Waste and Biomass Valorization (2020) 11:1711–1716
https://doi.org/10.1007/s12649-018-0521-5

SHORT COMMUNICATION

Vitamin B12-Enriched *Yarrowia lipolytica* Biomass Obtained from Biofuel Waste

Monika Elżbieta Jach[1] · Maciej Masłyk[1] · Marek Juda[2] · Ewa Sajnaga[3] · Anna Malm[2]

Received: 1 June 2018 / Accepted: 19 November 2018 / Published online: 22 November 2018

Abstract
The aim of the study was to obtain vitamin B12-enriched biomass of non-conventional yeast *Yarrowia lipolytica* A-101. The cultivations were performed on waste from biofuel production in aerobic conditions at different temperatures (20–30 °C) and pH values (4.0–7.0) of the medium, with and without the addition of cobalt, L-methionine, molybdenum, δ-aminolevulinic acid, and niacin. A temperature of 30 °C and pH between 5 and 6 were the optimal conditions for obtaining B12-enriched yeast biomass on biofuel waste. In such conditions, *Y. lipolytica* A-101 contained 9 µg of vitamin B12 per 100 g of dried biomass. The addition of the ingredients mentioned above to the medium did not exert a significant effect on the B12 concentration. *Y. lipolytica* are able to accumulate this vitamin from the medium in a similar manner to animal cells. Additionally, it should be noted that the dried biomass of *Y. lipolytica* appeared to be safe for consumption. Taken together, these data showed that *Y. lipolytica* can be used as a nutritional supplement to increase the intake of vitamin B12, especially at risk of vitamin B12 deficiency.

Keywords Biofuel waste · Dried biomass · Non-conventional yeast · Vitamin B12 · *Yarrowia lipolytica*

Statement of Novelty

The aim of the presented study was to assess whether *Yarrowia lipolytica* biomass obtained from biofuel (biodiesel) waste could serve as a source of vitamin B12. The possibility to enrich yeast biomass in vitamin B12 is due to yeast being able to accumulate this vitamin from the medium. We found that B12-enriched *Y. lipolytica* biomass derived from cultures grown on biofuel waste can be regarded as a good source of vitamin B12 with proper bioavailability, given the high content of protein and essential amino acids in dried biomass. It can be included as an addition to food for people who avoid eating meat, especially vegans and vegetarians, or to feed for animals in order to prevent the risk of B12 deficiency. Probably, this is the first research of *Y. lipolytica* grown on biofuel waste as a vitamin B12 supplement for use in human diet.

Introduction

Vitamin B12 is one of the most important vitamins among the B complex group. It is a water soluble micronutrient also known as cobalamin [1]. Vitamin B12 is synthesized

✉ Monika Elżbieta Jach
monijach@kul.lublin.pl

Maciej Masłyk
maciekm@kul.lublin.pl

Marek Juda
marekjuda@umlub.pl

Ewa Sajnaga
esajnaga@kul.lublin.pl

Anna Malm
anna.malm@umlub.pl

1 Department of Molecular Biology, The John Paul II Catholic University of Lublin, Konstantynów Street 1I, 20-708 Lublin, Poland

2 Department of Pharmaceutical Microbiology with the Laboratory of Microbiology Diagnostics, Medical University of Lublin, Chodźki Street 4a, 20-093 Lublin, Poland

3 Laboratory of Biocontrol, Application and Production of EPN, Centre for Interdisciplinary Research, The John Paul II Catholic University of Lublin, Konstantynów Street 1J, 20-708 Lublin, Poland

in small amounts by some bacteria in the human and animal gastrointestinal tract and is then absorbed by the host. To cover to demand for this nutrient, vitamin B12 needs to be provided regularly with food. It is generally accepted that animal tissues are the best natural food sources of vitamin B12 [2, 3]. People who avoid animal products, for instance vegetarians, and especially vegans, and other groups with low intake of animal foods (e.g. populations who do not consume animal products through culture, poverty, conviction or those with restrictive dietary patterns) are at risk of B12 deficiency, which leads to serious health problems [1–4].

