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Review article

Influence of A biotic stress on biosynthesis of alga-chemicals and its relation to biological activities

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Present study is to illustrate the relationship between the environmental stress and defense pathway from algae to protection from this stress. Marine environments are characterized by broad fluctuations of environmental conditions that can be strong stress inducers in macroalgal populations, including extreme temperatures, rapid salinity and nutrient changes, desiccation, intense sunlight, and others. These factors are extremely important for the geographical distribution of macroalgae and also can be responsible for several physiological responses of these organisms. The current study conclude that, the effects of various stresses on algae led to change in gene expression and its related to of secondary metabolite production which have different biological activities as antioxidant, anticancer and antimicrobial activities.

[Keywords: Algae, Active ingredients, Stress factors, Biological activities]

Introduction

Algae are photosynthetic organisms that are able to rapidly generate biomass from solar energy, CO_2 and nutrients in bodies of water. This biomass consists of important primary metabolites such as sugars, oils and lipids, for which process path-ways exist for the production of high-value products including human and animal feed supplements, transport fuels, industrial chemicals and pharmaceuticals. Algae are capable of producing 30 times the amount of oils and lipids per unit area of land as compared to terrestrial oilseed crops[1].

Plants (algae included) are exposed to a range of environmental stresses and have to adapt physiologically to these as the local environment changes. The recognition and signaling pathways regulating the responses to a biotic stresses (*e.g*., drought, salinity, cold and heat) are similar to those used for responding to biotic stresses. The adaptation to one stress condition can therefore affect tolerance to other non-related stresses, a phenomenon referred to as cross-tolerance. The redundancy of some of the major signaling compounds, for example,

salicylic acid, calcium, reactive oxygen might form the regulatory basis for developing such multiple tolerance mechanisms. Recent findings reveal a role of abscisic acid in biological defense and involvement of salicylic acid in a biotic stress, thereby indicating that these compounds have a broader importance than previously anticipated. Furthermore, cellular responses often depend on the intracellular concentrations and fluxes of some of these signaling molecules can constitute a secondary stress themselves (2).

Cells exposed to stresses, undergo changes in their metabolism in order to adopt with changes in their environmental. Stress changes the morphological, physiological and biochemical response of plants. It affects adversely growth and development of cells (3). Antioxidant enzymes and osmolytes are known to occur widely in plants and other organisms in response

to environmental stress (4). Stress allevation in cyanobacteria has been known to be achieved through the production of variety stress proteins (5). Under stress conditions, cyanobacteria pigments, i.e. chlorophyll a, carotenoids and phycocyanin and adversely affected. Furthermore, cells produce more peroxide radicals which induce the enhancement of determination processes (6). In this regard, both secondary and primary metabolisms were studied as a prelude to future rational economic exploitation as show in Fig**.** 1.

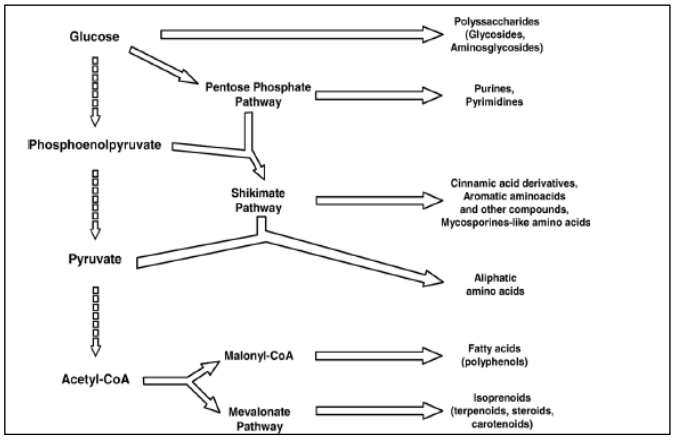

Fig. 1. Main pathways of some secondary and primary metabolites biosynthesis.

Marine environments are characterized by broad fluctuations of environmental conditions that can be strong stress inducers in macroalgal populations, including extreme temperatures, rapid salinity and nutrient changes, desiccation, intense sunlight, and others. These factors are extremely important for the geographical distribution of macroalgae and also can be responsible for several physiological responses of these organisms (7-8). It is reasonable to consider the possible existence of similar effects on the chemical defenses of these organisms or their secondary metabolism. Several species of macroalgae have the capacity to produce a diverse array of secondary metabolites, which play important and vital ecological roles such as defense and/or signal compounds (9). These chemicals can vary qualitatively or quantitatively within species; however, these intraspecific patterns remain largely unexamined.

