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# PRODUCTION OF IODIZED *SACCHAROMYCES CEREVISIAE* EDIBLE YEAST USING BROWN SEAWEEDS *SARGASSUM* SP EXTRACT MEDIA BY SUBMERGED FERMENTATION

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**ABSTRACT**
Brown seaweeds such as *Sargassum* are very rich in iodine, currently discarded as waste material leading to environmental pollution along Indian coasts of Tanzania. The ability of *Saccharomyces cerevisiae* edible yeasts isolated from mangrove sediment to absorb iodine from Sargassum extract was investigated and analyzed by standard iodine determination method. The effect of boiling *Sargassum* extracts as growth media for yeasts during submerged culture fermentation was determined by monitoring yeast growth on the media by using spectrophotometer. Results demonstrated the ability of *Saccharomyces cerevisiae* edible yeasts to bio-extract iodine from *Sargassum* brown seaweeds extract. The yeast cells biomass from non-boiled *Sargassum* extract recorded the highest concentration of (0.06 mg/l) followed by yeasts from boiled extracts (0.03 mg/l) and the lowest (0.02 mg/l) was recorded from yeasts grown in aquatic yeasts broth. This is the first feasibility study on production of iodized edible yeast *Saccharomyces cerevisiae* on Tanzanian *Sargassum* seaweeds. Therefore, production of iodized edible yeast could in future help alleviate the iodine deficiency problem through inclusion as dietary supplement. However, such unexploited potential needs further research on optimization of fermentation parameters, supplement formulation and biosafety issues such as dosage.

**KEYWORDS**: *Sargassum*, iodized edible yeast, submerged fermentation, brown seaweeds

## INTRODUCTION

The World Health Organization (WHO) and the International Council for Control of Iodine Deficiency Disorders (ICCIDD) have taken substantial measures to improve iodine nutrition worldwide. After the introduction of iodized salt and the inclusion of iodine in supplements during the early twentieth century, iodine deficiency was reduced in the United States (Pearce, 2007; Soldin, 2009). About 31% (1.9 billion) of the world's population is estimated to have insufficient iodine intakes and 312.9 million are in Africa (41.5% of the Africa's population) (WHO, 2007). Although there is worldwide programme for elimination of endemic goiter using "iodized salt", there are serious pockets of iodine deficiency related disorders here and there throughout in sub-Saharan Africa and iodine deficiency remains the main stay an important public health problem worldwide. In 2003, WHO estimated that 45% of the population of Eastern Africa was iodine insufficient (WHO, 2004). Tanzania moved from a situation where an estimated 25% of its population was vulnerable to iodine deficiency (Kavishe and Mushi, 1993) to one where 84% consumed I-salt and 94.5% of the 6 - 12 year olds had normal sized thyroid glands (WHO, 2007). Twelve years after the initiation of the USI in Tanzania, there was an impressive improvement in iodine nutrition in Tanzania **(**Assey *et al*., 2009**).** In spite of an adequate median iodine intake at the national level, the survey revealed large regional variation with evidence of iodine deficiency in some areas and of excessive intakes in other areas (Fig. 1).

Although iodine deficiency disorders (IDD) is almost completely eliminated in the group of under 10 years old, with the higher goitre prevalence (TGP) less than 5%, it remain above that level in the older age (>9.6% among 13 - 18 year olds) (WHO, 2007) and may remain so for some years after reaching adequate iodine nutrition (Zimmermann, 2004). The country still has pockets of moderate to mild iodine deficiency, which requires further action since the goal of universal salt iodation (USI) is not simply to increase urinary iodine concentrations (UIC) but to eliminate thyroid dysfunction caused by iodine deficiency (Zimmermann, 2004). Tanzania's IDD situation requires more attention in ensuring balance of optimal iodine nutrition.

