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Microbiological Assays on Edible Seaweed Ulva Lactuca (L.) Cultured in Outdoor Tanks

Ulviye KARACALAR* Gamze TURAN
Ege University, Fisheries Faculty, Dept. of Aquaculture, 35100 Bornova, Izmir,TURKEY

* Corresponding Author
e-mail: ulviye.karacalar@ege.edu.tr

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Abstract

In this study, microbiological analysis was performed on sun-dried Sea lettuce, *Ulva lactuca* (L.), cultured in outdoor seaweed tanks at Urla Marine Center of Ege University (Izmir, Turkey). A total of five microbial assays were performed between March and April 2005. Standard microbiological methods were applied for micro organisms, such as *Salmonella* spp., coliform, fecal coliform and *Vibriosis* spp., was examined. Results of microbiological analysis showed that *Salmonella* spp. and fecal coliform were absent in the dried samples of *U. lactuca* and the total plate count, coliform, and *Vibriosis* spp. were 8.18 (± 0.37) $\times 10^4$ cfu/g, 4.40 (± 4.72) cfu/g, and 4.80 (± 6.91) cfu/g, respectively. Since the total plate count and proportion of coliform , did not exceed the limits of the food quality standards, it was concluded that *U. lactuca* cultured in outdoor tanks and dried under the sun can be used as a raw material of food for animal and human consumption in Turkey.

Key words : *Ulva lactuca* ,seaweed, microbial analysis, aerobic plate count, coliform

INTRODUCTION

The use of marine algae, or seaweeds, in the human diet is a part of the lifestyle of many Far East and Pacific countries, particularly in Japan [1]. On average, the Japanese consume 1.4 kg seaweeds per person annually [2]. In Western countries, the principle use of seaweeds has been source of phycocolloids (alginates, carrageenan and agar), thickening and gelling agents for various industrial applications, including uses in foods [3]. However, in the past few years, interest in the use of seaweeds for human consumption has increased in many occidental countries [4]. More recently, there has been an interest in the consumption of this product based on anecdotal claims of it having beneficial health benefits, in particular due to its high mineral content [5]. Secondary or primary metabolites from seaweeds have been potential bioactive compounds of interest for the pharmacological industry [6]. Special attention has been reported for antiviral, antibacterial and/or antifungal activities related to marine algae against several pathogens [7-10]. There are numerous reports of compounds derived from seaweed products with a broad range of biological activities, such as anti-tumorals and anti-inflammatories [11], as well as neurotoxins [12].

The green algae *U. lactuca* has great potential as a commercial product because of its fruitful taste and varied chemical composition and quality. It is consumed as a vegetable in many countries [13,14] and among its nutritional benefits, it is also rich in dietary fibre [15-17]. Currently, it is authorized as vegetable and condiment in France with other 11 macroalgae and two microalgae [2].

With the consumption of any raw ready-to-eat product originating from an environment which may contain fecal and other human clinical pathogens, it is necessary to estimate the potential hazards and associated risks. Furthermore it is important to examine both safety and microbiological aspects relating to this particular product.

The aim of this study covers preliminary examination of the microflora of sun-dried *U. lactuca* cultured in outdoor tank to ascertain its potential safety, as well as determination of quantity of microbial species.

MATERIALS AND METHODS

U. lactuca Samples

U. lactuca samples were obtained from the biomass produced in outdoor tanks at Urla Marine Center of Fisheries Faculty, Ege University (Izmir, Turkey) between March and April 2005. A commercial fertilizer (TSP: Triple Super Phosphate) was used as a growth medium in the seaweed tanks where ambient water temperature was changed between 10 ˚C and 15 ˚C.

U. lactuca Sample preparation

For microbiologic analysis, *U. lactuca* samples were collected biweekly from the culture tanks. After sample collection, salts, epiphytes, shells and sands on the seaweed were removed using tap water. Afterwards, they were spread over a cement slab and sun-dried for three days. The dried seaweeds were ground well by using mixer grinder and were sieved using a nylon sieve in order to remove plant fibre. A sufficient quantity (25 g) of these seaweed particles were used for microbiological analyses.

Microbiological Analysis

Standard microbiological methods were applied for micro organisms such as *Salmonella* spp., coliform, fecal coliform and *Vibriosis* spp. First of all, portions of seaweed weighing 25 g were diluted with 250 ml of steril Buffer Pepton Water

(Oxoid, CM 509), blended for 2 minutes and subsequently diluted ten-fold with the same Buffer Pepton Water. Aerobic plate count was determined using Plate Count Agar (PCA) (Merck, 1.05463) after incubation for 24 h at 37˚C [18,19].

Colonies were counted and the total bacterial count was calculated by the following formula :

Number of colonies × dilution factor

Bacterial count (CFU g^{-1}) =

Weight of the sample (g)

Total and fecal coliforms were determined according to methods previously described by American Public Health Assocation-American Water Works Association [20] and Harrigan and McCance [21]. The diluted samples of 10^{-1}, 10^{-2} and 10^{-3} with Buffer Peptone Water were transferred to 3 series of test tubes each containing 10 ml of Modified Lauryl Sulphate Tryptose Broth (Merck, 1.10266). Following 24-48 h at 37˚C, positive tubes were transferred to tubes containing Brillant Green Bile Broth (BGLB) (Oxoid, CM 31) and incubated for 24-48 h at 37˚C. The number of test tubes giving positive results with the BGLB was noted. Results were given as MPN/g (Most Probable Number).

