Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on a request for initial assessment for marketing of the dried marine microalgae *Tetraselmis chuii* in food supplements under Regulation (EC) No 258/97 on novel foods and novel food ingredients

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Abstract

The company Fitoplancton Marino S.L. requested authorization to market dried microalgae *Tetraselmis chuii* in food supplements in the European Union. This would be an extension of use of the novel food authorized in March 2014 by a letter from the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) within the scope of Regulation (EC) No 258/1997 on novel foods and novel food ingredients.

The AECOSAN Scientific Committee takes the view that, according to the information provided, there is no indication that consumption of dried microalgae *Tetraselmis chuii* as a food supplement under the conditions proposed by the applicant, can produce adverse effects on health. The Committee concludes that the novel food presented for assessment meets the criteria for acceptance laid down by Regulation (EC) No 258/1997 concerning novel foods and novel food ingredients.

Key words

Microalgae, novel foods, food supplements, *Tetraselmis chuii*.

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1. Assessment of the novel food

Introduction

The company Fitoplancton Marino S.L. requested authorization to market dried microalgae *Tetraselmis chuii* in food supplements in the European Union (under trade name TetraSOD). This would be an extension of use of the novel food authorised in March 2014 by a letter from the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN).

The authorization was based on the initial assessment report issued in September 2013 by the Scientific Committee in which it concluded that the novel food presented for assessment by Fitoplancton Marino S.L. met the criteria for acceptance laid down by Regulation (EC) No 258/97 on novel foods and novel food ingredients (EU, 1997a) (AECOSAN, 2013).

Fitoplancton Marino S.L. was granted authorization to market dried *T. chuii* in the following food categories:

- Sauces, dried *Tetraselmis chuii* content of 20 % (250 mg of dried *Tetraselmis chuii*/day).
- Special salts, dried *Tetraselmis chuii* content of 1 %.
- Condiment, 250 mg/day.

In its new request, the applicant has included dried *Tetraselmis chuii* in Class 2 "complex novel foods obtained from non-GM sources", which includes intact microorganisms used as foods, and in Sub-class 2 "the source of the novel food has no history of food use in the Community". As a result of that classification (2.2) the request file has been dealt with in accordance with Commission Recommendation 97/618/EC, following the guidelines for that category (EU, 1997b).

Comments

The Scientific Committee agrees with the applicant's classification of the product as a food which had no history of consumption in the European Union prior to 1997.

I. Specification of the novel food

Tetraselmis chuii is a single-cell mobile marine microalgae between 10 and 15 µm in size, with an ellipsoidal form produced by longitudinal fission.

The taxonomic classification of microalgae *Tetraselmis chuii* Butcher (1959) is as follows:

- Kingdom, Plantae
- Phylum, Chlorophyta
- Class, Prasinophyceae
- Order, Chlorodendrales
- Family, Chlorodendraceae
- Genus, Tetraselmis
- Species, chuii

As the applicant certifies, the strain to be marketed comes from the collection of cultures of marine microorganisms at the Institute of Marine Science in Andalucía, belonging to the Consejo

Superior de Investigaciones Científicas (ICMAN-CSIC), where it has been kept as a culture since it was acquired from the Culture Collection of Algae and Protozoa (CCAP).

In the request assessed in 2013 the applicant presented studies identifying the strain used, results of the analysis of composition, including amino acids, minerals and fatty acids, and an analysis of heavy metals, pesticides and microbiological parameters of three batches of dried *Tetraselmis chuii* produced industrially by the company Fitoplancton Marino S.L.

The applicant keeps the same specifications (Table 1) and adds a series of parameters presented in table 2 to ensure the greater quality of the finished product.

Table 1. Initial specifications of dried *Tetraselmis chuii*

Parameter	**Specification**
Identity by nuclear marker rDNA 18 S according to the database of the National Center for Biotechnology Information (NCBI)	Not less than 99.9 %
Humidity	Not more than 7 %
Protein	35-40 %
Ash	14-16 %
Carbohydrate	30-32 %
Fibre	2-3 %
Fat	5-8 %
Saturated fatty acids	29-31 % of total fatty acids
Monounsaturated fatty acids	21-24 % of total fatty acids
Polyunsaturated fatty acids	44-49 % of total fatty acids
Iodine	Not more than 15 mg/kg

Tabla 2. Additional specifications of dried *Tetraselmis chuii* and results of the analysis of three batches				
Parameter	**Specification**	**Batch 060813100912**	**Batch 060304051013**	**Batch 120212031012**
Total aerobic count	$<10^3$ cfu/g	990 cfu/g	870 cfu/g	810 cfu/g
Moulds and yeasts	$<10^2$ cfu/g	Mould <10 cfu/g	Mould 10 cfu/g	Mould <10 cfu/g
		Yeast <10 cfu/g	Yeast 20 cfu/g	Yeast <10 cfu/g
Enterobacteriae	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Salmonella spp.	Absent in 25 g	Absent in 25 g	Absent in 25 g	Absent in 25 g
Staphilococcus aureus	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
Water activity	<0.21	**Batch 160423040813**	**Batch 260420050813**	**Batch 300420050814**
		0.196	0.195	0.196
		0.199	0.196	0.195
		0.195	0.197	0.196

