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Safety of *Lemna minor* and *Lemna gibba* whole plant material as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on *Lemna minor* and *Lemna gibba* whole plant material as a novel food (NF) pursuant to Regulation (EU) 2015/2283. *Lemna minor* and *Lemna gibba* are aquatic plants commonly named water lentils. The NF is produced by cultivation of *Lemna minor* and *Lemna gibba* plants, washing with water and heat treatment. The main constituents of the NF are water, protein and fibre. The Panel notes that the concentration of trace elements and contaminants in the NF is highly dependent on the conditions of cultivation of the plant and the fertiliser composition. The NF is intended to be used as a vegetable, similar to other leafy vegetables. The target population is the general population. The Panel considers that, with the exception of concerns related to the manganese intake, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous. The Panel considers that the risk that the consumption of the NF may trigger allergic reactions in humans is low. The Panel concludes that the NF, in consideration of its proposed uses and the concentration of manganese as compared to the normally present concentration of manganese in other leafy vegetables, may be of safety concern, therefore, the safety of the NF cannot currently be established.

Keywords: novel food, *Lemna minor*, *Lemna gibba*, water lentils, plant

Requestor: European Commission

Question number: EFSA-Q-2020-00512

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 20 May 2020, Wageningen Plant Research Institute submitted an application to the Commission in accordance with Article 10 of Regulation (EU) 2015/2283[1] to place on the EU market *Lemna minor* and *Lemna gibba* whole plant material.

The applicant requests to authorise the use of *Lemna minor* and *Lemna gibba* whole plant material to be used as food intended for the general population.

On 27 October 2020, in accordance with Article 10 (3) of Regulation (EU) 2015/2283, the European Commission asked the European Food Safety Authority to provide a scientific opinion on the safety of *Lemna minor* and *Lemna gibba* whole plant material.

1.2. Information on existing evaluations and authorisations

EFSA assessed previously the safety of other water lentil species, i.e. *Wolffia arrhiza* and *Wolffia globosa* as a traditional food (TF) from a third country (EFSA, 2021). The TF consists of fresh plants belonging to the species *Wolffia arrhiza* and *Wolffia globosa*, which have been consumed for more than 25 years in Asia (Myanmar, Laos and Thailand). In its technical report, EFSA did not raise any duly reasoned safety objection.

Additionally, the NDA Panel assessed previously the safety of powders produced from different species of water lentils as novel foods (NF):

i) Water lentil powder from Lemnaceae intended for consumption as food ingredient and food supplement (EFSA NDA Panel, 2021a), which consists of species from the *Lemna* genus (70%–100%) and the *Wolffia* genus (0%–30%); and
ii) *Wolffia globosa* powder intended for consumption as food ingredient and food supplement (EFSA NDA Panel, 2021b), which consists exclusively of *Wolffia globosa* species.

In the above-mentioned Scientific opinions, the Panel concluded that the increase in manganese intake from the NFs was substantial as compared to the background manganese dietary intake, and consequently, the safety of these NFs could not be established.

2. Data and methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information. During the assessment, the Panel identified additional data that were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all available (proprietary, confidential and published) scientific data (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application does not include a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283.

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the

[1] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001. OJ L 327, 11.12.2015, pp. 1–22.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71.

EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

Additional information, which was not included in the application, was retrieved by literature search following a search strategy and standard operating procedure as described by Dibusz and Vejvodova (2020).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF, which is the subject of the application, consists of cultivated species of *Lemna* genus, heat treated (steamed), which contain mainly water (92%–94%) with lesser constituents, including protein (2%–4%), dietary fibre (0.6%–2.6%) and ash (1.0%–1.6%). The NF is proposed to be used as a vegetable. The target population is the general population.

The applicant indicated that, as defined by Regulation (EU) 2015/2283, Article 3, the NF falls under the category 'Foods consisting of, isolated from or produced from plants or their parts, except when the food has a history of safe food use within the Union and is consisting of, isolated from or produced from a plant or a variety of the same species obtained by:

- traditional propagating practices which have been used for food production within the Union before 15 May 1997; or
- non-traditional propagating practices, which have not been used for food production within the Union before 15 May 1997, where those practices do not give rise to significant changes in the composition or structure of the food affecting its nutritional value, metabolism or level of undesirable substances'.

3.2. Identity of the NF

The NF is heat treated *Lemna minor* and *Lemna gibba* plant material. *L. minor* and *L. gibba* are members of the *Lemna* genus, Lemnoideae subfamily, and the Araceae family. *L. minor* and *L. gibba*, belong to the water lentil family, represented by species of five genera (*Lemna*, *Wolffia*, *Wolffiella*, *Landoltia* and *Spirodela*) and all species are commonly called water lentils or duckweeds (Cabrera et al., 2008). Water lentils are floating aquatic plants, growing mainly by asexual reproduction, wherein a new plant grows from the parent plant without a seed stage. Plants are composed of small leaves (fronds) and occasionally contain a small root (Leng, 1999). The presence of the roots depends on the culturing conditions and harvesting approach. The geographical distribution of *L. minor* and *L. gibba* is expanded worldwide, except deserts and permanently frozen areas. Nevertheless, optimal conditions for the growth of water lentils are in tropical and temperate zones (Leng et al., 1995). Consequently, the NF is intended to be cultivated in any region with long daylight and warm temperatures.

Following a request from EFSA, the applicant clarified that the plant strains are obtained from the 'Landolt collection' and verification of the identity of the plant is performed by partial DNA sequencing based on the Wang et al. (2010) publication.

3.3. Production process

According to the information provided, the NF is produced in line with Hazard Analysis Critical Control Point (HACCP) principles.

The *Lemna* plants used to start the cultivation of the NF are obtained from a sterile *in vitro* stock and they are initially grown indoor in trays with a layer of water at least 2–3 cm deep. During the vegetal growth, *Lemna minor* and *Lemna gibba* plants are kept separate to preserve their purity until enough material is obtained (~ 7 kg of *L. minor* and 10 kg of *L. gibba*) to start culturing in the greenhouse. No heating or artificial lighting is used during the plant growth. At the beginning of the cultivation, the *Lemna* plants are arranged in pools of 5 m^2 surface area filled with water from a local source, 60-m deep drilling; at this stage, the applicant does not apply water circulation. Once the entire pool surface is covered with a thick layer of *Lemna* plants, the pool is extended and pumps for water circulation and UV treatment for water disinfection are installed. According to the applicant, water circulation would reduce algae growth, spread nutrients and introduce some oxygen flow in the pools. Throughout the growing season, the *Lemna* plants are treated with fertiliser solutions to promote plant growth. The fertilisation is performed based on electrical conductivity (1.5) and pH (5.5–7), and the mineral content of fertiliser is modulated considering the chemical composition of water.

