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OPTIMIZATION OF FERMENTATION OF *Ulva* sp. HYDROLYSATE BY NOVEL YEAST *Cyberlindnera jadinii* MMS7 FOR ENHANCEMENT OF POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY

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ABSTRACT sp

This study is aimed to evaluate the total phenols (TP) content and antioxidant activity of green alga *Ulva* sp. hydrolysate by aerobic fermentation using novel yeast *Cyberlindnera jadinii* MMS7. Response surface methodology (RSM) was applied to optimize the ultrasound assisted hydrolysate preparation from *Ulva* sp and found that the ultrasound power density of 0.35 WmL^{-1} at 15 min as optimum for hydrolysate preparation by ultrasonic pretreatment. The maximum TP of 35.75 ± 0.12 mg PGE g^{-1} observed in these condition, hence, it was used for further investigation for enhancing the TP content of hydrolysate. By the classical method of optimization pH 5.5, temperature 35°C and agitation speed 150 rpm were found to be the optimum physical parameters for improving TP content (44.59±0.06 mg PGE g^{-1}) of *Ulva* sp. hydrolysate fermentation by *C .jadinii* MMS7. At these optimum conditions, 3.26 fold DPPH radical scavenging activity was observed in fermented extract than unfermented extract. Therefore, this study demonstrates that the RSM is an adequate approach for optimization of ultrasound assisted hydrolysate preparation from *Ulva* sp. In addition to this, the novel yeast strain *C. jadinii* MMS7 considered as potential candidate for fermentation of *Ulva* sp. hydrolysate for enhanceing TP content and antioxidant activity.

Keywords: Antioxidant activity, *Cyberlindnera jadinii*, fermentation, hydrolysate, RSM, total phenols, *Ulva* sp.

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INTRODUCTION

Nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone, and propyl-gallate have been used in various food industries to prolong the shelf life of polyunsaturated fats contained foods. However, the incorporation of these synthetic antioxidants in food preparations has been questioned due to their potential health risks and toxicity[1]. Therefore, day by day increasing the interest to explore the substances which having antioxidant properties that are given to humans and animals as food components or as preventative pharmaceuticals[2]. Because the antioxidants are considered as protection agents for reducing oxidative damage in human body, when the enzymatic mechanisms are failed or are inadequately efficient[3].

The activities of free radicals have been implicated in aging, destruction of DNA, obstruction of arteries, cancer, strokes, cardiac and central nervous system (CNS) disorders which have led to an increase in the investigation of substances that can protect against these reactive oxygen species and thus may play a role in disease prevention[4,5]. The plant kingdom is a good source to produce a wide range of natural antioxidants. Mostly the herbal infusions, commonly used as home medicines have antioxidative and pharmacological properties related to the presence of phenolic compounds, especially phenolic acids derivatives and flavonoids. The secondary metabolites of plants, namely antioxidant phenolics, and flavonoids are commonly found in various fruits, vegetables, stems, leaves and herbs and they have been shown to provide a fruitful defense against oxidative stress from oxidizing agents and free radicals[2,6]. However, still there is not enough knowledge and data about the

practical usefulness of other plants to meet out the requirement due to rapid population development.

Like other plants, seaweeds also contain various inorganic, organic substances, minerals, dietary fiber and phenolic compounds, which can benefit human health[7]. These marine natural products provide a rich source of pharmacologically active metabolites and potentially useful new therapeutic agents[8]. Generally, algae have higher antioxidant activity due to presences of non-enzymatic antioxidant components like ascorbic acid, reduced glutathione, phenols and flavonoids[9]. As a result, many marine biosources have gained much attention in the search for various natural bioactive compounds to develop new drugs and healthy foods. Compounds with antioxidant, antiviral, antimicrobial, antifungal, antitumor and anti-inflammatory activities have been found in green algae, brown algae and red algae[10].