When the environmental conditions are extreme and microalgae grow under stress, they synthesize and produce various secondary metabolites (Fig. 2).

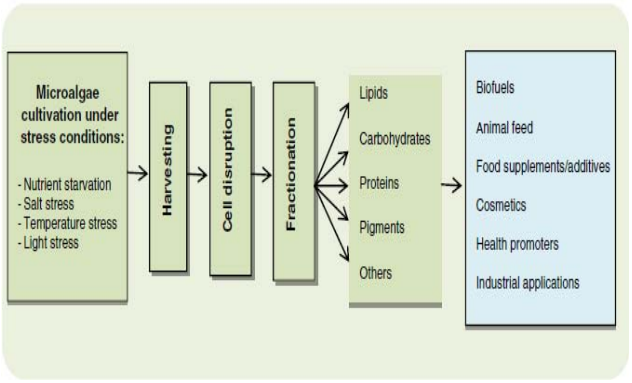

Fig. 2 . General scheme of microalgal biorefinery concept.

The synthesis of these secondary metabolites is believed that function as attempt of the microorganisms to retain their growth rates or to increase the possibility of surviving under these unfavorable environmental conditions. Secondary metabolites refer to those compounds which are not used for microalgal primary metabolism (i.e. cell division and metabolism) and they include compounds that act as antioxidants, hormones, antibiotics, allelochemicals and toxins (10-11). Some of these secondary metabolites have particular interest because they constitute high-value products with several applications (12-14),

A serious concern about the cultivation of microalgae under stress conditions is the decrease or the arrest of growth rates and consequently the decrease of the total production and productivity. In some cases it is possible that the productivity of an accumulated compound cannot reach the productivity under regular conditions because of the decrease in the growth rates (15). However, this negative effect might be mitigated applying various techniques. One of the most suggested techniques is the cultivation of microalgae in multiple-stage process, in which in each stage optimum or appropriate conditions are applied. The most frequently suggested multiple-stage technique is the cultivation in two-stage systems, in which in

the first stage optimum conditions are applied aiming the maximization of biomass production, while in the second stage, stress conditions are applied aiming the accumulation of the desired compound(s). Nevertheless, the cultivation in multiple stages might consume more energy in comparison to the one stage systems, especially in those systems, in which harvesting of biomass is essential for forwarding it to the next stage (16). The topic of the optimization of a desirable compound under stress conditions is of particular significance and more research is needed. Since the productivity of compounds could be regulated by an appropriate technique, this review will focus on the absolute effect of the specific a biotic stress conditions on biosynthesis of different active compounds (Fig. 3).

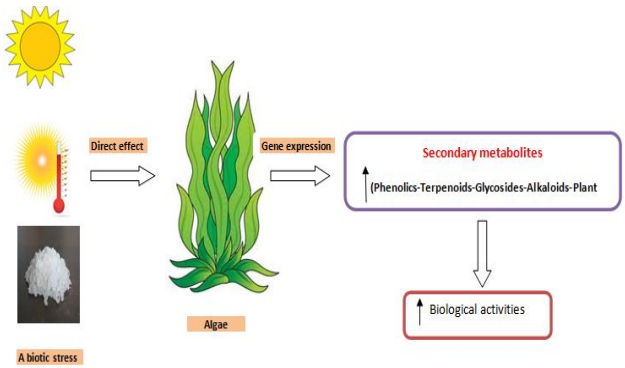

Fig.3. The effect of different a biotic stress factors on biosynthesis of secondary metabolites in algal cells.

The production of secondary metabolites in plants is generally a plastic trait that can be influenced by a number of biotic and a biotic factor (17). Temperature has been reported to affect physiological responses of the secondary metabolism (18), including variation in concentrations of secondary metabolites of macroalgae (19). Few studies have shown that phlorotannin contents in brown macroalgae increase with increasing salinity in their habitats (20). Some authors have described the influence of the nutrient regime on phlorotannin (21) and terpenoid contents in macroalgae (22). The influence of irradiance on macroalgal secondary metabolites has been extensively studied although the results are contradictory. For example, UV radiation can induce significant increases in phlorotannin concentrations of *Ascophyllum nodosum* (23), but this effect was less intense in a further study (17). It is indubitable that the light environment has great importance in predicting the phlorotannin content for some species (24). Field experiments manipulating light intensity also show contradictory effects on terpenoid compounds (25). However, if the resources available to organisms are finite, they must be allocated to many life processes, including the production of defensive chemicals.

