RAMBOLL

ENVIRONMENT
& HEALTH

Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740.

RE: GRAS NOTIFICATION FOR HINOMAN'S "MANKAI" STRAIN OF *WOLFFIA GLOBOSA*

Date October 21, 2020

On behalf of Hinoman Ltd., I am pleased to submit this Notification of the Generally Recognized as Safe (GRAS) Determination for Hinoman's "Mankai™" strain of *Wolffia globosa*. This Notification contains the expert evaluation report on the GRAS Status of Mankai™ prepared by Joseph V. Rodricks, PhD, DABT and Duncan Turnbull, DPhil, DABT of Ramboll US Corporation, who are "experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food" according to the criteria of 21 CFR 170.30.

All of the information supporting the GRAS status of Mankai™ cited in this document is publicly available.

Also attached is a CD containing an electronic copy of this letter and the GRAS Notice, and copies of the publicly available documents supporting the GRAS status of Mankai™.

Please contact me (703-516-2308; dturnbull@ramboll.com) if you have any questions about this submission.

Yours sincerely

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Prepared for
Hinoman, Ltd.

Document type
GRAS Notice

Date
October 2020

Generally Recognized as Safe (GRAS) Assessment of Hinoman's "Mankai™" strain of *Wolffia globosa*

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CONTENTS

TABLES AND FIGURES

1. SIGNED STATEMENTS AND CERTIFICATION

1.1 GRAS Notice Submission

In accordance with 21 CFR Part 170, Subpart E,[1] Ramboll US Corporation ("Ramboll"), on behalf of Hinoman Ltd. ("Hinoman"), submits this Generally Recognized as Safe (GRAS) notice to the U.S. Food and Drug Administration ("FDA") for Hinoman's "Mankai" strain of *Wolffia globosa* (a registered strain; US Patent No. PP29977 (Patent application No. 15/330,249)), one of several species of small flowering aquatic plants commonly known as duckweed. The use of Mankai™ described herein is exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FD&C Act) for food additives because it is GRAS through scientific procedures, as established in Section 201(s) of the FD&C Act and 21 CFR 170.3. The GRAS evaluation has been conducted by Ramboll US Corporation.

1.2 Name and Address of the Submitter

[redacted]

October 20, 2020
Date

Duncan Turnbull, DPhil, DABT
Ramboll US Corporation
4350 N. Fairfax Drive, Suite 300
Arlington, VA 22203

Submitted on behalf of:

Miri Lapidot, PhD
Chief Scientific Officer,
Hinoman Ltd.
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Rishon LeZion 7546302,
Israel

1.3 Name of Notified Substance

The subject of this GRAS notification is "Mankai™", a selected, cultivated strain of *Wolffia globosa* (a registered strain; US Patent No. PP29977 (Patent application No. 15/330,249)).

1.4 Intended Conditions of Use of the Notified Substance

Mankai™ is intended to be marketed for human consumption as food and as a food ingredient in fresh, dried, & frozen form. Its proposed uses in a variety of food products are described in detail in Part 3.

1.5 Statutory Basis for Conclusion

The use of Mankai™ described herein is GRAS through scientific procedures, as described below (21 CFR 170.30):

> "(a) General recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. The basis of such views may be either (1) scientific procedures or (2) in the case of a substance used in food prior to January 1, 1958, through experience based on common use in food."

[11] https://www.govinfo.gov/content/pkg/CFR-2018-title21-vol3/pdf/CFR-2018-title21-vol3-part170-subpartE.pdf.

General recognition of safety requires that "there is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use." (21 CFR 170.3(i)).

1.6 Exemption from Premarket Approval Requirements of the FD&C Act

The use of Mankai™ described herein is GRAS and is therefore not subject to the premarket approval requirements of Section 409 of the FD&C Act for food additives.

1.7 Availability of Data and Information to FDA

Should FDA ask to see the data and information that are the basis for the conclusion of GRAS status, Ramboll and Hinoman will Permit FDA to review information at the Ramboll office in Arlington, VA or be provided copies if requested.

1.8 Freedom of Information Act (FOIA)

None of the data and information in this GRAS notice is exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

1.9 Certifications

To the best of the knowledge of Hinoman and Ramboll, this GRAS notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to Hinoman and Ramboll and pertinent to the evaluation of the safety and GRAS status of Mankai™.

1.10 Name, Position, and Signature of Certifier

Based on an evaluation of relevant data laid out within this report, the submitter has determined that Hinoman's Mankai™ is safe for its intended uses and generally recognized as safe (GRAS) under the terms of 21 CFR 170.30.

