Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Production and diagnosis of Vitamin B12 from Lactobacillus reuteri LBIQ1 and its application as a therapeutic food supplement

Abdulmutalib Abdulla Mohammed 1
1 Branch of Fundamental Science, College of Nursing, University of Basrah, 60014, Basrah, Iraq

Email: talib.abdulla75@gmail.com

Abstract. Vitamin B12 is an essential biological nutrient with multifaceted roles in human physiology and the diet is the main source of it because the human body cannot synthesize it. The photo stability nature of vitamin B12 is a major challenge, as its stability is affected by exposure to light in various industries. Absorption of VB12 also needs to be mediated by internal gastrointestinal factors, and abnormalities of the stomach or lack of such factors result in impaired absorption of VB12. Innovative strategies are therefore required to maintain its stability, effectiveness and ease of absorption, as the current strategies are generally very expensive and are still in the development stage. Thus, The objectives of the current study are to produce and test the therapeutic efficacy of the vitamin in a dietary supplement to the yogurt product, to help increase its intestinal absorption and enhance its optical stability, which can be produced in a formula with a balanced cost. Methods: VB12 was produced in the fermented medium of Lactobacillus reuteri LBIQ1 isolate and then extracted and diagnosed using infrared spectroscopy with the standard vitamin before being added to a laboratory yogurt product and subjected to sensory evaluation to be applied as a therapeutic dietary supplement to a group of skin eczema patients and followed up for three months for exploring its therapeutic effects. Results: Vitamin B12 was successfully produced and extracted from the fermentation medium of the bacterial isolate. The infrared absorption spectrum also demonstrated a high similarity between the extracted and standard samples. Sensory evaluation showed no significant difference between the vitamin-fortified yogurt product and the unfortified control product. The results of applying the fortified product to patients, during the treatment period, proved its effect in reducing the symptoms of skin infections by ≤ 25% in the first month, and (50-75%) in the second month , and (75-100%) at the end of the third month, which gives the possibility that the product has significantly enhanced the intestinal absorption of vitamin B12 and led to stabilization and increased effectiveness ($P \leq 0.05$). Conclusion: The preparation and production of vitamin B12 in a fortification yogurt supplement can increase its stability and intestinal absorption and enhance its therapeutic effectiveness as a simple and inexpensive method that can be adopted for commercial and pharmaceutical manufacture.

Highlights:

1. Production: VB12 was fermented, extracted, and added to yogurt.
2. Efficacy: Improved stability, absorption, and reduced eczema symptoms significantly.
3. Application: Affordable supplement for commercial and pharmaceutical use.

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Keywords: Vitamin B12 ; Lactobacillus reuteri LBIQ1 ; Therapeutic food supplement.

Introduction

Vitamin B12 (Cobalamin) plays an important role in body cell metabolism, erythrocyte formation, nerve function, and the formation of ribonucleic acids. This vitamin is un organic metal compound that naturally forming , highly water soluble and containing cobalt, which acts as a catalyst for the function of two enzymes in the human body. It is a structurally complex vitamin with a molecular weight of over 1000. Vitamin B12 consists of a corrin ring that consists of four inhomogeneous aromatic rings with cobalt at the center of the ring. Vitamin is made by certain bacterial species in approximately thirty enzymatic steps. No fungi, plants, or animals can produce vitamin B12, but only bacteria, as well as archaea , have the enzymes necessary to build it [2, 13].

