

# Citrus peel, Yeast, and Azolla as natural nutraceuticals

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Received on 5 May 2021/ Accepted on 25 May 2021

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## Abstract

Citrus plants belong to the rutaceae family, which include fruits such as orange, mandarin, lemon, sour orange and grapefruit, which are known to be a promising source of many beneficial nutrients for humans. Due to a large amount of peel produced, the processing of citrus by-products is potentially a rich source of phenolic compounds and dietary fibre. These residues of citrus fruits, which are usually discarded as environmental waste, can act as potential nutraceutical resources. The use of these rich bioactive citrus residues can provide an efficient, cost-effective and environment friendly platform for the production of novel nutraceutical and for the improvement of older products. *Saccharomyces cerevisiae*, which has shown to have health-promoting effects due to its high content of vitamins (especially B-complex) and minerals, as well as its role in the production of microbial proteins,  $\beta$ -glucans and mannans. The intake of the yeasts of the brewer as a nutritional supplement is therefore popular with vegans and health-conscious people. In addition, several species of yeasts have the properties necessary to consider these microorganisms as a probiotic. The market for dietary supplements is abundant in products containing minerals and vitamin B complex yeasts, as well as valuable amino acids, glucans and mannans. Vegetarian community often suffers from deficiency of vitamin B as plant sources lack vitamin B. *Azolla* contains both macro nutrients like protein and micronutrients like vit. B12, vit. B9 and non-enzymatic antioxidants that can be used in various foods fortification and enrichment products which would ultimately cater the needy population.

**Keywords** Citrus Peel • Yeast • Azolla

## Introduction

Nutraceuticals are food or part of food that provides medical or health benefits including the prevention and/or treatment of a disease. Nutraceuticals have an advantage over the medicine because they have side effect, act as natural dietary supplement, etc. Nutraceuticals; on the basis of their natural source, Pharmacological conditions and chemical constitution.

Nutraceuticals are prescribed because a healthy diet is hard to find. Many people like to think that they eat a healthy diet. They think they have everything their bodies need for good health. Studies have shown that many people can describe a healthy diet. But when they write down what they actually eat, it's not a nutritionally complete diet.

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## Citrus Peel

Citrus is one of the world's most popular fruit crops with active phytochemicals capable of improving health and immunity. In addition, it offers a wide vitamin C, folic acid, potassium and pectin supply. The contribution of citrus species to life-threatening diseases has been evaluated and citrus fruits, citrus fruit extracts and citrus flavonoids have a wide range of promising biological properties due to their phenolic profile and antioxidant properties. [1-5,7-8]

Several studies have identified the presence of polyphenols, vitamins, minerals, dietary fibers, essential oils and carotenoids that make citrus a fruit-friendly health benefiting phytoconstituent. Several examples of the use of citrus fruits as therapeutic remedies can be cited in this regard: oranges for the scurvy cure. orange, lime, and lemon juices as remedies for the prevention of kidney stones formation, grapefruits as agents capable of lowering blood pressure and to interfere with calcium channel blockers, citrus flavonoids as effective in vivo agents able to modulate hepatic lipid metabolism, orange juice to prevent and modulate inflammatory processes, polyphenolic kumquat peeling as an effective antioxidant, anti-genotoxic grapefruit juice and many others. [11, 13-14]

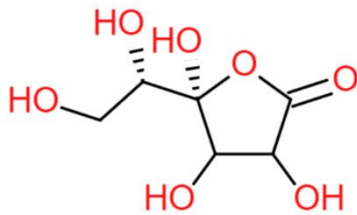


Figure 1: Structure of Vit. C (Ascorbic acid)

Phenols, amino acids, essential oils, pectin, carotenoids, flavonoids and vitamin C in citrus fruits are increasingly accepted as having beneficial effects in preventing degenerative disc disease, like a herniated disc. Antioxidants are currently used to retard the formation of compounds that lead to a reduction in sensory and nutritional quality, such as butylated-hydroxy anisole, butylated hydroxytoluene (BHA, BHT). [9-10]

In-Vivo studies have shown that these synthetic antioxidants, such as BHA, stimulate cancer cell development. These findings have shifted both researchers and consumers from their synthetic analogues to natural foods and food ingredients which are believed to be healthy and unadulterated. Thus, identification and isolation of bioactive compounds from by-products of the food processing industries can result in value addition.[12]