We have also concluded that other "experts qualified by scientific training and experience to evaluate the safety of food and food ingredients" would agree.

Joseph V. Rodricks, PhD, DABT
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2. IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

2.1 Scientific Data and Information that Identifies the Notified Substance

2.1.1 Substance Name, Origin, Composition, and Other Characteristic Properties

"Mankai™" is an aquatic plant, part of the family of plants known as duckweeds. Mankai™ is categorized as belonging to the plant family *Lemnaceae*, genus *Wolffia* and species *globosa* as described by (Roxburgh) Hartog & Plas.

https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=161118 (http://www.discoverlife.org/mp/20q?search=Wolffia+globosa; http://waynesword.palomar.edu/wogl.htm; http://plants.jstor.org/stable/10.5555/al.ap.flora.fna022_wolffia_globosa).

USDA – Natural Resources Conservation Services (https://plants.usda.gov/core/profile?symbol=WOGL3)
Wikipedia (https://en.wikipedia.org/wiki/Lemnoideae)
Wikipedia – Wolffia globosa (https://en.wikipedia.org/wiki/Wolffia_globosa)
US National Plant Germplasm System - GRIN – Wolffia globosa (https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?406374)
Rutgers University, New Jersey USA (http://www.ruduckweed.org/uploads/1/0/8/9/10896289/iscdra-duckweedforum_issue14-2016-07-corrected.pdf)
Japan International Research Center for Agricultural Sciences (https://www.jircas.affrc.go.jp/project/value_addition/Vegetables/105.html)
Plants For A Future Org. (https://pfaf.org/user/Plant.aspx?LatinName=Wolffia+globosa)

Duckweeds are flowering aquatic plants which float on or just beneath the surface of still or slow-moving bodies of water (Landolt 2014a). The duckweeds are described taxonomically as belonging to their own plant family, the *Lemnaceae*. However, some taxonomists classify the *Lemnaceae* as a subfamily of the *Araceae* due to results from comparative analysis of chloroplast DNA (Duvall et al. 1993). The genera in the *Lemnaceae* are: *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia* (http://www.mobot.org/jwcross/duckweed/duckweed.htm).

The duckweeds are simple, lacking an obvious stem or leaves. They are composed of a "frond" structure which is only a few cell layers thick and contain air pockets which allow it to float on or just under the water surface. Depending on the species, each plant may have no roots or may have one or more simple rootlets (Landolt 2014a).

Reproduction is mostly by asexual budding, which occurs in 1 or 2 pouches, or from a meristem enclosed at the base of the frond. Seldom, one to two tiny "flowers" consisting of two stamens and a pistil are produced, by which sexual reproduction occurs. The flower of the duckweed genus *Wolffia* is the smallest known, measuring only 0.3 mm long. The fruit produced through this occasional sexual reproduction is an utricle, and a seed is produced in a sac containing air that facilitates flotation. Due of the rarity of flowering and fruiting, many specimens are identified using only vegetative characteristics (Landolt 2014a).

2.2 Information Identifying Biological Material

2.2.1 Wolffia

The genus *Wolffia* was named for Johann Friedrich Wolff, a German physician who lived from 1778 to 1806. The description of the characteristics of the genus *Wolffia* (Horkel ex Schleiden) as published in Beitrage zur Botanik 1:233, 1844, is given below (Landolt 2014b):

> Roots absent. Fronds floating or submersed (only turions sink to bottom), 1 or 2, coherent, each frond globular, ovoid, or boat-shaped, 3-dimensional, smaller than

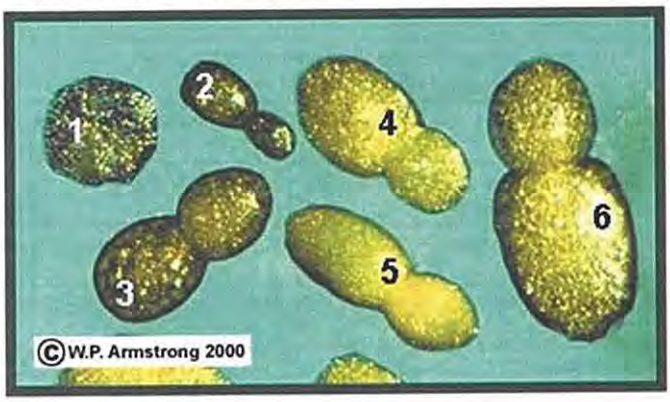
1.6 mm, margins entire; air spaces not in tissue; reproductive cavity terminal, at base from which daughter fronds (but no flowers) originate, conic, lower side of cavity with short tract of elongated cells along median line forming connection between node and attachment to mother frond; veins 0; scale at base of frond absent; anthocyanins absent; in some species pigment cells present (visible in dead fronds as brown dots); turions light green, globular, smaller than growing fronds. Flowers 1 per frond, originating in cavity ± on median line of upper frond surface, not surrounded by utricular scale; stamen 1, 2-locular. Seeds 1, nearly smooth. x = 10, 20, 21, 22, 23.