A common form of vitamin B12 in nature is the adenosyl-Cobalamin form which found in mitochondria in eukaryotes, and serves as a catalyst for the enzyme methyl- malonyl CoA mutase. The other common form of B12 is that of methyl Cobalamin, the dominant form of the cytosol, which serves as a catalyst for the enzyme methionine synthase [12].Vitamin B12 deficiency affects the general health of the world's population, especially vegetarians, the elderly persons, pregnant women and infants, and also causes pernicious anemia and neurological disorders, such as numbness and multiple sclerosis, as well as memory loss, infertility, heart disease, sleep disturbances and muscle fatigue. therefore, is widely used in the medical and food industries [15,17]. Lactic acid bacteria have a great ability to manufacture the B group vitamins (foliate , thiamine, riboflavin and Cobalamin). Enriching foods, especially dairy foods, with B12 is one of the best ways to provide a good source of the vitamin. Lactobacillus reuteri is a Gram-positive, hetero fermented lactic acid bacterium that is a member of the digestive environment of humans and other animals. These bacteria have a distinguish probiotic characteristics , such as lowering cholesterol levels in rat blood, stimulating anti-inflammatory activity in human cells, and a condition generally known to produce Cobalamin [9, 19]. L. reuteri bacterium has been demonstrated to use glycerol during the fermentation of glucose without external Cobalamin in a vitamin B12 free fermentation medium. Consequently, this indicates L. reuteri ability to produce

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Cobalamin on its own and also its ability to supply vitamin B12 [13] requirements. In our study, we focused on producing and extracting vitamin B12 from the isolation of LBIQ1 and adding it to the yogurt product as a therapeutic supplement for people with skin eczema

Methods

Media and cultures: the growth and fermentation experiments were conducted in the Department of food science ,college of Agriculture, University of Basrah. The materials used in the experiment were as follows : Lactobacillus reuteri LBIQ1 , was isolated in a previous study [14] and characterized using 16-23SrRNA sequencing (NCBI accession number: MT259030) ; yogurt starter mixture of Streptococcus thermophiles and Lactobacillus bulgaricus ;Yeast Extract Lactone Agar Medium (YELA) ; MRS Medium.

Production, extraction, and diagnosis of vitamin B12

L. reuteri LBIQ1 isolate, which stored in glycerol at -80°C, was activated in 5 ml of MRS broth medium and incubated at 30°C for 3-4 days under anaerobic growth conditions to be ready for the fermentation process. The Fermentation method of [4] was using to prepare 1 liter medium of cheese serum supported by yeast extract (6.4 pH) , including 10 g yeast extract, 13 g sodium lactate solution (w/w60%), 0.1 g Tween 80, 0.2 g magnesium sulfate, 0.02 g manganese sulfate and 100 ml potassium phosphate.5 mg/100 ml of cobalt chloride ,sterilized with 0.2 µm filter, was added to the medium. The fermentation process was carried out by placing 225 ml of the growth medium in 6 conical flasks with a cotton plug, then the fermentation medium was inoculated with 25 ml of activated starter culture, and the plug was closed to ensure anaerobic fermentation, then incubated at 30°C. for 4 days in an anaerobic incubator [20]. After fermentation was completed, the starter was stopped using 10 ml of sodium hydroxide solution and acetic acid pH4.5.Vitamin B12 was then converted to precipitate in the extraction process by adding sodium cyanide, then the media were placed in a boiling water bath for 30 minutes and then refrigerated in an icy water bath and, finally, centrifugal separation at 10,000 rpm . The precipitate was then kept in a dark place until it dried up, after which it was collected and the weights obtained in each flask were measured. The extracted vitamin B12 was then diagnosed against a standard vitamin sample using IR spectrum absorption test.

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Manufacture of Vitamin B12-fotified yogurt

Skim milk powder was purchased locally and used to make yogurt. The starter culture consisted of a 1:1 mixture of Streptococcus thermophiles and Lactobacillus bulgaricus. The therapeutic yogurt was prepared and produced according to the method described by [6,7], about 600 grams of skimmed milk powder was dissolved in water and then heated (pasteurized) to 80°C for 15 minutes, after which it was cooled to 42-45°C before the starter culture was added. The milk was then divided into three parts: control yogurt (CY) without added vitamin, yogurt with added vitamin B12 at a concentration of 200 mg , and yogurt with added vitamin B12 at a concentration of 400 mg. The three portions of yogurt product were filled into 100 g plastic cups and then incubated at 43°C for 10-12 hours until the coagulation process is complete and the pH reaches (4.3-4.5). Then, refrigerate the product at 4-6°C for a period of 14 days .

