THE ROLES OF *Candida tropicalis* TOWARD PEPTIDE AND AMINO ACID CHANGES IN CHEESE WHEY FERMENTATION

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ABSTRACT

Whey is a by-product of cheese processing and is comprised of nearly 90% of the milk used. The protein content in cheese whey has the potential to create peptide and amino acids which have a functional effect in biological activity. Peptides and amino acids can be produced through fermentation with *Candida tropicalis* into native whey from cheese whey. The study aims to determine fermentation time in producing peptide and amino acid profiling in the fermentation of native cheese whey by *Candida tropicalis*. Cheese whey fermented with *C. tropicalis* was compared to a naturally fermented cheese whey as control at an ambient temperature for 48 hours. Peptide content identified by Folin–Ciocalteu methods and the amino acid profile is determined by high performance liquid chromatography (HPLC). Fermentation results showed that the maximum content of peptides needs a 24-hour fermentation in 10.42 ppm. Peptide content decreased with further fermentation caused by the degradation of peptides into amino acids. The amino acids that increased were aspartate, glutamate, threonine, valine, isoleucine, and lysine, while those that decreased were serine, histidine, glycine, arginine, alanine, tyrosine, and methionine.

Keywords: Amino acid; *Candida tropicalis*; Native cheese whey; Peptide content

1. INTRODUCTION

As a by-product of cheese processing, whey can be harmful to the environment because of the value of Biochemical Oxygen Demand (BOD) that can exceed 35,000 ppm and a Chemical Oxygen Demand (COD) of more than 60,000 ppm. Based on that statement, the environmental harm of 4,000 L of whey can be compared to 1,900 L of human feces (Smithers, 2008). In mozzarella cheese production, almost 90% of raw materials become whey. As a result, 100 L of milk can produce 80–90 L of whey (Božanić et al., 2014). Based on the amount and the harmful effect to the environment, whey as an agro-waste biomass needs to be handled before disposal (Hawashi et al., 2019).

Despite being a by-product, whey still has many nutritional benefits. Whey has 55% of the total

nutrients contained in milk (Andrade et al., 2017). Specifically, whey contains 93.7% (w/w) water, 0.1–0.5% (w/w) fat, 0.8% (w/w) protein, 4.9% (w/w) lactose, 0.5–0.8% (w/w) ash, and 0.1–0.4% (w/w) lactic acid (Božanić et al., 2014). Because of those nutritional characteristics, 70% of whey can be utilized to become another product, such as whey powder, that can be used in various products such as pastry, sweets, jams, and melted cheese. The added cost and technology to turn whey into whey powder can be a reason it isn't often applied in rural areas. That's why only 30% of whey is only used as animal feed and fertilizer, while the rest is thrown away into the rivers or sea (Jelen, 2011).

A mozzarella producer in Bandung District, KPBS Pangalengan, was one of the producers that can't turn whey into whey powder. One utilization of whey that has seen some popularity is the conversion into bioethanol and liquid organic fertilizer. This process can reduce the BOD of whey to 1,920 ppm, with emissions of CO_2 to 13.65% (422,219.67 kg/CO_2eq/year), and results in a positive community perception toward the environmental, social, and economic impacts (Utama et al., 2019). For further research, the nutritional component in whey has the potential to be utilized in more ways. That's because, besides nutrition, whey also has some functional properties derived from amino acids and peptides.

Amino acids are protein monomers and peptides are components that contain two or more amino acids which are bound together by peptide bonds (Dullius et al., 2018). Essential amino acids in whey are higher than in eggs, casein, meat, and soybeans (Smithers, 2008). The functional properties of essential and nonessential amino acids are beneficial to human health, including the immune system, neurological system, anti-oxidative responses, protein synthesis, reproduction, and growth (Wu, 2010). Peptides in whey also have some functional properties such as antioxidant, antimicrobial, antihypertensive, anticancer, opioid, and immunomodulatory functions (Dullius et al., 2018). The presence of amino acids and peptides in whey can be enhanced by enzymatic reactions.