Some a biotic stress factors and its effect on algal species

Physical factors

a-Light

Frequent exposure of seaweeds during tide periods imposes considerable environmental stress especially on intertidal seaweeds due to high irradiance levels, temperature changes and desiccation, which generates acute physiological stress. The ability of seaweeds to overcome these challenges and survive in such a harsh environment has been linked to elevated altered metabolism with an increase of Reactive oxygen species (ROS). Logically, seaweeds which are sensitive or intolerant to ambient stresses inhabit the lowermost intertidal zone (where emersion at low tide is brief and/or absent), while those found at the supralittoral zone (spray zone) usually possess higher tolerance to environmental fluctuations. It is likely that such spatial separation within the intertidal zone is the result of morphological (i.e. thallus shape, size and thickness, Chapman, 1986), physiological and biochemical variations among seaweed species (26). Prolonged exposure of seaweeds to high light intensities can damage the photosynthetic system and contribute to decreases in both quantum efficiency and maximum photosynthetic rates.

Many intertidal seaweeds have developed mechanisms to prevent/avoid lethal physiological damages incurred and maintain physiological integrity during and throughout emersion. The generation of reactive oxygen

species (ROS) like superoxide, hydrogen peroxides, hydroxyl radicals and singlet state oxygen is routine during —normal" photosynthetic and respiratory metabolism. However, during periods of elevated physiological stress such as emersion, ROS formation can escalate rapidly. In both algae and higher plants, major defense systems have evolved to protect them against superoxide radicals and the subsequent formation of hydrogen peroxide (H_2O_2). With the exception of singlet excited O2, all ROS are derived from superoxide and peroxides. Production of antioxidant enzymes as Ascorbate peroxidase (APX), the superoxide dismutase (SOD), peroxidase (POD) and catalases (CAT) are therefore the primary defensive enzymes against excessive ROS damage (27).

A drastic decrease of underwater light intensity may lead to physiological stress in submersed algae, including reduction in their growth rates, increased tissue nitrogen content, lower production of carbon-based secondary chemicals such as sugar and phenols, and restrained carbon allocation to root symbionts and soil-dwelling microbes. Moreover, low light weakens the stress-tolerance of submersed macrophytes and further leads to the decrease of submersed plants in aquatic ecosystems, especially in shallow eutrophic lakes (28).

The intertidal areas of estuaries are characterized by a large variability in environmental conditions caused by the exposure to air and direct sunlight during low tide periods, alternating with submersion throughout high tide. During diurnal low tide, organisms living in intertidal sediments subjected or exposed to potentially stressful conditions that include high light intensities, extreme low or high temperatures, high salinities and wind. These variables and extreme environmental conditions are likely to affect the communities of benthic microalgae, or microphytobenthos, which form highly dense biofilms on the surface of intertidal sediments (29). During low tide, the prolonged exposure to wind and direct sunlight favors the evaporation in the uppermost layers of sediment, frequently exposing the benthic microalgae to an intense process of de-watering. Desiccation may be expected to cause important limiting effects on photosynthetic activity of microphytobenthos, as shown for other photoautotrophs such as macroalgae (30).

b- Temperature

A decrease from the optimal temperature for growth (30oC) to suboptimal (18oC) temperatures induced β-carotene synthesis and increased lipid content in *Dunaliella salina* cells, thereby promoting the formation of lipid-carotene globules in the chloroplast periphery. The content of polyunsaturated fatty acids was higher in cells cultured at low temperature. Therefore, the induction of carotenogensis and accumulation of total lipids (31; Fig. 4). Especially, polyunsaturated fatty acids (are mechanisms of acclimation to unfavorable environmental condition for growth (32).

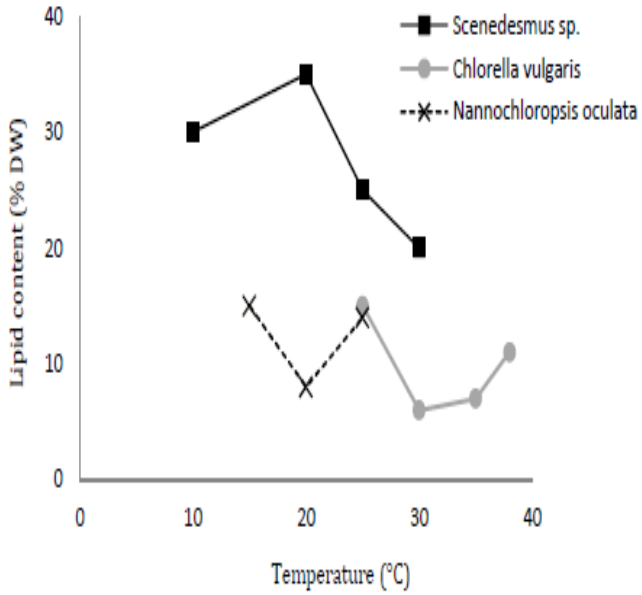

Fig. 4. Temperature effect on lipid content in different microalgae species.

