Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Potential of *Laurencia obtusa* as a substrate for the development of a probiotic *Saccharomyces cerevisiae*

F. Caf[1*], Ö. Yilmaz[2], N. Şen Özdemir[3]

[1] Technical ScienceVocational High School, Bingöl University, 12000 Bingol, Turkey
[2] Department of Biology, Faculty of Sciences, Fırat University, 23119 Elazığ, Turkey
[3] Department of Fisheries, Faculty of Agriculture, Bingöl University, 12000 Bingöl, Turkey

Correspondence to: f.baydas23@hotmail.com

Received April 20, 2017; **Accepted** August 10, 2017; **Published** August 30, 2017

Doi: http://dx.doi.org/10.14715/cmb/2017.63.8.16

Abstract: *Laurencia obtusa* (Ceramiales, Rhodophyta) has tremendous nutritional value, being high in proteins, oligosaccharides, vitamins, essential minerals, and fatty acids, and it is a rich source of amino acids and trace elements. In this study, *L. obtusa* was extracted and subjected to phenolic, sugar and flavonoid analyses.The fatty acid, vitamin and phytosterol contents in *Saccharomyces cerevisiae* were evaluated when it was incubated with *L. obtusa* dry biomass. The fatty acids in the lipid extract were analysed after converting them into methyl esters using gas chromatography, and vitamin concentrations were measured using high-performance liquid chromatography (HPLC). According to the achieved results, the total fatty acid levels and vitamin contents of the *S. cerevisiae* prepared with algal extract increased at different rates. Our results showed that α-tocopherol decreased in the group in which the *S. cerevisiae* was added the algal extract. When compared to the control group, ergesterol increased in the group in which *L. obtusa* extract was added. Additionally, when compared to the control group in which *L. obtusa* extract was added, stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) increased in the other groups. Palmitoleic acid (16:1) increased in the *L. obtusa* culture medium, but palmitic acid decreased in the *L. obtusa* culture medium. In conclusion, it was determined that the *L. obtusa* extract added to the development medium of *S. cerevisiae* caused differences in the synthesis of some vitamins and fatty acids.

Key words: Fatty acids; Nutritional value; Probiotic/prebiotic; *Laurencia obtusa*; *Saccharomyces cerevisiae.*

Introduction

Recently, significant research has been conducted on the dietary modulation of gut microflora, and evidence has shown that some foods, food ingredients or biochemical compounds have an effect on gut microbiota. Researchers have focused on dietary prebiotics (indigestible carbohydrates) as useful substances for gastrointestinal health. Potentially, polysaccharides (PS) can be exploited as functional prebiotic ingredients for both human and animal health applications (1). PS, or their derivatives, namely oligosaccharides or low-molecular-weight (LMW)-PS, are considered extremely important as they include dietary fibres (2). Some of these PS can be found in various seaweeds and microalgae. In fact, the fibre content of some seaweed varieties is higher than that of most fruits and vegetables (3, 4). Prebiotics are the foods of probiotic microorganisms (5). Probiotics are live microorganisms, which, when ingested, have a positive effect on the host. These microorganisms have been studied a long time and are used as alternatives in the prevention and treatment of gastrointestinal disorders because of their resistance to anti-micro biotic substances (6). Today, major species of yeast, such as *S. boulardii* and *S. cerevisiae*, are used *S. cerevisiae* is a single-celled yeast and is one of the most frequently researched organisms for industrial applications and genetic studies (7). Milk, fermented foods and fruits are examples of important sources of probiotic *S.cerevisiae* (8, 9). Considering the risk of using antibiotics in large amounts and excessive doses, several laboratories and research centres, encouraged by the World Health Organization (WHO), are seriously investigating probiotics (6).

In particular, seaweeds are rich in PS that can potentially be used as prebiotic functional substances in both human and animal health practices (1).Various researchers have shown that algae and their products are potential sources for promoting the growth of probiotic microorganisms (1, 10, 11, 12, 13). Red algae of the genus *Laurencia* J. V. Lamour exist in tropical and subtropical regions of the world and are an extremely rich source of secondary metabolites with diverse structural features, mainly halogenated sesquiterpenes and C15 acetogenins (14, 15). Ji et al. (16) confirmed that *Laurencia* is a rich source of valuable secondary metabolites which exhibit a variety of biological capabilities, such as antioxidant, antimicrobial, antifeedant, anthelmintic and cytotoxic activities. Also, PS has been determined to be abundant in *L. obtusa* (17).