Comments

In its first report, the Scientific Committee took the view that the molecular and composition studies presented accurately identified the novel food to be marketed. Likewise it considered that the applicant had provided sufficient information to demonstrate the absence of contaminants. The Committee reviewed the data provided in the first assessment request and, given that the production system and the origin of the strain of microalga used have not changed, it is considered that there are no new grounds for concern.

With regard to the new specifications which set criteria for microbiological parameters and water activity, those specifications are met according to the results of the analysis of three batches submitted in the initial request assessed in 2013 (Table 2).

II. Effects of the production process applied to the novel food

In its first request, the applicant described the process for producing the novel food ingredient in detail and stated that it is similar to that used to obtain *Chlorella* and *Odontella aurita*, which are microalgae authorised for human consumption in the European Union. It also described the checks carried out on the finished product and provided data to demonstrate the product's stability.

The applicant states that the system of production (cultivation, harvesting, drying and packaging) has not changed, that the same checks are performed and that a Hazard Analysis and Critical Control Points (HACCP) system is employed.

The proposed food supplement will take the form of a measurable powder or a tablet. With regard to the stability of the finished product in the form of measurable powder reference is made to the tests carried out at 25 ± 2 ºC and 60 ± 5 % relative humidity and submitted in the first

request, since the food supplement format is equivalent to the condiment format. A stability study of a tablet form in accelerated conditions (40 ± 2 ºC and 75 ± 5 % relative humidity) is now added.

Comments

In its first assessment, the Scientific Committee approved the description provided of the production process, which the applicant says has not changed. The culture in photobioreactors isolated from the environment precludes external contamination and the production monitoring and hygiene systems (HACCP and the hygiene plan) laid down by the company were deemed to be adequate.

The stability studies indicated no significant variations in the product during the period of observation, in the forms in which the applicant currently markets it.

The proposed novel use as a food supplement in measurable powder form does not involve any significant changes compared to the characteristics and stability of the dried product.

In relation to the accelerated stability study of the product in tablet form, the tests performed show that the product does not undergo any change in physical appearance, physical/chemical properties or microbiological properties.

III. History of the organism used as the source of the food

Fitoplancton Marino argued in the request assessed in 2013 that, although there are no references to direct consumption by humans, the species *Tetraselmis chuii* has been introduced indirectly into the human food chain.

Comments

The Committee reviewed the information and made no comments on this section. At the time of the first assessment the species *Tetraselmis chuii* was not present in human food, but since the marketing of dried *Tetraselmis chuii* was authorised as a novel food in accordance with Regulation (EC) No 258/97 there is now some history of consumption.

IX. Anticipated intake/extent of use of the novel food

It is intended to market the product in powder form in food supplements with a recommended maximum daily intake of 250 mg. The target population is healthy adults.

In view of its organoleptic characteristics, the applicant laid down a daily intake of the dried *Tetraselmis chuii* for the uses already authorised of 250 mg. The estimated intake is as follows:

- Sauces. The intake per person/day of sauce with 20 % of the product would be a portion of 1.25 g. This would contain 250 mg of the dried microalgae. The applicant packages in containers labelled with the number of portions.
- Salt prepared with 1 % of the dried *T. chuii.* Maximum recommended intake of salt by the World Health Organization (WHO) is 5 g/day and the envisaged consumption of the dried microalgae would be 50 mg/day (WHO, 2003).
- Powder. Direct use of the dried powder as a condiment. The envisaged intake would be 250

mg/day. The applicant packages the product in single-dose containers or in larger containers for the food industry.

In the existing uses the applicant has estimated a maximum daily intake of dried *Tetraselmis chuii* of 550 mg, assuming that a consumer chooses to take the maximum quantity of all the approved categories of foods.

The novel use as a food supplement means the addition of a further 250 mg to the envisaged intake, thus resulting in an intake of 800 mg/day in the case of a consumer who chooses to consume all of the products for which the novel food is authorised.

Comments

The Scientific Committee considers the estimates of intake made by the applicant to be appropriate.

The true salt intake of the European population may be higher than the WHO's maximum recommended intake of salt. However, given its flavour, it is not likely that the consumer would add salt containing dried *Tetraselmis chuii* to all foods.

XI. Nutritional information on the novel food

In the initial request, the applicant presented analyses of the nutritional profile of three batches which showed that it was made up mainly of proteins and carbohydrates and, to a lesser extent, fat (Table 3).

