ADVISORY COMMITTEE FOR NOVEL FOODS AND PROCESSES

TRADITIONAL FOOD NOTIFICATION RP128- GO WOLFFIA

Issue

- 1. A notification for Go Wolffia (*Wolffia arrhiza* and *Wolffia globose*), a traditional food from a third country, has been received under Regulation (2015/2283) (EU retained law).
- 2. The Committee is asked whether there are safety concerns with the proposed use of this traditional food in the UK market. The information from the Committee will provide the basis for risk management decisions made by the UK.

Background

- 3. On the 21st of January 2021, the FSA received a notification from GreenOxyn Limited for authorisation of Go Wolffia. The applicant intends to market the product as such as fresh produce
- 4. The FSA and FSS has four months to provide reasoned safety objections to the Traditional Foods sale in the UK. If authorised, the authorisation will be open to any company subject to the specification and conditions of use detailed in the dossier. A risk assessment on the safety of this traditional food is requested to inform this process.
- 5. The notification dossier is attached as **Annex A**. Relevant supporting information is attached as **Annex B**. Both contain confidential information.

Identification

- 6. Go Wolffia is a product made from *Wolffia arrhiza* and *Wolffia globose*, green spherical aquatic plants (1mm in size) which grows naturally in quiet water bodies such as ponds, floating on the water's surface. Traditionally, this plant is cultivated in pond systems in local aquatic farms. The applicant classifies the Go Wolffia as food consisting of, or isolated from or produced from plants or their parts.
- 7. The applicant states that the geographical origin of Wolffia is in Asia, including, Myanmar, Laos, and Thailand. Go Wolffia refers to the plant species *Wolffia arrhizal and Wolffia globose*. These are the only two edible varieties in the genus of 11 species. Other common names for the plant are Khai-nam, Kai-Pum and Kai-nhae.

Production Process

- 8. The applicant explains that traditionally *Wolffia* is grown in local aquatic farms, in open pounds, that require intensive daily management, where it is harvested twice a week from November through to July (9-months).
- 9. Due to issues in controlling contamination acting as a barrier to scaling up production using traditional methodology the applicant explains that they intend to produce their product using indoor aquatic vertical farm, designed to provide optimal growth conditions and a controlled environment.
- 10. The production facility is closed and controlled with all inputs sterilised or subject to controls to minimise microbial contamination before use. The applicant explains that the nutrients provided to the plants are tightly controlled and this prevents contamination of the plants with heavy metals.
- 11. The control process in place, quality assurance processes and HACCP procedures are described in detail. See production process section pages 18-21.

Composition

12. The applicant explains that they have analysed samples of the product dried state on advice of the analysis laboratory to gain accurate measurements for some of the parameters. The results have then been adjusted based on moisture content to produce the calculated values for the wet product. It is the wet product that is intended to be consumed by consumers.

Table 1 – Proximate analysis in fresh Go Wolffia

Test	Unit	Sample A	Sample B	Sample C	Sample D	Sample E
Calories (Total)	kcal/100 g	NM	12	11	11	12
Calories from fat	kcal/100 g	NM	1	2	1	1
Moisture	g/100 g	96.665	96.585	96.595	96.520	96.277
Carbohydrates*	g/100 g	NM	0.9	1.0	0.4	0.6
Protein	g/100 g	1.89	1.67	1.46	2.10	2.13
Total fat	g/100 g	0.10	0.15	0.17	0.13	0.14
Saturated fat	g/100 g	0.03	0.03	0.04	0.03	0.03
Polyunsaturated fat	g/100 g	0.06	0.11	0.12	0.09	0.10

Fibre	g/100 g	1.3	1.0	0.9	2.5	2.4
Ash	g/100 g	0.739	0.686	0.766	0.812	0.809

NM- not measured

13. The applicant has considered the nutritional content of Go Wolffia in relation to vitamins and minerals these are presented in Table 2.