In a later stage, the applicant clarified that the *Lemna* plants used in the production of the NF are harvested at regular intervals along the entire growing season (from April to September), washed with running tap water for 1 min, steamed at 100°C for 3–4 min and then stored in closed boxes at −20°C.

Both source water and pool water with growth medium are analysed at different stages of the NF cultivation. The pools are cleaned during and after the growing season of the NF. The applicant removes the sediments on the bottom of the pools (e.g. root abscission of *Lemna* plants) by using a pool vacuum cleaner.

The Panel considers that the production process is sufficiently described.

3.4. Compositional data

The NF is a heat-treated *Lemna minor* and *Lemna gibba* plant material and it is composed by water (92%–94%), proteins (2%–4%), dietary fibre (0.6%–2.6%), ash (1.0%–1.6%).

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical data on chemical and microbiological parameters for ten independent batches of the NF (five for *L. minor* and five for *L. gibba*). Certificates of accreditation for the laboratories that conducted the analyses were provided by the applicant. Analytical data were produced using validated methods and, whenever in-house methods were employed, a full description of the method as well as results of the respective validation procedures have been provided.

The applicant submitted analytical data for proximate parameters as presented in Table 1.

Table 1: Batch-to-batch analysis of the NF (expressed on a FW basis)

Parameter	Unit	*Lemna minor* batches					*Lemna gibba* batches					Method of analysis
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Moisture	%	93.3	91.6	92.5	92.8	91.9	93.3	93.9	93.2	94.0	93.1	Thermo-gravimetry (internal)
Proteins (N × 6.25)	%	3.0	4.1	3.3	3.1	4.0	3.4	3.1	2.4	2.8	3.4	Kjeldahl (internal)
Carbohydrates	%	2.1	2.3	2.7	2.7	2.8	1.7	1.1	3.0	2.0	2.4	Calculation (by difference)
Sugars[(a)]	%	< 0.2*	< 0.2*	< 0.2*	< 0.2*	< 0.2*	< 0.2*	< 0.2*	< 0.2*	< 0.2*	< 0.2*	IC-PAD (internal)
Dietary fibre	%	1.8	0.6	2.6	2.3	2.3	1.3	1.3	1.8	0.9	1.7	Adapted from AOAC method 985.29, Enzymatic-gravimetry
Ash	%	1.56	1.51	1.47	1.25	1.22	1.09	1.34	1.28	1.01	1.02	Gravimetry (internal)
Fat	%	< 0.3*	0.3	< 0.3*	< 0.3*	< 0.3*	0.4	0.5	< 0.3*	< 0.3*	< 0.3*	Gravimetry, microwave method (internal)

IC-PAD: ion chromatography with pulsed amperometric detection; AOAC: Association of Official Analytical Collaboration; FW: fresh weight.

*: Limit of quantification.

(a): Sugars: sum of monosaccharides (i.e. fructose, glucose) and disaccharides (i.e. sucrose, lactose, maltose).

The variability of minerals, trace elements, heavy metals and vitamins among the ten batches of the NF is presented in Table 2.

Table 2: Concentration of minerals, trace elements, heavy metals and vitamins in ten batches of the NF (expressed on a FW basis)

Parameter	Unit	*Lemna minor* batches					*Lemna gibba* batches					Method of analysis
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Minerals, trace elements and heavy metals												
Calcium	mg/kg	4,100	3,300	3,000	2,300	1,700	2,400	2,900	2,000	2,300	1,600	According to NEN-EN 13805 & 17294-2, ICP-MS
Potassium	mg/kg	1,500	1,700	2,300	2,900	2,300	3,200	2,100	2,200	2,500	2,600	According to NEN-EN 13805 & 17294-2, ICP-MS
Sodium	mg/kg	34	56	62	60	60	97	97	64	71	54	According to NEN-EN 13805 & 17294-2, ICP-MS
Magnesium	mg/kg	280	270	270	240	230	240	270	260	300	260	According to NEN-EN 13805 & 17294-2, ICP-MS
Phosphorus	mg/kg	3,000	2,300	2,200	1,900	1,700	2,000	2,200	1,700	2,000	1,600	According to NEN-EN 13805 & 17294-2, ICP-MS
Copper	mg/kg	2.4	2.2	1.7	1.5	1.3	1.3	1.3	2.6	3.6	2.3	According to NEN-EN 13805 & 17294-2, ICP-MS
Iron	mg/kg	53	46	34	29	20	30	21	22	26	25	According to NEN-EN 13805 & 17294-2, ICP-MS
Manganese	mg/kg	45	32	33	17	25	27	25	29	33	22	According to NEN-EN 13805 & 17294-2, ICP-MS
Molybdenum	mg/kg	1.5	1.2	0.97	0.77	0.51	0.67	0.39	0.56	0.61	0.65	According to NEN-EN 13805 & 17294-2, ICP-MS
Selenium	mg/kg	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	According to NEN-EN 13805 & 17294-2, ICP-MS
Zinc	mg/kg	24	16	17	9.7	12	15	10	13	15	8.7	According to NEN-EN 13805 & 17294-2, ICP-MS
Arsenic	mg/kg	< 0.02*	< 0.02*	< 0.02*	< 0.02*	< 0.02*	< 0.02*	< 0.02*	< 0.02*	< 0.02*	< 0.02*	According to NEN-EN 13805 & 17294-2, ICP-MS
Boron	mg/kg	23	20	20	11	16	11	24	18	21	15	According to NEN-EN 13805 & 17294-2, ICP-MS
Cadmium	mg/kg	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	According to NEN-EN 13805 & 17294-2, ICP-MS
Lead	mg/kg	0.030	0.020	0.013	< 0.01*	< 0.01*	0.012	< 0.01*	< 0.01*	< 0.01*	< 0.01*	According to NEN-EN 13805 & 17294-2, ICP-MS
Mercury	mg/kg	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	According to NEN-EN 13805 & 17294-2, ICP-MS
Vitamins												
beta-Carotene	mg/kg	23.8[(a)]	17.9[(a)]	23.7[(a)]	24.2[(a)]	20.7[(a)]	27.6	29.4	23.6	14.5	17.2	EN 12823-2:2000, LC-DAD