As an example of enzymatic reaction, fermentation needs several components such as a carbon source (Febrianti et al., 2017). Cheese whey has enough carbon with the presence of lactose, a component that is widely used in bioprocess media needing a lactic microorganism (Utama et al., 2017a). Fermentation by microorganisms has shown to be a cost-effective method and is widely used in the dairy industries (Daliri et al., 2017).

Fermentation of mozzarella whey can be accomplished with native yeast, because it lives naturally in mozzarella whey (Hossain et al., 2017). Six colonies were isolated from mozzarella cheese whey, three of them were identified as *C. tropicalis* and the rest were two isolates of *Trichosporon beigelii*, and one isolate of *Blastoschizomyces capitatus* (Balia et al., 2018). Therefore, *C. tropicalis* is the native yeast from mozzarella whey and has the potential to create the enzymatic reaction (Utba et al., 2018). *C. tropicalis* produces several proteinase and peptides can be produced by secretions of aspartyl proteinases families such as pepsin, cathepsin, and chymosin (Balia et al., 2018; Utba et al., 2018).

The fermentation time and the peptide-amino profile during fermentation need to be observed. The longer the fermentation, the more peptides will be converted into amino acids because of further enzymatic activity (Wang et al., 2017). The yeast released its proteolytic compounds into the protein material to discharge peptides and amino acids from the parent proteins (Daliri et al., 2017). The approximation of amino acids during protein hydrolysis didn't allow for adequate evaluation of the concentration change to have the option to analyze hydrolysis and fermentation rates (Duong et al., 2019). Therefore, the research aimed to determine the optimal fermentation time in producing peptides and observed the amino acid profiles in native cheese whey before and after fermentation.

2. METHODS

This research was conducted using experimental methods with descriptive analyses. Two fermentation treatments were carried out with a *C. tropicalis* starter and without a starter. Observation of peptide levels was examined every 12 hours with four replications (a modification of Rochín-Medina et al., 2018). Amino acid profiles was examined in three treatments, before fermentation, after fermentation, and after fermentation without starter addition. Observations of amino acids were observed with the duration of fermentation which produced the most optimal peptide levels (Bartolomeo & Maisano, 2006).

Materials used were whey samples that were taken from KPBS Pangalengan, Bandung District. The starter for fermentation was *C. tropicalis* isolated from whey based on Utba et al.'s (2018) research. Other materials used were Yeast Mold Agar (YMA) (*Oxoid*), Chloramphenicol (*Sanbe*), Nutrient Broth (NB) (*Merck*), NaOH 0.1 N (*Emsure, Merck*), Na_2CO_3 (*Emsure, Merck*), $C_4H_4KNaO_6.4H_2O$ (*Emsure, Merck*), $CuSO_4$ (*Merck*), Bovine Serum Albumine (BSA) (*Merck*), Folin Ciocalteu (*Merck*), Trichloroacetic Acid (TCA) (*Emsure, Merck*), Aquadest, HCl (*Merck*), Alcohol 75% (technical grade, *Seino*), HCl (*Merck*), Ortoftalaldehyde (OPA) (*Merck*), Methanol (*Merck*), Potassium borate (*Merck*), Sodium acetate (*Merck*), Sodium Ethylene-diamine-tetraacetic Acid (Na-EDTA) (*Merck*), Tetrahydrofuran (THF) (*Merck*), and HP water.

Tools used were Spectrophotometer 1000 nm 4 nm Ultraviolet Vision Ble (FRU), Sorvall Legend Micro 17 Microcentrifuges (*Thermo Scientific*), HPLC ODS-2 Hyersil (*Thermo Scientific*) with mobile phase 1 mL / minute and fluorescence detector, Rotary Evaporator (*Buchi*), magnetic stirrer with heater 7–91 (*RRC*), acrodisc 0.45 μm (*Minisart, Sartorius*), Millipore paper (*Merck*), stove, pan, spatula, test tube, 1 mL measuring pipette, bulb pipette, petri dish, beaker glass (*Pyrex*), and stirring rod.