Recently more interest has been increased considerably in producing antioxidants from various natural sources by fermentation for use in foods or medicinal products to replace chemically extracted natural antioxidants, which are being restricted due to their cost. Fermentation is one of the oldest and most effective strategies of food production and preservation. It provides a natural way to reduce the volume, to destroy undesirable components, to enhance the nutritive value of foods through the biosynthesis of vitamins, essential amino acids and appearance of the food, to reduce the energy required for cooking, and to make safer products[11]. Furthermore, fermentation improves micronutrient bioavail-ability and aids in the degradation of anti-nutritional factors[12].

The antioxidant activity of several seaweeds has been reported[7,13]. Compared to the terrestrial plants, seaweeds have no distinct organs. This character makes the whole plants available for exploitation[14]. To the best of our knowledge, there is no publication on the antioxidant activities on ultrasonic pretreated *Ulva* sp. hydrolysate fermented by novel yeast *C. jadinii* MMS7 isolated from soil samples of sugarcane biogases dumping sites. Hence, the major objectives of this study are to (i) optimize parameters affecting the ultrasonic assisted hydrolysate preparation from *Ulva* sp. biomass by RSM, (ii) optimize the hydrolysate fermentation conditions using novel yeast *C. jadinii* MMS7 for enhancement of polyphenol content and antioxidant activity, (iii) to study the in vitro antioxidant activity.

MATERIALS AND METHODS

Materials

Yeast Peptone Dextrose (YPD) agar and other chemicals with highest purity or analytical grade was used for this study and was purchased from Himedia Chemicals (Mumbai, India), Sigmae-Aldrich (Bommasandra, India) and Merck Chemicals Ltd.,(Mumbai, India).

Yeast strain and Inoculum preparation

The novel yeast *C. jadinii* MMS7 (GenBank Accession Number MK942589) used in the present study was isolated from soil samples of sugarcane biogases dumping sites. It was stored at 4^{o} C on YPD (Peptone 20 gL^{-1}, yeast extract 10 g L^{-1}, dextrose 20 gL^{-1}, Agar 20gL^{-1}) agar slants and subculture every month. Inoculum was prepared by transferring a loop full of *C. jadinii* MMS7 from YPD agar slant into 50 mL of YPD broth at aseptic condition. Then the inoculated broth was maintained under aerobic conditions at 30 ^{o}C with agitation speed at 120 rpm in an orbital shaking incubator (REMI laboratory Instruments, India) for 48 h. The medium pH was adjusted at 6.0 with 1N HCl/NaOH. The newly prepared inoculum was used as seed culture for fermentation process.

Seaweed collection and sample preparation

Seaweed green alga *Ulva* sp. was freshly collected from coastal region of Kovalam (Latitude 12^{o}79'25'' N; Longitude 80^{o}25'30" E) Tamil Nadu, India and thoroughly washed with normal water followed by distilled water to freshwater to remove sands, salts and epiphytes Next, the *Ulva* sp. biomass was desalinated by soaking in double distilled waste for 1 h, again repeated the same procedure was repeated for two times. Then cleaned *Ulva* sp. biomass was dried to a constant weight at 50 - 60 ºC in hot air oven. After drying, the dried biomass sample was chopped into small pieces and grounded to powder using grinder. A 200 mg of *Ulva* sp. powder was mixed with 100 mL of distilled water and then this sample was used for hydrolysate preparation by ultrasonic treatment.