To that effect alternative complimentary solutions are imperative. The oceans are the single largest source of biogenic iodine, which are biosynthesized as organohalogens by myriad seaweeds, sponges, corals, tunicates, bacteria, and other marine life (Gribble, 2003). Organohalogens are organic compounds that contain one or more halogen atoms (fluorine, chlorine, bromine or iodine). The halogen atom(s) is covalently bonded to a carbon atom. Relatively few organohalogen compounds are found in the terrestrial organisms of nature, but organohalogen compounds are much more common within marine organisms. An organohalogen with an essential role in human biochemistry is thyroxin, the iodine-containing hormone that helps to regulate human metabolism. Edible marine algae, also called sea vegetables and seaweeds such as *Sargassum* are major coastal resources, which are valuable to human consumption and environment in many countries. In Tanzania, *Sargassum* is underutilized genetic resource and its biomass is cast on the beach, often leading to environmental pollution of coastal sea water due to rapid break down resulting in offensive smell and accumulation

of large amounts of organic sediments in mud (Oliveira *et al*., 2005). On the other hand brown seaweeds such as *Sargassum*, *Undaria*, *Laminaria* species and various kelps are known to contain many other beneficial dietary components such as large amounts of vitamins especially (B vitamins including $B_{12}$, A and C) and essential minerals (iodine, zinc, selenium, potassium, sodium etc), medicinal products and protein. Many species are harvested for use as human food supplements (McHugh, 2003; Teas *et al*., 2004).