## Yeast

Yeasts can be regarded as the oldest industrial microorganisms. Due to their multiple health effects and industrial applications (e.g., as dietary supplements with a high nutritional value or as yeast extracts), Yeasts attracted attention. *Saccharomyces cerevisiae* (also known as the yeasts of the brewer or the yeasts of the baker) is one of the well-known and commercially important yeasts. [16-19]

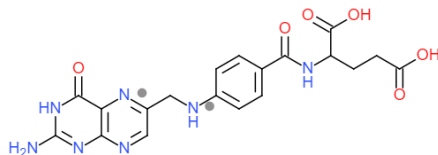


Figure 2: Structure of Vit. B9

The brewer's yeasts are often used as an abundant source of a vitamin B complex, including B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B9 (folic acid), and B7 (biotin), which plays an important role in human body. These essential nutrients help to convert the ingested food into energy, allowing the human body to remain energized all day long. While all B vitamins work together to maintain good

health, healthy eyes, heart, liver, skin, nails and hair, it also helps in preventing memory loss and migraine. [23-25] Vitamin B also support the nervous system and help in maintaining the digestive tract 's smooth muscles. It should be noted that the yeast of the brewer does not contain vitamin B12. Vegetarians with a low diet of vitamin B12 often mistakenly consume them in the hope that the yeasts of the brewer will compensate for the lack of meat in their daily diet. [20-22]

## Azolla

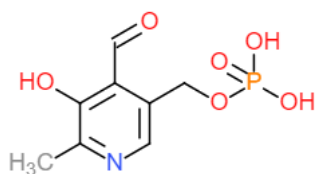


Figure 3: Structure of Vit. B6

*Azolla* are spore-producing (heterosporous), free-floating vascular fresh water plants with no seeds and flowers. It can be found in calm waters, bassins, ditches, canals and paddy fields. The nitrogen- fixing endosymbiont provides enough nitrogen for itself and its host (*Azolla*). [43-44]

*Azolla* contains many beneficial phytochemicals such as flavonoids, steroids, alkaloids, phenols, triterpenoid compounds, amino acid varieties and fatty acids.[45] The high crude protein content (more than 20%) & presence of essential amino acids like lysine, vitamin A which is a precursor beta-carotene, vitamin B12 and minerals like iron calcium, phosphorous, potassium and magnesium made *Azolla* useful feed supplement for livestock, poultry and fish.

Vitamin B12 is essential for DNA synthesis and for cellular energy production. *Azolla* is a good source of Vit B12 for Vegetarians as they are at risk of deficiency of vitamin B12 or other groups with low intakes of animal foods or those with restrictive dietary patterns.[54] Incorporation of *Azolla* in bread can target the affected population. It was revealed by texture analysis that there was only slight increase in hardness in case of bread fortified with 5% *Azolla*. Sensory analysis revealed bread fortified with 15% of *Azolla* powder has shown acceptance because of its colour.[55]

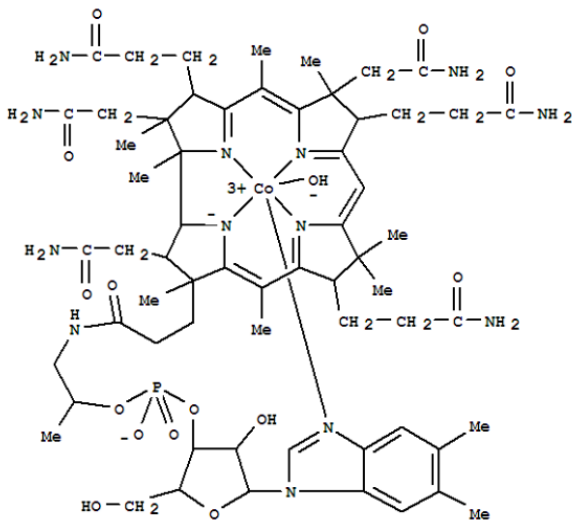


Figure 4: Structure of Vit. B12

### Briefing on extraction method for Citrus peel, Yeast, and Azolla

**Citrus Peel.** Citrus peel extracts have been prepared by refluxing the dried soil skin with ethanol, methanol, acetone, hexane, diethyl ether and dichloromethane. With methanol, a maximum amount of citrus peel extract has been obtained. [12, 15]