RSM Optimization of *Ulva* sp. hydrolysate preparation

Ultrasound assisted hydrolysate preparation was carried out using a probe system sonicator (Lark Innovative Fine Teknowledge, Chennai, India) combined with a transducer and a metallic probe of 2 mm. The ultrasonic pretreatment process parameters such as ultrasound power density (0.2 to 0.5 WmL^{-1}), and treatment time (5 to 30 min) having an impact on fermentation of *Ulva* sp. hydrolysate to TP yield were investigated through by RSM using central composite design (CCD). The experimental design includes 13 runs; experiment was carried out separately for each run by taken 150 mL of sample in a 250 mL stainless steel beaker and dipped the probe in the sample upto 1.5 cm depth. During sonication, the desired temperature was controlled by placing the beaker in a water bath[15]. Hydrolysate prepared by each pretreatment run was used as sole medium for fermentation. The experiment was carried out in replicates for each run and the TP was taken as a dependent variable or response. The regression analysis was performed on the data obtained. This resulted in an empirical model that related the response measured, to the independent variables of the experiment. For any system, the model equation is represented as follows,

$$Y = \beta o + \Sigma \beta i Xi + \Sigma \beta i Xi^2 + \Sigma \beta ij XiXj$$

Where Y is the response of TP, βo the intercept, βi the linear coefficient and βij is the interaction coefficient. The statistical significance and analysis of variance (ANOVA) was performed, and three dimensional response surface curves were plotted to study the interaction among these factors.

Fermentation of hydrolysate by yeast *C. jadinii* MMS7

The hydrolysate prepared by ultrasonic pretreatment was used for fermentation by *C. jadinii* MMS7. The experiments were carried out in 250 mL Erlenmeyer flask contained 100 mL of hydrolysate as a sole medium. Prior to inoculation, the hydrolysate pH was adjusted to 5.0 using 1N NaOH or HCl and autoclaved at 121 ^{o}C for 15 min with 15 psi. Then the medium was inoculated with 1 % v/v inoculum of *C. jadinii* MMS7 and incubated in an orbital shaker incubator at 28±2 °C for 72 h under constant shaking at 120 rpm. Every 12 h once the sample was withdrawn and estimated the biomass and the TP content. The untreated *Ulva* sp. extract was used as a control. Based on the maximum TP content of *Ulva* sp. hydrolysate the optimum ultrasonic pretreatment conditions were found and used for hydrolysate preparation for further study.

Optimization of Fermentation of hydrolysate by yeast *C. jadinii* MMS7

The maximum amount of TP content produced hydrolysate (experimental run) was selected and used for further optimization of the following fermentation process conditions by classical method of optimization (One parameter at-a-time), pH (4 to 6), temperature (25 to 40 ^{o}C) and agitation speed (100 to 300 rpm). All the experiments were carried out in triplicate to check the reproducibility.

Biomass Estimation

The biomass was estimated by gravimetrical method. 10 mL of samples was added to pre-dried (105°C in oven, overnight) and pre-weighed conical bottom glass centrifuge tube and then centrifuged at 6000 rpm for 10 min. The pellet was washed twice with deionized water and the centrifugation process was repeated. Then washed biomass was dried at 105 °C until the constant weight was obtained. After drying, it was allowed to cool in a desiccator and the final weight was recorded using an analytical balance (S234, Denver Instrument, Bohemia, NY). The loss of weight was calculated as grams of dry weight per litre. The biomass estimation was performed in triplicate and all values are represented as mean ± SD of three replications.

Estimation of total phenolic content

The TP content present in the fermented hydrolysate was determined by Folin-Ciocalteu method[16]. The phloroglucinol was used as standard. The samples were diluted to match the measurable range of the spectrophotometer. A 100 µL aliquot of sample was mixed with 500 µL of 1 N Folin-Ciocalteu reagent. Then 0.4 mL of Na_2CO_3 (20%) was added to this mixture and incubated in the dark at room temperature for 45 min. Fermented sample was centrifuged at 6000 rpm for 10 min and the collected supernatant was measured at 765 nm using UV-Visible spectrophotometer (UV-1601, Shimadzu Corporation, Tokyo, Japan). The OD value of sample was interpolated in the standard graph and calculated the TP content. The total phenolic contents in milligram phloroglucinol equivalents per gram of extract.