Table 2 -Vitamin analysis for 5 batches of fresh Go Wolffia

Nutrient	Unit	Sample A	Sample B	Sample C	Sample D	Sample E
Vitamin B2 (Riboflavin)	mg/100 g	0.080	0.100	0.080	0.090	0.100
Vitamin B6 (Pyridoxine)	mg/100 g	0.032	0.038	0.038	0.034	0.043
Vitamin E (α-tocopherol)	mg/100 g	0.826	0.887	0.444	0.448	0.572
Vitamin K1	μg/100 g	149.2	131.3	141.4	115.0	162.0
Total beta-caroten (RAE)	e µg/100 g	152	185	170	138	192

14. The applicant has analysed the mineral content of Go Wolffia in dry samples to improve accuracy and back calculated the levels in wet samples based on the moisture content. The resulting values are presented below.

Table 3 - A Mineral Analysis of Go Wolffia in Wet Samples

	Mineral content calculated for wet weight (mg/100g)										
Mineral	Sample G	Sample H	Sample I	Sample J	Sample K	Sampl e L	Sample M				
Iron	0.718	0.850	0.792	1.07	0.911	0.905	0.877				
Potassium	403	284	351	402	526	521	527				
Magnesium	10.2	6.67	8.77	6.86	11.6	13.7	13.4				
Manganese	3.06	4.59	4.11	4.57	3.01	3.06	2.97				
Phosphorus	79.6	67.8	79.2	93.68	66.1	68.5	72.0				
Zinc	0.235	0.202	0.238	0.28	1.86	1.20	1.35				
Calcium	34.8	37.3	30.7	33.85	34.1	38.6	45.1				

	Mineral content calculated for wet weight (mg/100g)										
Mineral	Sample G	Sample H	Sample I	Sample J	Sample K	Sampl e L	Sample M				
Selenium	0.0029 5	0.0039 4	0.0048 9	0.0047 4	NM	NM	NM				
Sodium	1.21	1.55	1.34	2.03	5.08	5.08	4.72				
Copper	0.0264	0.0295	0.0309	0.0343	<0.055 6	0.0614	<0.054 1				

NM – not measured

15. The applicant has identified Iodine as a potential component of interest in the safety assessment and this was analysed in 5 batches.

Table 4 - Iodine analysis in Dry Samples of Go Wolffia

Mineral	Unit	BL20	GR20	PK20	RD20	WH20	YL20
Moisture	g/100 g	12.3	12.3	11.3	9.85	11.4	9.02
Iodine (DW)	μg/kg	120	119	120	117	160	167
Iodine (WW)	μg/100g	13.68	13.57	13.53	12.98	18.06	18.36

16. The applicant has identified heavy metal contamination as a parameter of concern based on the findings from the traditional cultivation practices for Wolffia. Therefore, an analysis of 5 samples of the dry product were analysed and the wet values calculated based on the moisture content.

Table 5 - Heavy Metal Analysis Wet Samples of Go Wolffia

Heavy	Heavy metal content calculated for wet weight (µg/100g)										
metal	Sample G	Sample H	Sample I	Sample J	Sample N	Sample O					
Mercury	<0.11	<0.11	<0.11	<0.11	0.043	0.043					
Arsenic	<0.56	<0.56	<0.56	<0.56	<0.054	<0.054					
Cadmium	<0.28	<0.28	<0.28	<0.28	<0.054	<0.054					
Lead	<0.56	<0.56	<0.56	<0.56	0.15	0.42					
Chromium	0.49	0.71	0.72	2.81	0.25	0.23					

17. The applicant identified microbial contamination as a parameter of concern based on experience of traditional cultivation of Wolffia. A microbiological analysis of 7 batches was performed on the product as would be sold.