Changes in temperature may vitally affect algae, such as the acute response observed at 35 ºC, but they can also promote chronic responses due to their effect on the rates of photosynthesis,

growth and reproduction (33). Different species and even different populations of a given species may have different optimal temperatures for photosynthesis (34) and growth (33). Although the clones that we used were acclimated at 24 ºC for more than 6 months under laboratory conditions before being used in the experiments, they had the same genetic characteristics as their own natural population, which experiences a broader annual temperature range.

Studies investigating the relationship between temperature and amount of secondary metabolites in macroalgae are few. Here, we observed a parabolic response of the elatol amounts according to temperature changes. However, this factor did not influence the concentrations of secondary metabolites in *L. okamurae* (35) and *Plocamium cartilagineum* (19), when temperatures ranged from 15 to 25 and 11 to 18 ºC, respectively. Observation of seasonal variations in concentrations of secondary metabolites attributed to temperature changes is a more common approach; for example, temperature positively influenced the production of chemical defenses in *Caulerpa taxifolia* (36).

Chemicals factors

a) Nitrogen

Sharenkova and Klyachko-Gurvich (37) claimed that the fatty acid composition did not change, even after 10 days in the absence of nitrogen; lipid synthesis was reduced and eventually even stopped. It appeared that since growth was arrested and no new lipids were synthesized after the third day of nitrogen starvation, the fatty acid composition could not change. But noticeable change would probably have been observed by gradual nitrogen depletion. Thus, varying the nitrogen concentration cannot be used as a mean for manipulation of the content and composition of lipids and fatty acid in cyanobacteria.

The patterns of soluble proteins of *Spirulina maxima* (blue-green algae) grown at various nitrogen concentration levels in all molecular weight regions clearly indicated that several protein bands were present in soluble protein of Spirulina cells and many of which were detected as new protein bands (Fig. 5 and Table 1).

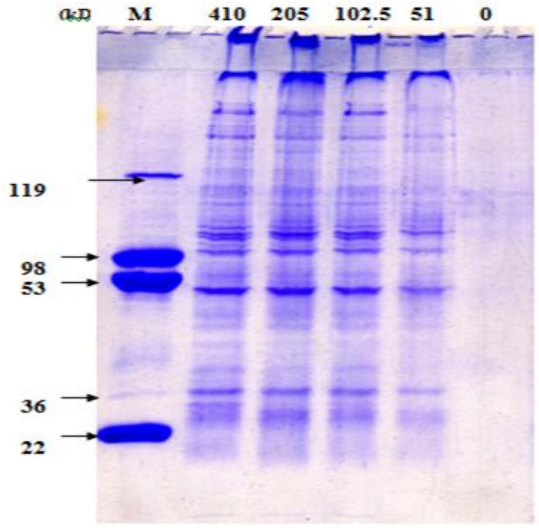

Fig.5. SDS-PAGE for separation of soluble protein from *Spirulina maxima* grown under various nitrogen concentration.(ppm).

In contrast, only three protein bands were appeared in soluble protein of *Spirulina maxima* cell grown in free nitrogen media these bands were found in high molecular weight regions with molecular weight of 115, 112 and 107 kDa. The band with MW of 112 kDa was appeared as a new protein, and phycocyanin protein was detected as a trace bands. In all Spirulina cells grown at different anew N condition, characterized with two bands with MW of 42 and 37 kDa, and were identified as c-phycocyanin and allophycocyanin protein respectively.

The relative intensities of the protein bands were alternated markedly as a result of nitrogen concentration variation. For instance, the percentage of c-phycocyanin and allophycocyanin (in paranthese) protein in *Spirulina maxima* grown in media contained 410, 205, 102.5 and 51 ppm of nitrogen were 4.87 (3.74), 4.74 (6.51), 4.19 (5.93) and 3.8% (5.0%). Therefore, the c-phycocyanin and allophycocyanin showed negative and positive correlation, respectively with nitrogen concentration.