In Vitro antioxidant activity

2,2,-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed as described by Qureshi et al [17]. 1 mL of fermented hydrolysate was mixed with 1 mL of ethanolic solution of DPPH (0.2 mM). To this mixer, 1 mL of ascorbic acid (200 µgmL^{-1}) in ethanol was added. After mixed thoroughly for 30 s, it was incubated in dark for 25 min at 37°C. Then the DPPH radical scavenging activity was measured at 517 nm using UV-Visible spectrophotometer (UV-Corporation, Tokyo, Japan) using mixture of 1 mL ethanol and 1 mL of DPPH as blank. The synthetic antioxidant Butylated Hydroxy Toluene (BHT) used as positive control. Assay was performed in triplicates and the mean value was used to calculate the DPPH radical scavenging activity using the following equation,

Radical scavenging activity =

[(Abs control - Abs samples)/Abs control] X 100

Statistical analysis

All the experimental results were presented by the average value of triplicates with standard deviation (SD). The ultrasonic pretreatment parameters were statistically optimized at $p \leq 0.05$ confidence level using RSM and the data were further analyzed using one-way analysis of variance (ANOVA) using MINITAB 12 software.

RESULTS AND DISCUSSION

RSM optimization of ultrasound assisted hydrolysate preparation for enhancement of TP content by fermentation using *C .jadinii* MMS7

RSM optimization of process parameters for ultrasound assisted hydrolysate preparation from *Ulva* sp. powder sample was studied using CCD. In order to find the optimum level of ultrasound power density and treatment time for this study, the TP content was considered as an indicator for these parameters optimization. A 13 experiments were designed for these two independent factors and all the experiments were carried out in duplicate. Table 1, showed the design matrix included two variables and the predicted and experimental values of response TP content. The CCD analysis was done using coded units (Table 2). The model was expressed by the following linear regression equation for the TP content (Y) as a function of the effect of ultrasound power density (X_1) and treatment time (X_2) on the *Ulva* sp. powder sample for hydrolysate preparation for fermentation by *C .jadinii* MMS7.

$$Y_{CODED} = 35.742 - 0.1509X_1 - 0.4094X_2 - 0.9479X_1^2 - 0.9329X_2^2 + 5525X_1X_2$$

where Y is the TP content (mg PGE g^{-1}), X_1 and X_2 are the coded value of ultrasound power density and treatment time time respectively. The RSM results were plotted as the three dimensional response surface curves to find out the optimum level of independent variables for *Ulva* sp. sample pretreatment to prepare hydrolysate and make it as a suitable medium for fermentation by *C .jadinii* MMS7 for maximizing yield of TP content. From the surface plot (Fig. 1), it was found that the ultrasound power density of 0.35 WmL^{-1} and treatment time 15 min as the optimum conditions for converting *Ulva* sp. sample into hydrolysate by ultrasonic pretreatment, because at these conditions the yeast strain *C .jadinii* MMS7 fermented the hydrolysate and gave the maximum TP of 35.75 ± 0.12 mg PGE g^{-1}. This may be because of the high dissolved nutrient contents in the hydrolysate, which were liberated from *Ulva* sp. sample during the ultrasonic pretreatment at these conditions.

ANOVA was performed to examine the significance and adequacy of second-order polynomial equation. The ANVOA results were given in the Table 3. The coefficient of determination (R^2) value of 0.996 was closer to 1. Therefore, the correlation is better between the experimental and predicted values by the second order polynomial model[18]. Similarly, adjusted R^2 value of 0.993 was also very close to R^2 value. Hence, the model is well fitted to represent the effect of variables (ultrasound power density and treatment time) on ultrasonic pretreatment using RSM.