Studies reported elsewhere [5–7] revealed that yeast biomass, or so-called nutritional yeasts, can be a valuable addition for diet poor in animal products. Yeast like *Saccharomyces cerevisiae* and non-conventional species e.g. *Candida utilis* or *Yarrowia lipolytica* are known as a rich natural source of nutritional components such as proteins (e.g. single cell protein, SCP), peptides, trace minerals and fats, especially polyunsaturated fatty acids (PUFAs). Yeast biomass is also rich in essential amino acids, e.g. lysine and methionine, which occur in limited amounts in most plant and animal foods [5, 6, 8–11]. The use of nutritional yeast biomass as a supplement to the regular diet can help solve the problem of food scarcity in ever-growing human population, especially in developing countries like India [6, 7]. Now, *Y. lipolytica* is used as a high-quality protein source for livestock feeding, as a biotechnological production host for several substances like organic acids, PUFAs, carotenoids or enzymes as well as for bioremediation purposes [12].

Yeasts, e.g. *S. cerevisiae*, contain vitamins, including the B-complex. However, vitamin B12 has not been detected in this yeast [13]. It has been shown that *Y. lipolytica* has the ability to bind some minerals on the cell surface or accumulate minerals and vitamins e.g. B12 from aqueous solutions. A metabolic pathway B12 production is involved in ex novo accumulation of vitamin B12 (from substrates) in biomass produced by yeast. In contrast to de novo B12 synthesis by bacteria, ex novo B12 production is an anabolic process, occurring simultaneously with cell growth [13–17]. Being environmental friendly, *Y. lipolytica* utilizes inexpensive non-conventional feedstock and waste to produce biomass [6–12]. In this respect, yeast occupies an important place in pharmaceutical, feed, and food industry [7]. In 2010, *Y. lipolytica* biomass obtained from biofuel production waste medium was authorized by the European Feed Manufacturers' Federation as a feed additive [15].

The main aim of our project was to assess the potential of biofuel waste in the production of *Y. lipolytica* A 101 as a source of SCP and amino acids for human intake [6]. To date, there are a few reports on the vitamin B12 content in *Y. lipolytica*. The aim of the presented study was to assess whether *Y. lipolytica* biomass obtained from biofuel waste could serve as a source of vitamin B12 for human consumption and animal nutrition. Additionally, we established the optimal growth conditions to obtain vitamin B12-rich yeast biomass.

Materials and Methods

Microbial Strains

During the research, we used yeast *Y. lipolytica* A-101 obtained from Skotan S.A. Poland. The reference yeast strain *Y. lipolytica* ATCC 9793 obtained from LGC Standards was included in some experiments.

Production, *Y. lipolytica* Biomass Harvesting, and Variations of Fermentation Parameters

Yarrowia lipolytica was cultures as previously described in two trial media: the YPD medium (Difco) and the industrial SK medium, which is a waste from biofuel production (a mix of vegetable oils and degumming and glycerol fractions formed during biofuel production) [6]. The SK medium was provided by Skotan S.A. (Poland). For cultivation on the SK medium, the biofuel production waste was replaced with a partially refined, desalinated, and methanol-free by-product from biodiesel manufacture (from Lotos Group, Poland to Skotan S.A.). The SK medium contains mean 6.2 (± 0.5) µg vitamin B12/100 mL. The sterile media in the Erlenmeyer flasks (150 mL) were supplemented with cobalt(II) nitrate, L-methionine, or ammonium molybdate (VII) in a concentration range from 0 to 100 mg/L of the growth medium. Mixtures of ingredients such as cobalt (10 or 50 mg/L), L-methionine (50 mg/L), δ-aminolevulinic acid (50 or 100 mg/L), and niacin (1 mg/L) were also added to the culture medium. Biomass obtained from a biofermentor (100 L) was transferred into a tumble dryer and dried at 165–175 °C for 1 h; this yielded dried biomass called Yarrowia powder.