Table 6 – Analysis of the Microbiological parameters of Go Wolffia

Parameter	Unit	BL30	RD30	YL30	GR30	OR30	WH30	PK30
Coliforms	CFU/g	<10	<10	<10	<10	<10	<10	<10
Yeast	CFU/g	<10	<10	<10	<10	<10	<10	<10
Moulds	CFU/g	<10	<10	<10	<10	<10	<10	<10
Total Count	CFU/g	<10	<10	<10	<10	<10	<10	<10
Salmonella	per 25 g	Neg.						
Escherichia Coli	g	Neg.						
Staphylococcus aureus	CFU/g	<50	<50	<50	<50	<50	<50	<50
Listeria monocytogenes	per 25 g	Neg.						
Enterobacteriacea	CFU/g	<10	<10	<10	<10	<10	<10	<10

- 18. The applicant has identified Oxalic content as a consideration for Go Wolffia. Oxalic acid was measured in one sample and calculated as 0.26% fresh weight, equivalent to 260 mg/100 g dry weight.
- 19. A further 8 samples of Go Wolffia (5 dried samples and 3 fresh samples) were analysed for oxalic content. The analysis was performed using several methods. The results the applicants suggest leads to an average Oxalic content in fresh Go Wolffia of 31 mg/100 g fresh weight (range of 14 50 mg/100 g fresh). The variability between the later samples and the initial sample was not explained.

Table 7 - Oxalic Acid Content in 9 samples of Go Wolffia

Parameter	Sample code								
	AN08	JD09	UK02	CR99	TL104	AM40	OC40	AG50	Р
Sample state	Dry	Dry	Dry	Dry	Dry	Fresh	Fresh	Fresh	Dry
Moisture %	7.97	7.78	6.87	10.6	5.52	-	-	-	7
Oxalic acid content									

Dry weight: mg/100 g	326	325	288	934	996	-	-	-	280
Fresh weight: mg/kg	-	-	-	-	-	400	320	460	-
Calculated fresh weight: mg/100g	16.30	16.27	14.39	46.70	49.80	40.00	32.00	46.00	13.98

Stability

The applicant has undertaken a 28-day stability test of Go Wolffia as would be sold, sealed packed samples stored under chilled conditions for 28 days (refrigerated at 2 - 8°C). The samples were tested for parameters including energy, macronutrients, ash, fatty acids, and vitamins). In general, the applicant considered that all parameters examined remained stable over the 28-day period. They suggested that the results of the microbiological analysis indicate no growth of any bacteria or fungus in the stability test.

Specifications

20. The applicant has identified a specification for Go Wolffia that considers nutritional, microbiological, and heavy metal contaminants levels to be used as controls on the final product.

Table 8 - The proposed specifications:

Parameter	Specification – We weight	tMethods of analysis
Nutrient profile		
Fat, g/100g	0.1 – 0.3 g	AOAC: 996.06
Protein, g/100g	1.0 – 3.0 g	AACC 46-30; AOAC 992.15
Dietary fibre, g/100g	0.5 – 3.0 g	AOAC 991.43
Microbiological		
TPC, cfu/g	<10,000	ISO 4833-1
Yeast/mould, cfu/g	<100	ISO 21527-2
Enterobacteriaceae, cfu/g	<30	ISO 21528-2
Coliforms, cfu/g	<30	ISO 9308
E. coli	Negative/10g	ISO 21528
Salmonella	Negative/10g	ISO 6579
Listeria monocytogenes	Negative/10g	ISO 11290-1
Staphylococcus aureas	Negative/10g	ISO 6888-1
Chemical compounds		
Lead, mg/kg	NMT 0.05	ICP-MS, AOAC: 993.14, 2006.03
Arsenic, mg/kg	NMT 0.05	ICP-MS, AOAC: 993.14, 2006.03
Mercury, mg/kg	NMT 0.05	ICP-MS, EPA 7473
Cadmium, mg/kg	NMT 0.05	ICP-MS, AOAC: 993.14, 2006.03
Chromium, mg/kg	NMT 1	ICP-MS, AOAC: 993.14, 2006.03