The small P-values indicate the higher significance of the corresponding variable. Therefore, the model obtained for this study was significant ($P \leq 0.05$) and suggesting that the ultrasonic pretreatment independent variables power density and treatment time could play a synergistic role on liberating the nutrient contents from the *Ulva* sp. sample which was fermented by *C .jadinii* MMS7 for enhancement of TP content. The statistical results of this study demonstrated that the ultrasonic pretreatment is a significant factor in prior utilization of *Ulva* sp. sample for fermentation by novel yeast strain *C .jadinii* MMS7 for enhancement of TP content and antioxidant activity

Experimental verification

Ultrasound assisted *Ulva* sp. hydrolysate was prepared by pretreatment at 0.35 WmL^{-1} for 15 min. 100 mL of

hydrolysate was taken in 250 mL Erlenmeyer flask as sole fermentation medium and inoculated *C. jadinii* MMS7 (1% v/v). The cultures were incubated at 28±2 °C for 72 h under constant shaking at 120 rpm. Every 6 h once the sample was collected and estimated the biomass and PT content. The maximum amount of biomass and PT was found to be as 15.05±0.04 g dwL^{-1} and 35.75±0.05 mg PGE g^{-1} respectively at 36 h. These results were very close to the results obtained in the experiment run 9 to 13 (Table 1). In this study, PT content of *Ulva* sp. hydrolysate after fermentation using *C. jadinii* MMS7 was 7.04 fold higher than the methanolic extract of *U.clathrata* (5.080 mg GAE g^{-1}) reported by Farasat et al[19]. Whereas in this study, very less TP content of 6.12±0.05 mg PGE g^{-1} was obtained from untreated *Ulva* sp. sample. This study proves that the RSM as an adequate approach for optimization of parameters for ultrasound assisted hydrolysate preparation from *Ulva* sp. powder. Moreover, the novel yeast strain *C. jadinii* MMS7 was considered as potential candidate for fermentation of *Ulva* sp. hydrolysate for enhancement of TP content and antioxidant activity.

Table 1: CCD matrix of two variables with experimental and predicted values of TP content of fermented *Ulva* sp. hydrolysate

Run Order	X_1	X_2	TP content (mg PGE g^{-1})	
			Experimental	Predicted
1	0.200000	5.0000	34.84	34.9740
2	0.500000	5.0000	33.57	33.5673
3	0.200000	25.0000	32.96	33.0502
4	0.500000	25.0000	33.90	33.8535
5	0.137868	15.0000	34.20	34.0596
6	0.562132	15.0000	33.58	33.6329
7	0.350000	0.8579	34.53	34.4553
8	0.350000	29.1421	33.31	33.2972
9	0.350000	15.0000	35.75	35.7420
10	0.350000	15.0000	35.75	35.7420
11	0.350000	15.0000	35.72	35.7420
12	0.350000	15.0000	35.75	35.7420
13	0.350000	15.0000	35.74	35.7420

Table 2: Estimated regression coefficients of second order polynomial model for TP content of fermented *Ulva* sp. hydrolysate

Variables	Estimated Coefficients	t-value	*p*-value
Model	35.7420	884.042	<0.001*
X_1	-0.1509	-4.720	<0.002*
X_2	-0.4094	-12.809	<0.001
X_1^2	-0.9479	-27.654	<0.001*
X_2^2	-0.9329	-27.216	<0.001*
X_1X_2	0.5525	12.223	<0.002*

R-Sq = 99.6% R-Sq(adj) = 99.3% * Significant

Table 3: ANOVA for TP content of fermented *Ulva* sp. hydrolysate

Source	Degree of freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	F-value	*p*-value
Regression	5	13.7210	3.0211	334.01	<0.001*
Linear	2	1.54	0.8001	93.20	<0.001*
Square	2	11.02	5.5001	670.91	<0.001*
Interaction	1	1.31	1.2210	150.40	<0.001*
Residual Error	7	0.061	0.0090		
Lack of fit	3	0.063	0.0190	110.91	<0.001*
Pure Error	4	0.0007	0.0002	-	-
Total	12	14.01	-	-	