The USDA, ARS, Natural Resources Conservation Service plants database (https://plants.usda.gov/) lists multiple species of *Wolffia* including:

- *Wolffia arrhiza* (L.) Horkel ex Wimm.
- *Wolffia borealis* (Engelm. ex Hegelm.) Landolt ex Landolt & Wildi
- *Wolffia brasiliensis* Weddell
- *Wolffia columbiana* Karst.
- *Wolffia cylindracea* Hegelm.
- *Wolffia globosa* (Roxb.) Hartog & Plas

While all six species of Wolffia have the same general shape, they differ in the overall size. *Wolffia globosa* is the species most commonly consumed by humans in Asia. As presented by Professor Armstrong, *W. globosa* is the smallest and slightest of the six species, while *W. arrhiza* is the largest and bulkiest (Armstrong) (see Figure 1).

Figure 1. Six Species of *Wolffia*



Six Species Of Wolffia

Dorsal view of six **Wolffia** species: 1. **W. microscopica** (India); 2. **W. globosa**; 3. **W. columbiana**; 4. **W. brasiliensis**; 5. **W. borealis**; 6. **W. arrhiza** (Germany). [Species 2 through 6 are all known to occur in the state of California, USA.]

2.2.2 Wolffia globosa

The description of the characteristics of the genus *Wolffia globosa* (Roxburgh) Hartog & Plas as published in Blumea. 18:367 (1970), is given below (Landolt 2014b).

Fronds ovoid, 0.4-0.8 mm (x 0.3-0.5 mm), 1.3-2 times as long as wide, 1-1.5 times as deep as wide, rounded or slightly pointed at apex, papilla absent; adaxial surface transparently green, with 1-10(-30) stomates; pigment cells absent in vegetative tissue. 2n = 30, 60.

Flowering (very rare) late spring--fall. Mesotrophic to eutrophic, quiet waters in warm-temperate to tropical regions with mild winters; 0--600 m; probably introduced; Calif., Fla.; South America (Colombia, Ecuador); E. Asia; Pacific Islands (Hawaii).

The US National Plant Germplasm System (https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?406374) reports that *Wolffia globosa* is native to temperate and tropical Asia (e.g. China, Japan, India, Thailand, Vietnam, and Malaysia), the Pacific (Hawaii), South America (Columbia, Ecuador) and North America (Florida, Arkansas and California in the United States) (USDA 2019). *Wolffia arrhiza* is reported as native to Africa (throughout the continent), Asia (Iran, Israel, the Caucasus and the Indian subcontinent), Europe and South America (Brazil). It is reported to be naturalized in Japan, the Czech Republic, Slovenia, California and Brazil (USDA 2019).

2.2.3 Food-grade duckweed product

Parabel, Inc. ("Parabel") was the first company to have launched a food-grade duckweed product, with their brand Lentein™. Lentein™ is made from different species of duckweed, including *Wolffia* species, which are cultivated in Florida, USA. This product is cultivated in open ponds with different species of duckweeds (See Table 1) throughout the year.