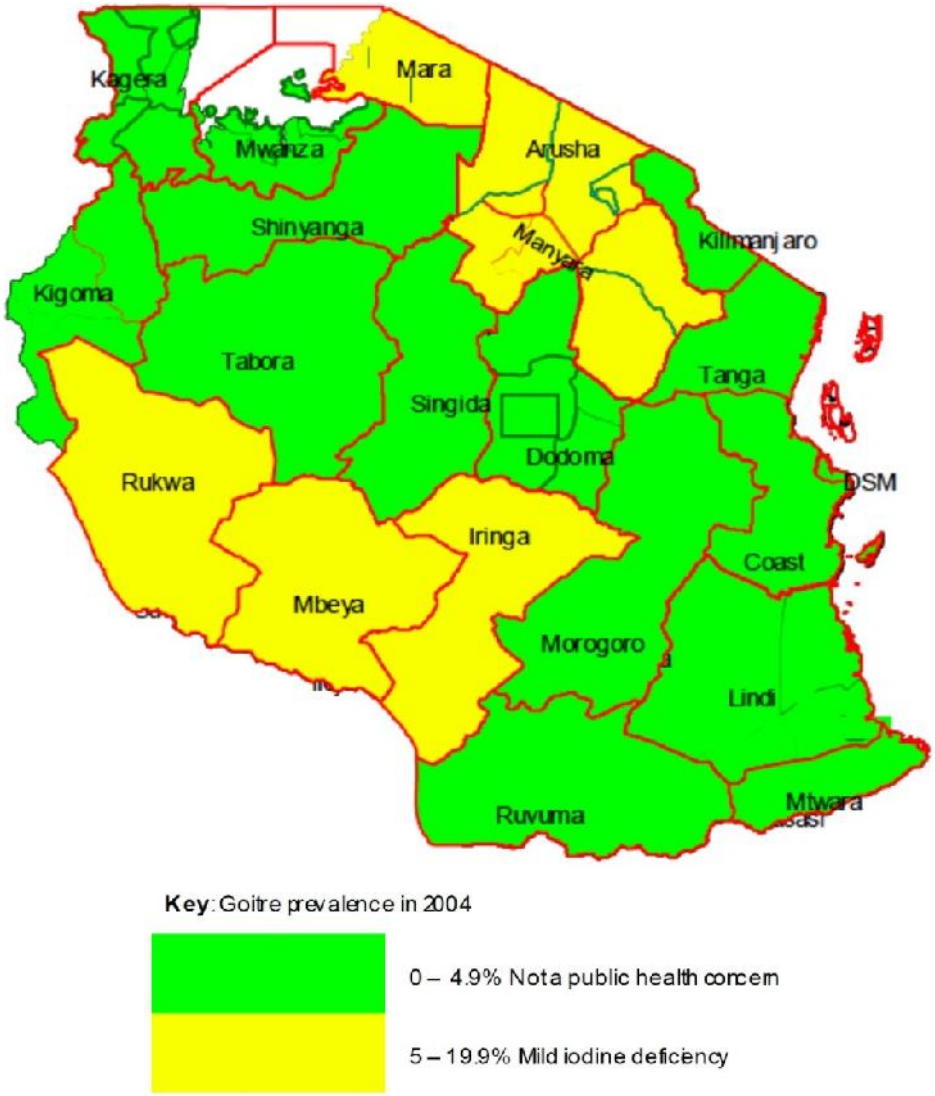


**FIGURE 1.** Total goitre prevalence by region before and after USI intervention in Tanzania (Assey *et al.,* 2009)

Iodine is the single most important element provided by seaweeds and is more abundant in seaweeds, than any land plants and animals (Teas *et al*., 2004; Smit, 2004). In seaweed, iodine species varies widely with the species of seaweed. In brown seaweed, most of iodine exists as iodide; while in green seaweed, iodine is mainly bound to organic molecules, such as protein and polyphenol (Huo, 2009). Iodine content in seaweeds has been reported to vary with species, age of the tissue, locality and the treatments the seaweed receive before analysis (Dharmananda, 2002; Teas *et al*., 2004). Simply eating unprocessed dried seaweeds can yield many essential minerals gain benefits. However, due to high iodine content in seaweeds direct consumption of seaweed may lead to hyperthyroidism (Teas *et al*., 2004). Also, high seaweed consumption has been associated with the risk of thyroid cancer (2012). Furthermore, some people are oral adverse to the tastes, smells, and/or textures of seaweeds. There is a need to find ways to make this rich health resource safely accessible for human consumption.

One such attempt is the incorporation of iodine-rich seaweed extract as a substrate to grow a **w**ild edible yeast *Saccharomyces cerevisiae* local isolate. This is mainly due to absorptive nature of yeast cells, if grown on substrate with high iodine levels the cells may also have high levels of iodine content. Such an absorptive ability of yeast cells is vital for bioextraction of iodine from *Surgasssum* seaweeds that could be used to improve nutritional and medicinal values of the yeast cells (Gao *et al*., 1993). This

may offer an alternative solution to the wide spread problem of goiter and related iodine deficiency disorders that are common throughout sub-Saharan Africa (Molloy *et al*., 1999; Staji *et al*., 2005). However, it has never been explored, tapped and investigated in Tanzania or elsewhere before. Fungi have provided food for man, primarily in the form of fruit bodies of basidiomycetes and a few ascomycetes, for thousands of years. The yeasts are eukaryotic unicellular microfungi that are widely distributed in the natural environment, and can also be found in more specialized or extreme environments, such as low temperatures, low oxygen availabilities and water potential (Gerngross, 2004). Furthermore yeast are fast-growing organism, can be easily cultivated to high cell densities (HCD) in non-expensive media, changeable growing conditions which can ensure the quantity produced in a better way. The 'food' yeasts are highly acceptable for the production of pharmaceuticals having GRAS affirmation (i.e. 'Generally Recognized as Safe'). Moreover, yeast is able to secrete large amounts of functional protein into the culture broth and is easy to handle at large scale and during product recovery. In addition, yeasts (e.g. *Saccharomyces cerevisiae*) have provided dietary supplements (e.g. vitamins) in the form of beer, wine and bread (Kovar and Meyer, 2005).

Fermentation processes are used for production of a vast number of valuable bioproducts using various fermentation media (substrates) and fermenting organisms. In submerged fermentation (SmF); which is also known as submerged liquid fermentation (SLF) or liquid-state fermentation (LSF) or submerged cultivation (SmC); the substrate is solubilized or suspended as fine particles in a large volume of water (Tang *et al*., 2007). SmF techniques have been developed for a variety of fungi and offers the possibility of high biomass production in a compact space, shorter time and with fewer chances of contamination (Friel and McLoughlin, 2000; Tang *et al*., 2007). Since seaweeds such as *Sargassum* absorb a wide range of inorganic mineral nutrients dissolved in the water where they grow, and accumulate them in their tissues, these mineral nutrients such iodine can be bio-extracted through bioprocess such as SmF. Submerged propagation of yeast cells in shaken flasks or fermentors is an easy way of producing large quantities biomass rich in iodine. The present study aimed at producing iodine *Saccharomyces cerevisiae* by cultivating on iodine-rich *Sargassum* seaweeds extract submerged culture fermentation.