Detection of the Vitamin B12 Concentration in Wet and Dry Yeast Biomass

The analyses were carried out using a microbiological assay with a VitaFast® B12 Microbiological Microtiter Plate Test for determination of vitamin B12 (Cyanocobalamin) (R-Biopharm) as described by AOAC Official Methods 960.46 [18]. Microbiological quantification of vitamin B12 was performed in both dry and wet *Y. lipolytica* biomass. A selected sample of dry biomass was also analyzed with the high-performance liquid chromatography (HPLC) method [19].

Statistical Analysis of Data

All data are expressed as a mean ± SD (standard deviation) of three independent experiments. The differences between the concentrations of vitamin B12 in biomass obtained in the different conditions were compared to *Y. lipolytica* A-101 cultivated in the YPD medium at 30 °C and pH 6.0 with two-sided student's *t*-test using Statistica software version 12.0. The P value < 0.05 was considered statistically significant.

Results and Discussion

Influence of Culture Conditions on the Vitamin B12 Concentration in *Y. lipolytica* Biomass

In both media, i.e. YPD and SK, the cultivation of *Y. lipolytica* A-101 at 30 °C (pH 6.0) resulted in the highest vitamin B12 content, i.e. 3.3 µg and 3.0 µg/100 g of wet biomass, respectively (Fig. 1a). Lower incubation temperature in the range from 20 to 25 °C (pH 6.0) resulted in a decrease in the vitamin B12 concentration in the biomass of this strain cultivated in both media. These differences were statistically significant ($P < 0.05$ or $P < 0.01$). In contrast, the reference strain *Y. lipolyti*ca ATCC 9793 contained a higher vitamin B12 concentration when cultivated at temperatures from 20 to 25 °C (pH 6.0) than at a temperature of 30 °C both in the YPD and SK medium at the same pH value.

In the case of *Y. lipolytica* A-101 grown in the SK medium at 30 °C, the changes in pH (5.0 or 7.0) did not influence significantly the B12 concentrations (Fig. 1b), which were comparable in these fermentation samples (2.9 µg or 2.8 µg/100 g of wet biomass, respectively). The differences were not statistically significant ($P > 0.05$). The reference strain *Y. lipolytica* ATCC 9793 did not grow at low pH 4.0 or 5.0 in the SK medium, in contrast to the growth in the YPD medium. The analyses revealed that the temperature of 30 °C and pH 7.0 were the optimal conditions for cultivation of this strain in both media, i.e. YPD and SK, in terms of the B12 content (3.5 µg and 3.0 µg/100 g of wet biomass, respectively).

These results suggest that, irrespective of the culture conditions, both *Y. lipolytica* strains accumulated vitamin B12 in their biomass. However, the concentration of vitamin B12 in yeast biomass depends to some extent on the strains and culture conditions employed. The highest levels of vitamin B12 in the fermentation carried out by *Y. lipolytica* A-101 were obtained in the SK medium at a temperature of 30 °C and pH 6.0 (3.0 µg/100 g of wet biomass), although a promising result was obtained at pH 5.0 as well (2.9 µg/100 g of wet biomass). These differences were not statistically significant. Our previous results of SPC production revealed that temp. 30 °C and pH 5.0 were more suitable for cultivation of *Y. lipolytica* A-101 in SK medium than pH 6.0 at the same temperature value [6]. This data supports the thesis that the cultivation conditions of 30 °C and pH 5.0 are efficient in providing *Y. lipolytica* A-101 biomass rich in both protein and vitamin B12. Other media containing ingredients from biodiesel production (crude glycerol), like the SK medium, were reported to induce *Propionibacterium freudenreichii* spp. *shermanii* to produce vitamin B12 and to accumulate vitamin B12 inside cells, as in the case of *Y. lipolytica* [15, 16].

Influence of Cobalt, L-Methionine, and Other Factors on the Vitamin B12 Concentration in *Y. lipolytica* Biomass

The addition of cobalt(II) nitrate (up to 100 mg/L) did not influence the vitamin B12 concentration in the *Y. lipolytica* A-101 biomass (Fig. 2a). The differences were not statistically significant in comparison to the B12 content of this strain grown in the media without cobalt supplementation.