**Yeast.** In order to manufacture a yeast formulation rich in bioavailable organic forms of microelements, the yeasts should be cultured in a medium supplemented with a selected mineral (Cr, Se, Mg, Cu, Zn, Mn etc.). Yeast cultivation in a selenium-rich medium produces the so-called selenium-enriched yeasts. [26-27]

**Azolla.** Production of biomass under natural conditions. The efficiency of production can be improved by reducing

pollution and competition with other algae. The crops were often subculture in order to maintain the constant availability of the cultures.[48] A water body is made, preferably under the shade of a tree, with the help of a silpauline sheet. A pit of 2 x 2 x 0.2 m is dug as a first step. The pit is covered with plastic gunnies to prevent the roots of the nearby trees piercing the silpauline sheet, which is spread over the plastic gunnies.

About 10 – 15 kg of sieved fertile soil is uniformly spread over the silpauline sheet. Slurry made of 2 kg cow dung and 30 g of Super Phosphate mixed in 10 liters of water, is poured on to the sheet. More water is poured on to raise the water level to about 10 cm. About 0.5 – 1 kg of fresh and pure culture of *Azolla* is placed in the water. This will grow rapidly and fill the pit within 10 – 15 days. From then on, 500 – 600 g of *Azolla* can be harvested daily. A mixture of 20 g of Super Phosphate and about 1 kg of cow dung should be added once every 5 days in order to maintain rapid multiplication of the *Azolla* and to maintain the daily yield of 500 g. [49, 52] A micronutrient mix containing magnesium, iron, copper, Sulphur can also be added at weekly intervals to enhance the mineral content of *Azolla*.[51]

### *Saccharomyces cerevisiae* as a dietary supplement

They are usually in powder form (administered in food or in a small amount of water or juice), capsules and tablets or in a liquid form. In these preparations, raw herbal ingredients (i.e., mint, lemon balsam, horsetail, nettle) or vitamins A, C and E are often added. The yeasts of Brewer can also be found in food products such as fermented milk. Pure yeast drinks are also popular. However, regular breaks from the use of dietary supplements are usually recommended. Furthermore, products containing high amounts of protein can burden the kidneys and are therefore not recommended for continuous use.

Although the side effects of dietary supplements containing the yeasts of the brewer are usually mild, these preparations are intended primarily for adults. There are no sufficient data on children's safety. [30-37]

### **The nutritional supplements based on other yeast species**

Due to their unique properties, a number of other yeasts is also reported to be beneficial to human health. A non-pathogenic *Y* is one of them. *Lipolytica* species has been successfully used in industry as biocatalyst. It also falls under the purview of GRAS (generally regarded as safe) guidelines. *Lipolytica* is mainly found in foods containing large amounts of fats and/or proteins, especially fermented dairy and meat products (e.g., Cheese, butter, cream, margarine, yogurt, salami and sausages with Spanish fermentation).

The beneficial properties of lactic yeast traditionally grown on cheese whey, i.e., *Kluyveromyces marxianus*, have been examined, apart from the well-known probiotic *Saccharomyces boulardii*. It has been demonstrated that this microorganism can be regarded as probiotic. [28-29, 38-39]

**Note.** The plants were sterilized on the surface with a mercury chloride solution (0.1 percent) for thirty seconds and were immediately immersed in a large volume of distilled water. Plants were then transferred to double distilled water plastic tanks and washed three times. The high chlorophyll content of *Azolla* would affect *Azolla*'s nutritional analysis Therefore, dechlorophyllized *Azolla* has been treated.[53]

### **Conclusory remarks and discussion**

Recent researches have enumerated on the functional properties of citrus by-products, especially peel. Due to the low cost and easy availability of fruit residues, which would otherwise be discarded as waste in the environment. Potential nutraceutical resources should be considered capable of offering significant nutritional supplements at low cost. These unwanted casts of production, rich in bioactive compounds, could be recycled as value-added food supplements that provide advantageous dietary fibres and polyphenols. They act as non-caloric bulking agents, improve water and oil retention, improve emulsion and prevent a wide variety of oxidative stress-related diseases. Fruit peel extracts are promising sources of bioactive compounds in the food industry. Furthermore, the established use of the citrus peel would also help to reduce the pollution problems caused by the poor disposal of these residues. More research is needed to establish bioavailability and the real benefits of the citrus peel extracts.[6]