Table 3. Results of the analysis of composition of three lots of dried *Tetraselmis chuii* produced industrially by Fitoplancton Marino S.L. (average of the three batches ± standard deviation)

Analysis		**Result**
Humidity (%)		6.3 ± 0.02
Proteins (%)		37.6 ± 0.40
Ashes (%)		15.5 ± 0.05
Carbohydrates (%)		31.6 ± 0.38
Dietary fibre (%)		2.3 ± 0.00
Fats (%)		6.7 ± 0.25
Kcal/100 g		337 ± 1.35
Kjoules/100 g		1 408 ± 5.66
Amino acid (%)	Valine	2.27 ± 0.12
	Tryptophan	0.61 ± 0.01
	Threonine	1.81± 0.13
	Tyrosine	1.38 ± 0.15
	Serine	1.63 ± 0.09
	Methionine	0.87 ± 0.12
	Lysine	2.03 ± 0.15
	Leucine	3.08 ± 0.09
	Isoleucine	1.57 ± 0.11
	Histidine	0.65 ± 0.13
	Glycine	2.25 ± 0.14
	Phenylalanine	1.95 ± 0.07
	Arginine	2.66 ± 0.09
	Alanine	2.79 ± 0.17
	Glutamic acid	4.67 ± 0.12
	Aspartic acid	3.71 ± 0.25
Mineral (mg/g)	Calcium	33.80 ± 0.26
	Magnesium	5.06 ± 0.09
	Iron	2.01 ± 0.04
	Phosphorus	6.27 ± 1.87
	Sodium	14.33 ± 4.16
	Potassium	10.40 ± 0.56
	Chlorides	17.77± 0.25
	Copper	0.006 ± 0.00
	Iodine (mg/kg)	5.03 ± 5.78*
Fatty acids (% fat)	Saturated	30.27 ± 0.50
	Monounsaturated	22.97 ± 0.90
	Polyunsaturated	46.77 ± 1.36

Source: (AECOSAN, 2013).

*Four batches were used for analysis of the iodine concentration. The variability between the batches was attributed to seasonal changes: cultures in summer provided lower results (0.45 and 0.47 mg/kg) than the two batches from winter cultures (6.7 and 12.5 mg/kg).

The applicant also provided the results of the iodine analysis by inductively coupled plasma mass spectrometry (ICP-MS), conducted in two different laboratories. In one of them iodine was not detected in any of the three samples of the different batches analysed at a detection threshold of 54 mg/kg and in the other laboratory with a detection threshold of 0.01 mg/kg and a quantification limit of 0.019 mg/kg, values of between 12.5 and 0.45 mg/kg were detected in the four batches analysed.

Taking the highest value obtained in the analyses conducted by the latter laboratory (12.5 mg/kg) and the consumption estimated by the applicant (250 mg), the quantity of iodine ingested would be 3.1 µg/day, which would be 2.1 % of the recommended daily intake (RDI) of iodine for adults (EFSA, 2006). For a population ingesting a daily portion of sauce with 250 mg of the microalgae, 5 g of salt and a dish seasoned with 250 mg of the client product, the uptake of iodine from this novel food would be 4.6 % of the RDI for iodine.

Based on these data, the new amount of 250 mg per day of dried *Tetraselmis chuii* as a food supplement would mean an additional intake of 3.1 µg/day, which when added to the intakes for uses already authorised would represent 7.4 % of the iodine RDI for adults.

The applicant states that there are many similarities between *Chlorella* and the microalgae *Tetraselmis chuii* which it wishes to market in dried form as a food supplement.

Comments

The Scientific Committee believes that the nutritional value of the novel food is limited, but that it is similar to that of other microalgae such as *Chlorella* which are already consumed as a food supplement in the European Union, and it is not therefore a nutritional disadvantage for this microalga to replace other microalgae of a similar composition.

XII. Microbiological information on the novel food

The Scientific Committee considered the documentation presented in the first request to be adequate as the microbiological analyses did not show the presence of pathogenic organisms and the company had submitted its HACCP system and hygiene plan.

Comments

Since the production system and the origin of the strain of microalga have not changed, it is considered that there are no new grounds for concern except those arising from the possibility of contamination during the production of the supplement. In this regard, if its marketing as a food supplement is authorised, the product must comply with all of the food legislation applicable to it and, once the product is on the market, the operator must ensure that undesirable microorganisms are absent or are present at levels below the maximum limits laid down.

XIII. Toxicological information on the novel food

The microalga to be marketed is used in aquiculture for the industrial cultivation of crustaceans, molluscs and fish larvae without any toxic effects having been detected. In its initial request, the applicant declared that the species of algae likely to produce toxins are members of 7 of the 76 orders of algae microorganisms, none of which belong to the Plantae kingdom, a kingdom in which no toxinogen has yet been described.