Sensory evaluation of the yogurt products

The sensory evaluation of natural and B12 supported yogurt was conducted by a ten-member expert team using a 10-grade assessment paper included five sensory criteria: appearance, color, taste, flavor, texture, and overall acceptance of product , for possible application of it as a therapeutic supplement to people with skin eczema, and follow-up of their response to treatment , after ingestion, for a specified period of time to assess its therapeutic effectiveness .

Evaluation of the yogurt supplement in treating eczema

The effectiveness of B12 supported yogurt supplement was determined in treating a sample of 30 donor patients with symptoms of dermal eczema، were they divided into three groups of 10 patients: a control group, a 200 mg B12 treatment group and a 400 mg B12 treatment group. Their dermatological symptoms and severity were diagnosed before and after treatment. A dose of daily intake of two packs of product, morning and evening, was applied for each patient's group, followed for three months without intake of any other drug. The changes in the dermatological inflammatory symptoms were then determined and compared among the three groups.

Statistical analysis

The results were statistically analyzed using the One Way Anova table and the F test to compare sample means, using the version of 26 SPSS at $P \leq 0.05$.

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Result and Discussion

Results

The production amount of extracted vitamin B12 in the six conical flasks ranged from (20.85-25.93) mg , with a production rate of 23.23 mg/250 ml, the quantity which opposite to 92.92 gm in liter size of production medium.

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR absorption spectrum for the extracted VB12 and the standard sample have been analyzed with explanation of their prominent functional groups (Table 1) (Figure 1). The main results show a great approximation between the two samples, represents by a small peaks ranging between 2700 cm-1 and 3000 cm-1 , corresponding to typical C-H stretch vibrations. The O-H group of extracted VB12 showed a broad peak at 3290 cm-1.The figure also showed a sharp peak at 2134 cm-1, corresponding to Co-C and C-N stretching vibration. Further peaks were observed in 1639 and 1033 cm-1 referring to the groups of C=O, C-C respectively, and no further distinct absorption peaks were discovered. The above results clearly indicate that the absorption waves for functional groups in the extracted sample spectrum were approximate to the standard sample spectrum [8].

Table1:Absorption peaks and functional groups in FT-IR spectrum for standard and extracted Vitamin B12.

Functional groups	Wavelength from 400 to 4000 cm-1		
	Vitamin B12 standard	Vitamin B12 extracted	Vibration
Corrin ring	1545-1575	1415	stretching
C=O	1630-1675	1639	stretching
Co-C , C-N	2130	2134	stretching
O-H	3200-3400	3290	stretching
C-H	2700-3000	2949	stretching
C-C	947-1033	1033	stretching

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

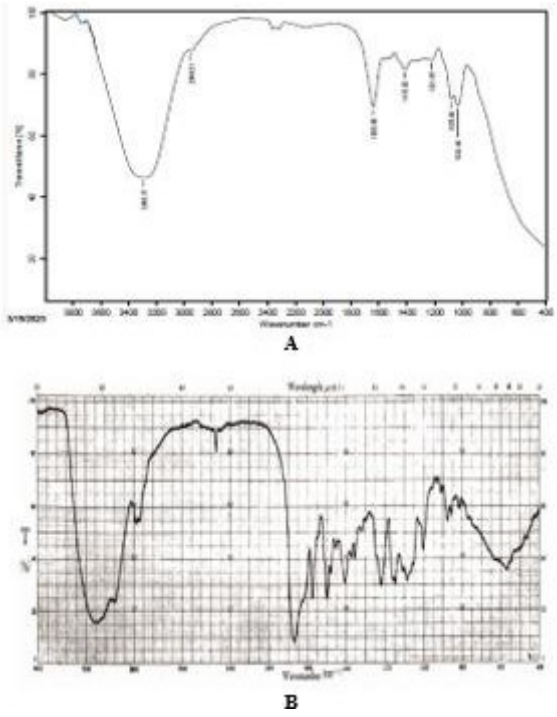

Fig.1: Graph of FT-IR spectrum for extracted VB12 (A) and standard VB12 (B).