Yeasts have been used more frequently in recent years as nutritional supplements. The most famous and commercially important yeasts is *Saccharomyces cerevisiae* while interests in other closely related species are also increasing. Yeast biomass has been found to be an important starting material in the pharmaceutical industry for the production of dietary supplements, especially for vegetarians and young adults entering the puberty age. There is no doubt that yeasts, especially the brewer's yeasts, is a rich source of exogenous amino acids, microbial proteins, vitamins and minerals that are bioavailable. [40-42] Some of the yeast-based preparations may be especially suitable for people recovering from an illness or medical treatment, muscle builders or those who focus on maintaining a healthy body weight. Patients with cancer or glucose metabolism disorders can benefit from the selenium or chromium-enriched yeasts. In the traditional technological process, the deliberate use of yeasts in diet opens up new fields. [28-29]

*Azolla*'s nutritional profiling showed that fern is rich in enzyme-free antioxidants and micro-nutrients. *Azolla microphylla* is rich in essential amino acids including Histidine, Lysine, Methionine and Valine. Quantitative analysis of B-vitamins showed that *Azolla microphylla* is rich in Vit B12 and Vit B9. Regular intakes of Vit. B12 and B9 in stressed adults have been shown to improve memory. [46-47, 50] It was found that *Azolla* is packed micronutrients like VitB12, VitB9 and non-enzymatic antioxidants that are very useful for fortification of foods and enrichment of food products. [55]

