ANTIOXIDANT ACTIVITY OF FERMENTED BUFFALO MILK USING SELECTED LACTIC ACID BACTERIA

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Thesis submitted in fulfillment for the degree of
MASTER OF SCIENCE

UNIVERSITI SAINS ISLAM MALAYSIA

June 2023

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AUTHOR DECLARATION

I hereby declare that this thesis entitled "Antioxidant activity of fermented buffalo milk using selected lactic acid bacteria." is my own unless specified and duty acknowledged by quotation.

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ACKNOWLEDGEMENTS

In the name of Allah, the most Gracious and the most Merciful.

All praises return back to the Almighty Allah for giving me this opportunity to complete my thesis writing after a long journey as a master student at Universiti Sains Islam Malaysia (USIM). Firstly, I would like to thank Faculty of Science and Technology (FST) and Centre for Graduate Studies (CGS) for giving me opportunity to pursue my study in this university as well as for their help and guidance during my master journey.

During my master journey, I would like to express my deepest gratitude to special person which are Associate Prof. Dr. Zaiton binti Hassan, a former supervisor and Dr. Nur Huda binti Faujan, a recent supervisor for their endless supports, patient, guidance, positive feedback, sincere advises and great concern regarding my master's study journey from the beginning until the end. I am so pleased to have them both as my supervisors and I consider myself very lucky and honour to work under their supervision. Through a lot of difficulties due to my health issue, I still survived and keep on progressing even I admitted my progress work was slow and both of them patiently tried their best to follow my rhythm.

Not forgetting to all my laboratory friends and staffs for their help, guidance and advises especially when I did my laboratory work.

A sincere gratitude, love and thankfulness to parents, parents-in-law, husband, children, siblings, relatives and friends for their strong supports, understanding, sacrifice each one of them had made for me so that I can finish my study.

Thank you.

Not a single word can express my gratefulness, except *Alhamdulillah*.