2.1. *C. tropicalis* Starter

The *C. tropicalis* culture was obtained by isolation from the whey mozzarella (Utba et al., 2018). Culture propagation was carried out on the media to YMA with an added 10 ppm chloramphenicol to prevent bacterial growth (modification of Suryaningsih et al., 2018). The culture decayed using sterile Aquadest, then transferred to NB (1:9) with the amount of NB was 3% of the total whey sample (modification of Belem et al., 1999). Total plate count (TPC) result with the Bacteriological Analytical Manual (BAM) calculations of the starter produced 1×10^6 cfu/ml.

2.2. Cheese Whey Fermentation

Fermentation was carried out at 26–28°C for 48 hours to pasteurized whey at 60°C for 30 minutes. Fermentation without the starter was also carried out as a control (modification of Belem et al., 1999). Furthermore, peptide content was observed every 12 hours during fermentation.

2.3. Standard Curve of BSA

The test used Reagent C which is a mixture of Reagent A (50 mL NaOH 0.1 N + Na_2CO_3 to 2%) and Reagent B (1 mL 2% sodium potassium tartrate + $CuSO_4$ to 1%). Each solution of BSA with concentrations of 4, 8, 12, 16, 20, and 24 ppm was taken by 0.5 mL, then filled with distilled water up to 1 mL. Each solution had 5 ml of Reagent C added and was incubated for 10 minutes. After incubation, 0.5 mL of dilute Folin–Ciocalteu reagent (1:1) was added and incubated again for 30 minutes. Absorbance was observed with a spectrophotometer with a wavelength of 610 nm. Plotting the absorbance value (y) with concentration (x) will produce the expected standard curve linear equation (modification of Rochín-Medina et al., 2018). The BSA standard curve resulted with the equation $y = 0.0387x + 0.0469$. The value of R^2 was good enough, which is 0.988. The curve was then used to calculate ppm levels of peptides in sample by entering the absorbance value at y.

2.4. Peptide Content Determination

Fermented whey samples were tested by the same procedure as BSA at the standard curve making stage. Whey samples were prepared by reacting samples with 12% (w/v) TCA (1:1). Samples were centrifuged at 11,000 rpm for 30 minutes at 4°C. The supernatant was filed using acrodisc 0.45 μm, then 5 mL of the sample produced was diluted with Aquadest up to 10 mL (modification of Rochín-Medina et al., 2018).

2.5. Observation of Amino Acid Composition

Samples containing 6 mg of protein were prepared by adding 2 mL of 6N HCl, flowed to nitrogen gas for 0.5−1 minute, and hydrolyzed using a 110°C oven for 24 hours. The sample was cooled then evaporated. Once dry, 0.01 N HCl to 10 mL was added to the sample, followed by analysis preparation using HPLC. For the preparation of the sample, it was treated by filtration using Millipore paper, mixing with a potassium borate buffer pH 10.4 (1:1), 25 μL of OPA reagent, and 1-minute incubation for derivatization. Finally, 5 μL of the prepared sample was injected into the HPLC with a process time of 25 minutes (Bartolomeo & Maisano, 2006).

Orthoftalaldehyde (OPA) reagent was made with 25 mg OPA, 2 mL methanol, 0.020 mL mercaptoetanol, 0.050 brij-30 30%, and 0.5 mL borate buffer 1M with pH 10.4. The HPLC was used with mobile phase formed by 1-liter Buffer A (0.02% Na-acetate pH 6.5; 0.005% Na-EDTA; 9% methanol, 1.5% THF) and Buffer B (95% methanol and HP water). Samples were poured into the HPLC with Hyersil Thermo Scientific ODS-2 columns, flow rate of mobile phase 1 mL / minute, fluorescence detector and Buffer A and Buffer B as mobile phases with gradient of 5% (0.01 minutes), 35% (13 minutes), 70% (20 minutes), up to 100% (25 minutes) (Bartolomeo & Maisano, 2006). Calculation of amino acid concentrations was calculated by Equation 1.

$$[AA] \ (\mu mol \ AA) = \frac{Sample \ peak \ area}{Standard \ peak \ area} \times [Std] \qquad (1)$$

Amino acids in ppm was calculated by Equation 2.