## References

1. M. Elleuch, D. Bedigian, O. Roiseux, S. Besbes, C. Blecker, H. Attia, Dietary fibre and fibre rich by-products of food processing: characterisation, technological functionality and commercial applications: a review, *Food Chem.*, 4 (2011), pp. 411-421
2. A. Moure, J.M. Cruz, D. Franco, J.M. Dominguez, J. Sineiro, M.J. Nunez, J.C. Parajo, Natural antioxidants from residual sources, *Food Chem.*, 72 (2001), pp. 145-171
3. R. Cozzi, R. Ricordy, T. Aglitti, V. Gatta, P. Petricone, R. DeSalvia, Ascorbic acid and b-carotene as modulators of oxidative damage, *Carcinogenesis*, 18 (1997), pp. 223-228
4. R.S. Farag, A.Z. Badei, F.M. Hewejj, G.S.A. El-Baroty, Antioxidant activity of some spices essential oil on linoleic acid in aqueous media, *J. Am. Oil Chem. Soc.*, 66 (1986), pp. 792-799
5. N. Ito, S. Fukushima, A. Hasegawa, M. Shibata, T. Ogiso, Carcinogenicity of butylated hydroxyanisole in F344 rats, *J. Nat. Cancer Inst.*, 70 (1983), pp. 343-344
6. S.M. Burlow, Toxicological aspects of antioxidants used as food additives, B.J.F. Hudson (Ed.), *Food antioxidants*, Elsevier, Amsterdam (1990), pp. 253-268
7. L. Wang, J. Wang, L. Fang, Z. Zheng, Z. Dexian, S. Wang, S. Li, C.T. Ho, H. Zhao, Anticancer activities of citrus peel polymethoxyflavones related to angiogenesis and others, *Biomed. Res. Int.* (2014)
8. R. Guimarães, L. Barros, J.C.M. Barreira, M.J. Sousa, A.M. Carvalho, I.C.F.R. Ferreira, Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, lemon, lime and orange, *Food Chem. Toxicol.*, 48 (1) (2009), pp. 99-106
9. J.A. Manthey, K. Grohmann, Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses, *J. Agric. Food Chem.*, 49 (7) (2001), pp. 3268-3273
10. E. Magiorkinis, A. Beloukas, A. Diamantis, Scurvy: past, present and future, *Eur. J. Int. Med.*, 22 (2011), pp. 147-152
11. I. Alvarez-Gonzales, E. Madrigal-Bujaidar, V.Y. Sanchez-Garcia, Inhibitory effect of grapefruit juice on the genotoxic damage induced by ifosfamide in mouse, *Plant Foods Hum. Nutr.*, 65 (2010), pp. 369-373
12. E.S. Sadek, D.P. Makris, P. Kefalas, Polyphenolic composition and antioxidant characteristics of kumquat (*Fortunella margarita*) peel fractions, *Plant Foods Hum. Nutr.*, 64 (2009), pp. 297-302
13. C.R.C.L. Assis, H.H.M. Hermsdorff, J. Bressan, Anti-inflammatory properties of orange juice: possible favorable molecular and metabolic effects, *Plant Foods Hum. Nutr.*, 68 (2013), pp. 1-10
14. J.Y. Cha, Y.S. Cho, I. Kim, T. Anno, S.M. Rahman, T. Yanagita, Effect of hesperedin, a citrus flavonoid, on the liver triacylglycerol content and phosphatidatephosphohydrolase activity in oroticacidfed rats, *Plant Foods Hum. Nutr.*, 56 (2001), pp. 349-358
15. Citrus peel extract – A natural source of antioxidant, *Food Chemistry Volume 99, Issue 3, 2006, Pages 450-454*
16. Barbulescu I.D., Rusu N., Rughinis R., Popa O. and Stefanu A., Casarica A. (2010) Obtaining yeast biomass enriched Yeast dietary supplements 87 with copper, zinc and manganese.
17. Romanian Biotechnology Letters. 15: 5034-5041. Chen J. and Raymond K. (2008).
18. Beta-glucans in the treatment of diabetes and associated cardiovascular risks.
19. *Vascular Health and Risk Management* 4:1265-1272.
20. Chen L.X. and Schumacher H.R. (2008) Gout: an evidencebased review.
21. *Journal Clinical Rheumatology* 14: 55-62.
22. Clark L.C., Dalkin B., Krongrad A., Combs G.F. and Turnbull B.W. (1998).
23. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial.
24. *British Journal of Urology* 81: 730-734. Combs G.J. and Lu J. (2006) Selenium as a cancer preventive agent.
25. In: Hatfield, D., Berry, A. (Eds) *Selenium: Its molecular biology and role in human health*, (New York: Springer), pp. 246-264.
26. Corrigan A. Horrgan K. and Murphy R.A. (2011).
27. Effect of dietary supplementation with a *Saccharomyces cerevisiae* mannan oligosaccharide on the bacterial community structure of broiler cecal contents.
28. *Applied and Environmental Microbiology* 77: 6653-6662. Delimaris I. (2013).
29. Adverse Effects Associated with Protein Intake above the Recommended Dietary Allowance for Adults.
30. *International Scholarly Research Notices Nutrition* 2013:1-6.
31. Dobrzański Z., Korniewicz A., Dolińska B., Górecki H., Jamroz D. and Ryszka F. (2003) The chemical content and feeding value of enriched with chromium, selenium and zinc yeast *Saccharomyces cerevisiae*. *Proceedings XI International Congress ISAH*. 23-27 February 2003, Mexico.
32. Duffield-Lillico A.J., Reid M.E., Turnbull B.W., Combs G.F., Jr., Slate E.H., Fischbach L.A., Marshall J.R. and Clark L.C. (2002).
33. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention Cancer Trial.
34. *Cancer Epidemiology, Biomarkers & Prevention* 11: 630–639. Duffield-Lillico A.J., Slate E.H., Reid M.E., Turnbull B.W., Wilkins P.A., Combs G.F. Jr., Park H.K., Gross E.G., Graham G.F., Stratton M.S., Marshall J.R. and Clark L.C. (2003).
35. Nutritional Prevention of Cancer Study Group. Selenium supplementation and secondary prevention of nonmelanoma skin cancer in a randomized trial.
36. Ferentinos, L., Smith, J. and Valenzuela, H. feed for ducks, pigs, chickens, and fish U. Cooperative Extension Service. 2002; 2:1–3.
37. K.Selvaraj,Ranjana Chowdhury, Chiranjib Bhattacharjee. A green chemistry approach for the synthesis and characterization of bioactive gold nanoparticles using *Azolla microphylla* methanol extract. *Front. Mater.Sci.* 2014; 8(2):123-135.