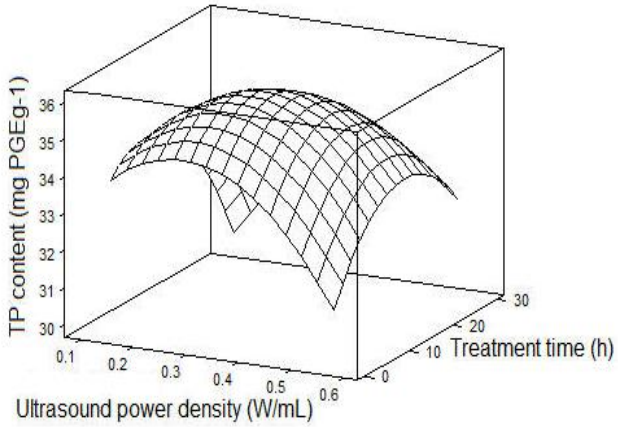

Figure 1. Response surface plot for TP content of *Ulva* sp. hydrolysate fermented by C. jadinii MMS7

Effect of pH on fermentation of *Ulva* sp. hydrolysate

The medium pH level can have a significant influence on yeast cell physiology since there are variations in growth and metabolism of yeast cells. Yeasts generally grow very well when the initial culture medium pH is between 4 - 6, However, some yeast are also capable of growth over quite a wide range[20]. The effect of medium initial pH on fermentation of *Ulva* sp. hydrolysate by *C. jadinii* MMS7 studied. The fermentation was carried out at different pH range from 4 to 6 with an increment of pH 0.5. The pH conditions of fermentation process were maintained using 1N NaOH/HCl. As shown in Figure 2, the maximum of 38.45 mg PGE g^{-1} TP content of *Ulva* sp. hydrolysate was enhanced by fermentation using *C. jadinii* MMS7 at pH 5.5 because the growth of *C. jadinii* MMS7 (Biomass 15.03±0.02 g dwL^{-1}) was well favoured in fermentation process at this pH level. The results obtained in this work agreed with the result of Ezekiel and Aworh[21] which revealed that *C. utilis* can be cultivated successfully on cassava peel slurry at an initial pH of 5.5. Based on the TP content, pH 5.5 was found to be an optimum pH and used for further optimization study.

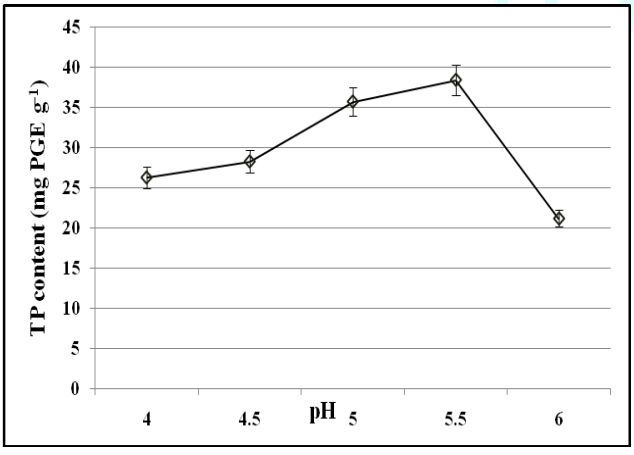

Figure 2: Effect of pH on TP content of *Ulva* sp. hydrolysate fermentation by *C. jadinii* MMS7