Sensory evaluation

The results of sensory assessment showed that the samples of vitamin B12 fortified yogurt were got convergent high scores with the vitamin free control sample when they fresh after manufacture as well as after the storage period (7 and 14 days at 5±1˚C) in terms of flavor, texture, appearance, color and acidity, as no significant difference was shown between them. The final overall acceptance score was 9.2 for the vitamin subsidized product compared to 9.4 for the unsubsidized natural product (table 2), which demonstrated no significant difference and that vitamin B12 supplementation in the yogurt industry did not affect the overall sensory characteristics of product.

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Table 2: Sensory evaluation of natural and B12-supported yogurt products

Product	Appearance (10)	Colour (10)	Taste (10)	Flavor (10)	Texture (10)	Overall Acceptability (10)
Yogurt without B12	9.5	9.5	9.4	9	9.6	9.4
Yogurt with 200 mg B12	9.5	9.3	9.1	9	9.3	9.2
Yogurt with 400 mg B12	9.5	9.1	9	8.9	9.3	9.2

P>0.05 = no significant difference.

Effectiveness of B12 supported yogurt in treating eczema

The results of the patient groups intake of the free and VB12 fortified yogurt products , after three months , showed a clear reduction in the index of dermatitis symptoms in the T2 and T3 groups with a significant difference from the T1 control group, which showed no response to recovery. The response to treatment in the T3 group was the best, with rates of ≤ 75% and ≤ 100% after two and three months ,respectively, which was significantly different from the T2 group, whose members gave a response of ≤ 50% for the same treatment period, in spite of the two groups had an equal response rate in the first month of treatment, which amounted to ≤ 25% (Table 3) with a standard deviation ranging between (2.25±1 - 2.71±1). Fig. 2 and Fig. 3 show the relative decline of symptoms as irritation, itching and pus which decreased by (<50%) after 6 weeks, except for allergic dermatitis, which decreased for these rate after 8 weeks. Finally, skin symptoms was fade away in patients to rate between 0-5% after 12 weeks of treatment.

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Table 3: Assessment of the therapeutic response of eczema patients through 3 months of consuming vitamin B12 fortified yogurt

Treatment (T) groups	Diagnosed eczema symptoms	Patient No.	Therapy Response After 1month	Therapy Response After 2month	Therapy Response After 3month	P-Value
T1 Yogurt without B12	Skin irritation Skin itch Allergic dermatitis Skin pus	2 4 1 3	-	-	-	
T2 Yogurt with 200 mg B12	Skin irritation Skin itch Allergic dermatitis Skin pus	1 5 2 2	+	++	++	0.000
T3 Yogurt with 400 mg B12	Skin irritation Skin itch Allergic dermatitis Skin pus	1 6 1 2	+	+++	++++	
Total		30				Sig.

∗(-)=No response;(+) ≤ 25% response;(++) ≤ 50% response;(+++) ≤ 75% response;(++++) ≤ 100% response.

Table 4: One-way Anova statistics for assessment of effectiveness of a B12-yogurt product in treating eczema

Product	Mean	SD	Total	Mean difference
Yogurt free of B12	1.52	2.25±1	10	2.81
Yogurt with 200 mg B12	4.31	2.26 ±1	10	3.18
Yogurt with 400 mg B12	4.54	2.71 ±1	10	3.45