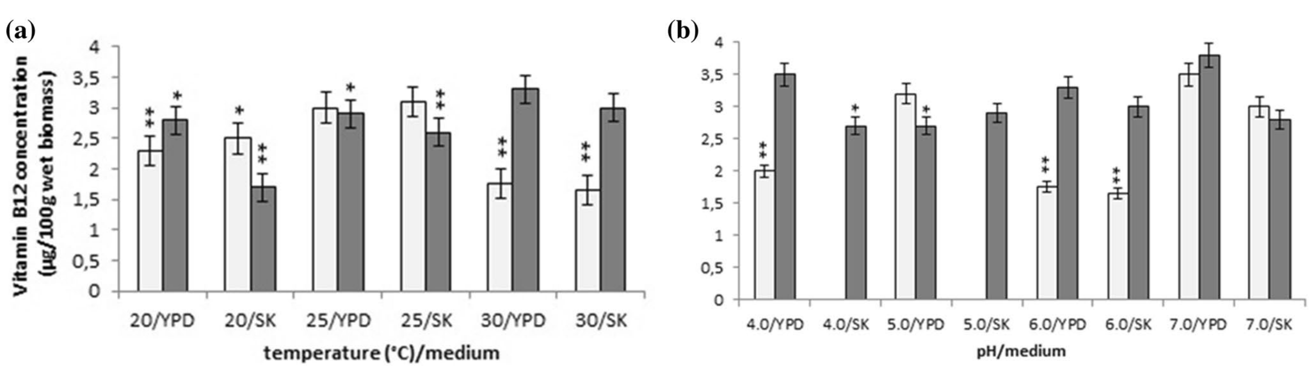

Fig. 1 Concentration of vitamin B12 in *Yarrowia lipolytica* strains in various culture conditions and growth media on a laboratory scale: effect of temperature at pH 6.0 (**a**); effect of pH at temperature 30 °C (**b**); symbols (for **a, b** panels): *Y. lipolytica* ATCC 9793 (open square); *Y. lipolytica* A-101 (filled square); $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate a significant difference in comparison with the reference cultivation (*Y. lipolytica* A-101, YPD, 30 °C, pH 6.0)