38. Hertog, M.G.L., P.C.H. Hollman, and M.B. Katan. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agr. Food Chem.* 1992;40:2379–2383.
39. Erik Sjödin. Azolla the Cooking and Cultivation Project. 2012;24 (6):134-138.
40. Smith, P.K., Krohn, R.L., Hermanon, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. Measurement of protein using bicinchoninic acid. *Am J. Biochem.* 1985;150:76-85
41. Shahnaz Perveen, Arfa Yasmina and Khalid Mohammed Khanb. Quantitative Simultaneous Estimation of Water Soluble Vitamins, Riboflavin, Pyridoxine, Cyanocobalamin and Folic Acid in Nutraceuticals Products by HPLC, *The Open Journal Analytical Chemistry*, 2013;3:1-5.
42. Singh, O. V., S. Labana, G. Pandey, R. Budhiraja and R. K. Jain. Phytoremediation: An overview of metallic ion decontamination from soil. *Appl. Microbiol. Biotechnol.* 2003;61:405-412.
43. Luciana Alves de Oliveira, Ronielli Cardoso Reis, Hannah Miranda Santana, Vanderlei da Silva Santos, José Luiz Viana de Carvalho. Development and sensorial acceptance of biofortified dehydrated cassava chips. *Semina: Ciências Agrárias, Londrina*, 2017; 38:6; 3579-3590.
44. Singh, G., Kawatra, A., & Sehgal, S. Nutritional composition of selected green leafy vegetables, herbs, and carrots. *Plant Foods for Human Nutrition.* 2001;56:359– 364.
45. Taweesak Khuantairong and Siripen Traichaiyaporn. Enhancement of carotenoid and chlorophyll content of an edible freshwater alga (Kai: *Cladophora* sp.) by supplementary inorganic phosphate and investigation of its biomass production. *Maejo International Journal of Science & Technology*, 2012;6(01):1-11.
46. E.M. Mostafa and M. M. Ibrahim. HPLC analysis of nonenzymatic antioxidants in *Azolla caroliniana* (Pteridopsida) subjected to UV-B. *J. Biolog. Sci.*, 2012;3(1):19- 30.
47. Bickoff, E. M.; Booth, A. N.; Fremery, D. de.; Edwards, R. H.; Knuckles, B. E.; Miller, R. E.; Saunders, R. M.; Kohler, G. O. Nutritional evaluation of alfalfa leaf protein concentrate. In *Protein nutritional quality of foods and feeds*; Friedman, M., Ed.; Dekker: New York, 1975.
48. Hertog, M.G.L., P.C.H. Hollman, and M.B. Katan. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agr. Food Chem.* 1992; 40:2379–2383.
49. K.Selvaraj, Ranjana Chowdhury, Chiranjib Bhattacharjee. A green chemistry approach for the synthesis and characterization of bioactive gold nanoparticles using *Azolla microphylla* methanol extract. *Front. Mater. Sci.* 2014; 8(2):123-135.
50. M Mohamed, Ibrahim, M Eazaz and Mostafa, UV-B Effect on Constituents of *Azolla caroliniana*. *Zeitschrift fur Naturforschung - Section C Journal of Biosciences.* 2006; 62(3-4):246-252.
51. Miller RO, Jacobsen JS, Skogley EO. Aerial accumulation and partitioning of nutrients by hard red spring wheat. *Commun Soil Sci Plant Anal.* 1993; 24:2389–2407.
52. Nishinari K, Kohyama K, Kumagai H, Funami T, Bourne M. C.. Parameters of Texture Profile Analysis. *Food Sci. Technol.. Res.* 2013, 19 (3), 519 – 521,
53. Kamalasanana Pillai, P, S. Premalatha, S. & Rajamony, S. 2001. *Azolla* – a sustainable feed substitute for livestock. *LEISA India*, Volume 4 number 1, March 2002
54. Fiona O’Leary and Samir Samman\*, Vitamin B12 in Health and Disease, *Nutrients.* 2010 Mar; 2(3): 299–316. Published online 2010 Mar 5. doi: 10.3390/nu2030299, PMID: PMC3257642, PMID: 22254022
55. Mohd Azhar, Sadaf Pervez, Bibhu Prasad Panda, Sushil Kumar Gupta, Development, Processing and Evaluation of *Azolla* Enriched Breads for Nutraceutical Application, *International Journal of Biotech Trends and Technology (IJBTT)*, © 2018 by IJBTT Journal, Volume - 8 Issue – 2, Year of Publication: 2018