## MATERIALS AND METHODS

### Yeast strain

A number of yeasts species are found in marine environment (Morris, 1968; Hagler and Ahearn, 1987). *Saccharomyces cerevisiae* local isolate has been isolated from Mtoni Kijichi mangrove sediment, Indian Ocean coast in Dar es Salaam, Tanzania. It was used throughout this study in attempt to absorb iodine from *Sargassum* brown seaweeds extract during SmF. It was obtained from the Department of Molecular Biology and Biotechnology, College of Natural and Applied Sciences, University of Dar es Salaam, Tanzania culture collection bank. It was stored in liquid nitrogen.

### Collection of *Sargassum* brown seaweed

Fresh *Sargassum* which was used in preparation of extract for growing yeast was collected from Msasani beach, Indian Ocean coast in Dar es Salaam, Tanzania. The samples were washed with seawater at the spot to remove the mud, sand and attached particles. They then were put into clean polythene bags, transferred to the laboratory and preserved at -20°C until when used.

### Medium extraction from *Sargassum*

Prior to extraction the samples *Sargassum* were defrosted at room temperature and cut into pieces of 0.5-cm length and blended using a laboratory blender (Snijders Scientific, Tilburg, Holland, Waring blender,Torrington, CT, USA) into small pieces. Two portions each 200g of grated *Sargassum* were carefully weighed using an Adventurer TM balance (Ohaus Corp. Pine Brook, NJ, USA) transferred into 800 ml of distilled water to make a slurry. One of the portions was not boiled while the other portion was boiled at 100 $^{o}$C in a pan using a table hot plate (E.G.O. (Elektro-Gerätebau GmbH), Germany) for 10 minutes. The slurry was left to cool to room temperature then together with the one not boiled were each separately strained through two different sets of two layers of cheese cloth to extract the liquid. The solid was discarded while the filtrate (extract/liquid) was used for medium preparation. The two medium prepared were sterilized in an autoclave (Koninklijke AD Linden JR.BN-Zwijinderect, Holland) at 121 $^{o}$C and for 20 minutes. For yeast growth monitoring in addition to the two media above (boiled and non-boiled *Sargassum* media extract, $(AYB)^{a}$ and distilled water as control were prepared and sterilized as above.

### Yeasts growth monitoring

The inoculum of *Saccharomyces cerevisiae* used for growth monitoring and iodine bioextraction from *Sargassum* extract was prepared as follow: $AYA^{b}$ comprised of (Glucose 1%, Yeast extract, 0.5%, NaCl, 1%, $NaH_2PO_4$, 0.2%, $(NH_3)_2SO_4$, 0.5% and 2% agar) was sterilized. The clean plates were sterilized using an oven at 180$^{o}$C for 3hours before being used to prevent microbial contamination. Then under aseptic conditions in a laminar flow cabinet (Envair C-Flow, Envair Ltd, York Avenue, Haslingden, UK). Fifteen ml of the medium poured per plate of 90-mm diameter and allowed to solidify to produce solid used media for yeast growth solid media to be later on as an inoculum. For yeast growth monitoring four media two media (boiled and non-boiled *Sargassum* media extract, third $AYB^{a}$ (Glucose 1%, Yeast extract, 0.5%, NaCl, 1%, $NaH_2PO_4$, 0.2%, $(NH_3)_2SO_4$, 0.5%) and fourth one distilled water as control prepared, 50 ml of each were transferred in 250 ml shake flask bioreactors covered with cotton wool and aluminium foil and sterilized. Each of the four media had six replicates which were inoculated with ten plugs each of (9 mm diameter) of the prepared *Saccharomyces cerevisiae* inoculum aspectically. The inoculated media were incubated at ambient temperature of 28-30 $^{o}$C while shaking at 100 rpm using a laboratory orbital shaker (Edmund, Bühler, 7400 Tübingen, West Germany). Growth was followed spectrophotometrically by measuring the optical density at 600 nm using JANEWAY Model 6700 spectrophotometer (Milian, USA). The culture was grown for 24 h, during which yeast samples were taken and the results recorded.

