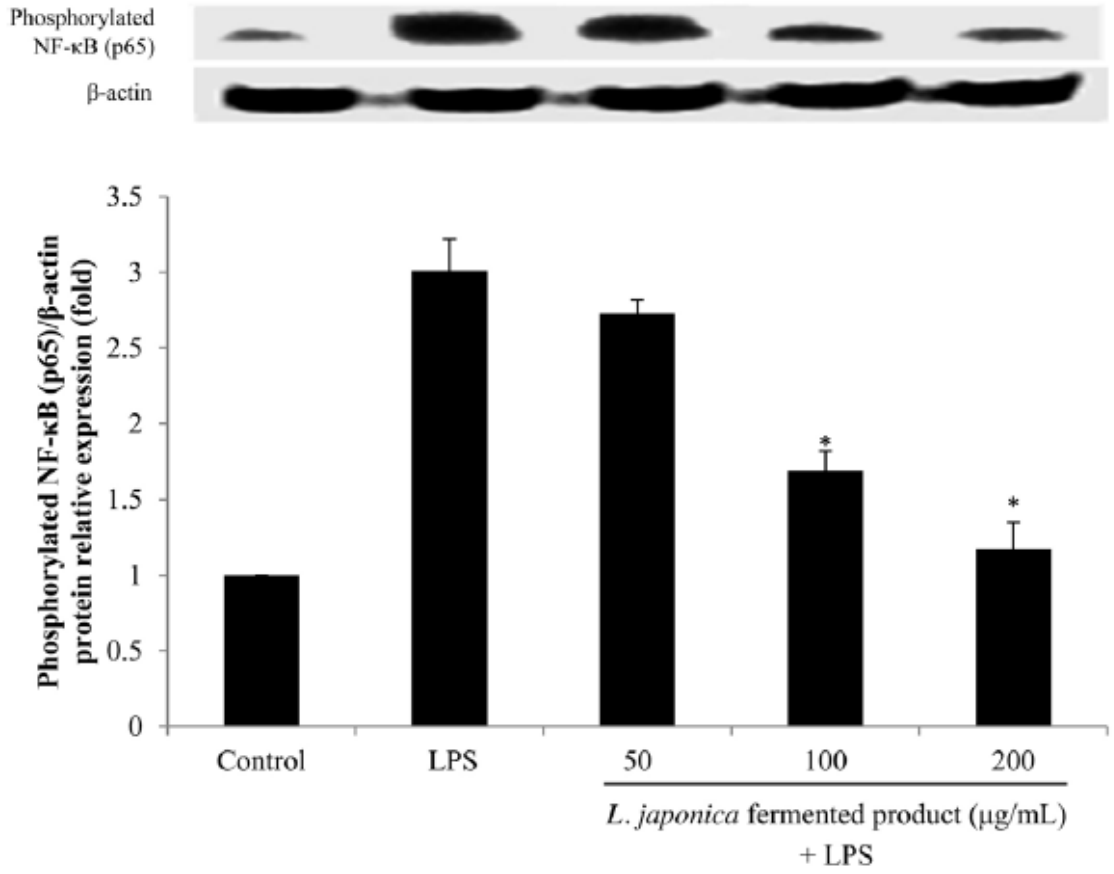


Figure 13. Effect of *L. japonica* fermented product on phosphorylated NF-κB (p65) in LPS-induced RAW 264.7 cells. Data are expressed as mean ± SD (n = 3). *p < 0.05compared to the LPS.

(Lin *et al*., 2016)