Parameter	Unit	*Lemna minor* batches					*Lemna gibba* batches					Method of analysis
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Retinol	IU/kg	< 700*	< 700*	< 700*	< 700*	< 700*	< 700*	< 700*	< 700*	< 700*	< 700*	EN 12823-12014, HPLC-UV/DAD
Thiamine	mg/kg	< 0.15*	< 0.15*	< 0.15*	< 0.15*	< 0.15*	< 0.15*	< 0.15*	< 0.15*	< 0.15*	< 0.15*	EN 14122-2014 mod., LC-FLD
Riboflavin	mg/kg	0.473	0.629	0.832	0.754	0.710	1.24	0.505	0.542	0.685	1.11	EN 14152:2014 mod., LC-FLD
Niacin	mg/kg	9.54	11.1	6.77	6.21	5.88	6.53	7.05	7.14	5.95	5.87	EN 15652:2009 mod., LC-FLD
Pantothenic acid	mg/kg	0.848	0.802	1.05	1.06	1.17	1.27	1.26	1.17	1.40	1.47	AOAC 2012.16, LC-MS/MS
Pyridoxine	mg/kg	0.582	0.639	0.882	0.956	0.789	< 0.1*	0.924	0.886	0.888	0.951	EN 14164:2014, LC-FLD
Biotin	μg/kg	89.7	105.0	106.0	107.0	99.9	100.0	92.7	109.0	89.3	101.0	Analog. to FDA method LST AB 266.1,1995, Nephelometry
Folate	μg/kg	276	283	338	364	314	NA	233	NA	255	253	NMKL 111:1985, Nephelometry
Cyanocobalamin	μg/kg	< 2.5*	3.18	16.3	3.9	< 2.5*	< 2.5*	< 2.5*	4.39	< 2.5*	3.56	J. AOAC 2008, vol 91 No 4, LC-UV/DAD
Ascorbic acid	mg/kg	6.39	16.1	< 5*	< 5*	< 5*	< 5*	< 5*	14.6	< 5*	< 5*	Food Chemistry, 94 (2006) 626–631, RP HPLC-UV
alpha-Tocopherol	mg/kg	13.7	17.5	11.8	9.4	13.2	7.5	18.0	13.8	8.9	7.8	EN 12822:2014, HPLC-FLD
Phylloquinone	μg/kg	390	340	327	346	282	291	281	216	323	452	EN 14148:2003 mod., RP HPLC-FLD

NEN: Nederlandse Norm; EN: Europese Norm; ICP-MS: inductively coupled plasma mass spectrometry; LC-DAD: liquid chromatography with diode array detection; HPLC-UV/DAD: high-performance liquid chromatography with ultraviolet–visible light/diode array detection; LC-FLD: liquid chromatography with fluorescence detection; AOAC: Association of Official Analytical Collaboration; LC–MS/MS: liquid chromatography tandem mass spectrometry; FDA: Food and Drug Administration; LST: Device Listing Number; NMKL: Nordic Committee on Food Analysis; LC-UV/DAD: liquid chromatography with ultraviolet–visible/diode array detection; RP HPLC-UV: reversed-phase high-performance liquid chromatography coupled with ultraviolet–visible light detector; (RP) HPLC-FLD: (reversed-phase) high-performance liquid chromatography with fluorescence detection; IU: international units; NA: not available.; FW: fresh weight.

*: Limit of quantification.

(a): These batches of the NF were reanalysed. The reported beta-carotene values are taken from the reanalysed batches.

In response to a request from EFSA to lower the concentrations and eventually specifications initially proposed for trace elements in the NF, the applicant remeasured the concentration of boron, molybdenum and manganese in the same ten batches of the NF. In addition, the applicant provided analytical data for chromium. All analyses were carried out on freeze-dried samples (different sample preparation protocol with respect to the first analysis) and the concentrations of boron, molybdenum, manganese and chromium were calculated on FW basis (Table 3). The Panel noted that results from reanalysis were considerably different from those of the initial analysis.

Table 3: Compositional data for boron, molybdenum, chromium and manganese in ten batches of the NF (expressed on a FW basis)

Parameter	Unit	*Lemna minor* batches[(a)]					*Lemna gibba* batches[(a)]					Method of analysis
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Boron	mg/kg	6	8	14	8	9	13	10	12	12	9	According to NEN-EN 13805 & 17294-2, ICP-MS
Molybdenum	mg/kg	0.15	0.44	0.71	0.45	0.49	0.38	0.36	0.32	0.50	0.37	According to NEN-EN 13805 & 17294-2, ICP-MS
Chromium	mg/kg	< 0.08*	< 0.08*	< 0.08*	< 0.08*	< 0.08*	< 0.08*	< 0.08*	< 0.08*	< 0.08*	< 0.08*	According to NEN-EN 13805 & 17294-2, ICP-MS
Manganese	mg/kg	4.8	15.3	20.7	9.8	15.8	16.9	10.9	15.3	15.3	14.2	According to NEN-EN 13805 & 17294-2, ICP-MS

NEN: Nederlandse Norm; EN: Europese Norm; ICP-MS: inductively coupled plasma mass spectrometry.
*: Limit of quantification.
(a): Analyses were carried out on freeze-dried samples and results were calculated on FW basis.

Following a request from EFSA, the applicant quantified the amino acids in the NF, according to ISO 13903:2005 and/or Commission Regulation (EC) No 152/2009[3] (Appendix A).

Moreover, the applicant investigated by gas chromatography coupled with a flame ionisation detector (GC-FID) the fatty acid profile in the ten batches of the NF. Polyunsaturated fatty acids were found to be on average ~ 0.25 g/100 g NF, saturated fatty acids ~ 0.08 g/100 g NF and monounsaturated fatty acids ~ 0.02 g/100 g NF. The average *trans*-fatty acid content in the NF was below the limit of quantification (LOQ < 0.05 g/100 g). The principal fatty acid found in the NF was alpha-linolenic acid (C18:3, n3), followed by palmitic acid (C16:0) and linoleic acid (C18:2, n6).

