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Species identification by SDS-PAGE of red algae used as seafood or a food ingredient

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Abstract

The identification, by SDS-PAGE, of four red algae used as sea vegetables or ingredients by the food industry, was performed. They were *Palmaria palmata* (Dulse), *Chondrus crispus* (Pioca), *Porphyra umbilicalis* (Nori), *Gracilaria verrucosa* (Ogo-nori). For each species, variations in protein patterns were observed, according to the season. However, for all species, some protein bands were always present during the yearly cycle of the plant. The reference pattern of *P. palmata* was composed of six protein bands with apparent molecular weights between 59.6 and 15.2 kDa. The *G. verrucosa* pattern was constituted of eight permanent bands. Two pattern bands, with apparent molecular weights of 49.1 and 45.9 kDa,differentiated the *G. verrucosa* profile from other seaweed patterns. *C. crispus* could be identified by a reference pattern composed of seven bands; three, with close molecular weights (49.3; 46.2 and 43.2 kDa), were characteristic of this species. Finally, the *P. umbilicalis* pattern showed seven bands with molecular weights between 73.1 and 15.9 kDa. The presence of a band with a molecular weight above 70 kDa appeared to be specific to the *Porphyra* pattern. So the SDS-PAGE seemed able to identify the four red species used by the food industry, but this analytical method appeared to be applicable only to raw material dried in mild conditions.

Keywords: Red algae; Species identification; SDS-PAGE

1. Introduction

The annual global aquaculture production of marine algae is 6.5×10^6 tonnes (fresh products) (FAO data, 1994). This plant resource is mainly used for processing of food additives (e.g. alginates) and sea vegetable production. Asian countries are the main markets for the use of seaweeds, such as marine vegetables. Japanese people are the first consumers with an average of 1.6 kg (dry weight) per year per capita (Fleurence, 1999).

In France, the use of algae for human consumption is subject to a specific regulation (Fleurence & Guéant, 1999; Mabeau & Fleurence, 1993). Only 13 macroalgae and one micro algae, *Spirulina* sp, are authorized for use by the food industry as sea vegetables or ingredients. Among the macroalgae, four species, belonging to the Rhodophyceae or red seaweeds, are affected by this regulation. They are *Palmaria palmata*, *Chondrus crispus,* G*racilaria verrucosa* and *Porphyra umbilicalis,* well known under the names of Dulse, Pioca, Ogo-nori and Nori, respectively. Generally, the seaweeds used as sea vegetables in Europe or in Japan, are dried in mild conditions (from 45 to 50°C for a long time in a hot–air chamber or sun-dried) (Nisizawa, Noda, Kikucki, & Watanabe, 1987). These processes are moderate and the proteins are little denatured in comparison with other heat treatments. The use of protein signatures for identification of species in fishery products is already described (Etienne et al., 2000, 2001; Rehbein, 1990). Most of the techniques used are urea IEF, native IEF and SDS-PAGE. Some reports describe the application of electrophoresis for isoenzyme purification (Mardsen, Callow, & Evans, 1981), genetic differentiation (Sosa & Lindstrom, 1999) or for the identification of seaweeds belonging to the genus *Fucus*, *Callithamnion* (Mardsen,

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Evans, Callow & Keen, 1984; Price, Pettit, & Russel, 1987) or *Gracilaria* (Fleurence & Guyader, 1995). In addition, SDS-PAGE was successfully applied, in some conditions, for the distinction between two *Ulva* species frequently consumed as sea vegetables in Europe (Fleurence, Le Coeur, Mabeau, Maurice, & Landrein, 1995). We have tested the application of SDS-PAGE as an analytical tool for identification of four red seaweed species authorised in France for human consumption. SDS-PAGE is easy to apply by food control laboratories and is less complex and cheaper than molecular biological methods.

2. Materials and methods

2.1. Algae sampling

The sampling was performed during 1997. The samples were collected at Pleubian (France) on the Brittany coast, the main region where the algae are harvested for industrial use as sea vegetables, food ingredients or additives. Every 2 months, the samples were collected to evaluate the eventual presence of variation in protein patterns according to the season. The species collected were *P. palmata* (Dulse), *G. verrucosa* (Ogo-nori), *C. crispus* (Pioca) and *P. umbilicalis* (Nori). The specimens, in the same development stage, were gathered to constitute a homogeneous batch.

2.2. Sample preparation

Epiphytes and sand were removed by successive washing with sea water and distilled water. Algae were dried at 45°C to a constant weight.

2.3. Extraction of protein fraction

Dried algae were ground with a Waring blender and then with an ultracentrifuge-type grinder, ZM 100 (Retsch), for 1 h. The powder obtained (100 μm) was suspended in deionized water (10 g of powder in 500 ml). This procedure allows cell lysis by osmotic shock and facilitates protein extraction (Fleurence et al., 1995). The suspension was gently stirred overnight at 4°C. After incubation, the suspension was centrifuged at 10 000 *g* for 1 h. The supernatant was filtered and freeze-dried. The freeze-dried powder was used as a raw material for SDS-PAGE.