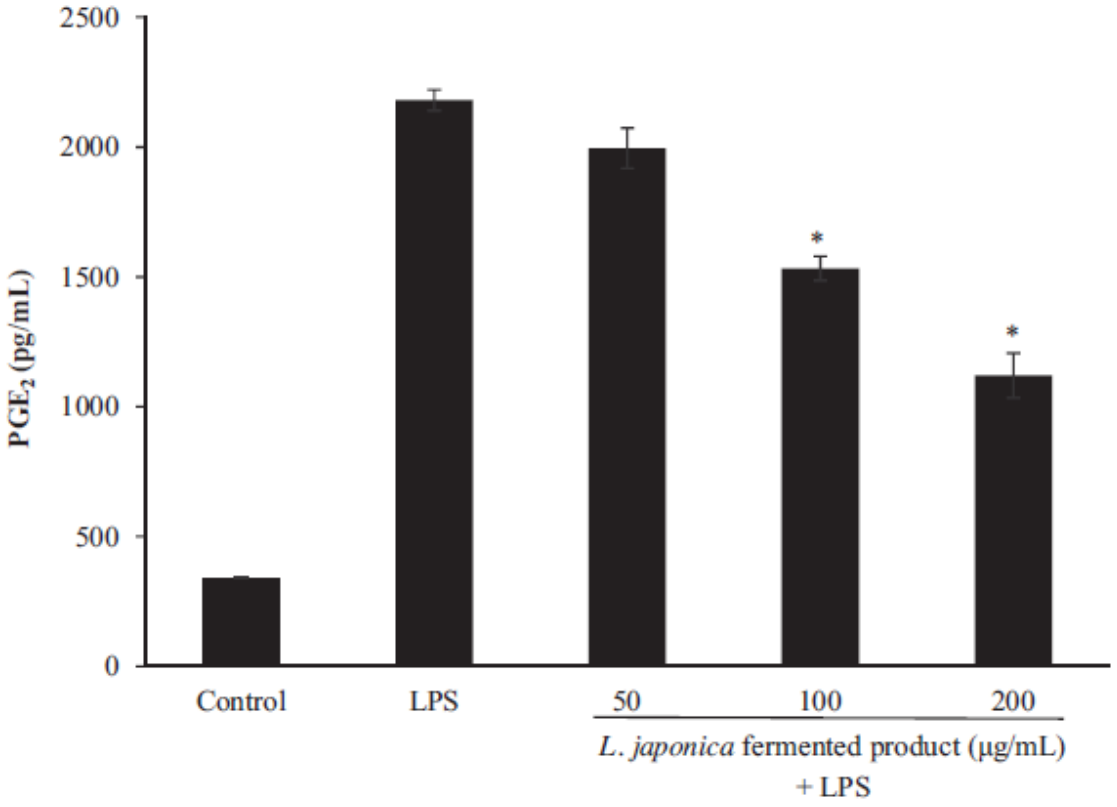


Figure 14. Effect of *L. japonica* fermented product on $PGE_2$ in LPS-induced RAW 264.7cells. Data are expressed as mean ± SD (n = 3). *p < 0.05 compared to the LPS.

(Lin *et al*., 2016)