The objective of this study was to investigate the effect of *L. obtusa* on the biochemical content of *S. cerevisiae* cell cultures.

Materials and Methods

L. obtusa samples were collected by hand from several rocky shores on the Lara coast (Antalya, Turkey)

in May 2012. To rid the samples of epifits, sediment and other organic matter, they were washed several times with sea water. Samples were then bagged and transported to the laboratory, where they were washed with distilled water. The dried algae material was powdered and stored in the dark. *L. obtusa* was selected for this study because it has a high nutritional content and is abundant in Turkey.

Development medium of the *S. cerevisiae*

20 mg/mL *L. obtusa* suspension was prepared in sterilized distilled water and added to the yeast extract, peptone and dextrose (YEPD) medium containing yeast cultures. The YEPD medium was used for the control group without the addition of *L. obtusa*. It was then incubated at 37 °C and vortexed at 50 rpm. The development of the yeast was periodically monitored by means of a spectrophotometer. After 72 hours, the yeast samples were centrifuged, and pellets were obtained. The yeast pellets were washed with 0.09% NaCl and separated for ADEK vitamin and fatty acid analysis.The study was performed with five parallel experiments.

Biochemical analysis

The yeast pellets were weighed and homogenized with a hexane isopropanol mixture. The homogenate was centrifuged, and the supernatant portion was used for lipophilic vitamin and fatty acid analysis (18).

Derivatization and analysis of fatty acid methyl esters (FAME)

5 mL H_2SO_4 (2%) solution was added in aliquot taken from the supernatant portion of the sample pellets. The sample was vortexed and kept for 12 h in a 50°C oven. 5 mL NaCl solution (5%) was added, and the sample was again vortexed. Extraction was performed with 2×5 ml hexane. Next, it was treated with a 5 mL $KHCO_3$ solution (2%). Nitrogen (N_2) was used to dry the lipids by evaporating the hexane. The dry lipids were dissolved in 1 mL of hexane. The FAME were analysed with a Shimadzu 17 version 3 gas chromatograph (GC; Shimadzu, Kyoto, Japan). The FAME were injected into a capillary column (Machery-Nagel, Duren, Germany). The column was 25 m long, had a 0.25 µm inner diameter and a film thickness of 25 µ. The column temperature was 120 °C-220°C. The column temperature ramped to 200°C at a rate of 5°C/min and 220°C at a rate 4°C/min. The final temperature was held for 8 min at 220°C. The injector temperature stayed constant at 240°C, and the detector temperature stayed constant at 280°C. N_2 was used as the carrier gas. Peaks were identified using retention times from the FAME standard injected into the samples before the analysis. Then, fatty acid peaks were integrated, thus achieving the necessary programming (19).

Derivatization and analysis of lipophilic vitamins and sterols

5 mL of supernatant from each of the samples were transferred to 25 mL tubes, and 5mL of KOH: methanol (1:10 v/v) were added to each. The tubes were then vortexed and kept in an oven at 85°C for 15 min. After cooling the tubes to room temperature, 5 mL of distilled water were added,and the tubes were shaken. Extraction of lipophilic vitamins was done using 2×5 mL of hexane. The hexane in the samples was evaporated with N_2. One mL of acetonitrile:methanol (50% + 50% v/v) was used in the dissolving process.

Sterols were analysed on a high-performance liquid chromatograph (HPLC; Shimadzu,Kyoto, Japan). An LC-10 ADVP UV visible pump, SPD-10AVP detector, CTO-10ASVP column, SIL-10ADVP auto sampler, DGU-14A degasser unit and Class VP software (Shimadzu, Kyoto, Japan) were used. Acetonitrile:methanol (60% +40% v/v) was used in the mobile phase. A UV detector and the Supelcosil LC 18 column (Sigma, USA) were used (25 m in length, 0.25 µm inner diameter and 25 µ film thickness). The column was 15×4.6 cm and 5 µm. Wave lengths of the detection were 326 nm for vitamin A, 202 nm for vitamin E and 265 nm for vitamins D and K(20).