□ Chi2= 0.23 ; df=1 ; p= 0.55

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

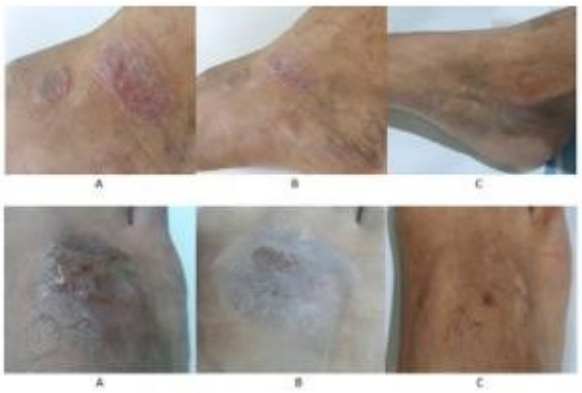

Fig. 2: Photos taken of some eczema patients who have been tested by intake of vitamin B12 fortified yogurt. A, Inflammation, itching, and pus during the first month. B, Pus dehydrated and inflammation diminished during the second month. C, All symptoms of dermatitis disappeared with the end of third month.

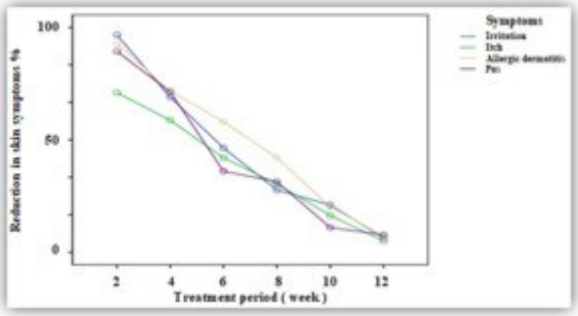

Fig.3: The relative reduction of dermal eczema symptoms in patients per every two weeks of treatment period.

Discussion

In this study, the ability of Lactobacillus reuteri LBIQ1 to produce vitamin B12 was demonstrated by providing the compounds needed for the fermentation medium under suitable conditions to stimulate bacteria to complete vitamin B12 formation. Infrared spectrum absorption was used to diagnose the vitamin B12 product, demonstrate the main functional groups of this biological compound and confirm the result of production, then proceed to the manufacture of yogurt with adding the vitamin for using it as a therapeutic dietary supplement to patients, This result is consistent with the study of [13] in which they demonstrated the ability of two wild strains of

Indonesian Journal on Health Science and Medicine
Vol 2 No 1 (2025): July
ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

Lactobacillus reuteri to produce α-(5,6-dimethylbenzimidazolyl) - cobamidcyanide or cyanocobalamin, which is the active form of vitamin B12

The overall result of the product sensory evaluation proved that the addition of vitamin B12 as a health promoting agent in yogurt manufacture did not imgrade yogurt's good sensory qualities, possibly due to the high solubility of vitamin B12 in water and its mixing with other yogurt components , this are in agreement with [18] who report that the use of probiotics or their products in dairy manufacturing gives better sensory properties than without them.

This study showed that a yogurt supplement containing vitamin B12 can be effective in reducing symptoms of dermal eczema. Two products with concentrations of 200 and 400 mg of vitamin were examined compared to a vitamin free control product. The 400 mg product had high anti-inflammatory and dry skin treatment capabilities compared to the 200 mg product [1,10] and was well accepted when used in the sample population with sensitive skin. This has led to the conclusion that the use of L. reuteri, or its vitamin product, as a nutritional supplement is beneficial and effective, and has no side effects on patients.

We also noticed from this study that the products in the treatments T2 and T3 containing the vitamin had a slight statistically significant effect on improving the skin symptoms index after 4 weeks of using the product (≤ 25%), based on their dermatological test assessments, compared to the control sample in the T1 treatment, which was not statistically significant in improvement (0%), which may be due to the low number of patients or the treatment period. However, after 8 weeks, the statistically significant effect of improvement increased between treatments, as treatment T3 showed the highest improvement at a rate of (≤ 75%), followed by treatment T2 at an improvement rate of (≤ 50%), with a significant difference from the control treatment. These results were similar to a study of [11] which showed that patients with Alzheimer and heart disorders, due to serum vitamin D deficiency, were increased their vitamin level and improved their clinical conditions, when they were intaked of vitamin supplement , more than untreated patients, making it useful and critical for patient case improvement.