History of Continued Use/Traditional Use

- 21. The applicant explains that Wolffia is a plant that has been eaten in parts of Asia (Laos, Thailand, India, Myanmar), that has not spread to the western world. They suggest that is has been studied both as potential alternative protein source as well as a source of nutrition.
- 22. The applicant provides a number of recipes where duckweed is used, mostly as a vegetable component in side dishes as well as combined to main dishes in weight ranges of 50-225g, noting that traditionally it is harvested from open and natural water sources and therefore needs thorough cooking before use.
- 23. The applicant references studies that investigated the role of Wolffia in the western diet, as well as its role as potential protein replacement, demonstrating that the plant was well tolerated and liked by participants. The applicant provides non-clinical data on studied duckweed species and extracts, noting that anti-proliferative, antioxidant, and cytotoxic activities had been investigated, showing favourable biological activities.
- 24. The applicant completed a systematic literature review on the human data that related to the intake of Wolffia. Some of the studies they found, dating back to the 1970s, recognise:
- a. Duckweeds are noted as a potential alternative protein and nutrition source.
 - b. A study found demonstrated that duckweeds do not possess any detectable anti-proliferative or cytotoxic effects on human cell lines.
 - c. That there were no negative effects seen when duckweed was supplement to a low meat diet.
 - d. A study showing that *Wolffia globsa* may serve as an alternative protein source that has potential postprandial glycaemic effects.
 - e. A study comparing against other protein sources, showing 3-hour blood concertation's increased in a range of amino acids like other protein sources (cheese and meat), as well as an increase in vitamin B12.
 - f. A study comparing *L.minor* against peas, there were lower concentration of blood amino acids, indicating less digestibility.
 - g. A paper on consumer attitudes towards duckweed, showing that people like the idea of an alternative, nutritious protein source, but were wary of the potential of duckweed to absorb heavy metals.

- 25. The applicant lists previous authorisations for Go Wolffia including GRAS Status in the USA, for consumption as a dried and wet plant for use as a vegetable component to beverages, frozen dairy mixes, grain products and pastas, plant protein products, snack foods, and soup and soup mixes and bases. When added to food or used as a dietary supplement, it will be added in an amount no higher than to achieve its intended purpose, *i.e.*, no more than 300 g per day wet weight, and 15 g per day dry weight.
- 26. The applicant provides information on a de-greened duckweed powder (DDWP) that was notified as GRAS for use as a source of protein in food at levels (3-20%). As well as recognition for Wolffia in Israel, and Thailand. The applicant also list uses to feed animals, such as fish, cows, pigs, and chickens.
- 27. The applicant notes that a number of food applications are under review by EFSA

Table 9 – Summary of active EFSA applications for Wolffia species

EFSA Q no.	Applicant	Novel food	Proposed uses
EFSA-Q-2019- 00695	Hinoman	Wolffia globosa powder	Unknown (public summary not available)
	Wageningen Plant Research	Lemna minor and Lemna gibba, whole plant material	Use as a fresh or frozen vegetable or as an ingredient (e.g. cooked dishes, smoothies)
EFSA-Q-2019- 00047	ABC Kroos BV	of Lemna gibba and Lemna minor	To be used as a protein rich ingredient with a broad application in a variety of food categories.
EFSA-Q-2018- 00995	Parabel Ltd.		To be used as a multipurpose food ingredient, particularly as a source of dietary protein, fibre, essential fatty acids and energy, and is intended to be used in a variety of food categories

Proposed Conditions of Use

- 28. The target population is the whole population, including children. The intended use is as a fresh vegetable, freshly packed, 200g per day wet weight. It is intended to be added to freshly added dished or to be cooked into dishes.
- 29. The applicant lists no restrictions of use for Go Wolffia. The applicant highlights oxalate as a possible concern in the application but note that it is not present in significant levels in the product.

Nutrition

30. The applicant provides a nutritional analysis of the Go Wolffia. The applicant as part of the history of use section explains the nutritional properties of the plant 20% protein, 44% Carbohydrate and 5% fat, explaining that due to its fast biomass

growth rate Wolffia could be used to produced 60 times the amount of protein per hectare compared to soy with a comparable amino acid profile.