Sugar analysis

Algae samples were thoroughly homogenized with distilled water. Next, the liquid portion was centrifuged, and pellets were formed. After this treatment, 1 mL of supernatant of each sample was mixed with 3 mL of acetonitrile and analysed with the HPLC. A mobile phase acetonitrile + water analysis (v/v) (75/25) was used. The Supelcosil™ LC-NH2 (250 times 4.6 mm, 5µ) HPLC column was used for analysis (21).

Total phenolic content

The concentration of phenolic compound was measured by the Folin-Ciocalteu method as described by Singleton et al. (22). The total phenolic content was determined by comparison with a standard curve prepared using gallic acid (0–200 mg/L). Experiments were repeated three times for every dilution, and a calibration curve was created.

Flavonoid analysis

Using a Prevail C18 (15x4.6 mm, 5µm) HPLC column, flavonoids in the extracts were analysed. A methanol/water/acetonitrile mixture(46/46/8, v/v/v) containing 1% acetic acid as the mobile phase was used (23). For the analysis of flavonoids, a PDA detector (SPD-M10A VP; Shimadzu, Kyoto, Japan) was used. Kamferol, naringenin, rutin, myricetin, morin and quercetin were quantified by a DAD detector following RP-HPLC separation at 280 nm for naringenin and 254 nm for rutin, myricetin, morin and quercetin. All chromatographic operations were carried out at an ambient temperature of 25°C.

$ABTS^{-+}$ cation radical scavenging

An ABTS test was performed according to Wu (24). Using the following equation, the scavenging capability of the $ABTS^{-+}$ radical (%AS) was calculated: %AS=100 (Acontrol–Asample)/Acontrol. The A control is the control absorbance obtained from the $ABTS^{-+}$ radical alcoholic solution, and the A sample is the radical absorbance in the presence of the sample or the trolox standard.

Antioxidant assay by DPPH radical scavenging activity

Free radical scavenging activities were assessed by

discoloration of the methanolic DPPH (2,2-diphenyl-1-picrylhydrazy) solution. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The following equation was used to calculate the DPPH radical scavenging ability: DPPH radical scavenging activity (%) = [(Abs control–Abs sample)]/(Abs control)]×100. The Abs control is the absorbance of the DPPH radical + methanol; the sample is the absorbance of DPPH radical + sample extract/standard (25).

Statistical analysis

Comparison between the control group and experimental group was made using analysis of variation (ANOVA) and least significant different (LSD) tests. The results were given as mean±standard error of the mean (SEM). A value of $p < 0.05$ was accepted as statistically significant, and $p < 0.001$was accepted as highly statistically significant.

Results

In agreement with the fatty acid analysis results when compared to the control group (p<0.001), it was observed that the palmitic acid (16:0) amount decreased in the algal extract group. It was observed that the amounts of myristic acid (14:0), palmiteoleic acid (16:1), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2) increased in the *S. cerevisiae* group incubated with algal extracts when compared to the control group (p<0.001). It was detected that the amounts of sterol and vitamins in *S. cerevisiae* incubated with algal extract varied when compared with the control group at different rates. It was observed that ergesterol and β-sitosterol increased significantly in the *L. obtusa* medium when compared with the control group (p<0.001). While the K1 vitamin amount increased to high levels, α-tocopherol decreased in yeast which was treated with algal extract (Table 1). HPLC analysis indicated the presence of fructose, glucose, maltose, arabinose and sucrose in the *L. obtusa* (Table 2). The amount of flavonoids belonging to *L. obtusa* in Turkey is shown in Table 3. Flavonoid analysis proved that rutin, myricetin, morin, quercetin, kamferol and naringenin were present in the *L. obtusa* extract. DPPH radical scavenging activity of the methanol extract was found to be high in the *L. obtusa.* On the other hand, to the ABTS radical cation decolourisation analysis, % activity of the extracts were high the activity of the methanol extracts. In addition, the ABTS analysis showed that *L. obtusa* had a somewhat stronger antioxidant activity. The same results observed in the case of the DPPH analysis were observed in the ABTS analysis (Table 4).