Effect of temperature on fermentation of *Ulva* sp. hydrolysate

Temperature is one of the most important physical factors, which influences on the growth of microorganisms and their product yield and productivity in the fermentation[22]. Effect of the temperature on fermentation of *Ulva* sp. hydrolysate by *C. jadinii* MMS7 was studied. The fermentation was carried out at different temperature range from 25 °C to 40 °C with an increment of 5 °C. As shown in Figure 3, the maximum TP content (42.15 mg PGE g^{-1}) of *Ulva* sp. hydrolysate was enhanced by fermentation using *C. jadinii* MMS7 at 35 °C. Similarly, Zhao et al[23] reported that the 35 °C as the optimum temperature for *C. utilis* to ferment the waste capsicum powder for single cell protein production. Further increase in the temperature there was a reduction in TP content because the temperature > 35 °C was unfavourable for the growth of *C. jadinii* MMS7. Hence, the fermentation gets affected. When increasing the temperature from 25 °C to 35 °C an increased in the *C. jadinii* MMS7 biomass from 12.21±0.09 g dwL^{-1} to 15.23±0.05 g dwL^{-1} was found. At 40 °C very less biomass (6.12±0.02 g dwL^{-1}) was observed. These results suggesting that the temperature above 35°C does not support the growth of *C. jadinii* MMS7 in the fermentation broth *Ulva* sp. hydrolysate. Therefore, the temperature 35 °C was found to be an optimum temperature for enhancing the TP content in *Ulva* sp. hydrolysate by fermentation using *C. jadinii* MMS7 and it was used for further optimization study.

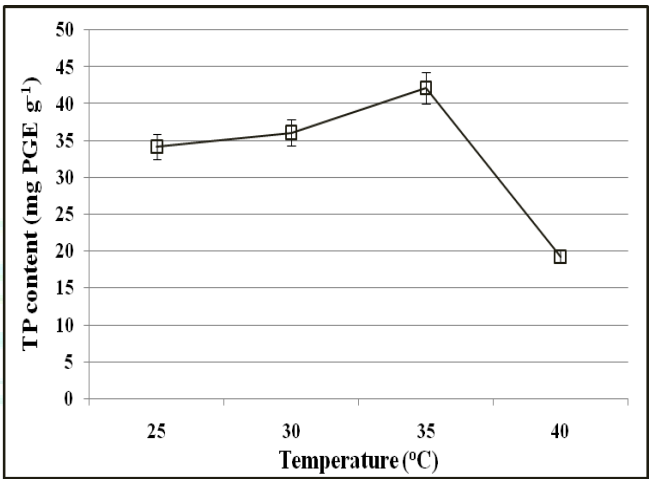

Figure 3: Effect of temperature on TP content of *Ulva* sp. hydrolysate fermentation by *C. jadinii* MMS7

Effect of agitation on fermentation of *Ulva* sp. hydrolysate

The agitation is an important parameter for the successful progress of the fermentation under laboratory conditions. It provides an adequate mixing of the nutrients present in the medium as well as a better dispersion of the cells thus facilitating the assimilation of nutrients by the yeasts[24]. However, the excess the agitation speed creates shear forces, which affect microorganisms in several ways, causing morphological changes, variation in their growth and product formation and damaging the cell structure[25]. In order to find the optimum agitation speed for enhancement of TP content in *Ulva* sp. hydrolyaste by fermentation using *C. jadinii* MMS7 experiments were carried out at different agitation speed range from 100 to 300 rpm with an increment of 50 rpm. The maximum PT content 44.59±0.06 mg PGE g^{-1} (Figure 4) and biomass (18.23±0.07 g dwL^{-1}) were found to be at agitation speed of 150 rpm because the maximum cell population reached under this condition. Further increase in the agitation speed there was no significant effect on fermentation.

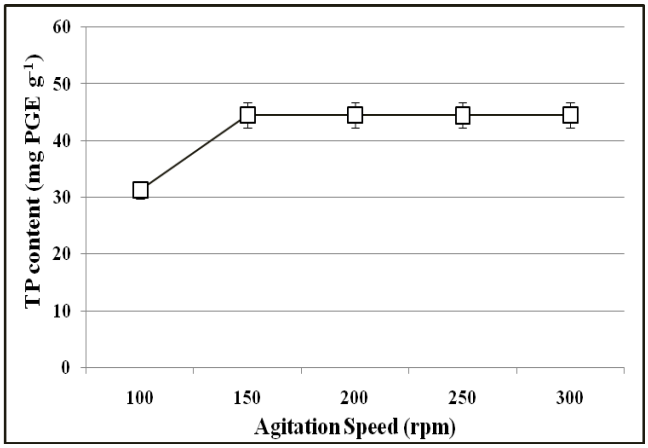

Figure 4: Effect of agitation speed on TP content of *Ulva* sp. hydrolysate fermentation by *C. jadinii* MMS7