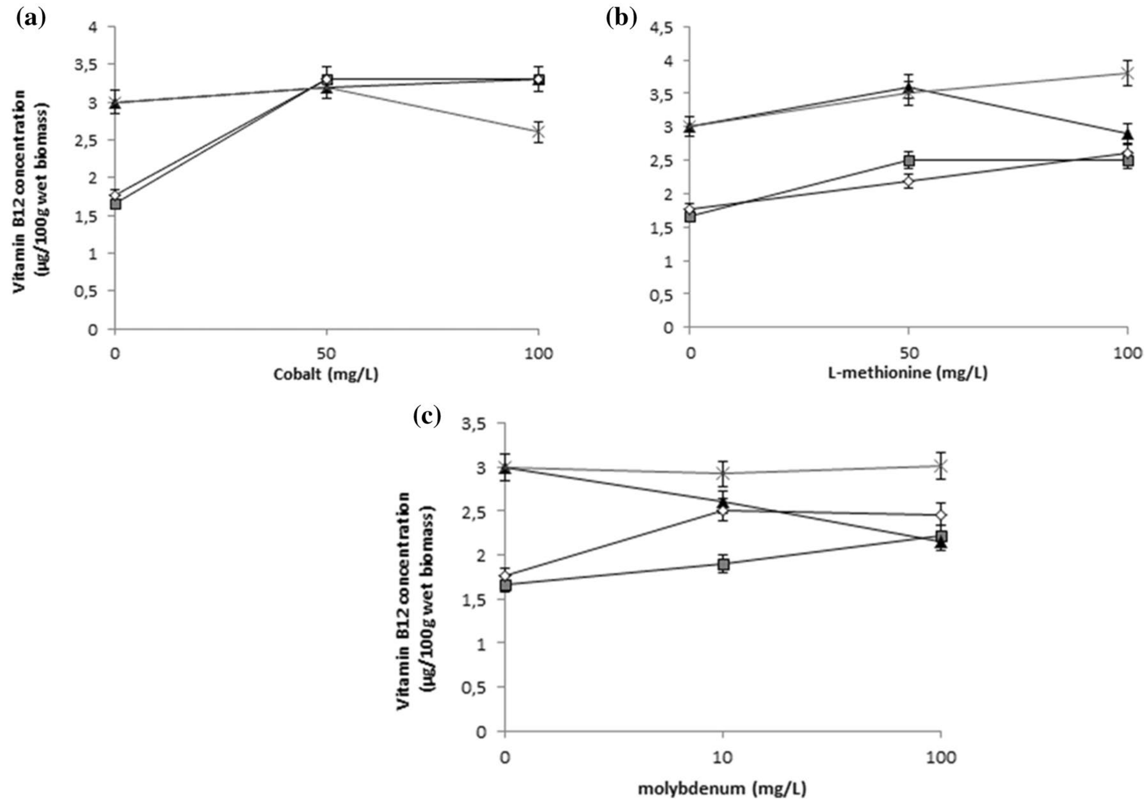

Fig. 2 Concentration of vitamin B12 in *Yarrowia lipolytica* strains in various culture conditions and growth media on a laboratory scale; after the addition of cobalt(II) nitrate (**a**), L-methionine (**b**) or ammonium molybdate (VII) (**c**), Symbols (for three panels); *Y. lipolytica* ATCC 9793 (filled square) grown in SK; *Y. lipolytica* A-101 grown in SK (filled triangle); *Y. lipolytica* ATCC 9793 grown in YPD (open triangle); *Y. lipolytica* A-101 grown in YPD (cross)

Cultivation of the reference strain *Y. lipolytica* ATCC 9793 in both media supplemented with the low concentration of cobalt(II) nitrate (50 mg/L) showed a statistically significant increase in the vitamin B12 content ($P<0.05$). Nevertheless, with the increasing cobalt concentrations, the vitamin B12 content did not increase (Fig. 2a).

The addition of L-methionine resulted in an increase in the vitamin B12 concentration in the biomass of both strains, especially at the low concentrations (0–50 mg/L) (Fig. 2b). These differences were statistically significant. The further increase in the L-methionine concentrations (up to 100 mg/L) did not cause statistically significant changes in the vitamin B12 level in the yeast biomass.

With regard to molybdenum, the vitamin B12 contents in the *Y. lipolytica* A-101 strain were slighty decreased after the addition of this factor. An opposite trend was observed in the case of the control strain *Y. lipolytica* ATCC 9793 (Fig. 2c).

We also tested the effect of the addition of factor mixtures to the media, such as cobalt (10 or 50 mg/L), L-methionine (50 mg/L), δ-aminolevulinic acid (50 or 100 mg/L), and niacin (1 mg/L), but there was no influence on the vitamin B12 concentrations in the biomass of both yeast strains. The B12 levels were comparable in all cultures (data not shown).

As reported by other authors [17, 20], an increase in the vitamin B12 content induced by the addition of ingredients like cobalt ions and further additions of potential factors such as δ-aminolevulinic acid or molybdenum indicated that these agents were necessary for efficient vitamin B12 biosynthesis. In our research, the addition of these factors did not cause a significant increase in the vitamin B12 concentration in the *Y. lipolytica* A-101 biomass. However, the addition of cobalt led to an increase in the vitamin B12 content (127%) in the reference strain *Y. lipolytica* ATCC 9793 biomass, which produced almost the same level of vitamin B12 as *Y. lipolytica* A-101. Although the addition of L-methionine caused an increase in the vitamin B12 concentration in the biomass of both strains, the incorporation of this vitamin was generally poor, as shown by the low yields. The supplementation with the mixture of such factors as cobalt, L-methionine, molybdenum, δ-aminolevulinic acid, and niacin did not have an effect on the vitamin B12 concentration obtained in the SK medium in the *Y. lipolytica* A-101 biomass. It seems that cells of both strains of *Y. lipolytica* yeast are able to accumulate this vitamin from the medium in a similar manner as animal cells.