Discussion

S. cerevisiae is a clinically proven yeast that is being used as a human probiotic. Our study shows that the biochemical content of *S. cerevisiae* changes in the presence of *Laurencia*. Bhowmik et al. (26) reported that the growth of lactic acid bacteria was enhanced in the presence of *spirulina*. It was observed that the palmitic acid (16:0) amount decreased in the algal extract group, when compared to the control group (p<0.001). It was also observed that the amounts of myristic acid (14:0), palmiteoleic acid (16:1), stearic acid (18:0), oleic

Table 1. Fatty acid and vitamins analysis results (µg/g).

Component	Control (*S. cerevisiae*)	*L. obtusa*+ *S. cerevisiae*	*L. obtusa (extract)*
14:0	78.23 ±2.23	359.32 ±6.67***	140±1.02
16:0	1020.76± 45.83	604.26 ±10.5***	930±20.03
18:0	250.12± 3.02	356.89±13.58**	333±10.23
16:1	45.13± 2.03	73.8 ±4.81***	212±2.23
18:1	60.45± 5.00	1276.1 ±5.14***	452±15.42
18:2	50.16± 6.95	431 ±14.88***	70.66±5.1
ΣSFA	1349.11±51.08	1320.47±30.77	1403±52.3
ΣUSFA	155.74±13.98	1780.9±24.8***	734.66±22.75
Total (mg/g)	1.59±0.10	7.03±0.56***	----------
Lipophilic vitamins and phytosterols			
Vitamin K_2	0.10 ± 0.00	0.10 ± 0.008	
α-tocopherol	65.26± 0.84	9.48± 0.99***	
Vitamin K_1	0.10±0.00	4.70± 0.71**	
Ergosterol	05.10 ± 0.00	29.30± 3.34***	
Stigmasterol	36.60± 4.03	50.98± 7.67*	
β-sitosterol	0.16± 0.05	1.22± 0.63**	

***: p<0.001, **: p<0.01, *: p<0.05

Table 2. Sugar contents of *L. obtusa* (mg/10 g).

Sugar	Arabinose	Fructose	Glucose	Sucrose	Maltose
Laurencia obtusa	77.13±0.027***	0.30±0.0006	0.47±0.016	0.5±0.01	0.6±0.001

***: p<0.001, **: p<0.01, *: p<0.05.

Table 3. Flavonoids (ng/g) and total phenolic content(mg/ GAE g) of *L.obtusa* extract.

Flavonoidler	Rutin	Myricetin	Morin	Quercetin	Kamferol	Naringenin	Total Phenolic
Laurencia obtusa	165±1.10	1±0.003	55.5±0.10	16.5±0.05	86.01±0.3	5.11±0.01	33.41±0.11

Table 4. ABTS and DPPH radical scavenging effects of *L. obtusa* samples (%).

	10µL	20µL	80µL	200µL
Lo (ABTS)	36±1.45	42±0.00	82.28± 0.71	92.47±0.54
Lo (DPPH)	41±1.23	62±0.23	76.28± 0.51	86.47±0.14
TOC	85.19± 0.05	83.44± 0.18	85.52± 0.09	85.72±1.32
BHA	83.81± 0.08	85.18± 0.22	85.33± 0.11	85.66±1.14
BHT	75.91± 0.03	79.42± 0.29	82.22± 0.15	83.58±1.05

*TOC: α-tocopherol BHA: Butil hidroksi anisol BHT: Butil hidroksi toluen, Lo: *Laurencia.*

acid (18:1) and linoleic acid (18:2) increased in the *S. cerevisiae* group that was treated with algal extract compared to those of the control group ($p<0.001$). We believe that the main reason for the synthesis of these fatty acids is related to the enzymes that have a role in the synthesis transcribed in *S. cerevisiae.* Various studies have reported that different extracts can be used as yeast growth (27, 28). However, we chose to study the effect of algal extract on yeast biochemistry. The higher fatty acid biosynthesis activity of *S. cerevisiae* may be related to the higher phenolic contents and the DPPH and ABTS radical scavenging activity (Table 1).