Table 1. Selected Species grown by Parabel for Lentein™ Product*

Strain	Defining Features	% composition**
Wolffia globosa	Plant body 0.4 -0.8 mm, longer than wide (ovoid-cylindrical), upper surface barely rounded	0-80% of the crop
Wolffia brasilensis	Plant body 0.7 -1.2 mm, ovoid- ellipsoid, upper surface flattened with a minute-prominent papule in the center; dead plants dotted with brown pigment cells.	
Wolffia columbiana	Plant body 0.8-1.2 mm, almost spherical, most of the upper surface clearly rounded, the uppermost top flat area	
Wolfiella gladiata	The fronds are 5-10 mm long, Wolfiella floats just beneath the surface of the water	0-30% of the crop
Wolffia arrhiza	Plant body 0.8-1.3mm, ovid to spherical. Distinctly flattened dark green dorsal. No brown pigment cells. Plant body.	
Landoltia punctata (Spirodela punctata)	2-4 roots per frond. Red/Purple ventral common	0-30% of the crop
Lemna minor	1 root, not reddish on lower, surface, can be red on upper. 3 veins	20-100% of the crop
Lemna gibba	1 root, gibbous on bottom	
Lemna japonica	Hybrid of L minor and L turionifera. 1 root, row of dorsal papillae	
Lemna obscura	Prominent apical papule on smooth dorsal surface	
Lemna turionifera	Shiny, red blotches on ventral side. Typically has dorsal papule row. Turions often present (seasonal)	
Lemna valdiviana	Plant bodies connected in 4-8 colonies, thin transparent with single vein.	

* GRAS notice to FDA GRN 742

** Composition depends on climate, season and other growing conditions.

Parabel submitted a GRAS Notice to the FDA (GRN-742), and in August 2018 the FDA issued an Agency Response Letter with "no questions at this time" for Lentein™, a mixed duckweed product made of duckweed grown outdoors which includes, as one of its components, *Wolffia globosa* (FDA 2018a).

2.2.4 Mankai™

The Hinoman strain was initially authenticated as *Wolffia arrhiza* (L.) Harkel ex Wimm by Mr. Winai Somprasong, Senior Agricultural Scientist at the Bangkok Herbarium (certificate dated April 11, 2011). Subsequently, Dr. Klaus J. Appenroth of the University of Jena, Germany, Head of the International Steering Committee on Duckweed Research and Application, and expert on duckweeds, examined the morphological structure of the Hinoman strain and in a letter dated May 21, 2014 stated that Hinoman's proprietary strain is *Wolffia globosa*. In addition, prof. (Emeritus) Marvin Edelman of the Weizmann Institute in Israel whose laboratory researched various aspects of duckweed biochemistry, physiology and molecular genetics for the past 39 years stated in a written opinion that the Hinoman strain is indeed *Wolffia globosa*, and either fresh or cooked, is eminently edible, nutritionally rich as a vegetable and well suited for regulated, non-toxic growth under modern agricultural management.

Figure 2. Comparison of Hinoman Strain with *Wolffia globosa* in the Literature

Hinoman's strain **Authentic *Wolffia globosa* (California)**

Lighting from below http://waynesword.palomar.edu/imgwogl.htm

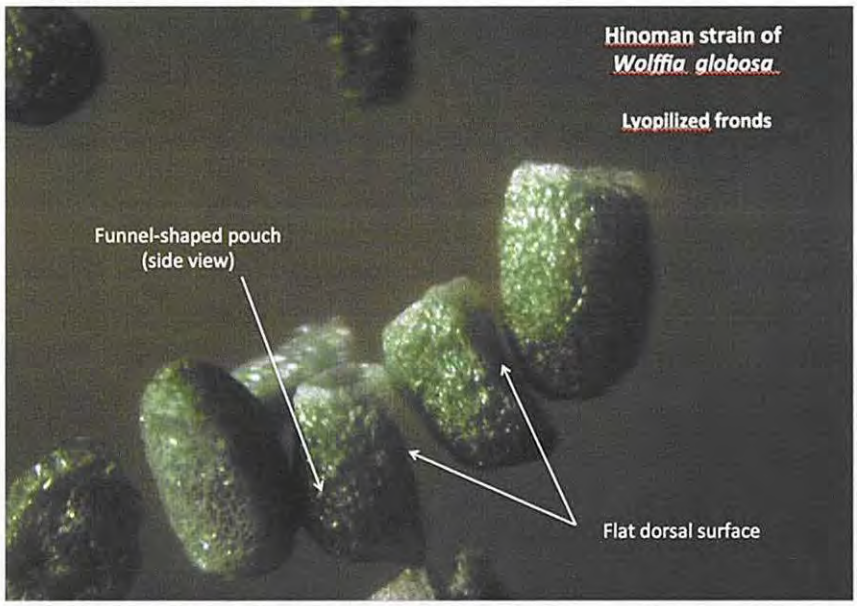
Photomicrograph of a fresh sample of the Hinoman strain in pool medium using a Nikon SMZ 1500 stereo microscope. Magnification provided by scale bar.