$$ppm \ AA = \frac{\mu mol \ AA \times Mr \ AA}{\mu gram \ of \ sampel} \times 1.000.000 \qquad (2)$$

3. RESULTS AND DISCUSSION

3.1. Peptide Content

The composition of mozzarella whey from KPBS Pangalengan was 3.16% lactose, 0.40% fat, 2.20% protein, and 93.66% water (Utama et al., 2017b). It has enough organic components for a fermentation with *C. tropicalis*. Based on the peptide content observed (Figure 1), the fermented whey with *C. tropicalis* showed the maximum number of peptides at 24 hours. Meanwhile, peptide contents in fermented whey without the addition of *C. tropicalis* did not reach that peak.

In the *C. tropicalis* fermentation, the increased peptide levels indicated the proteolytic activity from *C. tropicalis*. It's caused by the proteinase enzyme that is secreted by *C. tropicalis* such as pepsin, cathepsin, and chymosin (Korhonen & Pihlanto, 2003). This is also in accordance with other research by Chaves-López et al. (2012) which showed that 2 of 11 isolates of *C. tropicalis* produced bioactive peptides with 8.69−10.11% inhibitory properties of Angiotensin Converting Enzyme (ACE). The reduction of peptides after the 24th hour predicted by the converting of peptides into amino acids was due to further metabolizing of *C. tropicalis*.
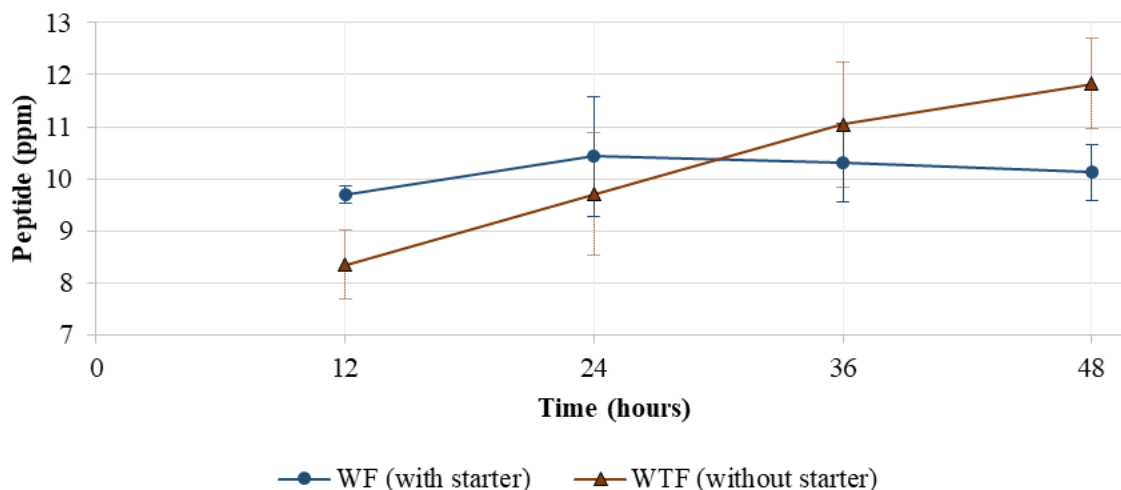
Figure 1 Peptide content curves

In the whey fermented without the starter, peptide contents increased without reaching a peak. This occurred due to the presence of indigenous microorganisms in the whey that are classified as thermophilic lactic acid bacteria. Some examples of lactic acid bacteria in whey mozzarella are *Streptococcus* sp., *E. durans*, *Streptococcus gallolyticus macedonicus*, *Aerococcus viridans*, and *E. faecium*. Fermentation with mixed cultures was able to stimulate proteolysis which enlarged the breakdown of peptides into amino acids, which was essential for the life of the microorganisms involved (KiBeom et al., 2014; Utba et al., 2018).

3.2. Amino Acids Profile

Observation of amino acid profiles was carried out on unfermented whey, fermented whey without the addition of *C. tropicalis* (control), and whey fermented with *C. tropicalis*. In general, the control whey showed that amino acid profiles increased in several amino acids such as aspartate, glutamate, threonine, valine, isoleucine, and lysine. On the other hand, fermentation with *C. tropicalis* showed amino acids, in general, had decreased, except isoleucine.