Linoleic acid isomers synthesized from *S. cerevisiae* are significant in the human diet (29). As Gurvitz et al. (29) explained, linoleic acid isomers have anticarcinogenic and antiarteriosclerotic effects and reduce anointment. In the present study, it was observed that *L. obtusa* has high antioxidant activity. The stimulatory effect of aqueous suspensions of this algal dry mass has also been detected on linoleic acid in the yeast. Linoleic acid is an essential fatty acid that is necessary for good health (30). Rodrigues et al. (30) studied the influence of fructo oligosaccharides on the free fatty acid composition of cheese. They found that conjugated linoleic acid content increased during the ripening process which suggests the benefit of adding prebiotics in probiotic cheese manufacture.

Sterols are the structural components of cell membranes which regulate membrane fluidity and permeability. They are composed of four rings (A–D) with a hydroxyl group at carbon-3, two methyl groups at C18 and C19 carbons and a side chain at C17. We found that the amounts of lipophilic vitamin and sterol varied in yeast prepared with algal extract when compared to the control group. While the K1 vitamin amount increased, α-tocopherol decreased in the *S. cerevisiae* prepared with algal extract according to the control group (Table 1) Vitamins play a key role in numerous bodily metabolic processes, and yeast has been reported to produce vitamins which provide a distinct advantage for a probiotic over bacteria (31). Kumari et al. (32) stated that the main sterols in seaweed are cholesterol, fucosterol, isofucosterol and clionasterol. When the amounts of sugars were compared, it was found that arabinose was at high levels ($p<0.001$), but not significantly different among other sugars. Jensen (33) reported that seaweeds contain galactose, glucose, mannose, fructose, xylose, fucose and arabinose. The monomeric sugar profile of *Kappaphycus alvarezii* strains from red algae, including the presence of anhydrogalactose, galactose, glucose, mannose and xylose, was determined by HPLC-MS analysis (34).

Flavonoids, the largest groups of phenolic compounds, are known to exhibit a wide range of chemical and biological activities, including antioxidant and free radical scavenging properties (35). Flavonoids are polyphenolic compounds present in plants (36), but information available on the distribution of flavonoids in seaweed is limited (37, 38, 39). The amount of flavonoids in *L. obtusa* algal extract in Turkey is given in Table 3. Flavonoid analysis proved that rutin, myricetin, morin, quercetin, kamferol and naringenin were present in *L. obtusa* extract. Quercetin and kaempherol have emerged as promising pharmacological agents in the treatment of cancer (40). A comparison showed that rutin, morin and kamferol had the highest content levels, whereas myricetin and naringenin were present at lower levels. Some researchers have reported that rutin, quercetin and kampferol are present in all of the studied algae samples, but they showed that these flavonoids are more abundant in red algae (*G. dendroides*) (39). Flavonoids such as rutin and quercetin exhibit high antioxidant activity (41). Johnson et al. (42) reported that flavonoids were only observed in the ethanolic extracts of *L. obtusa*. However, in our study, flavonoids were identified in methanol extract.

In the present study, *L. obtusa* extracts showed stronger ABTS and DPPH activity than synthetic antioxidants. Some countries have banned the use of synthetic antioxidants. Studies, such as the one by Suja et al. (43), have been conducted in an attempt to replace synthetic antioxidants with natural substances. DPPH radical scavenging activity of the solvent extracts of *L. obtusa*, according to other studies, is very low compared to synthetic antioxidants and ranges from about 1.5% to 12% (44). Phenolic compounds are widely found in plants, including seaweeds, and have been reported to exhibit a wide range of biological activities including antioxidant properties (45, 46). Johnson et al. (42) reported that *L. obtusa* has been elucidated in the ethanol solvent medium in an attempt to determine phenolic and flavonoid content in different solvents (ethanol, acetone, benzene, chloroform and petroleterium). In our study, the phenolic content was studied in water and methanol environments, and better results were obtained in the water environment. Their total of phenolic compounds was determined as 33.41±0.11 GAE mg/1g (Table 3). Nagai et al. (47) reported that phenolic compounds are one of the most effective antioxidants in brown algae. Phenolic compounds protect algal thalli from the adverse effects of UV radiation and exhibit antioxidant activity or activity that prevents free radicals' scavenging properties (44). Moreover, in many studies, it was found that the enzymes which make fatty acid synthesis from *S. cerevisiae* yeast were affected by various factors in the culture (28). Fatty acid biosynthesis in the *S. cerevisiae* cells was affected by the carbon source and antioxidant and bioactive compounds in the development medium (48). Prebiotics improve the function or viability of probiotics via their fermentation (49). We found that