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ABSTRAK

Bakteria asid laktik (LAB) dalam penapaian susu boleh menjana peptida bioaktif termasuk peptida antioksida. Walau bagaimanapun kajian masih terhad tentang penghasilan peptida bioaktif daripada penapaian susu kerbau yang dijanakan oleh LAB terpilih. Oleh itu, kajian ini dijalankan untuk menyaring aktiviti proteolitik pencilan LAB dan untuk menilai aktiviti antipengoksida wei susu skim menggunakan pencilan LAB serta untuk menilai kesan kaedah kultur dan masa penapaian pada aktiviti antipengoksida wei yang telah dijanakan oleh susu kerbau yang telah ditapai dengan LAB terpilih. Aktiviti proteolitik LAB telah disaring dengan kaedah agar susu skim (SMA) dan aktiviti antipengoksida susu skim dan susu kerbau tertapai telah diukur menggunakan analisis perencatan 1,1-difenil-2-pikrilhidrazil (DPPH) dan analisis pengkelatan ferus (FICA). Berat molekul (Mw) peptida daripada wei susu kerbau yang telah dihasilkan oleh LAB telah dianggarkan menggunakan teknik elektroforesis gel natrium dodesil sulfat berpoliakrilamida (SDS PAGE). Dua puluh lima pencilan LAB daripada pelbagai sumber (yogurt buatan sendiri, pisang, anggur, kurma, tanah, pekasam, dan budu) telah disaring kebolehannya untuk menghidrolisis protein susu skim. Kajian awal telah menunjukkan bahawa lapan (Yg, Bn2, WG2, BG, Dt, Pk2, S1, dan Bd2) daripada pencilan LAB menunjukkan hidrolisis protein ke atas SMA dengan diameter zon jelas antara 4.0 mm dan 14.5 mm. LAB ini telah dikulturkan ke dalam susu skim untuk menghasilkan wei yang mempunyai aktiviti antipengoksida dianalisis dengan DPPH dan FICA selepas 24 jam penapaian pada suhu 37 °C. Pencilan WG2, Pk2, S1, dan Bd2 menjana aktiviti antipengoksida pada wei susu skim dengan julat nilai DPPH antara 12.59 dan 19.04 % dan julat nilai FICA antara 78.61 dan 84.81 % berbanding pencilan lain seterusnya telah dinilai untuk potensi mereka sebagai probiotik untuk memastikan daya hidup dan fungsi LAB masih kekal ada. Keempat-empat pencilan LAB ini telah bertoleransi dalam persekitaran asid dan hempedu dengan sekurang-kurangnya pengurangan (maksimum <1.05 kitaran log) dalam jumlah daya hidup sel untuk kedua-dua analisis cabaran asid dan hempedu. Pencilan WG2 telah menunjukkan kebolehan untuk bertahan dalam tekanan berasid berbanding pencilan lain dengan kehilangan kiraan daya hidup yang kurang daripada 0.10 pengurangan log dalam persekitaran berasid pH 2.0 manakala pencilan Bd2 menunjukkan kenaikan dalam kiraan daya hidup dengan kitaran log 0.37 cfu/ml selepas pendedahan dalam persekitaran hempedu pH 6.5. Selain itu, pencilan LAB ini boleh bertindak sebagai aktiviti antimikrob dengan keluasan zon perencatan oleh kedua-dua kaedah lapisan kultur berpasangan dan telaga serapan agar menentang *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Staphilucoccos aureus* (*S. aureus*), *Staphilucoccus typhimurium* (*S. typhimurium*) and *Escherichia coli* (*E. coli*). Keempat-empat pencilan LAB sangat menghalang *S. typhimurium* dan *E. coli* dengan zon jelas berdiameter 85.00 mm dengan kaedah lapisan kultur berpasangan. Diameter terbesar zon jelas dengan kaedah telaga serapan agar telah diperhatikan pada pencilan LAB Pk2, S1, dan Bd2 menentang *E. coli* iaitu 13.83 mm. Pendekatan pengkulturan pencilan LAB dengan ketara mempengaruhi ($p<0.05$) nilai antipengoksida wei susu kerbau dengan analisis perencatan aktiviti radikal DPPH dan FICA. Wei yang telah dihasilkan oleh pengkulturan lansung LAB dalam penapaian susu kerbau menghasilkan nilai DPPH yang tinggi berbanding dengan wei yang telah dihasilkan oleh prapengkulturan LAB. Sebaliknya, wei yang telah dihasilkan oleh pengkulturan lansung LAB dalam penapaian susu kerbau menghasilkan nilai FICA yang rendah berbanding dengan wei yang dihasilkan oleh prapengkulturan LAB. Nilai DPPH wei susu kerbau yang telah