In the control whey fermentation, the increase of amino acids can be caused by the proteinase enzyme that was secreted from the whey's native microorganisms, such as pepsin. Pepsin can activate hydrochloric (HCl) becoming pepsinogen that converts protein into amino acids (Korhonen & Pihlanto, 2003). It can cleave peptide bonds between hydrophobic amino acids and it is the most bioactive peptide producer among other peptide releaser enzymes such as papain, bromelain, trypsin, etc. (Panjaitan et al., 2018).

The highest amino acid increase in the control fermentation was lysine, then glutamate. Lysine has the highest density in microbial hydrolysate cells, while glutamate is produced through deamination of glutamine by glutaminase enzymes from yeast and lactic acid bacteria (Shah et al., 2002; Liu et al., 2003). Aspartate, phenylalanine, valine, isoleucine, and leucine increased as a result of biosynthesis of amino-supported glutamate as the amine donor. That glutamate levels were still high showed that the conversion rate of glutamate was still lower than the rate of glutamate formation (Han et al., 2004).

C. tropicalis fermentation increased isoleucine due to proteolysis, in accordance with other studies which state 6 of 15 *C. tropicalis* indicated moderate levels of proteinase activity (Riceto et al., 2015). *C. tropicalis* has a proteolytic effect of 1.45–1.91 mg / ml and was able to increased amino acids by 0.08–0.18 mM during milk fermentation (Chaves-López et al., 2012). A decrease in some amino acid levels occurs in the control fermentation and *C. tropicalis* fermentation.
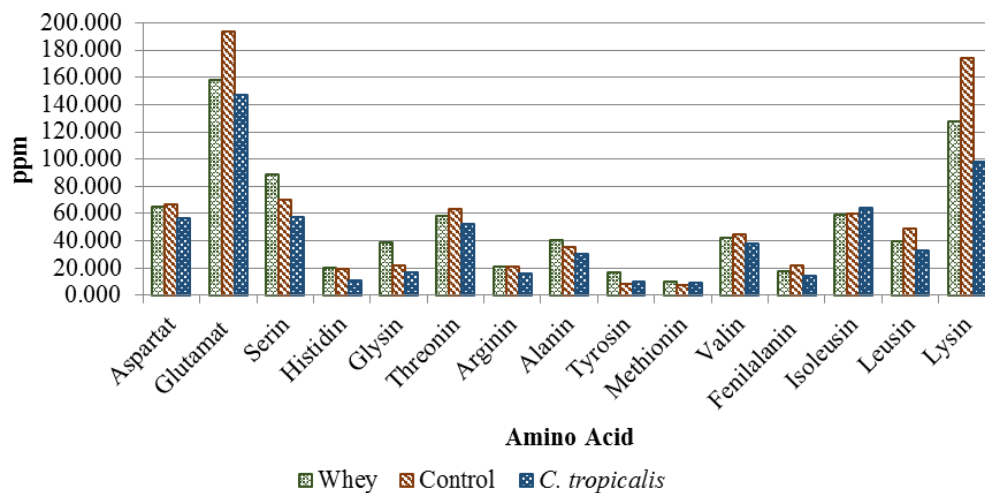
Figure 2 Amino acid profile

Control fermentation reduced serine, histidine, glycine, arginine, alanine, tyrosine, and methionine amino acids, while fermentation with *C. tropicalis* reduced all amino acids observed except isoleucine. Decreased levels of amino acids can be caused by various reactions that are capable of releasing amines such as amino acids that are required for *C. tropicalis* metabolism and fermentation. Serin, threonine, and histidine can become substrates for fermentation to produce NH_3, CO_2, and H_2. In addition, acetate can be formed from serine and histidine; propionate can also be formed from threonine.