Both of the subsidized products in this study significantly reduced the symptoms of irritation and itching of the skin to 25% and sepsis to 28%, while the

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

reduction of allergic dermatitis was 35% over 8 weeks to the end of treatment period, where the ratio decreased between (0-5%) at an accuracy level of ($P \leq 0.05$). Therefore, we can expect that with a larger patient sample size and a longer dosing period, the product may show greater significant differences. It has been proven in previous studies that L. reuteri species effect on the IgE gene, which associated with eczema [1], immunosuppression and pathogen inhibition [5]. It has also been recently observed to interfere with pain receptors such as TRPV1 receptor in the intestine, a prominent receptor for itching expressed in epidermal keratinocytes and other cells in the skin and is an important molecule of itching [3,16].

Conclusion

In this study, we demonstrated the ability of L. reuteri LBIQ1 strain to produce vitamin B12 by providing the required supplements for vitamin B12 synthesis in the fermentation medium, under suitable incubation conditions. Also, the study concluded that the supplement of vitamin B12- yogurt can be an important treatment to help in treat patients with eczema and reduce its symptoms and severity, a boot to the possibility that this strain may be a candidate for further studies of vitamin B12.

Acknowledgment

I am grateful to all the patients who participated in this study, as well as to the staff of the medical examination centers, who were all generous with their feelings and time to help complete the study to produce outstanding scientific results

References

[1]. T. R. Abrahamsson, T. Jakobsson, M. F. Böttcher, M. Fredrikson, M. C. Jenmalm, B. Bjorksten, and G. Oldaeus, "Probiotics in Prevention of IgE-Associated Eczema: A Double-Blind, Randomized, Placebo-Controlled Trial," Journal of Allergy and Clinical Immunology, vol. 119, pp. 1174–1180, 2007, doi: 10.1016/j.jaci.2007.01.007.

[2]. A. A. Alturfan, Water-Soluble Vitamins: A Guide to Vitamins and Their Effects on Diseases. Newcastle upon Tyne, UK: Cambridge Scholars Publishing, 2023, pp. 71-74.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

[3]. J. G. Bonchak and R. A. Swerlick, "Emerging Therapies for Atopic Dermatitis: TRPV1 Antagonists," Journal of the American Academy of Dermatology, vol. 78, pp. S63–S66, 2018, doi: 10.1016/j.jaad.2017.12.023.

[4]. P. Deptula, A Multifaceted Study of Propionibacterium Freudenreichii, the Food-Grade Producer of Active Vitamin B12, Dissertation, Schola Doctoralis Scientiae Circumiectalis, Alimentariae, Biologicae, 2017, ISSN: 2342-5423.

[5]. S. E. Jones and J. Versalovic, "Probiotic Lactobacillus reuteri Biofilms Produce Antimicrobial and Anti-Inflammatory Factors," BMC Microbiology, vol. 9, no. 35, 2009, doi: 10.1186/1471-2180-9-35.

[6]. A. Fadawy, A. I. Hassan, K. G. Zaki, and A. M. Hamdy, "Improving the Functional Properties of Bio-Yogurt by Adding Whey Protein Concentrate and Arabic Gum," Assiut Journal of Agricultural Sciences, vol. 54, no. 1, pp. 19-33, 2023, doi: 10.21608/ajas.2022.169803.1190.

[7]. M. B. Guler-Akin, B. Goncu, and M. S. Akin, "Some Properties of Probiotic Yogurt Ice Cream Supplemented with Carob Extract and Whey Powder," Advances in Microbiology Journal, vol. 6, no. 14, pp. 297-301, 2016, doi: 10.4236/aim.2016.614095.

[8]. C.-Q. Jia, S.-Y. Wang, Y. Yuan, Y.-Q. Wu, Y. Cai, J.-W. Liu, and H.-Q. Ma, "The Passive Diffusion Improvement of Vitamin B12 Intestinal Absorption by Gelucire That Fit for Commercialized Production," Saudi Pharmaceutical Journal, vol. 31, pp. 962–971, 2023, doi: 10.1016/j.jsps.2023.04.024.