2.4. Protein assay

The protein content in samples was determined by the Kjeldahl method (N×6.25) (Indergaard & Minsaas, 1991). The protein content in an aqueous extract was also evaluated by the Kjeldahl method for the determination of the protein recovery yield. For the dropping in the wells of the gel, the protein concentration in extract was determined with bincinchoninic acid (BCA) protein reagent assay (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Bovine serum albumin was used as a reference.

2.5. Polyacrylamide gel electrophoresis

Each sample migration in SDS PAGE was repeated four times. SDS PAGE was performed using a Protean II xi cell electrophoresis unit (Biorad, Hercules, CA, USA) with stacking gel of 5% and separating gel of 12% acrylamide in Tris–HCl 25 mM, pH 8.3, Glycine 0.18 M and SDS 0.1%. The separation was carried out at 35 mA for 5 h. The polypeptides used as molecular mass markers were: phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and lactalbumin (14.4 kDa). After migration, protein bands of algal protein extracts were detected by silver staining.

2.6. Silver staining

Proteins were fixed with 40% methanol and 10% acetic acid for 1 h. The gel was washed three times with a 30% ethanol solution for 20 min. Proteins were reduced with 0.02% sodium thiosulfate for 1 min, then washed with ultrapure water three times for 20 s and stained with 0.02% formaldehyde for 20 min. The gel was washed with ultrapure water and developed with 3% carbonate, 0.05% formaldehyde, and 0.0005% sodium thiosulfate for 3 to 5 min and the gel was washed with ultrapure water twice for 30 min. The gel was then dried for 1 h (Gel dryer, Biorad).

2.7. Gel analyses

Gel stained with silver nitrate was read through a scanner [OmniMedia, XR, optical resolution 300 dpi (H)×600 dpi (v)] and analyzed by the software "Image Whole Band" (BioImage).

3. Results and discussion

3.1. Protein content of seaweeds and algal extracts

Strong variations of protein content were observed according to the season (Table 1). These effects were already described in the literature (Fleurence, 1999; Morgan, Wright, & Simpson, 1980). The efficiency of extraction procedure with the deionized water differs according to the species (Table 2). It is optimal for the species *C. crispus* (35% of protein yield recovery instead of 5% for *P. palmata*) and *P. umbilicalis*.

3.2. SDS-PAGE protein patterns

3.2.1. Gracilaria verrucosa (Ogo-nori)

Some variations in the protein pattern, according to the season, were observed (data not shown). However, eight bands were present throughout the year and constitute the reference protein pattern of *Gracilaria verrucosa* (Fig. 1, Table 3). These bands show apparent molecular weights located between 62.1 and 14.8 kDa (Table 3). The presence of two bands with apparent molecular weights of 46 and 49 kDa (Fig. 2, Table 3) is specific to the profile of *G. verrucosa* and allows these species to be distinguished by SDS-PAGE from other red seaweeds.

3.2.2. Palmaria palmata (Dulse)

The reference protein pattern of *P. palmata* was characterised by the presence of six permanent bands with apparent molecular weights between 15.2 and 59.6 kDa (Table 4). Among these bands, three, with apparent molecular weights of 15.2, 20.3 and 48.3 kDa, are predominant.

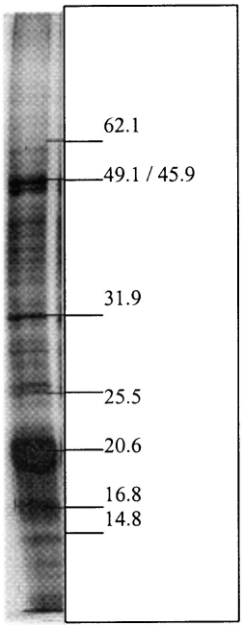

Fig. 1. *Gracilaria verrucosa* protein pattern in SDS-PAGE (October sample).

Table 1
Protein levels of red algae according to the season (expressed in % of dry weight, N× 6.2 5)

	Palmaria palmata	*Porphyra umbilicalis*	*Chondrus crispus*	*Gracilaria verrucosa*
February	22.0%±1.3	25.8%±1.5	25.5%±1.1	22.5%±1.1
April	20.4%±1.1	26.0%±1.1	25.4%±1.2	20.4%±0.8
June	10.1%±0.6	19.8%±0.9	7.9%±0.4	7.3%±0.5
August	9.5%±0.5	15.5%±1.0	7.7%±0.5	15.8%±0.6
October	13.7%±0.9	15.9%±0.9	11.9%±0.8	14.9%±0.3

Table 2
Efficiency of water extraction procedure according to the algal species[a]

Species	*Palmaria palmata*	*Gracilaria verrucosa*	*Porphyra umbilicalis*	*Chondrus crispus*
Yield of protein recovery (in% of total protein)	5±0.3	19±1.0	20±0.9	35±1.6

[a] On samples collected in February.