L. obtusa has a positive influence on linoleic and oleic acid in *S. cerevisiae*. In addition, it should be noted that 18:2 is a polyunsaturated omega-6 fatty acid and that it is an essential fatty acid; however, this fatty acid can not be synthesized by the human body from other food components. The major monosaccharide found in *L. obtusa* was arabinose, which was followed by maltose. Arabinose is a calorie-free sugar that can affect glucose and lipid metabolism, deter obesity and possibly lower body weight (50). In our work, it was detected that *L. obtusa* extract had positive effects on the development of *S. cerevisiae,* which is known to be a useful probiotic yeast. *L. obtusa* contains polyphenols and flavonoids called antioxidants which protect the body's tissues against pathogens, oxidative stresses and cancer. Also, the addition of algae or algal extracts to food products will help reduce the use of chemical protectors. In conclusion, the amounts of fatty acids and vitamins were affected when *L. obtusa* extract was used in a culture medium of *S. cerevisiae*. We propose that the prebiotic properties of seaweeds are not be restricted to their PS and fibre; they can also be linked to monosaccharides, vitamins, polyunsaturated fatty acids, antioxidant activity, polyphenols and the flavonoid contents of seaweed. We believe that the data obtained in this study will be a valuable contribution to the current literature.

Acknowledgments

This work was supported by Science Research Foundation of Fırat University of Turkey (project number: FÜBAP FF.13.02). This study does not require ethical approval.

Interest conflict

There is no conflict of interest.

References

1. O'Sullivan L, Murphy B, McLoughlin P, Duggan P, Lawlor PG, Hughes H, et al. Prebiotics from marine macroalgae for human and animal health applications. Mar Drugs. 2010; 8:2038–64.

2. Raposo MJ, Bernardo de Morais AMM, de Morais RMSC. Emergent Sources of Prebiotics: Seaweeds and Microalgae. Mar drug 2016; 14:1-27.

3. Fujii T, Kuda T, Saheki K, Okuzumi M. Fermentation ofwater-soluble polysaccharides of brown algae by human intestinalbacteria in vitro. Nippon Suisan Gakk 1992; 58:149–52.

4. Goni I, Guidel-urbano M, Bravo L, Saura-calixto F. Dietary modulation of bacterial fermentative capacity by edible seaweeds in rats. J Agric Food Chem 2001; 49:2663-68.

5. Manning TS, Gibson GR. Prebiotics. Best Pract Res Clin Gastroenterol 2004; 18:287-98.

6. Keila MRD, Luiz HG, Ana CKS, Issakowicz J, Rocha F, Thiago PG, et al. *Saccharomyces cerevisiae* Used As Probiotic: Strains Characterization And Cell Viability, IOSR-JAVS 2012; 1:17-19.

7. Branduardi P, Smeraldi C, Porro D. Metabolically engineered yeasts: 'Potential' industrial applications. J Mol Microbiol Biotechnol. 2008; 15:31–40.

8. Roostita LB, Fleet GH, Wendry SP, Apon ZM, Gemilang LU. Determination of Yeasts Antimicrobial Activity in Milk and Meat Products. Adv J Food Sci Technol. 2011; 3:442–5.

9. Sourabh A, Kanwar SS, Sharma OP. Screening of indigenous yeast isolates obtained from traditional fermented foods of Western Himalayas for probiotic attributes. J Yeast Fungal Res. 2011; 2:117–26.