dihasilkan oleh pengkulturan lansung LAB berjulat antara 22.61 dan 33.62% manakala nilai FICA wei susu kerbau yang telah dihasilkan oleh prapengkulturan LAB berjulat antara 59.41 dan 78.22% untuk pencilan WG2, Pk2, S1, dan Bd2. Pengecaman pencilan WG2, Pk2, S1, dan Bd2 telah dikenalpasti sebagai *Lactobacillus plantarum* (*L. plantarum* WG2), *Lactobacillus paracasei* (*L. paracasei* Pk2), *Lactobacillus plantarum* (*L. plantarum* Sl) dan *Enterococcus faecium* (*E. faecium* Bd2) menggunakan kaedah penjadualan rDNA 16S dengan 99 % persamaan. LAB ini telah dipelajari lebih lanjut dalam penapaian susu kerbau untuk menghasilkan wei dengan aktiviti antipengoksida dengan pendekatan pengkulturan yang berbeza dan masa penapaian yang berlainan. Penambahan LAB pada susu kerbau sama ada secara pengkulturan lansung atau secara prapengkulturan mempengaruhi aktiviti antipengoksida penapaian susu kerbau. Pengkulturan langsung LAB ke dalam susu kerbau telah menghasilkan wei dengan nilai DPPH yang tinggi berjulat antara 21.87 dan 55.03 % dan nilai FICA berjulat antara 50.13 dan 65.52 %. Walau bagaimanapun, prapengkulturan LAB pada susu kerbau telah menghasilkan wei dengan nilai DPPH yang lebih rendah berjulat antara 3.43 dan 12.28 % berbanding dengan nilai FICA berjulat antara 56.58 dan 84.45 % untuk *L. plantarum* WG2, *L. paracasei* Pk2, *L. plantarum* S1, dan *E. faecium* Bd2. Susu kerbau tertapai dengan prapengkuluran *L. plantarum* WG2 dan *E. faecium* Bd2 telah menghasilkan wei dengan nilai FICA yang tinggi dan dipengaruhi oleh masa penapaian. Selepas 24 jam proses penapaian, prapengkuluran oleh *E. faecium* Bd2 pada susu kerbau telah menghasilkan wei dengan nilai FICA 78.22 % tetapi selepas 48 jam proses penapaian, prapengkuluran oleh *L. plantarum* WG2 pada susu kerbau telah menghasilkan wei dengan nilai FICA paling tinggi dalam penemuan ini (84.45 %). Wei susu kerbau yang telah dihasilkan oleh prapengkulturan *L. plantarum* WG2 dan *E. faecium* Bd2 telah ditentukan aktiviti antipengoksida dengan separuh maksima kepekatan halangan (IC_{50}) pada ujian FICA yang berkeputusan dengan nilai IC_{50} berjulat antara 0.37 dan 0.41 mg/ml yang lebih rendah daripada IC_{50} nilai piawai EDTA (0.29 mg/ml). Dengan menggunakan analisis SDS PAGE, M_w peptida daripada wei susu kerbau yang telah dihasilkan oleh sama ada prapengkulturan *L. plantarum* WG2 atau prapengkulturan *E. faecium* Bd2 telah dianggarkan antara 150 dan 50 kDa, 20, dan 10 kDa untuk kedua-dua LAB, masing-masing. Kajian ini telah menyimpulkan bahawa *L. plantarum* WG2 dan *E. faecium* Bd2 boleh digunakan untuk menghasilkan peptida dari wei susu kerbau dengan aktiviti antipengoksida yang agak baik.