The Hinoman *Wolffia globosa* strain – Mankai™ - has the following characteristics which are consistent with those of *Wolffia globosa*:

- ***Size***: 0.4-0.9 mm long
- ***Veins***: None
- ***Budding Pouch Position***: One funnel-shaped pouch at basal end; budding pouch often with distinct collar of elongate cells at junction with daughter plant.
- ***Arrangement of Clonal Clusters***: Solitary or 2 connected
- ***Shape of Plant Body***: Ovoid or ellipsoid-cylindrical (longer than wide) and transparent green throughout (some populations, including the Hinoman strain, contain individuals with darker green dorsal surface); dorsal surface rounded on edges with upper central portion flattened,

floating with only the central portion of dorsal surface above water; without brown epidermal pigment cells.

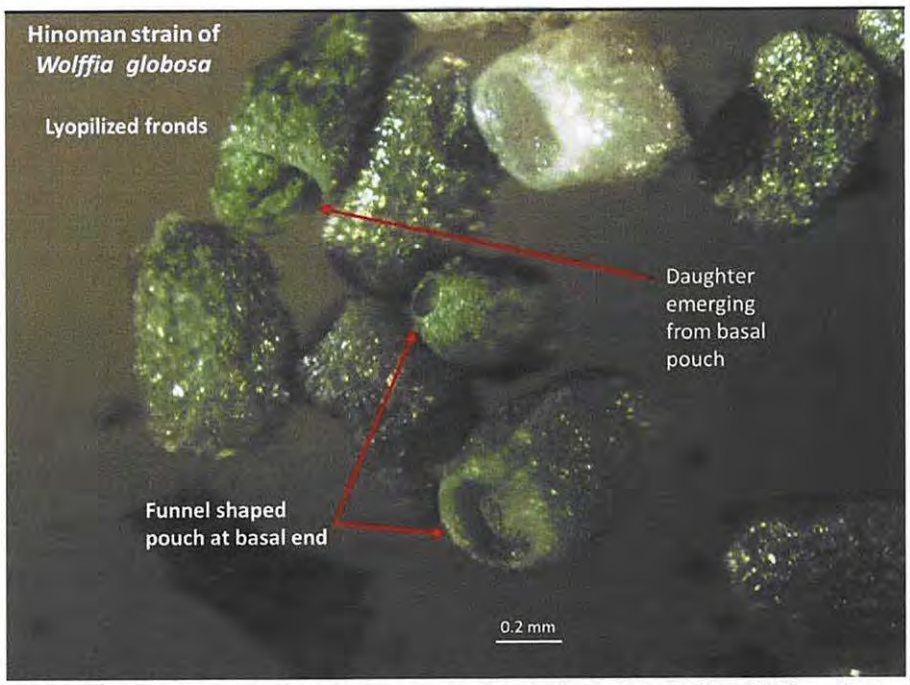
Figure 3. *Wolffia globosa* Hinoman Strain "Mankai™"



The photo of Mankai™ above (Figure 3) shows the funnel-shaped pouch and the dorsal flat surface characteristic of *Wolffia globosa*. The photo shows two specimens that have landed on their side allowing viewing of the flattened dorsal surface and the ventral pouch.

The photo below (Figure 4) shows the funnel shaped pouch at the basal end of the plant.

Figure 4. *Wolffia globosa* Hinoman Strain "Mankai™"



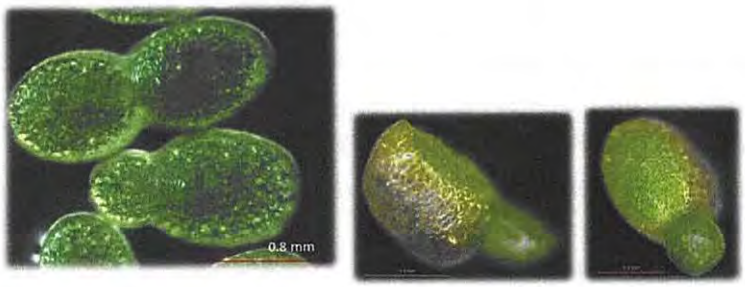
A sample of the Mankai™ grown in pool medium was harvested, drained, placed directly in liquid nitrogen and lyophilized at minus 95°C. It was photographed using a Nikon SMZ 1500 stereo microscope. The magnification is provided by the scale bar (0.2 mm). Illumination is from above and from the left.

Figure 5. *Wolffia globosa* and Hinoman Strain "Mankai™"

Mankai™ is an isolated strain of *Wolffia globosa*.

It is a very tiny, oval-shaped plant with no leaves, stems, or roots.