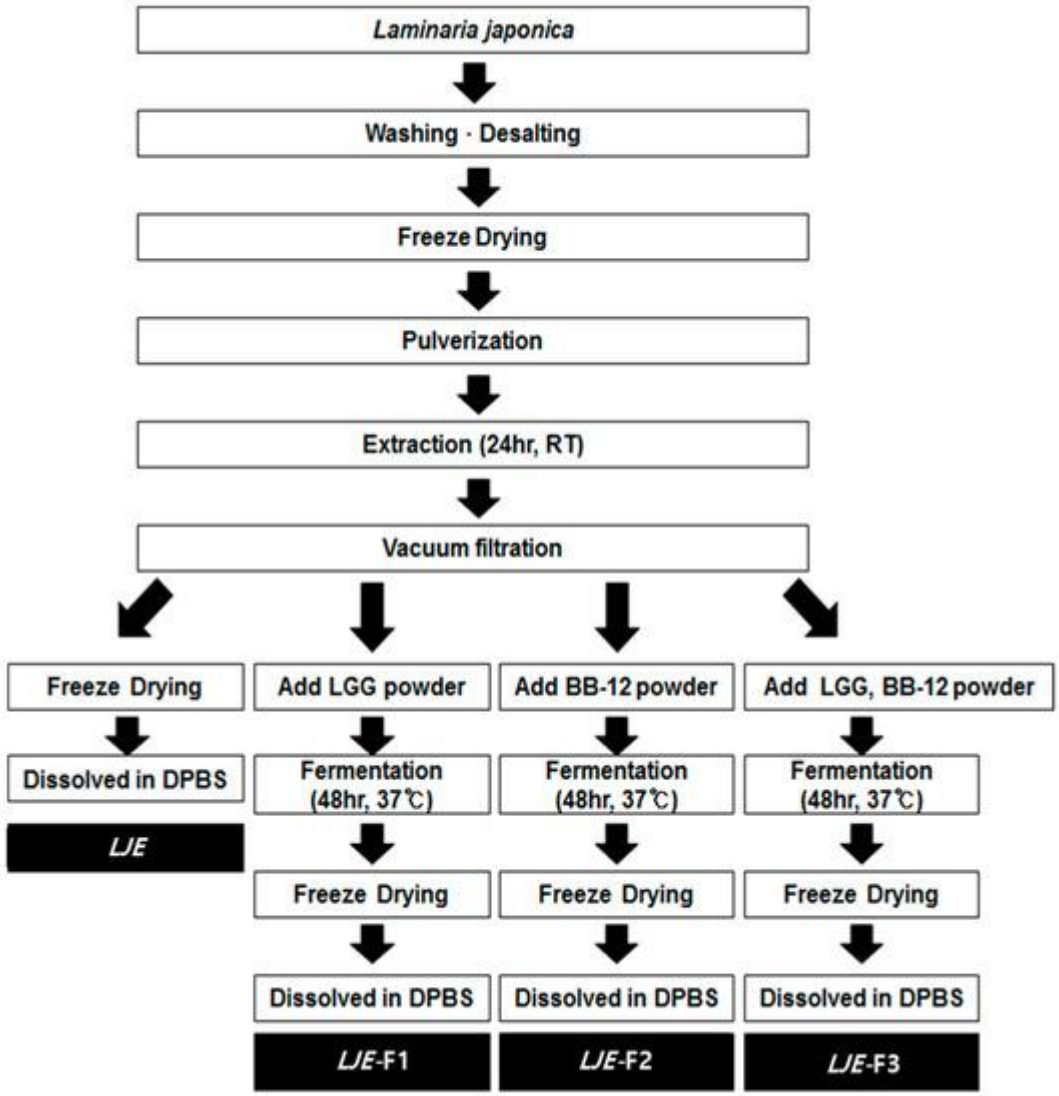


Figure 15. A flow diagram of *Laminaria japonica* (LJ) water extract (LJE) and fermented LJ water extract (LJE-F1, -2, and -3) preparation. Abbreviations: LGG; *Lactobacillus rhamnosus* LGG, BB-12; *Bifidobacterium animalis ssp. lactis* BB-12; DPBS, Dulbecco's phosphate-buffered saline; and RT, room temperature.

(Yang *et al*., 2019)

