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Research article

Radical scavenging capacity of RuBisCO bioactive peptides derived from *Dunaliella salina* and *Spirulina platensis*: An *in silico* and *in vitro* study

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Abstract: Microalgae have a large high-quality protein content that can be used as human protein supplements. *Dunaliella salina* and *Spirulina platensis* have been identified as rich sources of natural bioactive compounds. We aimed to examine the antioxidant properties of bioactive peptides using the enzymatic digestion of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme derived from *D. salina* and *S. platensis* microalgae. This was an in vitro and in silico study. Cell walls of *D. salina* and *S. platensis* were lysed, proteins were isolated, and RuBisCO fraction was concentrated. Then, the protein was enzymatically digested using pepsin, trypsin, and chymotrypsin. Finally, antioxidant activity was assessed at different stages (pre- and post-digestion). The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to assess the antioxidant potential of hydrolyzates both before and after digestion. Findings indicated that digestion over time, particularly by chymotrypsin, produced bioactive fragments with enhanced antioxidant properties. For the 0–35 protein fraction (which likely includes RuBisCO), the antioxidant potential of peptides derived from *S. platensis* was significantly greater than that from *D. salina.* We showed that chymotrypsin may be an appropriate enzyme to yield the highest peptide concentrations from the protein extracts of these microalgae with the highest antioxidant activity. Moreover, the results of digesting the RuBisCO sequence with digestive enzymes showed that antioxidant properties increased with the production of hidden bioactive peptides. This finding may lead to the application of RuBisCO protein and its derivative peptides in the food and pharmaceutical industries of *S. platensis* and *D. salina*.
Keywords: RuBisCO, microalgae; bioactive peptide; anti-DPPH; enzymatic digestion

Supplementary

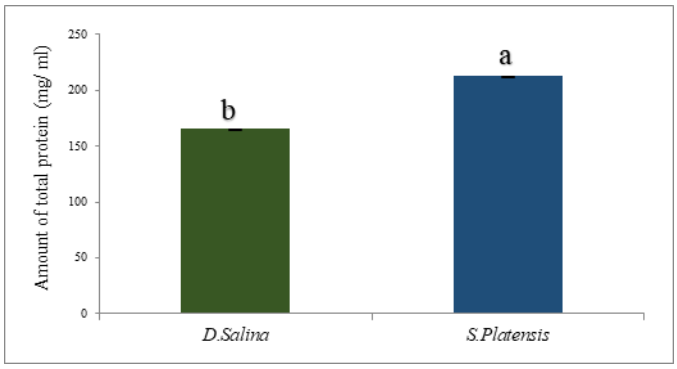

Figure 1S. Microalgae protein concentration (mg/mL).

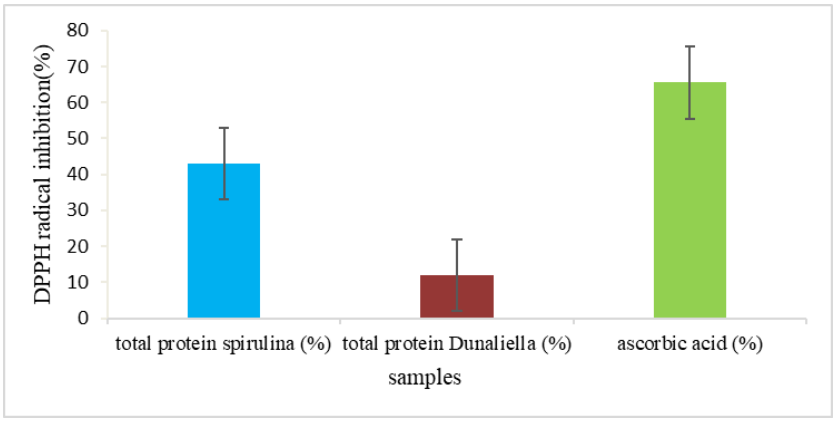

Figure 2S. DPPH free radical inhibition percentage of RuBisCO protein (F0–F35) from *S. platensis* and *D. salina*.

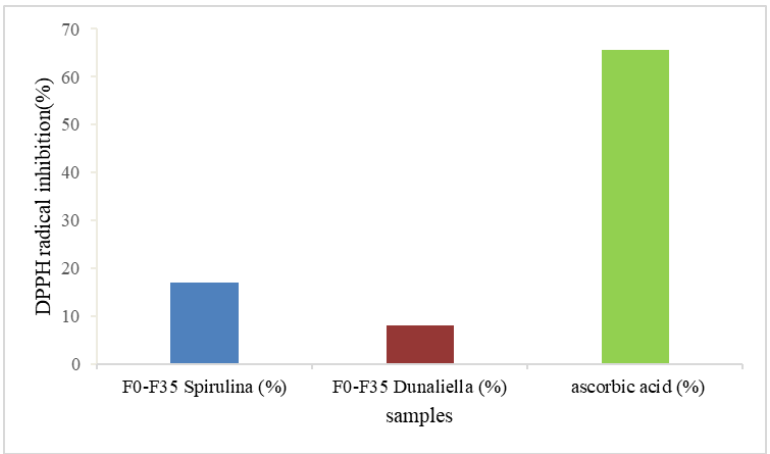

Figure 3S. DPPH free radical inhibition percentage of peptides derived from F0–F35 in *S. platensis* and *D. salina*.

Table 1S. Occurrence rate of hidden bioactive antioxidant peptides in the large subunit of RuBisCO.

Microalgae/RuBisCO large subunit	A	B
D. salina	0.1179	2.73
S. platensis	0.1261	4.17

Table 2S. Sequences 1 and 2 represent sequences of the RuBisCO large subunit of *D. salina* and *S. platensis*, respectively.

D. salina large subunit of RuBisCO	*S. platensis* large subunit of RuBisCO
G10	K11
F13	Y12
R21	K22
V30	P31
S31	K32
K79	R80
D86	H87
A99	C100
L115	**M116**
L138	M139
S141	**P142**
G168	P169
G245	P246
A247	C248
L252	M253
Q253	K254
H285	R286
Y286	W287
T317	C318
R339	K340
L343	M344
F354	H355

Table 3S. Similarity percentage of RuBisCO large subunit with the modeled subunit.

Microalgae/RuBisCO protein	GMQE	Sequence identity (%)
D. salina	0.96	95.16
S. platensis	0.94	84.81

Note: GMQE (global model quality estimate) is a quality estimate that combines properties from the target-template alignment and the template structure [47].

Table 4S. Best processing results from the docking of modeled RuBisCO protein with DPPH ligand.

Compound	Binding energy (Kcal/mol)	Interaction residues	H-bound
DPPH	−9.77	Gly 12, Lys 18, Lys 14, Ala 11	Gly 12, Lys 18, Lys 14