10. Wang Y, Han F, Hu B, Li J, Yu W. In vivo prebiotic properties of alginate oligosaccharides prepared through enzymatic hydrolysis of alginate. Nutr Res 2006; 26:597–603.

11. Ramnani P, Chitarrari R, Tuohy K, Grant J, Hotchkiss S, Philp K, et al. In vitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. Anaerobe 2012; 18:1-6.

12. de Caire GZ, Parada JL, Zaccaro MC, de Cano MMS. Effect of *Spirulina platensis* biomass on the growth of lactic acid bacteria in milk. World J Microbiol Biotechnol 2000; 16:563–5.

13. de Paula VC, Pinheiro IO, Lopes CE, Calazans GMT. Microwave-assisted hydrolysis of *Zymomonas mobilis* levan envisaging oligofructan production. Bioresour Technol 2008; 99:2466–70

14. Souto ML, Manriquez CP, Norte M, Fernandez JJ. Novel marine polyethers. Tetrahedron 2002; 58:8119-25

15. Blunt JW, Copp BR, Hu WP, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2007; 24:31-86.

16. Ji NY, Li XM, Ding LP, Wang BG. Two new aristolane sesquiterpenes from *Laurencia similis.* Chin Chem Lett 2007; 18:178-80**.**

17. Pereira L, Gheda SF, Ribeiro-Claro PJA. Analysis by Vibrational Spectroscopy of Seaweed Polysaccharides with Potential Use in Food, Pharmaceutical, and Cosmetic Industries. Int J Carbohydr Chem 2013: 1-7.

18. Hara A, Radin NS. Lipid extraction of tissues with a low-toxicity solvent. Anal Biochem 1978; 90:420–26.

19. Christie WW. Gas chromatography and lipids, The Oil Pres, Glaskow. 1990.

20. Katsanidis E, Addis PB. Novel HPLC analysis of tocopherols, tocotrienols, and cholesterol in tissue. Free Radic Biol Med 1999; 27:1137–40.

21. Ozsahin AD, Yilmaz O. Prunus armeniaca L. cv. Hacihaliloglu Fruits Extracts Prevent Lipid Peroxidation and Protect the Unsaturated Fatty Acids in the Fenton Reagent Environment. Asıan J Chem 2010; 22**:** 8022–32.

22. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. Meth Enzymol 1999; 299:152–78.

23. Zu YG, Li CY, Fu YJ, Zha CJ. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (Hippophae rhamnoides L.) leaves by RP-HPLC with DAD. J Pharm Biomed Anal 2006; 41:714-9.

24. Wu L, Chang L, Chen S, Fan N, Ho JA. Antioxidant activity and melanogenesis inhibitory effect of the acetonic extract of Osmanthus fragrans: A potential naturaland functional food flavoradditive. Food sci technol 2009; 42:1513-19.

25. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958; 181:1199 200.

26. Bhowmik D, Dubey J, Mehra S. Probiotic efficiency of Spirulina platensis-stimulating growth of lactic acid bacteria American-Eurasian. J Environ Sci 2009; 6:546-9

27. Aslan A, Can Mİ, Boydak D. Anti-Oxidant Effects of Pomegranate Juice on *Saccharomyces cerevisiae* cell growth. Afr J Tradıt Complem 2014; 11(4):14-8.

28. Guvenc M, Yılmaz O, Ozsahın AD, Aslan A, Tuzcu M, Kırecci OA. The Growth of Saccharomyces Cerevisiae in the Different Containing Grape Juices Environment Affects Fatty Acid Biosynthesis and Activities of Responsible Enzymes. TJST 2010; 5:43-51.