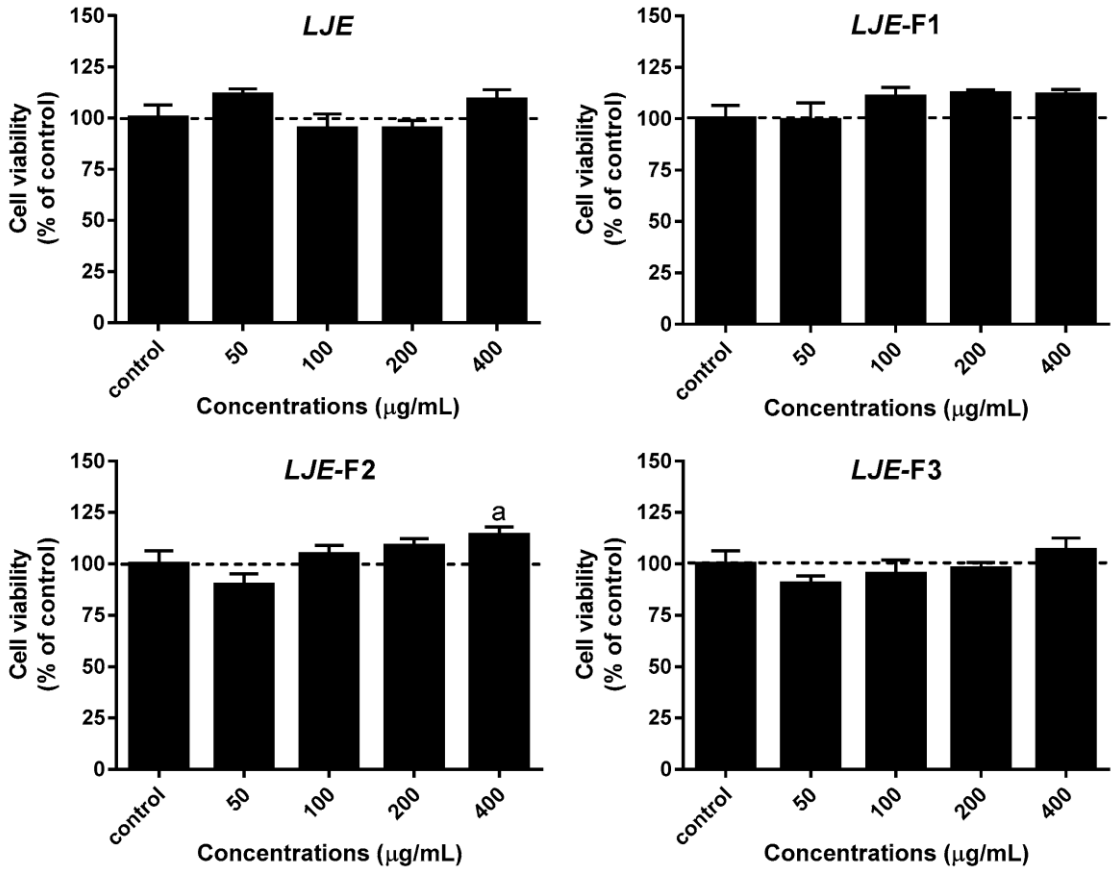


Figure 16. Effects of LJE and LJE-Fs on Caco-2 cell viability. Caco-2 cells ($1 \times 10^5$ cells/well) were seeded in a 24-well plate and treated with LJE or LJE-Fs (0, 50, 100, 200, or 400 μg/mL) for 24 h. Values are mean ± SEM of four independent experiments ($n$ = 3–6). [a] $p < 0.05$ compared with the control by one-way ANOVA with Tukey's comparisons test.

(Yang *et al*., 2019)

