

Nitrogen and protein contents in some aquatic plant species

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Abstract

Nitrogen and protein contents in higher aquatic plants deriving from a natural habitat were determined. The following plants were examined: *Spirodela polyrrhiza* (L.) Schleid., *Elodea canadensis* Rich., *Riccia fluitans* L. Total nitrogen and nitrogen of respective fractions were determined by the Kjeldahl method. Nitrogen compounds were fractionated according to Thimann et al. Protein was extracted after Fletcher and Osborne and fractionated after Osborne.

It was found, that total protein content in the plants under examination constitutes 18 to 25% of dry matter. Albumins and glutelins are the most abundant protein fractions.

INTRODUCTION

Unconventional sources of cheap protein feed have been sought for for many years (Bytniewska 1975). Aquatic plants not yet exploited can constitute an abundant protein reservoir (Popow 1964; Tamiya 1966; Hedén 1969; Boyd 1970, 1973; Maciejewska-Potapczyk et al. 1970, 1975). These plants are characterized by fast growth and intensive biomass production. They can be easily cultured under both natural and controlled laboratory conditions. Analysis of aquatic plants shows a high content of readily soluble protein in them. This problem has not been adequately considered in our inland investigations (Mowszowicz 1971) in spite of the need of their undertaking.

This research is concerned with nitrogen and protein contents in some aquatic plant species and preliminary analysis of protein isolated from them.

MATERIAL AND METHODS

The following aquatic plants deriving from a natural habitat were studied: *Spirodela polyrrhiza* (L.) Schleid. (Rostafiński, Seidl 1962; Kandeler 1973) was collected from a pond in Rogi near Łódź. This

plant has a short vegetation period and for this reason it was cultured in the laboratory under unsterile conditions. *Elodea canadensis* Rich. (Ros-tafiński, Seidl 1962) was collected each time from the Bobrówka river near the Okręt pond in Skierniewice voivodeship. *Riccia fluitans* (L.) (Rejment-Grochowska 1966, 1971) was obtained from an aquarium culture of the Zoological Garden in Łódź.

Unsterile culture of *Spirodela polyrrhiza*:

Spirodela was cultured in 2-liter glass crystallizers containing 1.5 l of a nutrient solution. Pirson and Seidel's (1950) solution 4-times diluted in my own modification consisting in the elimination of $\text{MnCl}_2 \cdot \text{H}_2\text{O}$ and H_3BO_3 and introduction of Hoagland A — Z solution. The composition of diluted Pirson and Seidel solution was as follows: KH_2PO_4 50 mg, KNO_3 100 mg, $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$ 153 mg, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ 75 mg, ferric citrate 12.5 mg. with 1 ml of A — Z solution and 0.6 ml of Na_2EDTA solution (18 mg) added. The whole was made up with distilled water to a 1 liter volume. pH was adjusted to 6.5 with NaOH. Day light was provided simultaneously with mercury lamps (3×400 Watt) for 15 hours. At 3—7 days intervals the plants were transferred to a freshly prepared solution.

Analytical methods:

Total nitrogen was determined by the Kjeldahl method in 300 mg samples of fresh material. Nitrogen-containing compounds were fractionated by the method of Thimann et al. (1957, 1960) which consists in the extraction of respective fractions with trichloroacetic acid (TCA) at various temperatures. Nitrogen content in these fractions was determined as above. Total protein was prepared after Fletcher and Osborne (1965) and determined by the Lowry et al. method (1951). Crystalline bovine serum albumin (Serva Entwicklungslabor Heidelberg) was used. Apart from that total protein was calculated on the basis of nitrogen content in the fraction obtained by the Thimann et al. method (1957, 1960) ($\text{N} \times \times 6.25$). Application of the 6.25 coefficient makes possible only approximate estimation of protein content in plant material (Boyd 1970; Boyd and Goodyear 1971; Betschart and Kinsella 1973). Total protein was fractionated after Osborne. Samples of fresh plant material (10 g) were homogenized and decolorized with ether. Albumins and globulins were extracted with 100 ml of 1 M NaCl in 0.05 M phosphate buffer pH 7.3 and dialysed against distilled water. The globulins precipitated during dialysis were dissolved in 0.2 M NaCl in 0.05 M phosphate buffer pH 7.3. Glutelins were extracted with 0.15% NaOH solution, prolamins — with

70% ethanol. The remaining protein was extracted with 1 M NaOH in a boiling water bath for 10 minutes. The protein content in the respective fractions was determined by the Lowry et al. method (1951).