Additionally, the applicant investigated the presence of antinutritional factors (i.e. oxalates and phytic acid) in the NF. Oxalates were found to range between 0.07 and 0.11 g/100 g expressed as oxalic acid and 0.10 and 0.16 g/100 g expressed as calcium oxalate and phytic acid was below the LOQ (< 0.14%) in the ten batches of the NF.

Gilbert et al. (2018) reported the presence of endophytic bacteria and related metabolites in water lentils, including toxic indole alkaloids (i.e. indole, indole-3-acetic acid, indole-3-lactic acid). Therefore, the applicant investigated the occurrence of indole-3-acetic acid (IAA) in the ten batches of the NF by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The applicant also tested blanched and frozen commercially available broccoli and spinach as reference material. Results indicated that IAA levels in the NF ranged between 58 and 463 μg/kg dry weight DW (3.4–23.1 μg/kg FW), and for spinach and broccoli IAA was found at 57 and 165 μg/kg DW, respectively. IAA levels in the NF were found below the maximum residue level of 100 μg/kg FW set for IAA in certain foodstuffs by Regulation (EU) 2016/71[4]. According to the applicant, pesticides or other treatments that may promote plant growth were not applied during the cultivation of *Lemna* plants, therefore, the presence of IAA in the NF was due only to the endogenous production of the plant and possibly to endophytic bacteria that may colonise the plant tissue or have symbiotic interactions with the plant.

[3] Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. OJ L 54, 26.2.2009, pp. 1–130.

[4] Commission Regulation (EU) 2016/71 of 26 January 2016 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 1-methylcyclopropene, flonicamid, flutriafol, indolylacetic acid, indolylbutyric acid, pethoxamid, pirimicarb, prothioconazole and teflubenzuron in or on certain products. OJ L 20, 27.1.2016, pp. 1–47.

Nitrate and nitrite were also analysed in the ten batches of the NF. Nitrate concentration ranged between 293.1 and 512.5 mg/kg, while nitrite concentration ranged between 0.5 and 1.5 mg/kg.

Accumulation of toxins from cyanobacteria in water lentils may represent a risk for the food chain and consequently to human health. In particular, microcystins have been reported to accumulate in *Lemna* species (Mitrovic et al., 2005; Saqrane et al., 2007). The applicant provided analytical data on the levels of microcystins, nodularins, anatoxin-a and saxitoxins in the fresh *Lemna* plant material. None of the above toxins were detected in the tested samples.

Pesticide residues (i.e. organonitrogen, organochlorine and organophosphorus pesticides) were also analysed in the NF by gas chromatography according to the DFG S-19 method. No pesticide residues were detected in the ten batches of the NF.

The applicant also provided data for dioxins, dioxin-like polychlorinated biphenyls (DL-PCBs) and non-dioxin-like polychlorinated biphenyls (NDL-PCBs) in fresh *Lemna* plant material. Dioxins were found to be on average 0.156 pg TEQ/g, DL-PCBs 0.065 pg TEQ/g and NDL-PCBs 3 ng/g (upper bound).

Microbiological data were also reported by the applicant. The total aerobic microbial count (TAMC) was measured in ten batches of the NF in concentrations within the specification limits initially proposed by the applicant (TAMC: m = 10^4 CFU/g, M = 10^5 CFU/g), except for B#10 (Table 4). Enterobacteriaceae were also found in B#10 higher than other NF batches. The applicant noted that this sample became contaminated during the shipment to the laboratory of analysis and therefore reanalysed B#10 in triplicate (#10a, #10b, #10c) for TAMC and Enterobacteriaceae (Table 5).

Table 4: Microbiological parameters in ten batches of the NF

Parameter	Unit	*Lemna minor* batches					*Lemna gibba* batches					Method of analysis
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
TAMC	CFU/g	10	30	250	510	580	220	270	2,100	200	> 300,000	AFNOR
Bacillus cereus	CFU/g	< 10*	< 10*	< 10*	< 10*	10	< 10*	< 10*	< 10*	< 10*	< 10*	ISO 7932
Clostridium perfringens	CFU/g	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	ISO 7937
Coagulase-positive staphylococci	CFU/g	< 100*	< 100*	< 100*	< 100*	< 100*	< 100*	< 100*	< 100*	< 100*	< 100*	AFNOR 3M 01/09-04/03 A mod.
Escherichia coli	CFU/g	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	AFNOR 3M 01/08-06/01 mod.
Enterobacteriaceae	CFU/g	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	100	AFNOR 3M
Moulds	CFU/g	< 10*	< 10*	< 10*	< 10*	< 10*	10	< 10*	< 10*	< 10*	< 10*	AFNOR
Yeasts	CFU/g	< 10*	< 10*	< 10*	< 10*	< 10*	10	< 10*	< 10*	10	< 10*	AFNOR
Listeria monocytogenes	per 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	AFNOR EGS 38/05-03/17 mod.
Salmonella	per 25 g	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	AFNOR EGS 38/01-03/15 mod.

TAMC: Total aerobic microbial count; CFU: colony forming units; AFNOR: French Standardization Association (Association Française de Normalisation); ISO: International Organization for Standardization; ND: Not detected; EGS: external guide sequence.
*: Limit of quantification.

Table 5: Reanalysis of B#10 in triplicate for aerobic plant count and Enterobacteriaceae

Parameter	Unit	*L. gibba* batches			Method of analysis
		#10a	#10b	#10c	
TAMC	CFU/g	370	160	240	AFNOR
Enterobacteriaceae	CFU/g	< 10*	< 10*	< 10*	AFNOR 3M

TAMC: Total aerobic microbial count; CFU: colony forming units; AFNOR: French Standardization Association (Association Française de Normalisation).
*: Limit of quantification.

The Panel considers that the information provided on the composition is sufficient for characterising the NF.

3.4.1. Stability

Upon a request from EFSA, the applicant indicated a shelf life up to 2 years for the NF.