The results showed that, the induction of new protein band was correlated with decreasing of nitrogen concentration in media. In free nitrogen media, the Spirulina did not induce any new protein bands. The soluble protein of Spirulina cells grown at medium N level 205 ppm N was distinguished by present of 4 new bands, of which 2 in HMW (156 and 113 kDa), one in MMW with MW 78 kDa and one in LMW with MW 46 kDa. At 102.5 ppm of N, Spirulina had three new bands similar in cells grown at 205 ppm N with MW of 156, 113 and 78 kDa. Whereas, at lower nitrogen levels (51 ppm N) the cells had 6 new protein bands, 3 of them similar that in cells grown at nitrogen level > 51ppm (158, 113 and 77 kDa) and the protein bands with MW of 140, 71, 64 and 30 were present as new proteins.

Data also indicated that, the Spirulina grown in media rich in nitrogen was characterized by have many protein band and high relative intensities of these bands when compared with that in Spirulina cells grow at low nitrogen levels. These data was agree with that obtained by Boussiba and Richmond (38) who reported that the concentration of phycobiliprotein was increased as a results of increasing nitrogen level in Spirulina grown media, and the phycobiliprotein content was decreased by decreasing N level in media, while other did not decrease. Thus, phycobiliprotein content exhibited positive correlation with nitrogen concentration in media. However, it could be concluded that:

1- The Spirulina cells grow in free nitrogen media had a few protein band mainly occurred in HMW regions.

2- The Spirulina cells grown under medium nitrogen level characterized by new protein band with MW of 46 kDa, which did not recorded in other Spirulina protein.

3- Spirulina grown in media with very low nitrogen level had several new protein bands in different MW regions.

b) Salinity

The response of *Spirulina platensis* cells to salinity stress was studied by Vonshak et al. (39). Who reported that salt-adapted cells have a modified biochemical composition; reduced protein and chlorophyll content, and increased level of carbohydrates.

The unicellular algae Dunaliella thrive in media with very high salt concentration, accumulated large amounts of commercially important chemicals such as β-carotene and glycerol. Dunaliella is cultivated commercially in large outdoor ponds, and harvested to produce high β-carotene dry algal meal and concentrated algal β-carotene in oil. This product is used by the health-food industry and for food coloring, respectively. The algal β-carotene differs from the synthetically available β-carotene in its stereoisomeric composition and may be of use as a pharmaceutical product (40).

Salt stress causes an imbalance of the cellular ions resulting in ion toxicity and osmotic stress, leading to retardation of growth either directly by salt or indirectly by oxidative stress induced by reactive oxygen species (ROS). Salinity can cause significant accumulation of compatible solutes which acts as enzyme producers, stabilizing the structure of macromolecules and organelles (41). Salinity stress may alter the metabolic pathways of stressed organism(s) leading to either enhancement or induction of biologically active compounds.

Salt stress conditions not only affected algal growth, pigment content but also protein and lipid production of the stressed alga. Analysis of soluble proteins (by SDS electrophorsis) of *S. platensis* cultivated under different salt concentrations and recorded in Fig. (6), revealed that, no protein bands of high molecular weights (190-117), were recorded at the highest NaCl conc. used (0.08 M). While two new highly intensive protein bands of molecular weights, 113, 77 were recorded only at higher NaCl conc (42). Also certain bands were present at low and moderate salt conc. (0.02 and 0.04 M) but absent (not detected) at higher ones (0.08 M). Moreover six protein bands were detected at low and/or moderate salt conc. but their intensities were highly increased at higher salt stress conditions (of M.wts 106, 90, 82, 67, 35 and 30). Absence

of either new protein bands or an increase in the intensity of 42 and 37 KDa bands confirmed the obtained results concerning the decrease in total phycobiliprotein pigments under salt stress conditions. The obtained results concerning protein analysis of salt stressed S. platensis was comparable to those of S. maxima cultivated under nitrogen stress condition (43). Both Spirulina species have two specific new protein bands of molecular weight 113 and 76 in addition to a highly intensive band at M.wt 103. Higher numbers of new protein bands were recorded in S. maxima at different nitrogen conc. and not equivalent to similar bands (of the same M.wt) produced by *S. platensis* under salinity stress conditions. These differences may be due to variable metabolic processes in both species and to the availability of nitrogen (essential for protein synthesis) in study of S. maxima and present only as normal medium constituent in experiments of *S. platensis*.

Conclusion

From the current review the authors conclude that, the effects of various stresses on algae led to change in gene expression and it's related to secondary metabolite production which has different biological activities as antioxidant, anticancer and antimicrobial activities.