4. CONCLUSION

Natural cheese whey fermentation with *C. tropicalis* can produce maximum amounts of peptides in 24 hours. The amino acid profile after fermentation decreased all amino acids observed except isoleucine. Fermentation without the addition of *C. tropicalis* continued to increase and showed no peak in peptides until the 48-hour mark. The amino acid profile produced showed an increase in aspartate, glutamate, threonine, valine, isoleucine, and lysine and a decrease in serine, histidine, glycine, arginine, alanine, tyrosine, and methionine.

5. ACKNOWLEDGEMENT

6. REFERENCES

Andrade, R.P., Melo, C.N., Genisheva, Z., Schwan, R.F., Duarte, W.F., 2017. Yeasts from Canastra Cheese Production Process: Isolation and Evaluation of Their Potential for Cheese Whey Fermentation. *Food Research International*, Volume 91, pp. 72–79

Balia, R.L., Kurnani, T.B.A., Utama, G.L., 2018. Selection of Mozzarella Cheese Whey Native Yeasts with Ethanol and Glucose Tolerance Ability. *International Journal on Advanced Science, Engineering and Information Technology*, Volume 8(4), pp. 1091–1097

Bartolomeo, M.P., Maisano, F., 2006. Validation of a Reversed-phase HPLC Method for Quantitative Amino Acid Analysis. *Journal of Biomolecular Techniques*, Volume 17(2), pp. 131–137

Belem, M.A.F., Gibbs, B.F., Lee, B.H., 1999. Proposing Sequences for Peptides Derived from Whey Fermentation with Potential Bioactive Sites. *Journal of Dairy Science*, Volume 82(3), pp. 486–493

Božanić, R., Barukčić, I., Lisak, K., Tratnik, L., 2014. Possibilities of Whey Utilisation. *Austin Journal of Nutrition and Food Sciences*, Volume 2(7), pp. 1–7

Chaves-López, C., Tofalo, R., Serio, A., Paparella, A., Sacchetti, G., Suzzi, G., 2012. Yeasts from Colombian Kumis as Source of Peptides with Angiotensin I Converting Enzyme (ACE) Inhibitory Activity in Milk. *International Journal of Food Microbiology*, Volume 159(1), pp. 39–46

Daliri, E.B.-M., Oh, D.H., Lee, B.H., 2017. Bioactive Peptides. *Foods*, Volume 6(5), pp. 1–27

Dullius, A., Goettert, M.I., de Souza, C.F.V., 2018. Whey Protein Hydrolysates as a Source of Bioactive Peptides for Functional Foods – Biotechnological Facilitation of Industrial Scale-up. *Journal of Functional Foods*, Volume 42, pp. 58–74

Duong, T.H., Grolle, K., Nga, T.T.V., Zeeman, G., Temmink, H., Eekert, M. van, 2019. Protein Hydrolysis and Fermentation Under Methanogenic and Acidifying Conditions. *Biotechnol Biofuels*, Volume 12, pp. 1–10

Febrianti, F., Syamsu, K., Rahayuningsih, M., 2017. Bioethanol Production from Tofu Waste by Simultaneous Saccharification and Fermentation (SSF) using Microbial Consortium. *International Journal of Technology*, Volume 8(5), p. 898–908

Han, B.-Z., Rombouts, F.M., Nout, M.J.R., 2004. Amino Acid Profiles of Sufu, A Chinese Fermented Soybean Food. *Journal of Food Composition and Analysis*, Volume 17 (6), pp. 689–698

Hawashi, M., Widjaja, T., Gunawan, S., 2019. Solid-State Fermentation of Cassava Products for Degradation of Anti-nutritional Value and Enrichment of Nutritional Value. *In:* New Advances on Fermentation Processes, Martínez-Espinosa, Rosa María (ed.)