All analyses were repeated at least 4—5 times. Mean value of the results and standard deviation of the mean are shown in the tables.

RESULTS

The determinations of nitrogen and protein contents in aquatic plants were preceded by estimation of the per cent of dry matter. *Elodea canadensis* is characterized by the highest level of dry matter namely $10.14\% \pm \pm 0.14$, *Spirodela polyrrhiza* $6.93\% \pm 0.07$, and *Riccia fluitans* $6.65\% \pm 0.09$. *Spirodela*, *Elodea* and *Riccia* contain respectively: 5.81 ± 0.08 , 4.76 ± 0.18 , 5.19 ± 0.13 mg of total nitrogen in 100 mg of dry matter (Table 1). The fraction most abundant in N protein fraction in all the plants under examination is prepared according to Thimann et al. (1957, 1960). This fraction comprises on the average: in *Spirodela* 4.12 ± 0.05 mg N in 100 mg of dry matter, in *Elodea* 2.98 ± 0.10 , in *Riccia* 2.87 ± 0.07 . The acid-soluble fraction contains in *Spirodela* 0.98 ± 0.03 mg N. The nucleic acids fraction in *Spirodela* as 0.74 ± 0.02 mg N in 100 mg of dry matter.

Table 1

Total nitrogen and nitrogen in respective fractions obtained by the method of Thimann et al.

| Material | mg N/100 mg dry matter | | | |
|-----------------------------|------------------------|--------------------------|--------------------------|--------------------|
| | total N | N acids soluble fraction | N nucleic acids fraction | N protein fraction |
| <i>Spirodela polyrrhiza</i> | 5.81 ± 0.08 | 0.98 ± 0.03 | 0.74 ± 0.02 | 4.12 ± 0.05 |
| <i>Elodea canadensis</i> | 4.76 ± 0.18 | 1.28 ± 0.05 | 0.46 ± 0.02 | 2.98 ± 0.10 |
| <i>Riccia fluitans</i> | 5.19 ± 0.13 | 1.62 ± 0.06 | 0.36 ± 0.04 | 2.87 ± 0.07 |

Among the plants under examination *Spirodela polyrrhiza* is characterized by the highest protein content (Table 2). It was found that this plant contains on the average 24.4 ± 0.47 mg of protein in 100 mg of dry matter as determined by the Lowry et al. method. This value, however, calculated on the basis of nitrogen content in the protein fraction is $25.8 \pm \pm 0.29$ mg. Depending on the method applied, *Elodea canadensis* contains 19.1 ± 0.75 mg or 18.6 ± 0.60 mg, *Riccia fluitans* 19.8 ± 0.46 mg or 17.9 ± 0.43 mg in 100 mg of dry matter.

Table 2

Total protein content determined on the basis of nitrogen in protein fraction or by the Lowry method

| Material | protein N x 6.25 | | Lowry et al. method | |
|-----------------------------|------------------|------------------|---------------------|------------------|
| | 100 mg | | 100 mg | |
| | fresh matter | dry matter | fresh matter | dry matter |
| <i>Spirodela polyrrhiza</i> | 1.78 ± 0.03 | 25.80 ± 0.29 | 1.69 ± 0.03 | 24.39 ± 0.47 |
| <i>Elodea canadensis</i> | 1.88 ± 0.06 | 18.64 ± 0.60 | 1.94 ± 0.07 | 19.14 ± 0.75 |
| <i>Riccia fluitans</i> | 1.19 ± 0.04 | 17.94 ± 0.43 | 1.31 ± 0.03 | 19.79 ± 0.46 |

Protein fractionation by the Osborne method has shown that albumins and glutelins are the most abundant protein mass in all the plants taken into consideration (Table 3). Probably albumins contain almost the whole readily soluble protein of vegetative plant parts. Albumin contents in the plants examined fluctuate within the limits 3.76 ± 0.12 to 10.34 ± 0.03 mg in 100 mg of dry matter. The highest level of these proteins was found in *Spirodela*. An average content of glutelins in all plant species examined is: 6.38 ± 0.32 to 7.95 ± 0.26 mg. Globulin and prolamin contents are low.