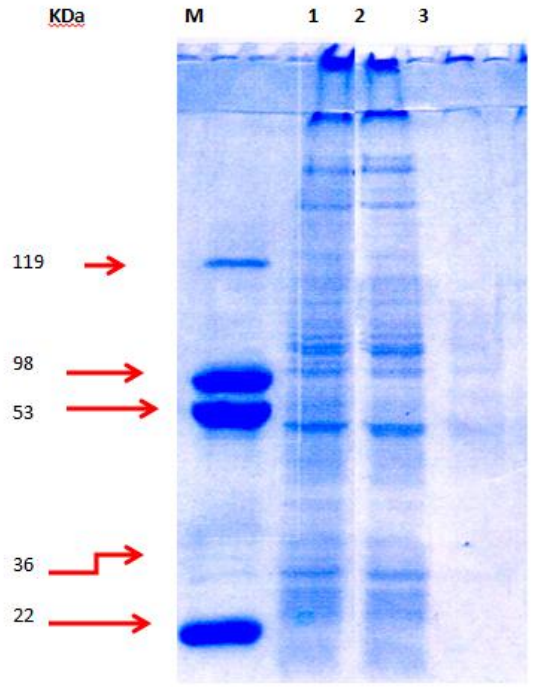

Fig.6: SDS-Electrophoretic analysis of soluble proteins produced by the salt stressed.

Table 1. Protein pattern of soluble protein of *Spirulina maxima.* grown at various nitrogen concentration levels.

Protein band	Molecular weight (kDa)	hRf	Concentration of nitrogen (ppm) 410	205	102.5	51	0.0
High molecular weight (HMW,%)							
1	189	0.9	2.53	4.21	8.33	6.06	-
2	185	2.1	3.41	4.73	-	-	-
3	167	8.1	1.27	-	2.27	-	-
4	161	10.2	3.06	4.37	4.13	4.60	-
5(n.p)*	156*	11.5	-	2.14*	2.17*	2.39*	-
6	152	13.3	1.09	2.03	1.30	2.71	-
7	149	14.3	1.10	-	-	-	-
8	145	15.7	2.79	3.72	3.29	3.84	-
9(n.p*)	140*	17.8	-	-	-	1.54*	-
10	137	18.8	3.29	4.20	2.57	3.90	-
11	132	20.9	1.16	-	1.80	-	-
12	124	23.9	3.56	-	2.77	-	-
13	115	27.8 29.0	5.69	-	4.04	2.08	74.41
14(n.p)*	113*		-	6.47*	1.35*	3.43*	20.45*

Protein band	Molecular weight (kDa)	hRf	Concentration of nitrogen (ppm) 410	205	102.5	51	0.0
15	106	31.8	2.14	2.43	2.57	4.42	5.14
16	103	33.3	1.31	-	-	-	-
17	101	34.3	1.25	-	-	-	-
Medium molecular weight (MMW,%)							
18	97	35.9	2.28	4.67	3.73	8.77	-
19	94	37.3	2.56	2.82	2.74	2.97	-
20(n.p)*	92*	38.5	-	-	3.04*	4.69*	-
21	91	39.2	2.32	6.20	2.97	-	-
22	89	40.0	2.58	-	2.28	-	-
23	83	43.0	4.19	4.95	4.75	4.13	-
24(n.p)*	77*	45.4	-	2.28*	1.99*	2.90*	
25	73	48.7	4.47	3.85	4.64	2.33	-
26(n.p)*	71*	49.7	-	-	-	1.81*	-
27	67	52.3	5.59	8.29	8.12	6.66	-
28(n.p)*	64*	54.3	-	-	-	2.20*	-
29	59	57.8	4.41	4.34	6.77	2.63	-
30	55	60.6	3.71	3.01	3.63	4.46	-
Low molecular weight (LMW,%)							
31	48	65.0	3.30	3.27	3.66	-	-
32(n.p)*	46*	67.3	-	2.79*	-	-	-
33	42	70.8	4.87	4.74	4.19	3.88	tr
34	37	76.3	3.74	6.51	5.93	5.00	tr
35	34	80.0	3.73	-	-	3.28	-
36	32	82.0	9.20	7.98	4.97	4.59	-
37(n.p)*	30*	85.1	-	-	-	4.82*	-
38	28	89.2	9.40	-	-	-	-
Total number			29	28	27	26	3
Total %			100.0	100.0	100.0	100.0	100.0

- *n.p: new protein band created as a result of the treatment

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