Table 3
Molecular weight distribution of bands composing the protein pattern of *Gracilaria verrucosa* in SDS-PAGE

Apparent molecular weight of bands (kDa)	Reference protein pattern
	Gracilaria verrucosa
Band 1: 62.1	75 kDa
Band 2: 49.1	
Band 3: 45.9	50 kDa
Band 4: 31.9	
Band 5: 25.5	30 kDa
Band 6: 20.6	
Band 7: 16.8	20 kDa
Band 8: 14.8	15 kDa

Table 4
Molecular weight distribution of bands composing the protein pattern of *Palmaria palmata* in SDS-PAGE

Apparent molecular weight of bands (kDa)	Reference protein pattern
	Palmaria palmata
Band 1: 59.6	62 kDa
Band 2: 48.3	50 kDa
Band 3: 32.7	
Band 4: 25.9	30 kDa
Band 5: 20.3	20 kDa
Band 6: 15.2	15 kDa

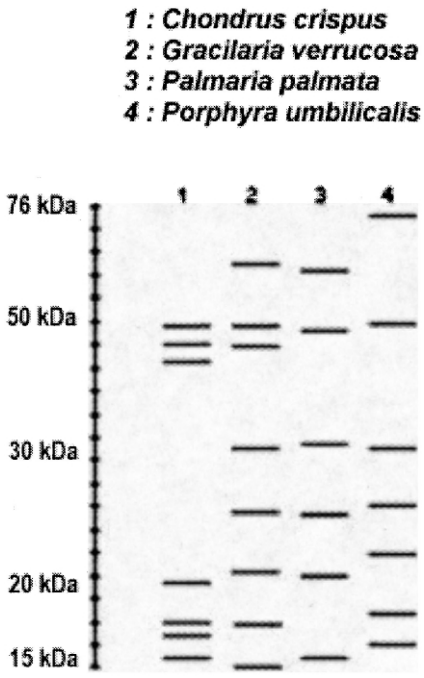

Fig. 2. Comparison between of protein pattern types of four red seaweeds *Palmaria palmata, Gracilaria verrucosa, Porphyra umbilicalis and Chondrus crispus*.

3.2.3. *Porphyra umbilicalis (Nori)*

The reference pattern of *P. umbilicalis* was composed of seven bands always present thoughout the year (Table 5). The apparent molecular weights of these bands were located between 73.1 and 15.9 kDa. The band with the apparent 73 kDa molecular weight was found only with the *Porphyra* pattern and it could therefore be considered as specific to this species.

3.2.4. *Chondrus crispus (pioca)*

For this species, seven bands with apparent molecular weights distributed between 49 and 15 kDa composed the reference pattern (Table 6). The pattern obtained can be distinguished from the others by the presence of triple bands with closely apparent molecular weights (49, 46 and 43 kDa) and the absence of protein bands between 20 and 40 kDa, unlike the other patterns (Fig. 2).

4. Conclusion

The four seaweeds in this study could be characterized by their protein patterns obtained in SDS-PAGE. The comparison between the profiles shows significant differences, notably for the species *C. crispus* and *G. verrucosa*. Therefore, SDS-PAGE appears to be a useful method for identification of red seaweeds authorized as sea vegetables or food ingredients. *C. crispus* and *G. verrucosa* are also exploited for the production of additives such as carrageenans and agar. Electrophoresis could be applied routinely for raw material used in food phycocolloid production. On the other hand, this methodology was tested only on raw material and not on processed products such as canned packs. In this last case, the use of DNA markers for identification of seaweeds (Broom, Jones, Hill, Knight & Nelson, 1999; Yamazaki, Kitade, Marvyama, & Saga, 1996) could be an interesting and alternative method in regard to the use of protein markers. The application of DNA markers could be also justified to remove the problems of seasonal variations reported with the protein pattern. However, in several cases the SDS-PAGE remains a more economic and more practical methodology than the molecular biological one for rapid control of the red alga species used by the food industry.

Table 5
Molecular weight distribution of bands composing the protein pattern of *Porphyra umbilicalis* in SDS-PAGE

Apparent molecular weight of bands (kDa)	Reference protein pattern
	Porphyra umbilicalis
Band 1: 73.1	76 kDa
Band 2: 49.6	60 kDa
Band 3: 32.1	
Band 4: 26.2	40 kDa
Band 5: 22.0	30 kDa
Band 6: 17.8	20 kDa
Band 7: 15.9	15 kDa

Table 6
Molecular weight distribution of bands composing the protein pattern of *Chondrus crispus* in SDS-PAGE

Apparent molecular weight of bands (kDa)	Reference protein pattern
	Chondrus crispus
Band 1: 49.3	50.00
Band 2: 46.2	
Band 3: 43.2	
	30.00
Band 4: 19.8	
Band 5: 17.2	
Band 6: 16.4	
Band 7: 15.2	16.00

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