The applicant provided analyses on the stability of the NF measuring cold tolerant microbial count upon heat treatments (blanching or steaming) at different temperatures and times and storing of the NF at −20°C up to 12 months. The Panel considers that the data provided are not relevant with respect to the stability of the NF because at −20°C microbiological growth is not expected. The applicant also provided stability data for food matrices where the NF is intended to be used as ingredient, such as soups and ready-to-use meals. However, the microbiological parameters tested in both experiments were not specified.

Based on the data provided, the Panel could not conclude on the shelf life of the NF.

3.5. Specifications

The specifications of the NF are indicated in Table 6.

Table 6: Specifications of the NF

Description: *Lemna minor* and *Lemna gibba* heat treated plant material consists of small green leaves with some minor white roots	
Source: *Lemna minor* and *Lemna gibba*	
Parameter	**Specification**
Moisture	91–95 g/100 g
Protein (N × 6.25)	1–4 g/100 g
Carbohydrates	1–3 g/100 g
Dietary fibre	0.5–3 g/100 g
Ash	1–2 g/100 g
Fat	0.2–0.6 g/100 g
Oxalates (as calcium oxalate)	< 1.6 g/kg
Beta-carotene	< 3,160 μg/100 g
Folate	< 38 μg/100 g
Phylloquinone	< 46 μg/100 g
Copper	< 2.5 mg/kg
Iron	< 53 mg/kg
Manganese	< 18 mg/kg
Molybdenum	< 0.5 mg/kg
Zinc	< 20 mg/kg
Chromium	< 1 mg/kg
Boron	< 15 mg/kg
Contaminants	
Nitrate	< 520 mg/kg
Nitrite	< 1.75 mg/kg
Lead	< 0.3 mg/kg
Cadmium	< 0.2 mg/kg
Mercury	< 0.05 mg/kg
Arsenic	< 0.05 mg/kg
Microcystins	< 23 μg/kg
Nodularins	< 7 μg/kg
IAA	< 0.1 mg/kg
Microbiological parameters	
TAMC	< 5,000 CFU/g
Escherichia coli	< 100 CFU/g

Bacillus cereus	< 100 CFU/g
Coagulase-positive staphylococci	ND in 10 g
Listeria monocytogenes	ND in 25 g
Salmonella spp.	ND in 25 g
TYMC	< 100 CFU/g

IAA: indole-3-acetic acid; CFU: colony forming unit; TAMC: total aerobic microbial count; TYMC: total yeast and mould count; ND: not detected.

The Panel noted that for copper and zinc few batches of the NF were above specification limits (B#8 and B#9 for copper and B#1 for zinc, Table 2), whereas for manganese and molybdenum several batches were above specification limits in the first set of results (Table 2) and one batch after reanalysis (B#3, Table 3). Additionally, boron was also found above specification limits in several batches of the NF in the first set of results (Table 2).

The applicant proposed a specification limit for TAMC as 'm = 10^4, M = 10^5 CFU/g'. The Panel notes that considering the NF production process (thermal processing) and compositional analyses of ten batches (Tables 4 and 5) a lower specification limit could be met. The Panel notes that TAMC and total yeast and mould count (TYMC) are indicators of hygiene and considers that these criteria ultimately also contribute to the safety of the NF and should be both included in specifications.

Additionally, the applicant proposed limits for *Bacillus cereus* and coagulase-positive staphylococci as 'm = 10^2, M = 10^3 CFU/g'. The Panel notes that both microorganisms are bacterial pathogens. Moreover, *Bacillus cereus* is a sporulated microorganism that can survive heat treatment and produce toxins. The Panel also notes that the values for these criteria in the compositional analysis are well below these proposed limits. Therefore, the Panel considers that these criteria should be lowered.

The Panel considers that the information provided on the specifications of the NF is sufficient (but see Section 3.9 nutritional information).

3.6. History of use of the source

Lemna minor and *Lemna gibba* have been recognised as a toxicological model in environmental research due to their fast-growing capacity and wide distribution (Wang, 1990). In addition, several studies available in the literature report the use of *Lemna* species for wastewater remediation, due to their capacity to accumulate heavy metals and metalloids from polluted wastewaters (Zayed et al., 1998; Hegazy et al., 2009; Megateli et al., 2009).

Besides their applications in the environmental field, water lentils are used widely as animal feed and specifically as feed for fish, poultry, ducks and pigs (Haustein et al., 1992; Leng, 1999; Ngamsaeng et al., 2004). The consumption of water lentils (*Wolffia* species) as food was reported in Southeast Asia in 1971. The article states that water lentils, known by the common name 'khai-nam' in Thailand, have been consumed for many generations in Myanmar, Laos and in Northern Thailand (Bhanthumnavin and McGarry, 1971). In particular, *Wolffia globosa* (cited as *W. arrhiza* initially by Bhanthumnavin and McGarry, 1971) has been traditionally consumed as a source of inexpensive protein in Southeast Asia. *Lemna gibba* is reported in the Germplasm Resources Information Network (GRIN) database as edible vegetable for humans (USDA, 2022). Other databases classify *Lemna* and *Wolffia* species as edible plants based on book citations (e.g. Plants for future (pfaf.org), Useful Tropical Plants (tropical.theferns.info)).

3.7. Proposed uses and use levels and anticipated intake

3.7.1. Target population

The target population proposed by the applicant is the general population.

3.7.2. Proposed uses and use levels

The NF is proposed by the applicant to be used as a vegetable, similar to other leafy vegetables currently available on the European market, e.g. spinach, lettuce, endive, kale. The applicant noted that the NF is expected to be sold as heat treated leafy vegetable with a broad application in a variety of food categories. The applicant indicated that the NF can be consumed as such or cooked or added in ready-to-eat products.

3.7.3. Anticipated intake of the NF

The possible future consumption of the NF is unknown. However, if used as spinach, as suggested by the applicant, the estimated chronic consumption at the highest 95th percentile would be 113 g/day for adults, 72 g/day for adolescents, 43 g/day for other children, 52 g/day for toddlers and 23 g/day for infants according to the EFSA Comprehensive European Food Consumption Database be (Level 4 – spinaches and similar) (EFSA, 2011).

3.8. Absorption, distribution, metabolism and excretion (ADME)

ADME data from Zeinstra et al., 2019 are considered and reported under the section 3.10.1 Human data.

The applicant notes that the NF consists of protein, fat, fibre and micronutrients (vitamins and minerals). The metabolic fate of the NF, consisted of common components of the diet, is expected to be similar to that of other leafy vegetables.