Vitamin B12 Concentration in Yarrowia Powder

In this study, we determined the vitamin B12 content in dried *Y. lipolytica* A-101 biomass obtained by standard production of seven independent batches on a pilot plant scale (Table 1). We earlier found that 30 °C and pH 5.0 were the optimal conditions for protein production from this strain in the SK medium (waste of biofuel production) in the biofermentor [6]. The Yarrowia powder obtained was an amorphous hygroscopic beige-coloured powder with a slight yeast odor.

The content of vitamin B12 in the yeast biomass detected by microbiological assays was confirmed using the HPLC method. It is believed that microbiological assays are not reference methods [2]. The vitamin B12 concentration in the dried *Y. lipolytica* biomass measured with the HPLC method was 10.18 ± 1.32 µg/100 g of dry weight of Yarrowia powder (Fig. 3). This result was comparable to that obtained with the microbial measurement – 8.7 µg/100 g of dry weight of Yarrowia powder.

We found that the dried *Y. lipolytica* biomass obtained by drying at high temperatures, which killed the yeast and destroyed its cell walls releasing the contents, improved the digestibility of the biomass [5]. In this respect, the bioavailability of dietary vitamin B12-enriched yeast biomass is an argument for inclusion thereof as a diet supplementation for both humans and animals. Vinson [21] reported that rats fed with an unspecified vitamin B12-enriched yeast product had 2.56-fold higher levels of vitamin B12 in the serum and 1.6-fold higher content of vitamin B12 in the liver than the control group receiving uncomplexed vitamin B12. Additionally, as shown by Paalme [22], *S. cerevisiae* has great capacity of uptake of other B group vitamins such as thiamine, niacin, and pantothenic acid.

Vitamin B12 in food is generally complexed with proteins [3]. In healthy humans, the absorption rate of vitamin B12 from foods has been shown to vary depending on the quantity and type of protein consumed [23]. 100 g of Yarrowia powder is sufficient to cover the recommended daily intake (RDI) of protein for adults [6]. The RDI of vitamin B12 is 2.5 µg a day [24]. However, it is estimated that habitual cobalamin intakes range from 3.5 to 9.3 µg a day among adults and the cobalamin absorption from diet ranges between 29 and 37% [25]. 100 g of Yarrowia powder supplied as vitamin B12-rich biomass may also be sufficient in prevention of vitamin B12 deficiency.

Table 1 Protein and vitamin B12 contents of dried *Yarrowia lipolytica* A-101 biomass (Yarrowia powder) obtained after culturing in SK medium (biofuel production waste) on a pilot plant scale

Batch number[a]	Protein (% of dry weight) [6]	Vitamin B12 concentration (µg/100 g of dry weight ± SD)
1	49.3	7.4 ± 0.37
2	41.9	9.9 ± 1.38
3	42.6	9.6 ± 0.03
4	43.7	8.6 ± 0.36
5	44.8	7.9 ± 0.40
6	42.0	8.7 ± 0.44
7	41.9	8.7 ± 0.44
Mean	45.6	8.7 ± 0.87

Conditions of cultivation: 100 L, 30 °C, pH 5.0, 40% oxidation, 12 h

Each batch was obtained from a different and independent culture

[a]A batch number (series number) is a designation given the each industrial product manufacturing, allowing the history of its production to be traced

Conclusions

The non-conventional yeast *Yarrowia lipolytica* has the ability to assimilate vitamin B12 from biofuel waste substrate containing B12. Vitamin B12-enriched yeast biomass derived from *Y lipolytica* cultures grown on biofuel waste

