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Nutritional value of duckweeds (Lemnaceae) as human food

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ABSTRACT

Duckweeds have been consumed as human food since long. Species of the duckweed genera, *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella* and *Wolffia* were analysed for protein, fat, and starch contents as well as their amino acid and fatty acid distribution. Protein content spanned from 20% to 35%, fat from 4% to 7%, and starch from 4% to 10% per dry weight. Interestingly, the amino acid distributions are close to the WHO recommendations, having e.g. 4.8% Lys, 2.7% Met + Cys, and 7.7% Phe + Tyr. The content of polyunsaturated fatty acids was between 48 and 71% and the high content of n3 fatty acids resulted in a favourable n6/n3 ratio of 0.5 or less. The phytosterol content in the fastest growing angiosperm, *W. microscopica*, was 50 mg g^{-1} lipid. However, the content of trace elements can be adjusted by cultivation conditions. Accordingly, *W. hyalina* and *W. microscopica* are recommended for human nutrition.

0. Introduction

Bhanthumnavin and McGarry emphasized already decades ago (Bhanthumnavin & McGarry, 1971) that "Khai-nam", literally meaning "eggs of the water" (duckweed) in Thai language, is "a possible source of inexpensive protein" that can be used as human food. They identified the species as *Wolffia arrhiza* and stressed on their high growth rate. This species is rather rare in Thailand (Landolt, 1986). We investigated three samples of the duckweeds sold in the market for human consumption from Northern Thailand in 2016, and identified all of them as *W. globosa*, which is in accordance with Landolt and Kandeler (1987) suggesting that these authors dealt with the more common species *W. globosa*. Bhanthumnavin and McGarry (1971) measured the protein, carbohydrate, and fat content of these plants and reported that "Khai-nam" was used as food of poor people for many generations in Laos, Thailand and Burma (now Myanmar). In many other South-Asian countries like India, Bangladesh and Pakistan, food is rich in carbohydrates but poor in proteins. Thus, protein-rich duckweed would be a perfect supplement to the rice-based staple food in these countries. Moreover, duckweed might add on to the protein content of the vegetarian or vegan diet as this life style becomes more and more popular in the developed countries.

In their landmark paper, Rusoff, Blakeney, and Culley (1980) investigated four species of duckweeds, *Spirodela polyrhiza*, *Landoltia punctata* (termed as *Spirodela punctata*), *Lemna gibba* and *Wolffia columbiana*, with respect to the protein and fat contents, and the amino acid composition. They already mentioned

Abbreviations: AA, amino acids; AAS, amino acid score; Ala, alanine; ALA, a-linolenic acid; Arg, arginine; Asp, aspartic acid; Cys, cysteine; DW, dry weight; EAA, essential amino acids; EAAI, essential amino acid index; essential AA/non-essential AA, EAA/NEAA; EPA, eicosapentaenoic acid; FA, fatty acids; FAO, Food and Agriculture Organization of the United Nations; FAME, fatty acid methyl esters; Glu, glutamic acid; Gly, glycine; Ile, isoleucine; LA, linoleic acid; LCFA, long-chain fatty acids; LC-PUFA, long-chain polyunsaturated fatty acids; Leu, leucine; Lys, lysine; MCFA, medium chain fatty acids; Met, methionine; MUFA, monounsaturated fatty acids; N, nitrogen; NPN, non-protein nitrogen; Phe, phenylalanine; PUFA, polyunsaturated fatty acids; SCFA, short-chain fatty acids; SD, standard deviation; SDA, stearidonic acid; SFA, saturated fatty acids; Thr, threonine; Trp, tryptophan; Val, valine; WHO, World Health Organization.

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the favourable amino acid composition of duckweed proteins as per the FAO recommendations. Later, a detailed investigation of the amino acid composition of the proteins in *W. arrhiza*, clone 9528 was performed by Appenroth, Augsten, Liebermann, and Feist (1982). Yan et al. (2013) investigated 30 out of the 37 duckweed species (cf. Sree, Bog, & Appenroth, 2016) and reported the presence of 11 saturated and unsaturated fatty acids. Recently, Tang, Li, Ma, and Cheng (2015) reported the starch and fatty acid content in four duckweed species (*S. polyrhiza*, *L. punctata*, *L. aequinoctialis*, and *W. globosa*), all from the lake Chao, China.

In the present study, we aimed to give an overview of the nutritional quality of different duckweed species for their possible use in human nutrition. However, as stressed by van der Spiegel, Noordam, and Fels-Klerx (2013), the concerned legal issues need to be considered before it can be commercially marketed as human food. As a step forward, it would be important to investigate the nutritive composition of duckweeds to prevent any unwanted effects on humans and also to make it more acceptable to the general public of the countries that do not have the tradition of consuming duckweeds. With our present report, we want to contribute to this issue. To have a broader view, we investigated six species representing all five genera, *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna gibba*, *Lemna minor*, *Wolffiella hyalina*, and the recently rediscovered species *Wolffia microscopica* (Sree, Maheshwari et al., 2015). Initially, we investigated dry weight, protein, fat, and starch content as well as their amino acid and fatty acid distributions. As a part of the detailed investigation, we focused on *W. microscopica* investigating other components having relevance to human consumption, i.e. content of minerals, antioxidants (carotenoids and tocopherols), phytosterols, fibre and ash content.