To investigate *Salmonella* spp.in the samples, 10 g portions of samples were pre-enriched with 100 ml pre-enrichment broth (Buffer Pepton Water) for 24 hour at 37˚C,1 ml of the pre-enriched culture was inoculated into 10 ml Tetrathionate Medium (Oxoid, CM 29) and another 1ml to 10 ml of Selenite Medium (Oxoid, CM 395) and incubated for 24-48 h at 37˚C. After 18-24 hour, loopful portions of these enrichment mediums were streaked to two small petri dishes each containing Brillant Green/Phenol Red Agar (Oxoid, CM 329) and Bismuth Sulphite Agar (Oxoid, CM 201) and incubated for 20-24 h at 37˚C.The culture was examined with respect to *Salmonella* spp.

Vibriosis spp was examined using Thiosulphate-citrate-bile salt-sucrose Agar (TCBS) (Oxoid, CM 333), cultures were left at 37˚C for 24-32 h. During the microbiological analysis, each one of the microorganisms were examined in triplicate.

Statistical Analysis

The data on microflora found on sun-dried *U. lactuca* was analyzed by using MEANS procedure in SPSS Statistical Program [22]. All the data was presented as mean ± standart deviation.

RESULT AND DISCUSSION

The results of the diversity of the microflora found on sun-dried *U. Lactuca* were summarized in Table 1. *Salmonella* spp. and fecal coliform were absent in the seaweed samples and the total aerobic count, coliform, and *Vibrio* spp. were 8.18 (± 0.37) $\times 10^4$, 4.40 (± 4.72) , and 4.80 (± 6.91), respectivelly (Table 1).

Table 1. Diversity of the microbial flora (mean ± sd) of sun-dried *U. lactuca* cultured in Outdoor tanks.

N	Total Aerobic Count (cfu/g)	Coliforms (cfu/g)	Fecal Coliforms (cfu/g)	*Vibrio* spp. (cfu/g)	*Salmonella* spp. (cfu/g)
5	8.18 (± 0.37) $\times 10^4$	4.40 (± 4.72)	0	4.80 (± 6.91)	0

Comparing the present study's result to the microbiologic quality standards for algae summarized by Becker [23] , it was seen that total aerobic count (8.18 ± 0.37 $\times 10^4$) did not exceed the counts determined in France (<0.1 X 10^6), Sweden (<10 X 10^6), or in Japan (0.005 X 10^6) and recomended paper (<0.1 X 10^6) (Table 2). Coliform number 4.40 (±4.72) was also in the limits investigated in France (<10) and Sweden (<100) (Table 2), but in Japan and recomended list show zero tolerans in the materials.

Table 2. Comparison of the microbiologic quality standards for algae and present study's results for *U. lactuca* cultured in outdoor tanks

Parameters	France	Sweden	Japan	Recomended	Present study
Standard Plate Count (number$X10^6$/g)	<0.1	<10	<0.005	<0.1	0.0818 ±0.37
Coliformes (number/g)	<10	<100	<100	Negative	4.40 ± 4.72
Fecal Coliforms (number/g)	-	-	-	-	Negative
Salmonella sp.	Negative	Negative	Negative	Negative	Negative
Vibrio spp.	-	-	-	-	4.80 ± 6.91

Initially, a conventional diagnostic approach employing culture was adapted, and this failed to detect *Salmonella* spp. in the laboratory analyses. Likewise, fecal coliform was not detected and the mean total plate count was 8.2×10^4cfu /g. Therefore, by categorizing edible seaweed as 'dried fruit and vegetables' and placing it in category 3, this product may be regarded as being 'acceptable', as interpreted by Public Health Laboratory Service [24].

The coliform level determined in samples appears to be connected with outdoor culture conditions of *U. lactuca*, but still it is in the safe limits. Furthermore, according to the results, there is no risk to consume this species as human and animal food in terms of total plate count, coliform and *Vibrio* spp in Turkey.

CONCLUSION

Results of microbiological analysis showed that *Salmonella* spp. and fecal coliform were absent in the dried samples of *U. lactuca* and the total plate count, coliform, and *Vibrio* spp. were 8.18 (± 0.37) $\times 10^4$ cfu/g, 4.40 (± 4.72) cfu/g, and 4.80 (± 6.91)

cfu/g, respectively. Since the total plate count and coliform did not exceed the limits of the food quality standards summarized by FAO [19]. With regard to microbial examination, this product can be used as a raw material of food for animal and human consumption.

The traditional basis for the identification of environmental and pathogenic organisms has been their isolation or propagation in the laboratory, where biochemical and morphological tests are used to help with their identification. However, with employment of such rRNA-based techniques should be gain increased popularity as a means of identifying phenotypically difficult-to-identify organisms. Coupled with this, it was the expectation of this study that any environmental organisms detected may be difficult to identify, as the majority of identification systems used in the clinical diagnostic laboratory, e.g. API kits, would not contain phenotypic profiles of such environmental genera and species in their databases. Therefore, all isolates should be identified by PCR in the future.

It is well known fact that *U. lactuca* has natural products such as anti-bacterial and anti-viral compounds of *U. lactuca*. In addition, it should be also examined in the future studies to see bacteria- *Ulva* and virus-*Ulva* relationships and their potential usage in drug industry and treatment of the diseases.

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