[9]. B. P. Kelleher and S. D. Broin, "Microbiological Assay for Vitamin B12 Performed in 96-Well Microtitre Plates," Journal of Clinical Pathology, vol. 44, no. 7, pp. 592-595, 1991.

[10]. T.-K. Lin, L. Zhong, and J. L. Santiago, "Anti-Inflammatory and Skin Barrier Repair Effects of Topical Application of Some Plant Oils," International Journal of Molecular Sciences, vol. 19, no. 70, 2017, doi: 10.3390/ijms19010070.

[11]. L. Lugović-Mihić, N. Mandušić, M. Dasović, N. Pondeljak, M. Kuna, and I. Pozderac, "Vitamin D Supplementation in Patients with Atopic Dermatitis, Chronic Urticaria, and Contact Irritant and Allergic Dermatitis - Possible Improvement Without Risk," Folia Medica Journal, vol. 64, no. 3, pp. 467-477, 2022, doi: 10.3897/folmed.64.e66166.

ISSN 3063-8186. Published by Universitas Muhammadiyah Sidoarjo

https://doi.org/10.21070/ijhsm.v2i1.122

[12]. F. Mancia, N. H. Keep, A. Nakagawa, P. F. Leadlay, S. McSweeney, B. Rasmussen, B. Diat, and P. R. Evans, "How Coenzyme B12 Radicals Are Generated: The Crystal Structure of Methylmalonyl-Coenzyme A Mutase at 2 Å Resolution," Structure, vol. 4, no. 3, pp. 339-350, 1996.

[13]. Y. Mohammed, B. Lee, Z. Kang, and G. Du, "Capability of Lactobacillus reuteri to Produce an Active Form of Vitamin B12 Under Optimized Fermentation Conditions," Journal of Academia and Industrial Research, vol. 2, no. 11, pp. 617-621, 2014, ISSN: 2278-5213.

[14]. A. K. Niamah, A. A. Mohammed, and N. A. Alhelfi, "Antibacterial Activity and Identification of Produced Reuterin from Local Lactobacillus reuteri LBIQ1 Isolate," Journal of Microbiology, Biotechnology and Food Science, vol. 12, no. 5, 2023, doi: 10.55251/jmbfs.4701.

[15]. R. Pawlak, "Vitamin B12 Status Is a Risk Factor for Bone Fractures Among Vegans," Medical Hypotheses, vol. 153, p. 110625, 2021.

[16]. A. Perez-Burgos, L. Wang, K.-A. M. Neufeld, Y.-K. Mao, M. Ahmadzai, L. J. Janssen, A. M. Stanisz, J. Bienenstock, and W. A. Kunze, "The TRPV1 Channel in Rodents Is a Major Target for Antinociceptive Effect of the Probiotic Lactobacillus reuteri DSM 17938," Journal of Physiology, vol. 593, pp. 3943–3957, 2015, doi: 10.1113/jp270229.

[17]. V. P. Singh and N. Sachan, "Vitamin B12: A Vital Vitamin for Human Health: A Review," International Journal of Biomedical Research, vol. 2, no. 3, pp. 1-10, 2011.

[18]. S. A. Soliman and K. G. Zaki, "Effect of Adding Probiotic Bacteria on White Soft Cheese Making," Al-Azhar Journal of Agricultural Research, vol. 27, pp. 379-392, 2016.

[19]. M. P. Taranto, M. Medici, G. Perdigon, A. R. Holgado, and G. F. Valdez, "Effect of Lactobacillus reuteri on the Prevention of Hypercholesterolemia in Mice," Journal of Dairy Science, vol. 83, no. 3, pp. 401-403, 2000.

[20]. M. I. Teixeira, J. C. R. Alva, and M. H. M. Rocha-Leão, "Whey Permeate Fermentation by Propionibacterium freudenreichii," Revista Eletrônica Perspectivas da Ciência e Tecnologia, vol. 8, no. 1, pp. 35, 2016.