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ABSTRACT

Lactic acid bacteria (LAB) in milk fermentation can generate bioactive peptides including antioxidative peptides. However limited studies about production of bioactive peptides from buffalo milk fermentation generated by selected LAB. Thus, this study was to screen the proteolytic activity and probiotic potentials of isolated LAB and antioxidative activity of skimmed milk whey using LAB isolates as well as to evaluate the effect of culturing methods and fermentation time on antioxidant activity of whey generated by buffalo milk fermented with selected LAB. Proteolytic activity of LAB was screened using skimmed milk agar (SMA) method and the antioxidant activities of whey skimmed and buffalo milk were measured using scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferrous chelating activity (FICA) assays. Molecular weight (M_w) of antioxidative peptides from whey buffalo milk produced by LAB was estimated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) method. Twenty-five LAB isolates from different sources (homemade yogurt, banana, grape, dates, soil, fermented shrimp, and fermented salted fish) were screened for their ability to hydrolyse protein in skim milk. Preliminary screening indicated that eight (Yg, Bn2, WG2, BG, Dt, Pk2, S1, and Bd2) of LAB isolates showed protein hydrolysis on SMA with diameter of clear zone between 4.0 mm and 14.5 mm. These LAB were fermented in skimmed milk produced whey had antioxidant activity analysed with DPPH and FICA assays after 24 h incubation at 37 °C. The WG2, Pk2, S1, and Bd2 isolates generated antioxidant activity in whey skimmed milk with DPPH values ranged between 12.59 and 19.04 % and FICA values ranged between 78.61 and 84.81 % compared to other isolates were further evaluated for their probiotic potentials to reassure LAB viability and functionality remained. All four LAB isolates tolerated in acid and bile environments with at least slight reduction (maximally <1.05 log cycle) in viable cell counts for both acid and bile challenge assays. Isolate WG2 showed more ability to survive in acidic stress than other isolates with viable count loss less than 0.10 log reduction in the acidic environment at pH 2.0 while isolate Bd2 showed an increment in viable count with the log cycle 0.37 cfu/ml after exposure in bile condition at pH 6.5. These LAB isolates able to act as antimicrobial activity with wide range of inhibition zone by both dual culture overlay and agar well diffusion methods against *Bacillus cereus* (*B. cereus*), *Bacillus subtilis* (*B. subtilis*), *Staphilucoccos aureus* (*S. aureus*), *Staphilucoccus typhimurium* (*S. typhimurium*) and *Escherichia coli* (*E. coli*), respectively. All four LAB isolates were greatly inhibited *S. typhimurium* and *E. coli* with the largest diameter of clear zone of 85.00 mm by dual culture overlay method. The largest diameter of clear zone by agar well diffusion method was observed for LAB isolates of Pk2, S1, and Bd2 against *E. coli* which was 13.83 mm. Culturing approach of LAB isolates significantly ($p<0.05$) affected antioxidative value of whey buffalo milk by scavenging DPPH radical activity and FICA assays. Whey produced by direct cultured LAB in fermented buffalo milk resulted in high DPPH value compared to whey produced by precultured LAB. In contrast, whey produced by direct cultured LAB in fermented buffalo milk resulted in low FICA value compared to whey produced by precultured LAB. The DPPH value of whey buffalo milk generated by direct cultured LAB ranged between 22.61 and 33.62 % while the FICA value of whey buffalo milk generated by precultured LAB ranged between 59.41 and 78.22 % for WG2, Pk2, S1, and Bd2 isolates. Identification of isolates WG2, Pk2, S1, and Bd2 were identified as *Lactobacillus plantarum* (*L. plantarum* WG2), *Lactobacillus paracasei* (*L. paracasei* Pk2), *Lactobacillus plantarum* (*L. plantarum* Sl) and *Enterococcus faecium* (*E. faecium*

Bd2) using 16S rDNA sequencing method with 99 % similarity. These LAB were further studied in fermentation of buffalo milk to generate whey with antioxidant activity by different culture approach and different fermentation time. Addition of LAB to buffalo milk either direct culturing or preculturing methods affected the antioxidant activity of whey buffalo milk. Direct culturing of LAB into buffalo milk produced whey with high DPPH values ranged between 21.87 and 55.03 % and FICA values ranged between 50.13 and 65.52 %. However, precultured LAB to buffalo milk produced whey with lower DPPH values ranged between 3.43 and 12.28 % compared to FICA values ranged between 56.58 and 84.45 % for *L. plantarum* WG2, *L. paracasei* Pk2, *L. plantarum* S1, and *E. faecium* Bd2. Buffalo milk fermented with precultured *L. plantarum* WG2 and *E. faecium* Bd2 produced whey with high FICA values and were influenced by fermentation time. After 24 h fermentation process by precultured *E. faecium* Bd2 produced whey buffalo milk with FICA value 78.22 % but after 48 h fermentation process, precultured *L. plantarum* WG2 produced whey buffalo milk with the highest FICA value in this finding (84.45 %). The whey buffalo milk generated by both precultured *L. plantarum* WG2 and *E. faecium* Bd2 were determined the antioxidant activity by half maximal inhibitory concentration (IC_{50}) in FICA assay resulted with IC_{50} values ranged between 0.37 and 0.41 mg/ml which was lower than IC_{50} value for standard EDTA (0.29 mg/ml). Using SDS PAGE analysis, the M_w of peptides from whey buffalo milk either by precultured *L. plantarum* WG2 or by precultured *E. faecium* Bd2 was estimated between 150 and 50 kDa, 20, and 10 kDa for both LAB, respectively. This study concluded that *L. plantarum* WG2 and *E. faecium* Bd2 can be applied to generate peptides from whey buffalo milk with relatively good antioxidant activity.