### Submerged yeast fermentation in shake flask bioreactors for iodine bioextraction from *Sargassum* extract

$AYB^{a}$ (Glucose 1%, Yeast extract, 0.5%, NaCl, 1%,

$NaH_2PO_4$, 0.2%, $(NH_3)_2SO_4$, 0.5%) submerged culture fermentation was carried out SmF bioreactors. One hundred ml of each media were transferred in SmF bioreactors made up of 500 ml Erlenmeyer flasks narrow neck flange-edged, graduated (Boeco, Germany), plugged with cotton wool and then covered with aluminium foil. The culture media was sterilized by autoclaving at 121 $^oC$ and 1atm for 20 minutes. The two sterilized culture medium were cooled to room temperature. The sterile media were inoculated aseptically in a laminar flow cabinet, with seven days old pure culture of *Saccharomyces cerevisiae* grown on $AYA^b$ solid media. Twenty plugs of 9-mm diameter of actively growing yeast cells were cut using a sterile cork borer were transferred aseptically into bioreactors. In this experiment three replicates SmF bioreactors were used for each media. In each set of medium there were three batch bioreactors without yeast cells inoculum (with medium only) as control. The experiment was conducted at 30 $^oC$ in the aforementioned SmF bioreactors by shaking at 100 rpm using a laboratory orbital shaker (Edmund, Bühler, 7400 Tübingen, West Germany). After 14 days of submerged fermentation of the yeast cells, their biomass was recovered by filtration under suction (Handy Aspirator, Model WP-25, Yamato Scientific Co. Ltd., Tokyo, Japan). The recovered biomass from each of the growth media type separately was measured (approx 5 grams from each). Then the presence of iodine in yeast biomass was determined spectrophotometically according to AOAC (1990).

## RESULTS AND DISCUSSION

### Yeast growth on *Sargassum* extract

Results from this study (Fig. 2, 3 and 4) demonstrated that all the growth media in which yeasts were grown, tend to support the growth of the *Saccharomyces cerevisiae* wild edible yeast isolated from mangrove sediment. This suggests that in *Sargassum* extracts (boiled and non-boiled), grown yeasts were depending on the minerals and nutrients present in *Sargassum*. The growth state of yeast namely; the non-growth phase and/or exponential growth phase affect mineral enrichment in yeast. Yeasts of marine origin as members of the marine microflora have been found to be epiphytic on decaying seaweeds. A preliminary survey indicated that yeasts *Candida parapsilosis, C. zeylanoides, C. crispus Rhodotorula rubra and R. lactosa* were epiphytic on nine species of seaweeds and that maximal populations occurred on the chlorophytes and rhodophytes especially during the periods of warmer water (Morris, 1968; Seshadri and Sieburth, 1971). That demonstrated that yeasts could utilize seaweeds natural substrate for growth. Previously Morris (1955ab) reported that various extracts from seaweeds as well as carbon-rich compounds isolated from seaweeds can be used as media or as media supplements for the cultivation of 46 strains of yeast, representing a wide range of yeast genera. In general it was found that extracts from *Laminaria cloustoni* (frond) were found to afford the most suitable media, and *Candida krusei*, *Candida solani*, *Nadsonia fulvescens*, *Pichia membranaefaciens* and *Oospora lactis* were the most prolific yeasts.