29. Gurvitz A, Hamilton B, Ruis H, Hartig A, Hiltunen JK. Degration of conjugated linoleic acid isomers in the yeast *Saccharomyces*

cerevisiae. Biochim Biophys Acta 2001; 1533:81-5.
30. Rodrigues D, Rocha-Santos TAP, Gomes AM, Goodfellow BJ, Freitas AC. Lipolysis in probiotic and symbiotic cheese: the influence of probiotic bacteria, prebiotic compounds and ripening time on free fatty acid profiles. Food Chem 2012; 131:1414-21.
31. Syal P, Vohra A. Probiotic attributes of a yeast-like fungus, *Geotrichum klebahnii*. Afr J Microbiol Res. 2014; 8:2037–43.
32. Kumari P, Kumar M, Reddy CRK, Jha B. Algal lipids, fatty acids and sterols: In Functional Ingredients from Algae for Foods and Nutraceuticals; (Eds), Domínguez, H. Cambridge, UK, 2013, pp 87–134.
33. Jensen A. Component sugars of some common brown algae, Norwegian Institute of Seaweed Research, Akademisk Trykningssentral, Blindern, Oslo. Report 1956; 9**:**1-8.
34. Masari, F, Cedeno FRP, Chavez EGS, Ezequiel de Oliveira L, Gelli VC, Monti R. Chemical analysis and biorefinery of red algae Kappaphycus alvarezii for efficient production of glucose from residue of carrageenan extraction process. Biotechnol Biofuels 2016; 9:122.
35. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999; 47:3954-62.
36. Qian JY, Mayer D, Kuhn M. Flavonoids in fine buckwheat (Fagopyrum esculentum Monch) flour and their free radical scavenging activities. Deut Lebensm-Rundsch 1999; 95:343-9.
37. Yoshie Y, Wang W, Hsieh Y, Suzuki T. Compositional Difference of Phenolic Compounds between Two Seaweeds, Halimeda spp. J Tokyo Univ Fish 2002; 88:21-4.
38. Yoshıe-Stark Y, Hsıeh Y, Suzukı T. Distribution of flavonoids and related compounds from seaweed in japan Halimeda spp. J Tokyo Univ Fish 2003; 89:1-6.
39. Al-Saif SSA, Abdel-Raouf N, El-Wazanani HA, Aref IA. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. Saudi J Biol Sci 2014; 21:57–64.
40. Matanjun P, Mohamed S, Mustapha N, Muhammad K, Ming C. Antioxidant activities and phenolics content of eight species of Seaweeds from North Borneo. J Appl Phycol 2008; 20:367-73.
41. Morel L, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Brisot P, et al. Anti-oxidant and iron-chelating activities of the flavonoids catechins, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem Pharmacol 1993; 45:13-9.
42. Johnson M, Babu A, Janakiraman N, Renisheya J, Jeba-Malar T. Phytochemical Studies On Laurencia Obtusa (Hudson) Lamourux. Int J of Biomed & Adv Res 2012; 03:225-32.
43. Suja KP, Jayalekshmy A, Arumughan C. Antioxidant activity of sesame cake extract. Food Chem 2005; 91:213-9.
44. Demirel Z, Yilmaz-Koz FF, Karabay-Yavasoglu, NU, Ozdemir G, Sukatar A. Antimicrobial and antioxidant activities of solvent extracts and the essential oil composition of *Laurencia obtusa* and *Laurencia obtusa var. Pyramidata*. Rom Biotech Lett 2011; 16:5927-36.
45. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from red alga, Polysiphonie urcelata. Food Chem 2006*;* 95:37-43.
46. Wang BG, Zhang WW, Duan XJ, Li XM. "In vitro antioxidative activities of extract and semi-purified fractions of the marine red alga, Rhodomela confervoides (Rhodomelaceae*).* Food Chem 2009; 113:1101-5.
47. Nagai T, Yukimoto T. Preparation and functional properties of beverages made from sea algae. Food Chem 2003; 81:327-32.
48. Ozsahin AD, Guvenc M, Yilmaz O, Aslan A, Tuzcu M. The effects of different sugar sources on fatty acid biosynthesis in the Saccharomyces cerevisiae cell culture. J Anim Vet Adv 2009; 8:424-9.
49. Rolım PM. Development of prebiotic food products and health benefts. Food Sci Technol 2015; 35:3-10.
50. Hao L, Lu X, Sun M, Li K, Shen L, Wu T. Protective effects of L-arabinose in high-carbohydrate, high-fat diet-induced metabolic syndrome in rats. Food Nutr Res 2015; 59:28886.