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الملخص

أن ينتج عن تخمير بروتين الحليب باستخدام بكتيريا حمض اللبنيك (LAB) البتيدات النشطة حيوياً والتي تفيد صحة الإنسان. وهكذا ، كانت هذه الدراسة لفحص نشاط التحلل للبروتين LAB المعزول وتقييم النشاط المضاد للأكسدة لمصل اللبن الخالي من الدسم باستخدام عزلات LAB وكذلك لتقييم تأثير طرق الاستزراع ووقت التخمير على نشاط مصل اللبن المضاد للأكسدة في مصل اللبن المتخمر مع LAB المحدد. تم فحص النشاط البروتيني لـ LAB باستخدام طريقة أجار الحليب الخالي من الدسم (SMA) وتم قياس أنشطة مضادات الأكسدة في الحليب الخالي من الدسم المخفوق وحليب الجاموس باستخدام تنظيف 1.1-ثنائي فينيل -2 بيكريل هيدرازيل (DPPH) ونشاط مخلبات الحديد (FICA) . تم تقدير الوزن الجزيئي (M_w) من البتيدات المضادة للأكسدة من مصل اللبن الجاموس الذي تم إنتاجه بواسطة LAB باستخدام طريقة الرحلان الكهربائي باستخدام سائل دوديسيل سلفونات بولي أكريلاميد الصوديوم (SDS-PAGE). تم فحص خمسة وعشرون عزلة LAB من مصادر مختلفة (الزبادي محلي الصنع والموز والعنب والتمر والتربة والروبيان المخمر والأسماك المالحة المخمرة) لقدرتها على تحلل البروتين في الحليب الخالي من الدسم. أوضح الفحص الأولي أن ثمانية (Yg، Bn2، WG2، BG، Dt، Pk2، S1 و Bd2) من LAB أظهرت تحلل بروتيني على SMA بقطر منطقة واضحة بين 4.0 مم و 14.5 مم. جنبا إلى جنب مع اثنين من السيطرة LAB من *Lactobacillus plantarum* ATCC 8014 .و *Lactobacillus casei* ATCC 393 ، وقد تم إنتاجها هذه LAB العشرة في اللبن الخالي من الدسم المنتج مصل اللبن لديها نشاط مضاد للأكسدة تحليلها مع المقايسات DPPH و FICA بعد 24 ساعة الحضانة. تولدت عزلات WG2 و Pk2 و S1 و Bd2 نشاط مضادات الأكسدة في مصل اللبن الخالي من الدسم مع قيم DPPH تراوحت بين 12.59 و ٪ 19.04 وتراوحت قيم FICA بين 78.61 و ٪ 84.81 مقارنة بالعزلات الأخرى تقييم لإمكاناتها بروبيوتيك.جميع LAB الأربعة عزلت في البيئات الحمضية الصفراوية مع انخفاض طفيف على الأقل (الحد الأقصى < دورة 1.05 سجل) في التهم خلية قابلة للحياة لكل من فحوصات التحدي حمض الصفراء. أظهر عزل WG2 قدرة أكبر على البقاء في الإجهاد الحمضي من العزلات الأخرى مع فقدان عدد قابل للحياة أقل من 0.10 تقليل سجل في البيئة الحمضية في درجة الحموضة 2.0 بينما أظهر عزل Bd2 زيادة في عدد قابلة للحياة مع دورة سجل 0.3 cfu/ml بعد التعرض في الصفراء حالة في درجة الحموضة 6.5. بالإضافة إلى ذلك ، فإن هذه العزلات LAB قادرة على العمل كنشاط مضاد للميكروبات مع مجموعة واسعة من منطقة تثبيط بواسطة كل من تراكب الثقافة المزدوجة وطرق نشر بئر أجار ضد *Bacillus cereus* (*B. cereus*) و *Bacillus subtilis (B. subtilis)* و *Staphylococcus aureus (S. aureus)*