3.9. Nutritional information

The applicant provided information on nutritional parameters of the NF. Compositional data on macronutrient and micronutrient content in ten independently produced batches of the NF were presented in Tables 1–3, Section 3.4. In brief, the NF consists of water (92%–94%), proteins (2%–4%), dietary fibre (0.6%–2.6%) and ash (1.0%–1.6%).

The amino acid profile of the NF is presented in Appendix A. All individual amino acids in the NF (average protein content 3%) were found in similar concentrations as compared to those reported in cooked spinach (average protein content 5%) when normalised to the protein content (Lisiewska et al., 2011), except for histidine, which is slightly lower.

Fatty acids were found in concentrations below 0.5% in the NF and their profile was investigated in the same ten batches as presented in Section 3.4.

The micronutrient content in the ten batches of the NF is presented in Tables 2 and 3, Section 3.4. Water lentils are known to accumulate trace elements, minerals and heavy metals from the aquatic environment, and thus their concentration in the NF depends on the composition and quantity of fertiliser used.

The concentration of manganese (Mn) in the NF ranges between 88 and 380 mg/kg in freeze-dried samples, which corresponds to approximately 5–21 mg/kg FW (results after reanalysis of samples, Table 3). A first analysis of the NF showed a Mn content between 17 and 45 mg/kg FW (Table 2). The concentration of Mn in leafy vegetables was reported in amounts around 2 mg/kg (green vegetables 2 mg/kg, Rose et al., 2010; vegetables excluding potatoes 1.34 mg/kg, Anses, 2011; vegetables 2.18 mg/kg, González et al., 2021). Among leafy vegetables, spinach was found to have the highest concentration of Mn, reported below 9 mg/kg in fresh weight [i.e. in a range between 2–8 mg/kg in mature spinach and 4–7 mg/kg in baby spinach (US Department of Agriculture[5]); 9 mg/kg in spinach, (Fordham and Hadley, 2003) and in a range between 1 and 6 mg/kg, (Holland et al., 1991)]. The Panel notes that the SCF (2000) reported that exposure to high levels of Mn by inhalation or oral intake of Mn may be neurotoxic. The SCF could, however, not set an UL for Mn and concluded that 'the margin between oral effect levels in humans as well as experimental animals and the estimated intake from food is very low. Given the findings on neurotoxicity and the potential higher susceptibility of some subgroups in the general population, oral exposure to Mn beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit' (SCF/NDA, 2006).

In consideration of the proposed uses of the NF, and the fact that the NF contains higher amounts of Mn compared to the concentration normally present in other leafy vegetables, the Panel considers that the addition or the substitution by this NF for other leafy vegetables may be of safety concern.

Antinutrients were also analysed in ten batches of the NF. Phytic acid was reported in all analysed batches below the limit of quantification (< 0.14%). Oxalic acid was found to range between 0.07 and 0.11 g/100 g expressed as oxalic acid and 0.10 and 0.16 g/100 g expressed as calcium oxalate. Oxalic acid in other commonly consumed leafy vegetables has been reported in higher amounts, e.g. spinach (0.7 g/100 g), rhubarb (0.8 g/100 g) and Brussels sprouts (1.5 g/100 g) (Duke, 1992).

The Panel considers that, with the exception of concerns related to the Mn intake, taking into account the composition of the NF and the proposed conditions of use, the consumption of the NF is not nutritionally disadvantageous.

[5] https://fdc.nal.usda.gov/fdc-app.html#/food-search.

3.10. Toxicological information

Water lentils are known to accumulate trace elements and heavy metals from the environment and, based on this attribute, are considered as a suitable plant for phytoremediation (Landesman et al., 2010). Therefore, in cases of uncontrolled cultivation conditions, and particularly when fertilisers, pesticides and other organic contaminants are present in high amounts in cultivation sites or in cases of algal/microbial contamination of the water, high concentration of contaminants or toxins in those plants may pose a potential risk for human health (Leng, 1999). However, the NF presented in this application was cultivated under controlled conditions (see Section 3.3).

The Panel notes that no toxicological studies with the NF were provided. Instead, the applicant referred to toxicological studies performed with powder from water lentils (conducted by Parabel, GRN No. 742, 2018). An oral 90-day toxicity study was performed in Wistar rats using a water lentil powder product produced from *L. minor* and *W. globosa*. The composition of the tested material was different from the NF, containing 47% proteins, 36% dietary fibre, 10% fat and 2% moisture. The study was conducted with 100 (50 male and 50 female) animals distributed randomly into six groups. Four groups of 20 animals, each received via gavage 0, 100, 500 and 1,000 mg/kg body weight (bw) of the water lentil powder product and two groups of 10 animals each received 0 or 1,000 mg/kg bw per day for an additional 28 days. The authors noted no treatment-related toxic signs or mortality were observed. In connection to this specific study, EFSA has previously noted that none of the parameters were changed in the course of the study in the control and treated animals, which is unusual, because often several findings are observed in toxicological studies in treated animals as well as in controls that are considered as incidental after thorough evaluation (EFSA NDA Panel, 2021a).

However, taking into account the nature of the NF and the history of use, the Panel considers that no toxicological studies are required on the NF.

3.10.1. Human data

The applicant performed and presented two studies involving human subjects and using *Lemna minor*, one of the water lentil species used as the NF. The applicant performed a randomised, cross-over trial with 12 healthy subjects. Two protein-based meals, containing either *Lemna minor* or green pea equivalent to 20 g of protein were used for this study (*Lemna minor* and green peas were freeze-dried, mixed with other ingredients, cooked for 10 min at 100°C and then served as meals). Subjects received protein-based meals after an overnight fast in randomised order with a washout period of 1 week. Blood samples were collected at baseline and 15, 30, 45, 60, 75, 90, 120, 150 and 180 min after consumption and measurements of blood amino acids, glucose and insulin levels were performed. Moreover, heart rate, blood pressure and aural temperature were measured before and after consumption and subjects were asked to report on gastro-intestinal discomfort for four subsequent days. A slower release of circulating amino acids was observed for subjects who consumed *L. minor* meal compared to green pea meal. Plasma glucose and insulin were lower post consumption of *L. minor* in comparison to green pea. For the rest, health parameters assessed in this study as well as gastro-intestinal complaints, no differences were observed between subjects that consumed freeze dried and cooked *L. minor* and green pea (Zeinstra et al., 2019).