The certificate from the Consejo Superior de Investigaciones Científicas (CSIC) was annexed mentioning that this species "neither produces nor accumulates toxins". The Australian microalgae collection, belonging to the Commonwealth Scientific and Industrial Research Organisation (CSIRO), indicates that the species *Tetraselmis chuii* is not toxic (CSIRO, 2017).

The applicant presented studies of acute toxicity (OECD No 423) and 90 day toxicity (OECD No 408) and reverse mutagenicity on bacteria or Ames test (OECD No 471) for dried *Tetraselmis chuii*.

The value of DL50 obtained in the acute toxicity test was greater than 2 500 mg/kg body weight and there were no signs of toxicity in the animals treated.

From the 90 day study on rats an NOAEL of 2 500 mg of the product/kg body weight/day was calculated, that being the maximum dose used in the study.

The result of the Ames test was negative in all the strains and at all the concentrations used.

In terms of allergenicity, the results of the analysis of sulphite content (15 mg/kg) in dried *Tetraselmis chuii* were presented. The product's labelling includes the indication "contains sulphites", as laid down in 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 (EU, 2011).

Furthermore, the applicant presented two studies on the sensitising capacity of the product in humans (prick test and patch test). Both studies were carried out following the guidelines of the Spanish Group for Research on Contact Dermatitis and Cutaneous Allergies (GEIDAC) and the European Society for Contact Dermatitis (ESCD). No positive reaction to the saturated solution of dried *Tetraselmis chuii* was observed in any individual.

From the 90 day study on rats a NOAEL of 2 500 mg of the product/kg body weight/day was calculated. Based on this value, the applicant calculates the Acceptable Daily Intake (ADI) for dried *Tetraselmis chuii* to be 25 mg/kg body weight/day (1 750 mg/day for a 70 kg adult).

The 250 mg/day increase in the intake of dried *Tetraselmis chuii* via food supplements means a total maximum intake, also taking account of the uses already authorised, of 800 mg/day, which is below the ADI calculated.

The existing toxicological information has been supplemented with a new chromosomal mutation test to assess the potential genotoxicity. A micronuclei test has been carried out with dried *Tetraselmis chuii* in accordance with OECD Guideline No 487 in CHO mammalian cells (Chinese Hamster Ovary). The number of micronuclei for the dried *Tetraselmis chuii* ASE was recorded at 1 000 ppm with and without metabolic activation. At this concentration, no genotoxic effect was observed in CHO cell lines.

Comments

The Scientific Committee has carried out a bibliographical search for possible cases of allergy following the marketing of this novel food and no such references have been found.

The Scientific Committee considers the harmlessness of the novel food to have been demonstrated, and that it is not altered by the novel use as a food supplement.

Conclusions of the Scientific Committee

The Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) takes the view that, according to the information provided, there is no indication that consumption of dried marine microalgae *Tetraselmis chuii* as a food supplement, under the conditions proposed by the applicant, can produce adverse effects on health. The Scientific Committee concludes that the novel food presented for assessment by Fitoplancton Marino S.L. meets the criteria for acceptance laid down by Regulation (EC) No 258/97 on novel foods and novel food ingredients.

References

AECOSAN (2013). Informe del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) en relación a una solicitud de evaluación inicial para la comercialización de la microalga marina *Tetraselmis chuii* en el marco de Reglamento (CE) Nº 258/97 sobre nuevos alimentos y nuevos ingredientes alimentarios. *Revista del Comité Científico de la AECOSAN*, 18, pp: 11-27.

CSIRO (2017). *Commonwealth Scientific and Industrial Research Organization. Australian National Algae Culture Collection* (ANACC), the CSIRO Collection of Living Microalgae. Available at: https://anacc-db-cdc.it.csiro.au/fmi/webd [accessed: 18-05-17].

EFSA (2006). European Food Safety Authority. Tolerable upper intake levels for vitamins and minerals. Available at: http://www.efsa.europa.eu/en/ndatopics/docs/ndatolerableuil.pdf [accessed: 18-05-17].

EU (1997a). Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. OJ L 43 of 14 February 1997, pp: 1-6.

UE (1997b). Commission Recommendation 97/618/CE of 29 July 1997 concerning the scientific aspects and the presentation of information necessary to support applications for the placing on the market of novel foods and novel food ingredients and the preparation of initial assessment reports under Regulation (EC) No 258/97 of the European Parliament and of the Council. OJ L 253 of 16 September 1997, pp: 1-36.

UE (2011). 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 of 22 November 2011, pp: 18-63.

WHO (2003). World Health Organization. Diet, nutrition and prevention of chronic diseases. WHO Technical Report 916. Available at: http://www.who.int/nutrition/publications/es/ [accessed: 18-05-17].