Table 10- Nutritional analysis of Go Wolffia

Nutrient	Unit	Fresh GO Wolffia (wet weight)				
	1	Per 100g	Per 200g	% Reference intake or Nutrient Reference Value (per 200 g)^		
Energy	kcal	11.5	23	1.2		
Protein	g	1.85	3.7	7.4		
Total fat	g	0.14	0.28	0.4		
Saturated fat	g	0.032	0.064	0.32		
Carbohydrate	g	0.73	1.46	0.6		
Calcium	mg	36.4	72.8	9.1		
Iron	mg	0.87	1.74	12.4		
Magnesium	mg	10.2	20.4	5.4		
Zinc	mg	0.76	1.52	15.2		
Potassium	mg	430.6	861.2	43.1		
Phosphorus	mg	75.3	150.6	21.5		
Sodium	mg	3.0	6.0	0.25¥		
Selenium	μg	3.92	7.84	14.3		
Copper	mg	0.036	0.072	7.2		
lodine	μg	15.03	30.06	20.0		
Beta carotene §	μg RAE	167.4	334.8	41.9		
Vitamin B6	mg	0.04	0.08	5.7		
Vitamin E	mg	0.64	1.28	10.7		
Vitamin K	μg	139.78	279.56	372.8		

31. The applicant provides a comparison analysis of their product to those in natural production.

Table 11 – Comparison of traditionally cultivated Wolffia and commercially produced Wolffia

Nutrient	Open ponds (a)	Natural pond (b)	Natural pond (c)		GO Wolffia (e)*	Different natural clones - lab analyses (f)**
Protein	19.8%	33.3%	28.7%	29.61%	35.7%	21.8 - 29.4%
Carbohydrate total	43.6%	N.R.	N.R.	N.R.	36%	N.R.
Fat	5.0 %	5.0 %	2.8%	N.R.	2.0 - 3.4%	~ 1.7 – 4.1 %
Fibre	13.3%	10.7%	13.5%	8.8%	27.1%	26.2 - 29.4%
Ash	18.3%	N.R.	18.2%	16.0%	13.7 – 16.2%	10.5 – 22.9%
Iron (mg/kg)	N.R.	N.R.	N.R.	N.R.	129 – 192	110 – 370
Zinc (mg/kg)	N.R.	N.R.	N.R.	N.R.	36.3 - 334	22.5 – 84.2

Calcium (mg/kg)	N.R.	N.R.	13,700		,	13,400 –
					8,330	25,700
Potassium (mg/kg)	N.R.	N.R.	N.R.	N.R.	51,100 –	42,100 –
					97,300	93,700
Manganese (mg/kg)	N.R.	N.R.	N.R.	N.R.	541 - 827	78.4 - 302
Copper (mg/kg)	N.R.	N.R.	N.R.	N.R.	4.74 – 11.2	2.32 – 4.43
Vitamin E (α- tocopherol) (mg/100g)	N.R.	N.R.	N.R.	N.R.	8.8 - 17.7	3.2-12.8
Total beta carotene (mg/100g)	N.R.	N.R.	N.R.	N.R.	23 - 46	13.4 - 36

N.R. = not reported

References: (a) Bhanthumnavin & McGarry (1971); (b) Ruekaewma, 2011; (c) Sirirustananun, 2018; (d) Chantiratikul *et al.*, 2010; (e) GO Wolffia; (f) Appenroth *et al.*, 2018

Conclusions

The applicant concludes that they demonstrate that the product has a safe history of use, that it used in local Asian cuisines. The applicant suggests they are improving the cultivation process by using an indoor vertical aquatic farm process rather than the traditional open pound growing process.

Committee Action Required

- Members are asked whether there are safety concerns that need to be managed with this traditional food and/or if additional information is required to assess it.
- The Committee's advice will form the basis for the UK's risk assessment for the traditional food and be used to inform the position of the FSA and FSS in developing any reasoned safety objections.

Secretariat May 2021

Annexes

Annex A-GO Wolffia Dossier

Annex B-Annexes for the Dossier (contained as a directory file)

^{*}For some of the composition data for GO Wolffia, these were converted from those based on the wet weight as analysed to dry weight, assuming 95% of water in fresh produce (i.e. fat, ash, vitamin E and beta-carotene) **Values reported for *W. arrhiza* and *W. globosa*