Hossain, N., Haji Zaini, J., Mahlia, T.M.I., 2017. A Review of Bioethanol Production from Plant-based Waste Biomass by Yeast Fermentation. *International Journal of Technology*, Volume 8(1), pp. 5–18

Jelen, P., 2011. Whey Processing: Utilization and Products. *In:* Encyclopedia Dairy Science, John W. Fuquay (ed.), Academic Press, Mississippi, USA, pp. 2739–2745

KiBeom, L., Ho-Jin, K., Sang-Kyu, P., 2014. Amino Acids Analysis during Lactic Acid Fermentation by Single Strain Cultures of Lactobacilli and Mixed Culture Starter Made. *African Journal Biotechnology*, Volume 13(28), pp. 2867–2873

Korhonen, H., Pihlanto, A., 2003. Food-derived Bioactive Peptides – Opportunities for Designing Future Foods. *Current Pharmaceutical Design*, Volume 9(16), pp. 1297–1308

Liu, S.-Q., Holland, R., Crow, V.L., 2003. The Potential of Dairy Lactic Acid Bacteria to Metabolise Amino Acids Via Non-transaminating Reactions and Endogenous Transamination. *International Journal of Food Microbiology*, Volume 86(3), pp. 257–269

Panjaitan, F., Gomez, H., Chang, Y.-W., 2018. In Silico Analysis of Bioactive Peptides Released from Giant Grouper (Epinephelus lanceolatus) Roe Proteins Identified by Proteomics Approach. *Molecules*, Volume 23(11), pp. 1–15

Riceto, É.B. de M., Menezes, R. de P., Penatti, M.P.A., Pedroso, R. dos S., 2015. Enzymatic and Hemolytic Activity in Different Candida Species. *Revista Iberoamericana de Micología*, Volume 32(2), pp. 79–82

Rochín-Medina, J.J., Ramírez-Medina, H.K., Rangel-Peraza, J.G., Pineda-Hidalgo, K.V., Iribe-Arellano, P., 2018. Use of Whey as a Culture Medium for *Bacillus clausii* for the Production of Protein Hydrolysates with Antimicrobial and Antioxidant Activity. *Food Science and Technology International*, Volume 24(1), pp. 35–42

Shah, A.H., Hameed, A., Khan, G.M., 2002. Fermentative Production of L-Lysine: Bacterial Fermentation-I. *Journal of Medical Sciences*, Volume 2(3), pp. 152–157

Smithers, G.W., 2008. Whey and Whey Proteins—from 'gutter-to-gold.' *International Dairy Journal*, Volume 18(7), pp. 695–704

Suryaningsih, V., Ferniah, R.S., Kusdiyantini, E., 2018. *Karakteristik Morfologi, Biokimia, dan Molekuler Isolat Khamir Ik-2 Hasil Isolasi dari Jus Buah Sirsak*, Annona muricata L. (Morphological, Biochemical, and Molecular Characteristics of Yeast Ik-2 Isolated from Soursop Fruit Juice). *Jurnal Biologi*, Volume 7(1), pp. 18–25

Utama, G.L., Januaramadhan, I., Dinika, I., Balia, R.L., 2019. Bioconversions of Cheese-Making Wastes to Bioethanol and Their Link to Sustainability. *In:* IOP Conferences Series: Earth Environmental Science, Volume 306, pp. 1–8

Utama, G.L., Kurnani, T.B.A., Sunardi, Balia, R.L., 2017a. Reducing Cheese-making By-product Disposal through Ethanol Fermentation and the Utilization of Distillery Waste for Fertilizer. *International Journal of GEOMATE*, Volume 13(37), pp. 103–107

Utama, G.L., Kurnani, T.B.A., Sunardi, S., Cahyandito, F., Balia, R.L., 2017b. Joint Cost Allocation of Cheese-making Wastes Bioconversions into Ethanol and Organic Liquid Fertilizer. *Bulgarian Journal of Agricultural Science*, Volume 23, pp. 1016–1020

Utba, F., Balia, R.L., Utama, G.L., 2018. The Presence of Indigenous Yeasts with Proteolytic Activity Isolated from Homemade-Mozzarella Whey. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development,* Volume 18(1), pp. 507–514

Wang, W., Xia, W., Gao, P., Xu, Y., Jiang, Q., 2017. Proteolysis during fermentation of Suanyu as a Traditional Fermented Fish Product of China. *International Journal of Food Properties,* Volume 20(sup1), pp. S166–S176

Wu, G., 2010. Functional Amino Acids in Growth, Reproduction, and Health. *Advances in Nutrition*, Volume 1(1), pp. 31–37