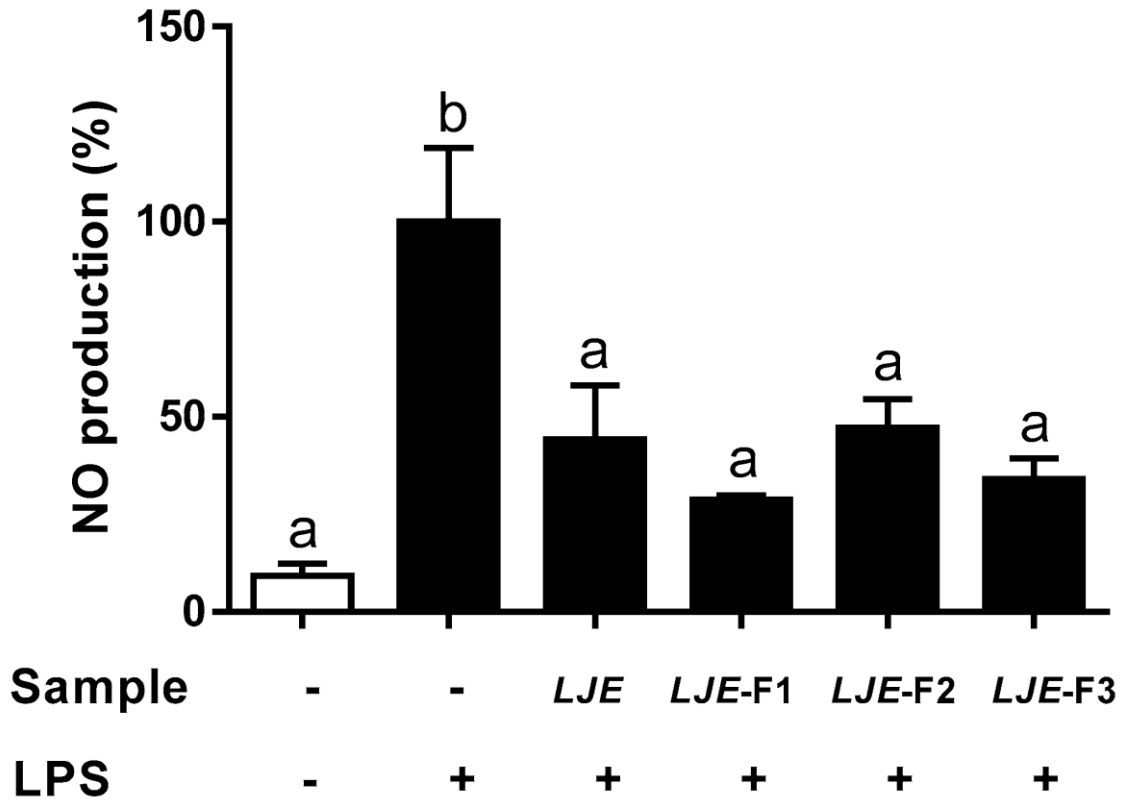


Figure 17. Effects of LJE and LJE-Fs on nitric oxide (NO) production in the lipopolysaccharide (LPS)-stimulated Caco-2 cells. Caco-2 cell monolayers were incubated for 6 h with 100 μg/mL LJE, LJE-F1, LJE-F2, or LJE-F3. Then, 1 μg/mL LPS was added, and the cells were incubated for an additional 24 h. Values are mean ± SEM of three independent experiments. Values that do not share the same superscript are significantly different by one-way ANOVA with Tukey's comparisons test ($p < 0.05$).

(Yang *et al*., 2019)

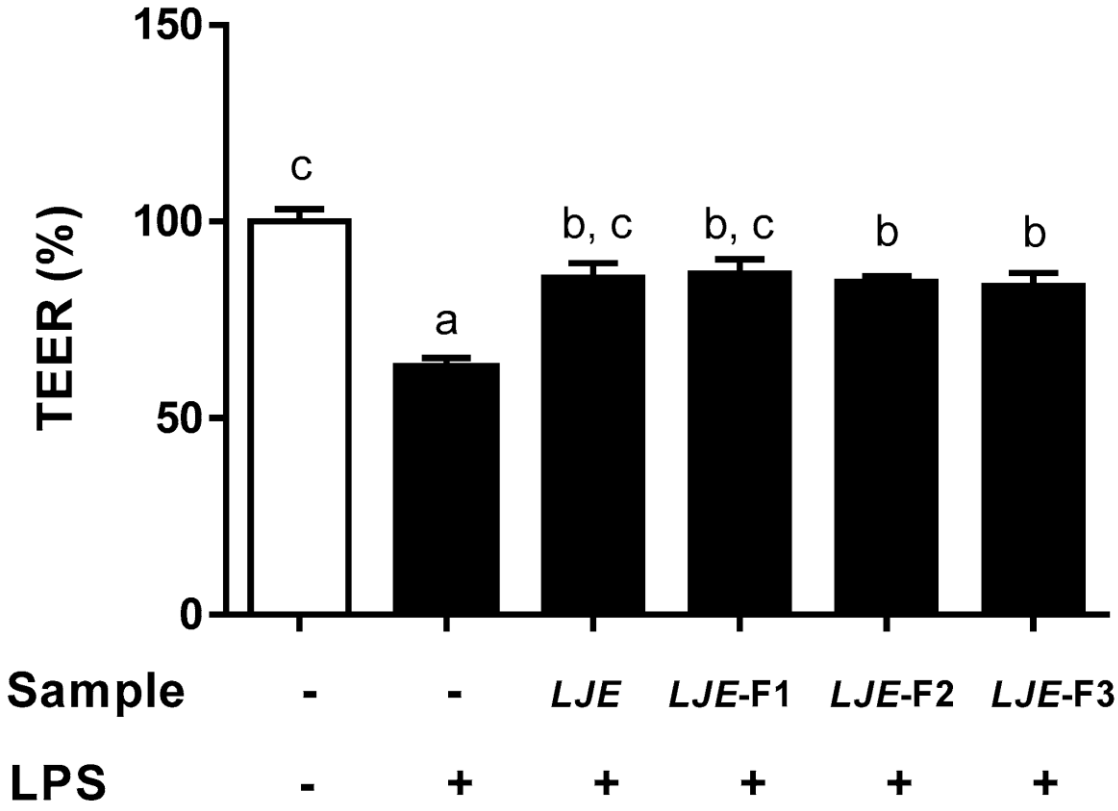


Figure 18. Effects of LJ and LJE-Fs on permeabilization of Caco-2 cell monolayers measured by transepithelial electrical resistance (TEER (%). Caco-2 cell monolayers were preincubated with 100 μg/mL LJE, LJE-F1, LJE-F2, or LJE-F3 for 6 h, and the cells were incubated for an additional 24 h with 1 μg/mL LPS. Caco-2 cell monolayer permeability was evaluated by measuring TEER. The results are shown as mean ± SEM of three independent experiments. Values that do not share the same superscript are significantly different by one-way ANOVA with Tukey's comparisons test ($p < 0.05$).