In a randomised controlled parallel trial, 24 healthy subjects consumed *Lemna minor* plant material or spinach as vegetable in a warm meal for 11 consecutive days. The intervention meals were prepared with 170 g fresh weight of spinach or *Lemna minor* added to basic meal ingredients, following different recipes each day. Fresh vegetables were washed with running tap water and frozen at −20°C until further use. Frozen products were heated in a steam oven (100°C) for 20 min and added to the basic meal ingredients that were equal for both intervention groups. Gastrointestinal complaints were recorded daily, while blood and urine samples were collected at the beginning and end of the study. Analysis of intestinal symptoms indicated some undesirable effect of *Lemna* intake on flatulence and constipation in comparison with spinach intake. Biomarkers for blood, kidney, liver, cardiovascular, inflammation and iron metabolism did not reveal any significant difference between the groups before and after the intervention. In this study, an increase in urinary oxalic acid (increase of 0.13 ± 0.17 mmol/L for *Lemna* and 0.08 ± 0.14 mmol/L for spinach) was observed post consumption of intervention meals, which was not different between the *Lemna* and the spinach group (Mes et al., 2022).

In addition, the applicant referred to human studies performed with another species belonging to water lentils, namely *Wolffia globosa*. In a human study, 294 subjects with abdominal obesity/dyslipidaemia were divided into three groups. One group consumed 100 g of *Wolffia globosa* plant daily for 6 months. The study was designed to examine systemic changes in iron status indicators concentrations (Yaskolka Meir et al., 2019). Another randomised controlled trial was carried out to assess protein bioavailability of *Wolffia globosa* powder in 36 men. Subjects were divided into three groups and received protein-based test meals of 30 g (soft cheese, green peas, *Wolffia globosa* powder). Circulating amino acids were measured at 0, 30, 90 and 180 min post-consumption (Kaplan et al., 2019). Moreover, the applicant noted that a randomised controlled crossover trial with 20 abdominally obese participants was performed to assess post-prandial glycaemic response after consumption of *Wolffia globosa*. In this study, participants received three meals of *Wolffia globosa* (75 g each) or low-fat yogurt for 3 days each. Glucose levels were monitored for 3 weeks (Zelicha et al., 2019). No adverse effects were reported in these studies.

The Panel notes that the human studies provided by the applicant were primarily designed to investigate putative beneficial effects and addressed only a limited number of safety-relevant endpoints. The Panel considers that no adverse events related to the consumption of the NF were reported. The Panel, however, notes that no conclusions can be drawn from these studies on the safety of the NF.

3.11. Allergenicity

The applicant noted that neither allergenicity nor cross-reactivity is described in the literature for *Lemna minor* and *Lemna gibba*.

According to the applicant, the protein content in the NF can range between 1% and 4% and the soluble proteins in the leaves of *Lemna* consist primarily of rubisco, a common protein found in commonly consumed vegetables, such as spinach and tomatoes (Rooijakkers, 2016).

The applicant provided *in silico* data (genomic and proteomic data). Homologies were found with the allergenic protein Api g 3 and with the putative allergenic proteins Cor a 10, Tri a 31 and Tri a 34. These proteins are found in allergenic foods such as celery (Api g 3), hazelnut (Cor a 10) and wheat (Tri a 31 and Tri a 34). *In silico* data were confirmed by proteomic analysis of the NF, which was performed by LC–MS/MS and publicly available databases.

The Panel noted that Api g 3 is identified as a food allergen in several databases, including WHO/IUIS database, while Cor a 10, Tri a 31 and Tri a 34 are reported as airway allergens. In addition, celery is reported as a food allergen in the Annex II of the Regulation (EU) No 1169/2011[6]. Therefore, the applicant assessed the potential cross-reactivity of the NF with wheat, celery and hazelnut by Dot Blot immunoassays. Analysis results showed that there is no cross-reactivity of the NF with the above allergenic foods.

The Panel considers the risk that the NF may trigger allergic reactions in humans is expected to be similar to other leafy vegetables and therefore low.

4. Discussion

The NF, which is the subject of the application, is *Lemna minor* and *Lemna gibba* heat treated plant material. The NF is proposed to be used as a vegetable. The target population is the general population.

The NF is produced by cultivation of *Lemna minor* and *Lemna gibba* plants, washing of plant material with water and heat treatment. The NF consists of water, protein, fibre, ash and micronutrients. The Panel notes that the concentrations of trace elements, as well as contaminants such as heavy metals, cyanobacteria toxins and microbiological parameters, in the NF highly depend on cultivation conditions of the plants and the composition of the fertiliser used.

Under the proposed uses, anticipated intake and compositional data, the intake of heavy metals, microcystins and micronutrients, except for Mn, does not raise safety concerns. For Mn no UL has been established in the EU.

[6] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304, 22.11.2011, p. 18–63.

The SCF/NDA 2006 stated that 'oral exposure to manganese beyond the normally present in food and beverages could represent a risk of adverse health effects without evidence of any health benefit'. The NF contains higher amounts of Mn as compared to the normally present concentration of Mn in other leafy vegetables, which may be of safety concern. Therefore, the Panel cannot conclude on the safety of the NF. The Panel notes that an assessment of an UL for Mn is ongoing (mandate No M-2021-00058).

5. Conclusions

The Panel concludes that the safety of the NF, *Lemna minor* and *Lemna gibba* whole plant material, cannot currently be established.