Escherichia coli (*E. coli*) و *Styphimurium* (*S. typhimurium*) على التوالي تم عزل جميع عزلات LAB الأربعة إلى حد كبير. التيفيموريوم و *E. coli* التي يبلغ قطرها أكبر من المنطقة الصافية 85.00 مم بواسطة طريقة التراكب المزدوج الثقافة. لوحظ أكبر قطر للمنطقة الصافية بواسطة طريقة نشر بئر أجار لعزل LAB من Pk2 و S1 و Bd2 ضد *E. coli* الذي كان 13.83 مم. نهج زراعة LAB يعزل بشكل كبير ($P<0.05$) أثرت القيمة المضادة للأكسدة من مصل اللبن جاموس عن طريق كشط نشاط DPPH جذرية ومقايسات FICA. نتج عن إنتاج مصل اللبن LAB المباشر في حليب الجاموس المخمر ارتفاعًا في قيمة DPPH مقارنةً بمصل اللبن الذي أنتجته LAB. في المقابل ، نتج عن إنتاج مصل اللبن LAB المباشر في لبن الجاموس المخمر انخفاض قيمة FICA مقارنة مع مصل اللبن الذي أنتجته LAB. تراوحت قيمة DPPH لمصل الحليب الجاموس الناتج عن LAB المزروعة مباشرة بين 22.61 و ٪ 33.62، بينما تراوحت قيمة FICA لمصل اللبن الجاموس المولدة بواسطة LAB المخصب بين 59.41 و ٪ 78.22 لعزل WG2 و Pk2 و S1 و Bd2. تم تحديد هوية العزلات WG2 و Pk2 و S1 و Bd2 على أنها *Lactobacillus plantarum* (*L. plantarum* WG2) و *Lactobacillus paracasei* (*L. paracasei* Pk2) و *Lactobacillus plantarum* (*L. plantarum* Sl) و *Enterococcus faecium* (*E. faecium* Bd2)، باستخدام طريقة تسلسل 16S rDNA . تمت دراسة هذه LAB بشكل إضافي في تخمير حليب الجاموس لتوليد مصل اللبن مع نشاط مضاد للأكسدة من خلال نهج الثقافة المختلفة ووقت التخمير المختلفة. أثرت إضافة LAB إلى حليب الجاموس إما في طرق الزراعة المباشرة أو المسببة للتأثير على نشاط مضادات الأكسدة في حليب الجاموس المخمر. تراوحت زراعة LAB المباشرة في لبن مصل اللبن المنتج مع قيم DPPH عالية بين 21.87 و ٪ 55.03 وقيم FICA تراوحت بين 50.13 و ٪65.52 ومع ذلك ، تراوحت من قبل مصل اللبن LAB إلى الحليب الجاموس المنتجة مصل اللبن مع انخفاض قيم DPPH بين 3.43 و ٪ 12.28 مقارنة بقيم FICA تراوحت بين 56.58 و ٪ 84.45 ل *L. plantarum* WG2، *L. paracasei* Pk2، *L. plantarum* Sl، *E. faecium* Bd2. حليب الجاموس المخمر بـ *L. plantarum* و *E. faecium* Bd2 أنتج مصل اللبن ذو قيم FICA عالية وكان متأثرًا بوقت التخمير. بعد عملية التخمر لمدة 24 ساعة ، أنتجت عملية تحضير حليب الجاموس بواسطة *E. faecium* Bd2 مصل اللبن بقيمة FICA ٪ 78.22 ولكن بعد عملية التخمير لمدة 48 ساعة ، استنباط حليب الجاموس بواسطة *L. plantarum* WG2 مصل اللبن مع قيمة FICA ٪. 84.45 تم تحديد مصل اللبن الجاموس الناتج عن كل من *L. plantarum* WG2 و *E. faecium* Bd2 في نشاط مضادات الأكسدة بنصف تركيز المثبطة الأقصى (IC_{50}) في مقايسة FICA مما أدى إلى تراوحت قيم IC_{50} من 0.37