(Yang *et al*., 2019)

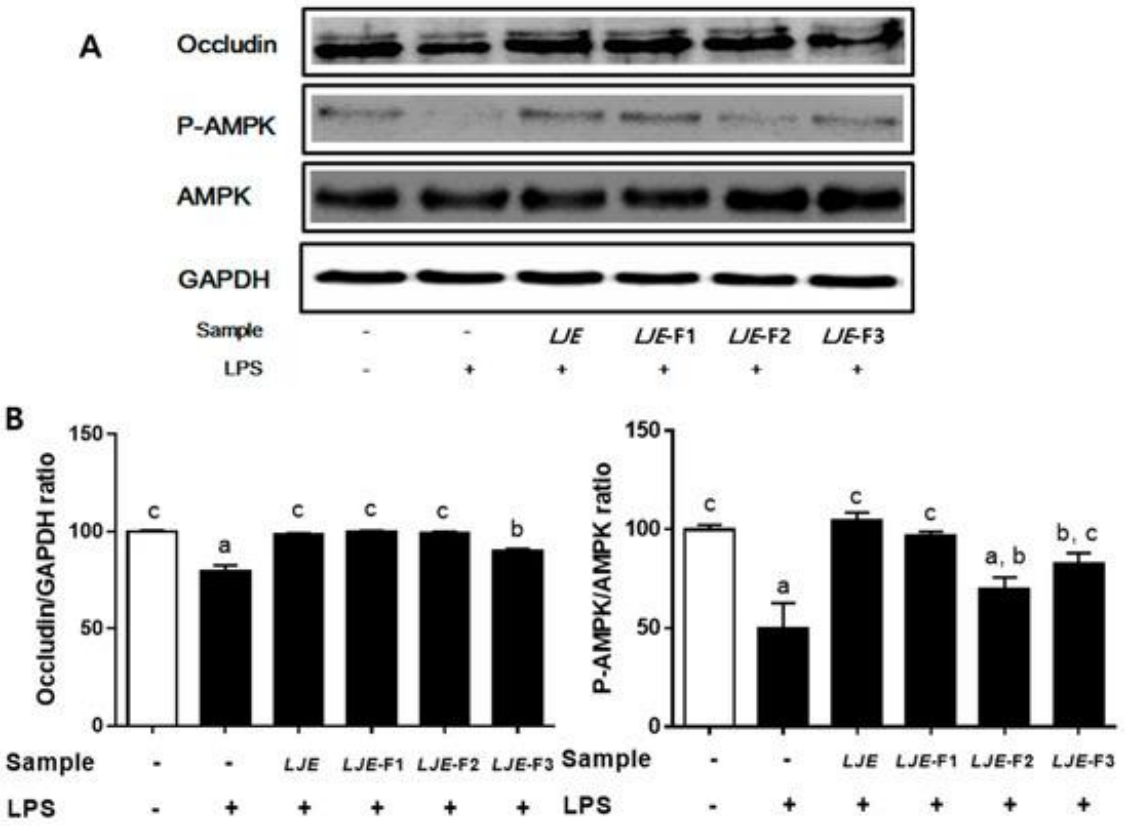


Figure 19. LJE and LJE-Fs differentially promoted intestinal epithelial barrier function via upregulation of the tight junction (TJ) in Caco-2 cell monolayers. (**A**) Representative immunoblot analysis of occludin, phospho-adenosine monophosphate-activated protein kinase (P-AMPK), total AMPK, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH); (**B**) intensity of occludin was normalized to GAPDH, and intensity of P-AMPK was normalized to total AMPK to account for apparent differences and confirm statistical significance. Caco-2 cells were pre-incubated for 6 h with 100 μg/mL LJE or LJE-Fs and then incubated for 24 h in the absence or presence of LPS (100 μg/mL). Values are mean ± SEM of three independent experiments. Values that do not share the same superscript are significantly different by one-way ANOVA with Tukey's comparisons test ($p < 0.05$).