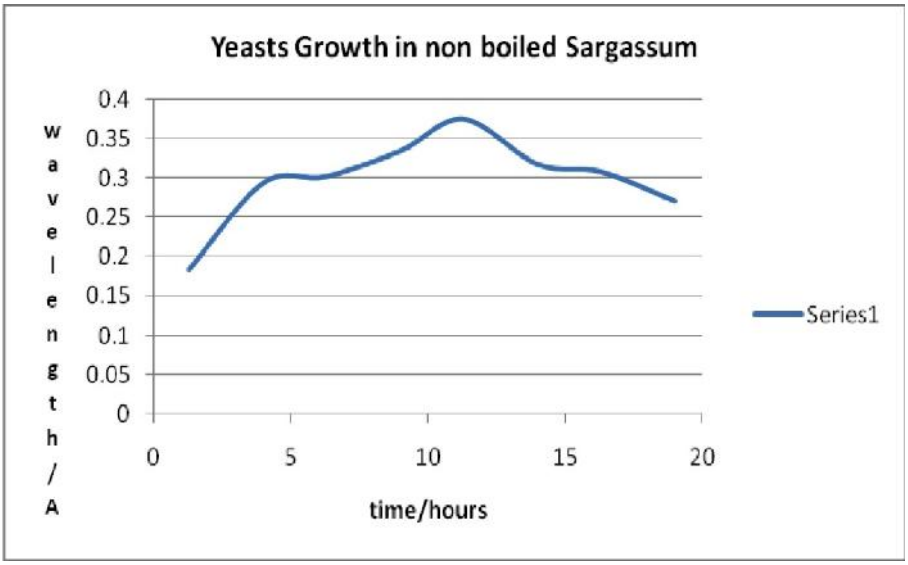


**FIGURE 2.** *Saccharomyces cerevisiae* growth on boiled *Sargassum* extract shown by wavelength (nm)vs time (hours).

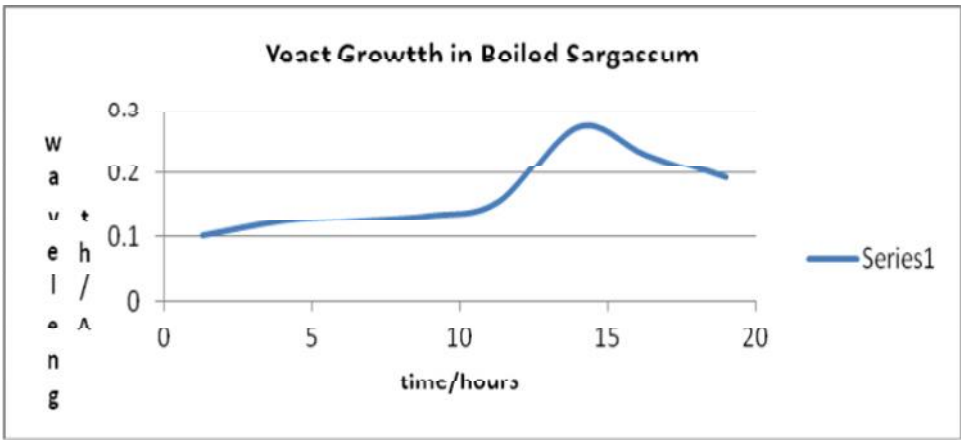


**FIGURE 3**. *Saccharomyces cerevisiae* growth on non-boiled *Sargassum* extract shown by wavelength (nm)vs time (hours).

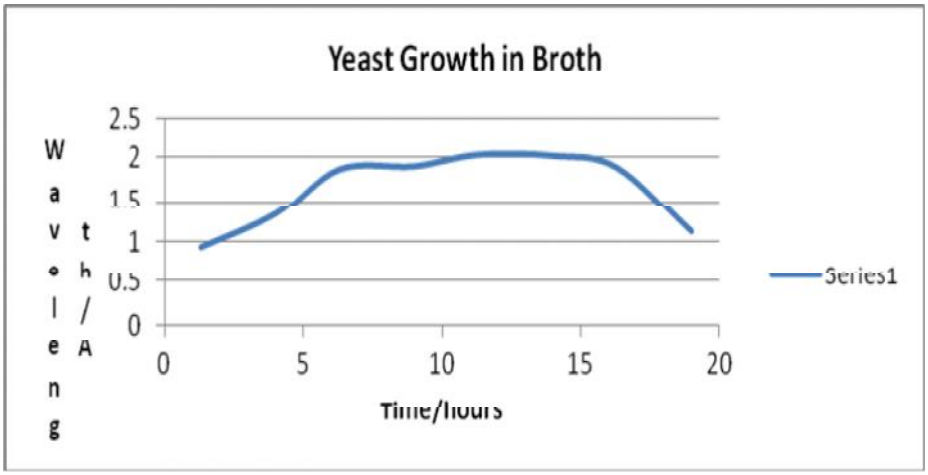


**FIGURE 4.** *Saccharomyces cerevisiae* growth on AYB[a] (Glucose 1%, Yeast extract, 0.5%, NaCl, 1%, $NaH_2PO_4$, 0.2%, $(NH_3)_2SO_4$, 0.5%) shown by wavelength (nm) vs time (hours).The presence of iodine in *Sargassum*