إلى ملغم / مل 0.41 والتي كانت أقل من IC_{50} قيمة EDTA القياسية (ملغ / مل 0.29). باستخدام تحليل SDS PAGE ، قدرت ميغاواط من الببتيدات من مصل اللبن الحليب إما عن طريق *L. plantarum* WG2 أو عن طريق *E. faecium* Bd2 المهدد ما بين 150 و 50 كيلو دالتون و بين 20 و 10 كيلو دالتون لكل من LAB ، على التوالي. وخلصت هذه الدراسة إلى أن *L. plantarum* WG2 و *E. faecium* Bd2 يمكن تطبيقها لتوليد الببتيد من مصل الحليب الجاموس مع نشاط مضاد للأكسدة جيد نسبيا.

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LIST OF ABBREVIATIONS

%	percentage
&	and
µm	Micrometer
16S	Sedimentation coefficient
$^1O_2$	Singlet oxygen
A. sojae	*Aspergillus sojae*
A. taiwanensis	*Actinomucor taiwanensis*
ABTS	2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)
ACE	Angiotensin I-converting enzyme
Ala	Alanine
ANOVA	Analysis of variance
API	Analysis Profile Index
APS	Ammonium persulfate
APX	Cycle ascorbate peroxidase
Arg	Arginine
as-GSH	Ascorbate glutathione
Asp	Aspartic acid
B.	*Bacillus*
B. cereus	*Bacillus cereus*
B. licheniformis	*Bacillus licheniformis*
B. subtilis	*Bacillus subtilis*
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
bp	base pairs
C	Carbon
℃	Degree celcius
$CaCO_3$	Calcium carbonate
CAT	Catalase
cfu/ml	colony-forming units per millilitre
CHL	*Lactobacillus* and related genera
Cu^{2+}	Copper ion
Cys	Cysteine
Da	Dalton
DHAR	Dehydroascorbate reductase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
E.	*Euphorbia*
E. coli	*Escherichia coli*
E. faecium	*Enterococcus faecium*
E. faecium	*Enterococcus faecium*
EDTA	Ethylenediamine tetra acetic acid
EFFICA	The European Food and Feed Cultures Association
EFSA	European Food Safety Authority
etc	Et cetera or so forth
f	Forward

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FAO	Food and Agriculture Organization
FICA	Ferrous chelating activity
Fe^{+2}	Ferrous ion
Fe^{+3}	Ferric ion
$FeCl_3$	Ferric chloride
FRAP	Ferric ion reducing antioxidant power
g	Gram
G. lucidum	*Ganoderma lucidum*
GBA	Gut microbiota brain axis
GI tract	Gastrointestinal tract
Glu	Glutamic acid
Gly	Glycine
GPX	Guaiacol peroxidase
GRAS	Generally recognized as safe
GSHPx	Glutathione peroxidase
h	Hour
H	Hydrogen
H_2O_2	Hydrogen peroxide
HAT	Hydrogen atom transfer
HCL	Hydrochloric Acid
His	Histidine
HNO_2	Nitrous acid
$HO^{\bullet}$	Hydroxyl
HOCl	Hypochlorous acid
HPLC	High performance liquid chromatography
Hys	Histidine
IC_{50}	Inhibitory concentration 50%
kDa	Kilo dalton
L.	*Lactobacillus*
L. acidophilus	*Lactobacillus acidophilus*
L. casei.	*Lactobacillus casei.*
L. curvatus	*Lactobacillus curvatus*
L. delbeurkii	*Lactobacillus delbeurkii*
L. gasseri	*Lactobacillus gasseri*
L. helveticus	*Lactobacillus helveticus*
L. jensenii	*Lactobacillus jensenii*
L. kefiri	*Lactobacillus kefiri*
L. lactis	*Lactobacillus lactis*
L. paracasei	*Lactobacillus paracasei*
L. paraplantarum	*Lactobacillus paraplantarum*
L. plantarum	*Lactobacillus plantarum*
L. rhamnosus	*Lactobacillus rhamnosus*
L.bulgaricus	*Lactobacillus bulgaricus*
LAB	Lactic acid bacteria
LC MS/MS	Liquid chromatography–tandem mass spectrometry
LDL	Low-density lipoprotein
Leu	Leucine
Leu. cremoris	*Leuconostoc cremoris*
Leu. lactis	*Leuconostoc lactis*