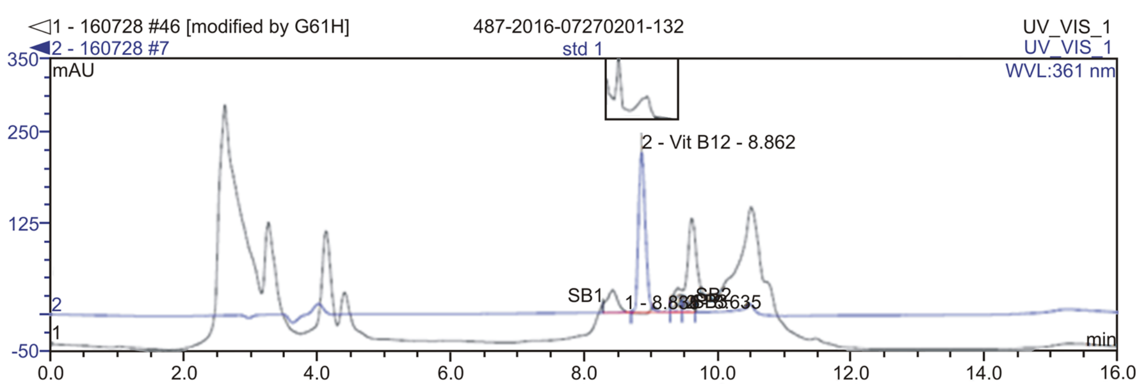

Fig. 3 Concentration of vitamin B 12 in dried *Yarrowia lipolytica* A-101 biomass (Yarrowia powder) obtained after culturing in SK medium (biofuel production waste) on a pilot plant scale. Conditions of cultivation: 100 L, 30 °C, pH 5.0, 40% oxidation, 12 h

can be regarded as a good source of vitamin B12 with proper bioavailability, given the high content of protein and essential amino acids in Yarrowia powder. It can be included as an addition to food for those who avoid eating meat, especially vegans and vegetarians, or to feed for animals in order to prevent the risk of B12 deficiency.

Acknowledgements This work was supported by the European Regional Development Fund under the Innovative Economy Operational Programme 2007–2013 [UDA-POIG-01.04.00-24-132/11-00] and Statutory project of John Paul II Catholic University of Lublin [8027].