In this study iodine was determined in fresh *Sargassum* biomass and was found to be present in detectable amount at (wavelength of 0.219 nm) that is equivalent to iodine concentration of 0.2 mg/l, when extrapolated from the standard iodine concentration (Table 1). This was not unexpected since marine environment is exceptional reservoirs of iodine and other bioactive natural products and minerals. The concentration of iodine 0.2 mg/l found *Sargassum* extract was similar to the concentration of iodine reported by Huo *et al*. (1997a) for *Sargassum Kjellmanianum* leachate. The presence of iodine in seaweeds is due to absorption of iodine due to their capacity to absorb inorganic substances from the ocean by polysaccharide structure of their surface. However, mineral composition and mineral contents are the functions of pretreatments (sun drying or washing or boiling of blades, freshness), maturity (age) and botanical fractions of blades, environmental, geographical and physiological factors (Huo *et al*., 1997; Edmondos and Morita, 1998; Kolb *et al*., 2004). These seaweeds are of nutritional interest as they are low calorie food, but rich in vitamins, minerals and dietary fibers. The seaweeds are known to concentrate various minerals in their tissues than that found from their surrounding sea water (Edmondos and Morita, 1998).

**TABLE 1**. Iodine concentration of the prepared standard solutions

| Conc (mg/l) | 1 | 0.8 | 0.4 | 0.2 | 0.1 |
|---|---|---|---|---|---|
| Wavelength/A | 0.294 | 0.232 | 0.229 | 0.217 | 0.195 |

**The presence of iodine in yeasts grown in *Sargassum* extracts**

Iodine found to be present in all the edible yeasts *Saccharomyces cerevisiae* biomass grown in AYB, boiled *Sargassum* extract, as well as boiled *Sargassum* extract, but in different proportional of wavelengths. The wavelengths of 0.118A, 0.065A and 0.040A were observed for the yeasts biomass from non boiled

*Sargassum* extract, boiled *Sargassum* extract and AYB, which are equivalent to the concentrations; 0.06 mg/l, 0.03 mg/l and 0.02 mg/l, respectively. The yeasts biomass grew in boiled *Sargassum* extracts found to have less iodine content as compared to the ones grown in non-boiled *Sargassum* extracts. It is not clear why boiling of the seaweed could result into less iodine absorption. However, it is so obvious that the chemical nature of the iodine in the *Sargassum* extracts was converted into a chemical form which is not available for absorption by the yeast cells on boiling; hence the amount of iodine presented in yeast cells growing on it was less as compared to non-boiled extracts. This study demonstrated that *S. cerevisiae* local isolate used could be bio-carrier for iodine from *Sargassum* extract medium. Although the quantities as lower than the recommended daily allowance (RDA) of 0.150 mg for adults (WHO, 1996). The pH and dissolved oxygen level in the culture medium (*Sargassum* extract) which are most important factors that influence incorporated forms of iodine in yeast can be optimized to attain the nutritional yeast of choice for food and ‘‘iodine enriched yeast’’ supplement applications. Iodine is one of trace elements essential to human. The iodine deficiency disorder (IDD) has been found in almost all of other countries, which can seriously threaten human health. The main reason for this disease is that the intake of iodine does not meet the physiological. The main routes from which people take up iodine are diet and air, for which diet accounts for more than 98% need (Huo *et al*., 1997b).

## CONCLUSION

The present preliminary data demonstrates the potential of *S. cerevisiae* edible yeast to bioextract iodine from *Sargassum* extract. This offers baseline data for further research on production of iodine enriched yeast (IEY) and various other supplements based on the common yeast *Saccharomyces cerevisiae.*

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