Table 3

Protein content in respective protein fractions determined by the Lowry method

| Material | mg/100 mg dry matter | | | | |
|-----------------------------|----------------------|-----------------|-----------------|-----------------|-------------------|
| | albumins | globulins | glutelins | prolamins | remaining protein |
| <i>Spirodela polyrrhiza</i> | 10.34 ± 0.03 | 0.82 ± 0.03 | 6.38 ± 0.32 | 0.60 ± 0.04 | 4.90 ± 0.10 |
| <i>Elodea canadensis</i> | 3.76 ± 0.12 | 0.95 ± 0.08 | 7.95 ± 0.26 | 0.26 ± 0.06 | 6.40 ± 0.09 |
| <i>Riccia fluitans</i> | 5.11 ± 0.19 | 1.01 ± 0.04 | 7.47 ± 0.15 | 0.41 ± 0.04 | 3.86 ± 0.12 |

DISCUSSION

The choice of material for this study was made after many considerations. This material was differentiated morphologically and anatomically, and therefore not uniformly susceptible to homogenization. A considerable growth intensity, a speedy biomass multiplication and a wide-spread prevalence of these plants in nature are their common features. The protein

level in them was examined by the Lowry et al. method and on the basis of nitrogen content in the protein fraction. Nitrogen of the protein fraction obtained according Thimann et al. (1957, 1960) constitutes 56—70% of total nitrogen in the plants under examination. Kolousek and Coulson (1955) have shown that protein N includes 75—85% of the total leaf nitrogen. The remaining 15—25% are contained in free amino acids, nucleotides, amides and nitrates. Singh (1964) reported that fresh alfalfa leaves contain 75% of protein N. According to Boyd (1970), 80—90% of N in plants are bound in the form of protein.

The data in the literature concerning the total protein content in aquatic plants are not complete. Maciejewska-Potapczyk et al. (1970) found that *Lemna minor* L. cultured under laboratory conditions contains 16% of protein. Boyd (1970) determined protein content in 11 species of higher aquatic plants, among them only *Typha latifolia* contained small amounts of protein (nearly 4%). The highest protein content was found by this author in *Nuphar advena* (21.6%). Protein content in the remaining species ranged within 10.5—17.1%. Some freshwater algae species contain a higher protein content than aquatic macrophytes. Boyd (1973) reports that such algae as: *Chara*, *Nitella*, *Spirogyra* contain 10—20% of protein, but blue algae: *Anabaena*, *Microcystis*, *Aphanizomenon* and also phytoplankter *Euglena* have 42—46% of protein. The red algae (*Porphyra*) cultured in Japan for consumption purposes contain nearly 30—50% of protein (Tamiya 1966). *Chlorella* can contain from 42% (Popow 1964) to nearly 50% of protein (Tamiya 1966). Abundant protein quantities were found in the leaves of fodder plants. Klyszejko-Stefanowicz et al. (1972) established that leaves of several clover species contain 19.2—20.0% of protein. Maciejewska-Potapczyk et al. (1973) found 11.9—15.2% of protein in the leaves of 5 clover varieties cultivated in Poland. Ponikiewska (1973) demonstrated high quantities of protein in the leaves of winter vetch (23.9—26.6%). Altschul (1965) reports that protein can constitute 10—41% of dry matter in the leaves. The aquatic plants examined in this study have as high protein content as the leaves of some fodder plants.

A comparison of protein level in the aquatic plants under examination with that in conventional foods is very interesting. *Spirodela*, *Elodea* and *Riccia* contain more (in relation to dry matter) total protein than cereal grains. They are surpassed in this respect only by some legume species, particularly soybean. This comparison is summarized in Figure 1.