1. Material and methods

1.1. Plant material and cultivation

Plant material was taken from the collection of duckweed ecotypes, or clones, of the Institute of Plant Physiology, University of Jena, Germany. The duckweeds in this stock collection, most of which stem from the collection of E. Landolt, ETH, Zürich, Switzerland, were maintained under axenic conditions on agar medium as described by Appenroth, Teller, and Horn (1996). Six species encompassing all five duckweed genera were selected: *Spirodela polyrhiza* 7498 (USA, NC), *Landoltia punctata* 9589 (India, Delhi), *Lemna minor* 9441 (Germany, Marburg), *Lemna gibba* 7742 (Italy, Sicilia), *Wolffiella hyalina* 9525 (India, Telangana), and *Wolffia microscopica* 2005 (India, Gujarat).

Duckweeds were pre-cultivated under axenic conditions at 25 ± 1 °C in 300 mL Erlenmeyer flasks containing 180 mL nutrient medium. They were exposed to continuous white light at 100 μmol m^{-2} s^{-1} (photosynthetically active radiation) from fluorescence tubes TLD 36 W/86 (Philips, Eindhoven, Netherlands) following the ISO 20079 protocol (Naumann, Eberius, & Appenroth, 2007). Accordingly, the plants were conditioned to the nutrient medium for four weeks during this pre-cultivation phase in order to ensure reproducible results. The nutrient medium was replenished every week to prevent nutrient limitation. In place of the Steinberg medium, specified by the ISO 20079 protocol, however, a modified Schenk-Hildebrandt medium was employed with the following composition: $CaCl_2{\cdot}2H_2O$ 0.68 mM, KNO_3 12.4 mM, $MgSO_4{\cdot}7H_2O$ 0.81 mM, $(NH_4)H_2PO_4$ 1.3 mM, $MnSO_4{\cdot}H_2O$ 30 μM, H_3BO_3 40 μM, $ZnSO_4{\cdot}7H_2O$ 1.74 μM, KI 3.0 μM, $CuSO_4{\cdot}5H_2O$ 0.4 μM, $Na_2MoO_4{\cdot}2H_2O$ 0.21 μM, $CoCl_2{\cdot}6H_2O$ 0.21 μM, FeNaEDTA 27.0 μM, and $Na_2EDTA{\cdot}2H_2O$ 2.74 μM. The pH of the medium was adjusted to 5.5.

The main phase of cultivation used for determining the biomass composition employed the same conditions as described for the pre-cultivation, except that plastic trays (area 60 × 40 cm) filled with 15 L Schenk-Hildebrandt medium and covered with glass plates were used. The main cultivation phase lasted for 14 days, during which the size of the inoculum ensured that the fronds did not completely cover the surface of the medium and thus avoiding a stress response. Thereafter, biomass was harvested and lyophilized for further analysis.

1.2. Analytical methods

Fresh duckweeds were analysed for dry weight (DW). After freeze-drying plants were finely ground and homogenised with a laboratory mill for small amounts for further use.

1.2.1. Lipid extraction and fatty acid separation

Total lipids were extracted from 1.0 g (or less) of the homogenised samples with methanol/chloroform/water in the ratio 1:2:1 (v/v/v). These lipid extracts were transmethylated using a combination of 0.5 N methanolic sodium hydroxide (Merck) and 10% (w/w, Supelco) boron trifluoride-methanol (at 100 °C for 5 min each). Subsequently, fatty acid methyl esters (FAME) were purified by TLC and dissolved in *n*-hexane for analysis. The separation of FAMEs was performed by GC (GC-17 V3, Shimadzu, Japan) equipped with a cooled auto sampler and a flame ionization detector. A fused-silica capillary column of medium polarity was used (DB-225MS/ 60 m × 0.25 mm i.d. with a 0.25 mm film thickness; Agilent Technologies, USA). The initial oven temperature was maintained at 70 °C for 2 min, then increased by 10 °C/min to 180 °C, further increased by 2 °C/min to 220 °C and held at this temperature for 5 min. Finally, it was increased by 2 °C/min to 230 °C and held for 15 min. The injector and detector temperatures were maintained at 260 °C. Hydrogen was used as carrier gas. The amount of the separated FAME was expressed as % of the total FAME. Various reference standards were used as FAME mix to identify fatty acid peaks/: No. 463, 674, (NU-CHEK PREP; INC., US), BR2, BR4, ME 93 (Larodan; Sweden), Supelco137 Component FAME Mix, PUFA No. 3. Lab Solutions software for GC (GC solution; Shimadzu, Japan) was used for peak integration.

1.2.2. Protein/amino acids (AA)

The protein content of freeze-dried duckweeds was calculated via the Kjeldahl procedure using the N factor 6.25. The quantitative determination of AA in duckweed samples was based on the chemical properties of the proteinogenic AA. Majority of the proteinogenic AA was determined after subjecting the samples to acid hydrolysis with phenolic hydrochloric acid. For the sulphur-containing AA, methionine and cysteine, an oxidation was performed before acid hydrolysis. The separation of AA occurred by means of ion exchange chromatography (Biochrom 30, Laborservice Onken, Gründau, Germany) and post column derivatization was carried out with ninhydrin.

1.2.3. Minerals

After acid digestion of the ash under pressure, macro and trace elements were determined using the following techniques: calcium, phosphorus, magnesium, sodium, potassium, iron, copper, zinc, and manganese with ICP-AES; arsenic, selenium with hydride-AAS, mercury with cold vapour AAS by direct mercury analyser, cadmium, lead with graphite furnace AAS and iodine after ammoniac extraction with ICP-MS.

1.2.4. Carotenoids/tocopherols

To determine the contents of carotenoids and vitamin E, the freeze dried duckweed samples were extracted three times under