In Vitro antioxidant activity

Ultrasound assisted *Ulva* sp. hydrolysate was prepared by pretreatment at 0.35 WmL^{-1} for 15 min. 400 mL of hydrolysate was taken in 1 L Erlenmeyer flask as sole fermentation medium and inoculated *C. jadinii* MMS7 (1% v/v). Prior to inoculate, the hydrolysate pH was adjusted to optimum pH 5.5 using 1N NaOH or HCl and autoclaved at 121 ^{o}C for 15 min with 15 psi. Then cultures were incubated at optimum temperature 35 °C for 48 h under constant shaking at optimum agitation 150 rpm. The fermented extract was used to measure the DPPH radical scavenging activity. Polyphenolic compounds are known to possess antioxidant activity[26]. The antioxidant activity of the hydrolysate before and after fermentation was measured. As shown in Figure 5, the highest DPPH radical scavenging activity of 78.57±0.03% was observed at fermentation time 36 h. Further increase in the fermentation time gradually decreased the DPPH radical scavenging activity. The DPPH radical scavenging activity of fermented extract was in accordance with their amount of TP content. Several reports were shown a close relationship between TP content and high antioxidant activity because the phenolic compounds are one of the most effective antioxidants in seaweed[27,28].

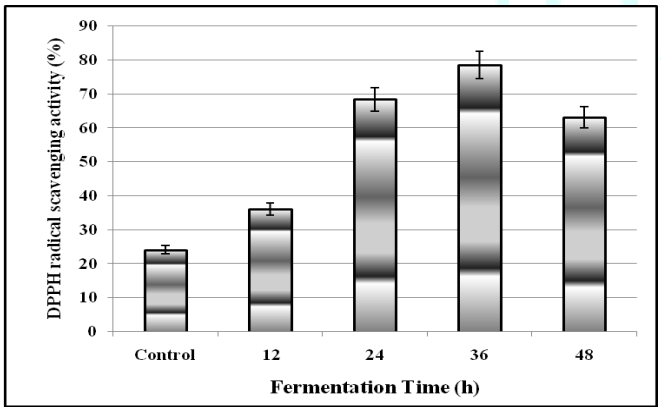

Figure 5: Effect of *C. jadinii* MMS7 fermentation on DPPH radical scavenging activity

CONCLUSIONS

The optimization of ultrasound assisted hydrolysate preparation from *Ulva* sp. was done using CCD of RSM. By this adequate approach found that the ultrasound power density 0.35 WmL and treatment time 15 min as the optimum condition for ultrasound assisted hydrolysate preparation. Because hydrolysate prepared at these conditions, gave the maximum biomass of 15.05±0.12 g dw L^{-1} and TP conten of 35.75 ± 0.12 mg PGE g^{-1} after fermentation by *C .jadinii* MMS7. The DPPH radical scavenging activity of 78.57±0.03 was observed in fermented extract at 36 h, which is 3.26 fold higher than unfermented extract. The optimum pH, temperature and agitation speed for enhancing TP content and antioxidant activity of *Ulva* sp hydroysate was 5.5, 35 ^{o}C and 150 rpm respectively. At these optimum conditions, the fermentation by *C .jadinii* MMS7 resulted in enhanced increased TP content and antioxidant activity of *Ulva* sp hydroysate prepared by ultrasound assisted. Hence, the novel yeast *C. jadinii* MMS7 considered as potential candidate for fermentation of *Ulva* sp. hydrolysate for enhancement of TP content and antioxidant activity.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest to publish this paper.

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