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Leu. mesenteroids	*Leuconostoc mesenteroids*
Lys	Lysine
M	Molarity
MDHAR	Monodehydroascorbate reductase
MFC	Microbial food cultures
mg/ml	Milligram per millilitre
min	Minute
ml	Millilitre
mm	Millimeter
mM	millimolar
MRS	de Man Rogosa Sharpe
MS	Mass spectrometry
MTT	3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
M_w	molecular weight
MWCO	Molecular weight cut of
N	Nitrogen
N_2O_3	Di-nitrogen trioxide
NBT	Nitroblue tetrazolium
nm	Nanometer
$NO^{\bullet}$	Nitric oxide
$NO_2^{\bullet}$	Nitrogen dioxide
$O_2^{\bullet-}$	Superoxide anion
O_3	Ozone
OD	Optical density
$ONOO^-$	Peroxynitrite
ORAC	Oxygen radical absorbance capacity
ORAC-FL	Oxygen radical absorbance capacity – fluorescein
P. pentosaceus	*Pediococcus pentosaceus*
P.acidilactici	*Pediococcus acidilactici*
PCR	Polymerase chain reaction
PG	Propyl gallate
pH	hydrogen ion concentration
Phe	Phenylalanine
PMS	Phenazin methosulfate
Pro	Proline
r	Reverse
rDNA	Ribosomal DNA
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
$ROO^{\bullet}$	Peroxyl
ROOH	Hydroperoxide
ROS	Reactive oxygen species
RP-HPLC	Reversed-phase high performance liquid chromatography
RPLC	Reversed phase liquid chromatography
S. aureus	*Staphylococcus aureus*
S. thermophilus	*Streptococcus thermophilus*
S. Typhimurium	*Salmonella Typhimurium*
SDS PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	Second

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Ser	Serine
SET	Single electron transfer
SMA	Skimmed milkagar
SOD	Superoxide dismutase
sp.	Species
ssp.	Subspecies
STREP	Streptococci and related bacteria
TAE buffer	Tris-acetate-EDTA buffer
TBHQ	Tert-butylhydroquinone
TEAC	Trolox equivalent antioxidant capacity
TEMED	Tetramethylethylenediamine
tetrazolium salt MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
Thr	Threonine
TPTZ-Fe^{2+}	Complex of ferric 2,4,6-tripyridyl-s-triazine
TRAP	Total radical trapping antioxidant parameter
Trp	Tryptophan
Tyr	Tyrosine
U/mg	Enzyme unit per milligram
U/ml	Enzyme unit per milliliter
UHT	Ultra-high temperature
UV	Ultraviolet
V	Unit of voltage
v/v	Volume per volume
Val	Valine
W. cibaria	*Weissella cibaria*
w/v	Weight per volume
WHO	World Health Organization
x g	Times gravitational force
α	Alpha
β	Beta
µg/ml	Microgram per millilitre
µmol	Micromole

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