The use of Osborne method for fractionation of proteins isolated from other plant material than seeds gave satisfactory results. Albumins and glutelins are the most abundant protein group in plants under examination. Fractionating *Lemna minor* L. proteins Maciejewska-Potapczyk et al. (1970) found that this plant is characterized by the highest albumins content. Analogous investigations performed in order to charac-

terize leaf proteins in 5 clover varieties led to the isolation of glutelins in amounts exceeding albumin content (Maciejewska-Potapczyk et al. 1973). Trace quantities of prolamins were found in both clover lea-

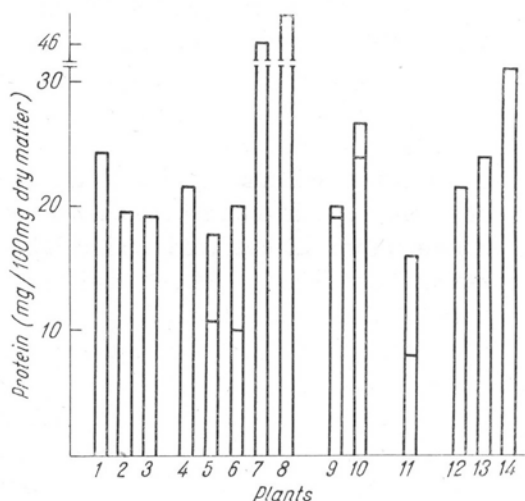


Fig. 1. Total protein content in aquatic plants under examination and in other common plant species

1 — *Spirodela polyrrhiza*, 2 — *Elodea canadensis*, 3 — *Riccia fluitans*, 4, 5 — *Nuphar advena*, aquatic plants (Boyd 1970), 6, 7 — green algae, blue-green algae (Boyd 1973), 8 — *Chlorella* (Tamiya 1966), 9 — clover (Kłyszajko-Stefanowicz et al 1972), 10 — vetch (Ponikiewska 1973), 11, 12, 13 — cereals, beans, peas (Johnson, Lay 1974), 14 — soybean (Altschul 1965)

ves and aquatic plants (apart from *Lemna minor* L.). The content of protein fractions extracted from vegetative plant organs is different from that in cereal grains and legume seeds (Dąbrowska Kączkowski 1971; Whitehouse 1973; Kakade 1974; Johnson and Lay 1974).

High total protein and the most readily soluble proteins (albumins) contents point to the aquatic plants under examination as a valuable source of fodder protein and also an interesting material for theoretical studies.

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Zawartość azotu i białka w kilku gatunkach roślin wodnych

Streszczenie

Materiał badań stanowiły wyższe rośliny wodne pochodzące ze środowiska naturalnego: *Spirodela polyrrhiza* (L.) Schleid., *Elodea canadensis* Rich., *Riccia fluitans* L. Badano zawartość azotu i białka całkowitego oraz frakcjonowanego. Azot oznaczano mikrometodą Kjeldahla. Wymienione rośliny posiadają średnio: $5,81 \pm 0,08$, $4,76 \pm 0,18$, $5,19 \pm 0,13$ mg N całkowitego w 100 mg suchej masy. Związki zawierające azot frakcjonowano zgodnie z metodą Thimanna i wsp. Najbogatszą w azot jest frakcja białkowa. Białko całkowite ekstrahowano metodą Fletchera i Osborne'a, a oznaczano metodą Lowry'ego. *Spirodela*, *Elodea* oraz *Riccia* zawierają średnio: $34,39 \pm 0,47$, $19,14 \pm 0,75$, $19,79 \pm 0,46$ mg białka w 100 mg suchej masy. Ponadto zawartość białka obliczono także na podstawie zawartości azotu we frakcji białkowej. Albuminy i gluteliny stanowią najobfitsze frakcje białkowe ekstrahowane metodą Osborne'a. Globuliny i prolaminy znajdują się w mniejszych ilościach.

Badane rośliny posiadają podobną zawartość białka jak np. koniczyna i wyka, więcej białka niż ziarniaki zbóż, natomiast mniej niż nasiona roślin motylkowych.