6. Steps taken by EFSA

1) On 27/10/2020 EFSA received a letter from the European Commission with the request for a Scientific opinion on the safety of *Lemna minor* and *Lemna gibba* whole plant material. Ref. Ares(2020)5981659 letter.
2) On 27/10/2020, a valid application on *Lemna minor* and *Lemna gibba* whole plant material, which was submitted by Wageningen Plant Research, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2020/1757) and the scientific evaluation procedure was initiated.
3) On 15/02/2021, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4) On 05/12/21, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
5) On 06/01/21, 18/08/2021, 28/09/2021, EFSA requested the applicant to provide clarifications on the information provided.
6) On 30/07/2021, 09/07/21, 30/11/2021, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7) On 19/01/2022, following a request from the applicant for providing additional information, EFSA opened the requested sections on the Commission e-submission portal and the scientific evaluation was suspended.
8) On 22/04/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
9) On 13/05/2022, EFSA requested the applicant to provide clarifications on the information proactively provided.
10) On 20/06/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
11) During its meeting on 28/09/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of *Lemna minor* and *Lemna gibba* whole plant material as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

(RP) HPLC-FLD	(reversed phase) high-performance liquid chromatography with fluorescence detection
ADME	absorption, distribution, metabolism and excretion
AFNOR	French Standardization Association (Association Française de Normalisation)
AOAC	Association of Official Analytical Collaboration
bw	body weight
CFU	colony forming unit
DFG	Deutsche Forschungsgemeinschaft
DL-PCBs	dioxin-like polychlorinated biphenyls
DW	dry weight
EGS	External Guide Sequence
EN	Europese Norm
EU	European Union
FDA	Food and Drug Administration
FW	fresh weight
GC-FID	gas chromatography coupled with flame ionisation detector
GRAS	Generally Recognized as Safe
GRIN	Germplasm Resources Information Network
HACCP	Hazard Analysis Critical Control Point
HPLC-UV/DAD	high-performance liquid chromatography with ultraviolet–visible light/diode array detection
IAA	indole-3-acetic acid
IC-PAD	ion chromatography with pulsed amperometric detection
ICP-MS	inductively coupled plasma mass spectrometry
ISO	International Organization for Standardization
IU	International Units
IUIS	International Union of Immunological Societies
LC-DAD	liquid chromatography with diode array detection
LC-FLD	liquid chromatography with fluorescence detection
LC–MS/MS	liquid chromatography tandem mass spectrometry
LC-UV/DAD	liquid chromatography with ultraviolet–visible/diode array detection
LOD	limit of detection
LOQ	limit of quantification
LST	Device Listing Number
NA	not available
ND	not detected
NDA	EFSA panel on Nutrition, Novel Foods and Food Allergens
NDL-PCBs	non-dioxin-like polychlorinated biphenyls
NEN	Nederlandse Norm
NF	novel food
NMKL	Nordic Committee on Food Analysis

pH	potential of hydrogen
RP HPLC-UV	reversed-phase high-performance liquid chromatography coupled with ultraviolet–visible light detector
SCF	Scientific Committee on Food
TAMC	total aerobic microbial count
TEQ	toxic equivalent
TF	traditional food
TYMC	total yeast and mould count
UL	tolerable upper intake level
USDA	United States Department of Agriculture
UV	ultraviolet
WHO/IUIS	World Health Organization/International Union of Immunological Societies

Appendix A – Batch-to-batch analysis of the amino acid profile of the NF

Parameter	Unit	*Lemna minor* batches					*Lemna gibba* batches					Method of analysis
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	
Tryptophan	%	0.0324	0.044	0.0361	0.0394	0.038	0.0459	0.0365	0.0351	0.0411	0.042	EU 152/2009
Cysteine + Cystine	%	0.022	0.030	0.021	0.023	0.020	0.024	0.020	0.024	0.023	0.024	ISO 13903:2005; EU 152/2009 (F)
Methionine	%	0.032	0.048	0.034	0.036	0.032	0.045	0.034	0.037	0.041	0.042	ISO 13903:2005; EU 152/2009 (F)
Aspartic acid	%	0.214	0.280	0.237	0.220	0.238	0.238	0.246	0.245	0.218	0.224	ISO 13903:2005, EU 152/2009 (F)
Threonine	%	0.0826	0.116	0.102	0.0944	0.107	0.108	0.0904	0.0971	0.0979	0.101	ISO 13903:2005, EU 152/2009 (F)
Serine	%	0.0889	0.130	0.110	0.104	0.114	0.115	0.0955	0.107	0.105	0.110	ISO 13903:2005, EU 152/2009 (F)
Glutamic acid	%	0.231	0.340	0.267	0.261	0.276	0.272	0.237	0.302	0.263	0.260	ISO 13903:2005, EU 152/2009 (F)
Proline	%	0.084	0.120	0.101	0.0935	0.107	0.110	0.0913	0.0985	0.0987	0.106	ISO 13903:2005, EU 152/2009 (F)
Glycine	%	0.0993	0.145	0.120	0.114	0.127	0.127	0.107	0.120	0.118	0.125	ISO 13903:2005, EU 152/2009 (F)
Alanine	%	0.109	0.165	0.136	0.13	0.143	0.145	0.122	0.136	0.134	0.138	ISO 13903:2005, EU 152/2009 (F)
Valine	%	0.101	0.147	0.125	0.115	0.132	0.133	0.109	0.123	0.123	0.125	ISO 13903:2005, EU 152/2009 (F)
Isoleucine	%	0.0777	0.113	0.0958	0.0879	0.101	0.102	0.0859	0.0935	0.093	0.095	ISO 13903:2005, EU 152/2009 (F)
Leucine	%	0.150	0.224	0.187	0.174	0.199	0.200	0.168	0.186	0.184	0.191	ISO 13903:2005, EU 152/2009 (F)
Tyrosine	%	0.0672	0.0973	0.0819	0.0783	0.0867	0.0891	0.0755	0.0802	0.0819	0.0835	ISO 13903:2005, EU 152/2009 (F)
Phenylalanine	%	0.0998	0.142	0.118	0.111	0.124	0.127	0.107	0.117	0.118	0.120	ISO 13903:2005, EU 152/2009 (F)
Lysine	%	0.122	0.178	0.150	0.139	0.154	0.158	0.134	0.144	0.146	0.151	ISO 13903:2005, EU 152/2009 (F)
Histidine	%	0.0409	0.0533	0.0459	0.0445	0.0483	0.0502	0.0421	0.0467	0.0477	0.0489	ISO 13903:2005, EU 152/2009 (F)
Arginine	%	0.169	0.204	0.163	0.158	0.176	0.172	0.180	0.187	0.168	0.183	ISO 13903:2005, EU 152/2009 (F)
Ornithine	%	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	ISO 13903:2005, EU 152/2009 (F)
Hydroxyproline	%	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	ISO 13903:2005, EU 152/2009 (F)

EU: European Union; ISO: International Organization for Standardization.
*: Limit of quantification.