References

1. Devi, A., Rush, E., Harper, M., Venn, B.: Vitamin B12 status of various ethnic group living in New Zealand: an analysis of the adult nutrition survey 2008/2009. Nutrients **10**, 181 (2018). https://doi.org/10.3390/nu10020181
2. O'Leary, F., Samman, S.: Vitamin B_{12} in health and disease. Nutrients **2**, 299–316 (2010)
3. Green, R.: Vitamin B12 deficiency from the perspective of a practicing hematologist. Blood **129**, 2603–2611 (2017)
4. Naik, S., Mahalle, N., Bhide, V.: Identification of vitamin B12 deficiency in vegetarian Indians. Br. J. Nutr. **15**, 1–7 (2018)
5. Adedayo, M.R., Ajibove, E.A., Akintunde, J.K., Odaido, A.: Single cell proteins: as nutritional enhancer. Adv. Appl. Sci. Res. **2**, 396–409 (2011)
6. Jach, M.E., Sajnaga, E., Świder, R., Baier, A., Mickowska, B., Juda, M., Chudzik-Rząd, B., Szyszka, R., Malm, A.: *Yarrowia lipolytica* grown on biofuel waste as a source of single cell protein and essential amino acids for human diet Saudi. J. Med. Pharm. Sci. **3**(12), 1344–1351 (2017)
7. Jach, M.E., Serefko, A.: Nutritional yeast biomass: characterization and application. In: Grumezescu, A., Holban, A.M. (eds.) Diet, Microbiome and Health. Handbook of Food Bioengineering. 1st ed, pp. 237–270. Academic Press, Cambridge (2018)
8. Bellou, S., Triantaphyllidou, I.-E., Aggeli, D., Elazzazy, A.M., Baeshen, M.N., Aggelis, G.: Microbial oils as food additives: recent approaches for improving microbial oil production and its polyunsaturated fatty acid content. Curr. Opin. Biotechnol. **37**, 24–35 (2016)
9. Lopes, M., Gomes, A.S., Silva, C.M., Belo, I.: Microbial lipids and added value metabolites production by *Yarrowia lipolytica* from pork lard. J. Biotechnol. **265**, 76–85 (2018)
10. Rywińska, A., Juszczyk, P., Wojtatowicz, M., Robak, M., Lazar, Z., Tomaszewska, L., Rymowicz, W.: Glycerol as a promising substrate for *Yarrowia lipolytica* biotechnological applications. Biomass Bioeng **48**, 148–166 (2013)
11. Uchakalwar, P.R., Chandak, A.M.: Production of single cell protein from fruits waste by using *Saccharomyces cerevisiae*. Int. J. Adv. Biotechnol. Res. **5**(4), 770–776 (2014)
12. Groenewald, M., Boekhout, T., Neuvéglise, C., Gaillardin, C., van Dijk, P.W.M., Wyss, M.: *Yarrowia lipolytica*: Safety assessment of an oleaginous yeast with a great industrial potential. Crit. Rev. Microbiol. **40**(3), 187–206 (2013)
13. Stewart, G.C., Russell, I.: The biology of *Saccharomyces*. In: Demain, A.L., Solomon, N.A. (eds.). Biology of Industrial Organisms. pp. 511–536. Benjamin Cummins Publishers, Menlo Park (1985)
14. Esmaeli, S., Khosravi-Darani, K., Pourahmad, R., Komeili, R.: An experimental design for production of selenium-enriched yeast. Mutat. Res. **496**, 105–115 (2012)
15. Juszczyk, P., Smolczyk, A., Gil, Z., Rywińska, A., Rymowicz, W.: Produkcja drożdży paszowych *Yarrowia lipolytica* wzbogaconych w aminokwasy selenowe i witaminę B12. Inż. Ap. Chem. **54**(4), 165–166 (2015)
16. Kośmider, A., Białas, W., Kubiak, P., Drożdżyńska, A., Czaczyk, K.: Vitamin B12 production from crude glicerol by *Propionibacterium freudenreichii* sp. *shermanii*: optimization of medium composition through statistical experimental designs. Bioresour. Technol. **105**, 128–133 (2012)
17. Martens, J.H., Barg, H., Warren, M.J., Jahn, D.: Microbial production of vitamin B12. App. Microbial. Biotechnol. **58**, 275–285 (2002)
18. AOAC Official Method 960.46. Vitamin assays, microbiological method Gaithersburg. The Official Methods of Analysis of AOAC International, 18th ed. Arlington, USA (2006)
19. Li, H.B., Chen, F., Jiang, Y.: Determination of vitamin B12 in multivitamin tablets and fermentation medium by high-performance liquid chromatography with fluorescence detection. J. Chromatogr. **A891**, 243–247 (2000)
20. Zhang, Y., Gladyshev, V.N.: General trends in trace element utilization revealed by comparative genomic analyses of Co, Cu, Mo, Ni and Se. J. Biol. Chem. **285**, 3393 (2010)
21. Vinson, J.A., Bose, P., Lemoine, L., Hsaio, K.: Relative bioavailability of trace elements and vitamins found in commercial supplements. In: Southgate, D.A.T., Johnson, I.T., Fenwick, G.R. (eds.) Nutrient Availability: Chemical and Biological Aspects, pp. 125–127. Royal Society of Chemistry, Cambridge (1989)
22. Paalme, T., Kevvai, K., Vilbaste, A., Hälvin, K., Nisamedtinov, I.: Uptake and accumulation of B-group vitamers in *Saccharomyces cerevisiae* in ethanol-stat fed-batch culture. World J. Microbiol. Biotechnol. **30**(9), 2351–2359 (2014)
23. Watanabe, F.: Vitamin B12 sources and bioavailability. Exp. Biol. Med. **232**, 1266–1274 (2007)
24. Regulation (E.U.) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers
25. Viñas, R., Ribas, B.L., Ngo, J., Gurinovic, M., Novakovic, R., Cavelaars, A., de Groot, L.C., van't Veer, P., Matthys, C., Serra Majem, L.: Projected prevalence of inadequate nutrient intakes in Europe. Ann. Nutr. Metab. **59**(2–4), 84–95 (2011)