(Yang *et al*., 2019)

Table 1. Monosaccharide composition, sulfate group content and glycosidic linkage of extracted polysaccharide fractions.

| Compositions | LJPA-P | LJPA-P1 | LJPA-P2 | LJPA-P3 |
|---|---|---|---|---|
| Monosaccharide composition (molar ratio, %) | | | | |
| Rhamnose | 2.7 | 1.9 | 4.3 | nd |
| Fucose | 3.6 | 3.1 | 5.5 | 4.4 |
| Xylose | 2.7 | 7.6 | 6.0 | nd |
| Mannose | 10.5 | 22.3 | 24.8 | 3.7 |
| Glucose | 15.8 | 36.4 | 4.9 | nd |
| Galactose | 64.7 | 28.7 | 54.5 | 91.9 |
| Sulfate group content (%) | 4.0 | nd[a] | nd | 12.7 |
| Uronic acid content (%) | | | | |
| Glucuronic acid | 3.6 | 3.4 | 4.7 | 4.1 |
| Galacturonic acid | nd | nd | 0.2 | 0.1 |
| Molar ratio of glycosidic linkage (molar ratio, %) | | | | |
| Galp-(1→ | 7.2 | 6.8 | 7.2 | 6.6 |
| Glcp-(1→ | 2.1 | 14.6 | 1.3 | nd |
| →3)-Galp-(1→ | 15.2 | 5.4 | 3.7 | 19.3 |
| →3)-Fucp-(1→ | 1.8 | 1.9 | 1.5 | 1.7 |
| →3)-Rhap-(1→ | 1.0 | 1.0 | 1.0 | nd |
| →6)-Galp-(1→ | 2.4 | 3.5 | 2.4 | nd |
| →6)-Glcp-(1→ | 1.8 | 4.1 | nd | nd |
| →3,6)-Manp-(1→ | 4.1 | 12.6 | 6.3 | 1 |

[a] nd: Not detectable.

(Cui *et al*., 2016)

Table 2. Changes of pH, total sugar contents, reducing sugar content, and total polyphenol contents in LJE-Fs by fermentation.

| Measurements | Fermentation | LJE-F1 | LJE-F2 | LJE-F3 |
|---|---|---|---|---|
| pH | Before | 7.30 ± 0.12 [1] | 7.31 ± 0.14 | 7.34 ± 0.10 |
| | After | 4.06 ± 0.09 * | 4.01 ± 0.11 * | 3.95 ± 0.15 * |
| Total sugar (mg D-glucose/mL) | Before | 4.40 ± 0.13 | 4.67 ± 0.02 | 4.93 ± 0.01 |
| | After | 3.34 ± 0.06 * | 4.35 ± 0.07 * | 4.04 ± 0.01 * |
| Reducing sugar (mg D-glucose/mL) | Before | 1.44 ± 0.03 | 1.39 ± 0.01 | 1.47 ± 0.03 |
| | After | 1.39 ± 0.09 * | 1.36 ± 0.03 * | 1.36 ± 0.03 * |
| Total polyphenols (μg gallic acid/mL) | Before | 1.14 ± 0.01 | 1.15 ± 0.07 | 1.17 ± 0.01 |
| | After | 0.90 ± 0.01 | 1.01 ± 0.01 | 1.11 ± 0.04 |

[1] Values are mean ± SEM of three independent experiments. * Values with an asterisk are significantly different by *t*-test (before vs. after fermentation, $p < 0.05$). Abbreviations: LJE-F1, LJE fermented by LGG; LJE-F2, LJE fermented by BB-12; and LJE-*F3*, LJE fermented by a combination of LGG and BB-12.

(Yang *et al*., 2019)

Table 3. Effect of LJE and LJE-Fs on TNF-α and IL-6 production in the LPS-stimulated Caco-2 cells.

| Sample | TNF-α Levels (pg/mL) | IL-6 Levels (pg/mL) |
|---|---|---|
| LPS | 21.75 ± 0.27 $^{b,1}$ | 5.47 ± 0.7 $^{c}$ |
| LPS+LJE | 18.36 ± 1.20 $^{a}$ | 4.16 ± 0.26 $^{b}$ |
| LPS+LJE-F1 | 18.16 ± 0.01 $^{a}$ | 0.92 ± 0.15 $^{a}$ |
| LPS+LJE-F2 | 19.82 ± 0.19 $^{b}$ | 0.91 ± 0.03 $^{a}$ |
| LPS+LJE-F3 | 19.11 ± 0.60 $^{b}$ | 0.77 ± 0.05 $^{a}$ |

[1] Values are mean ± SEM of three independent experiments. Values that do not share the same superscript are significantly different by one-way ANOVA with Tukey's comparisons test ($p < 0.05$). Caco-2 cells were pre-incubated for 6 h with 100 μg/mL LJE or LJE-Fs and then incubated for 24 h in the absence or presence of LPS (100 μg/mL).

(Yang *et al*., 2019)