Mankai™ (*Wollfia globosa*):
Size: 0.4-0.9 mm length,0.3-0.6 mm width



Confidential 1

Figure 5 – The upper picture provides a view of *Wolffia globosa* culture to demonstrate its small size. On the right-hand side - a microscopic photograph of Mankai™ using a Nikon SMZ 18 stereo microscope.

This photo on left-hand-side shows several stages within the life cycle of this plant. Magnification is provided by the scale bar (0.8 mm).

Figure 6. Arrangement of Clonal Clusters

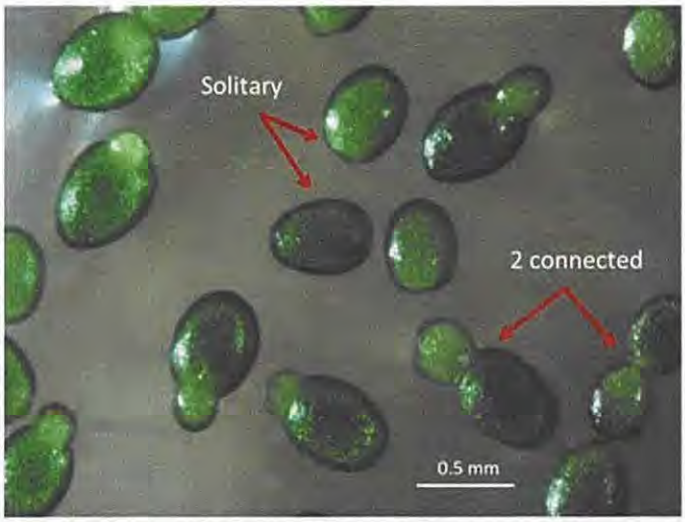


Figure 6 shows a sample of Mankai™ grown in pool medium and photographed using a Nikon SMZ 1500 stereo microscope. Magnification is provided by the scale bar (0.5 mm). Populations of Mankai™ show clusters of 2 connected plants (mother and daughter) and solitary plants.

2.3 Part of Plant Used as Source

Mankai™ consists of *Wolffia globosa* (US Plant Patent number PP29977) whole plants, marketed and consumed in a fresh, frozen, or dried form.

2.4 Known Toxicants Present in the Source

Wolffia species may contain oxalic acid but do not accumulate calcium oxalate crystals (Landolt and Kandeler 1987). See Section 6.1.5 for a discussion of the safety considerations of oxalic acid and duckweed. The amount of oxalic acid in Mankai™ samples analyzed by Covance, Inc. ("Covance") a global contract research organization, is reported in Table 9 (Section 2.7.1).

2.5 Method of Manufacture

Mankai™ is grown in a closely monitored and controlled cultivation basin covered by food grade PVC foil, equipped with a variety of controllers. In addition, a meteorological station monitors environmental factors including air temperature, humidity, sunlight, wind direction and wind speed (Figure 7).

The cultivation basins are either placed directly on the ground or in "floors" and are equipped with automatic/semi-automatic systems for water filling and fertilizing.

Sensors monitor the levels of pH, electro-conductivity (EC), oxygen, and the temperature of the growth medium.

Circulating water is supplied to the system routinely, filtered and analyzed for elemental content using ICP and other methods. Fertilizer is supplied via a dedicated fertilizing system, that can either function manually or be programed to function automatically, in a very precise way, from several tanks that contain stock solutions of nutrients.

Figure 7. Greenhouses Used to Culture Mankai™

The plants continuously grow in the cultivation basins with routine harvesting, mostly on a daily basis. A QC analysis is performed for the biomass in the basins, as well as the harvested biomass, which includes, the composition profile such as protein content, minerals content as well as safety aspects such as heavy metals content, and microbiology analysis.

Harvesting is routinely performed automatically without human hand contact. During the harvesting process the biomass passes through several washing steps:

- During the collection of the biomass from the basin, the plants are washed with tap water (harvesting from the basin performed with our propriety automatic harvesting system that separates growth medium and the plant biomass while washing the collected plant with tap water during the collection step).

- At the harvesting farm collecting center, the harvested biomass passes an additional washing step where the excess water is removed via filtration while the biomass is collected into containers and transferred to a cooling room (post harvesting).

Post harvesting biomass is organoleptic, texture and color tested according to the QA validated process.

The post harvesting system and the harvesting collection center is CIP (Cleaning in Process) treated at the end of each harvesting cycle.

The washed biomass is transferred to the processing unit under cooling conditions for further processing of the final product.

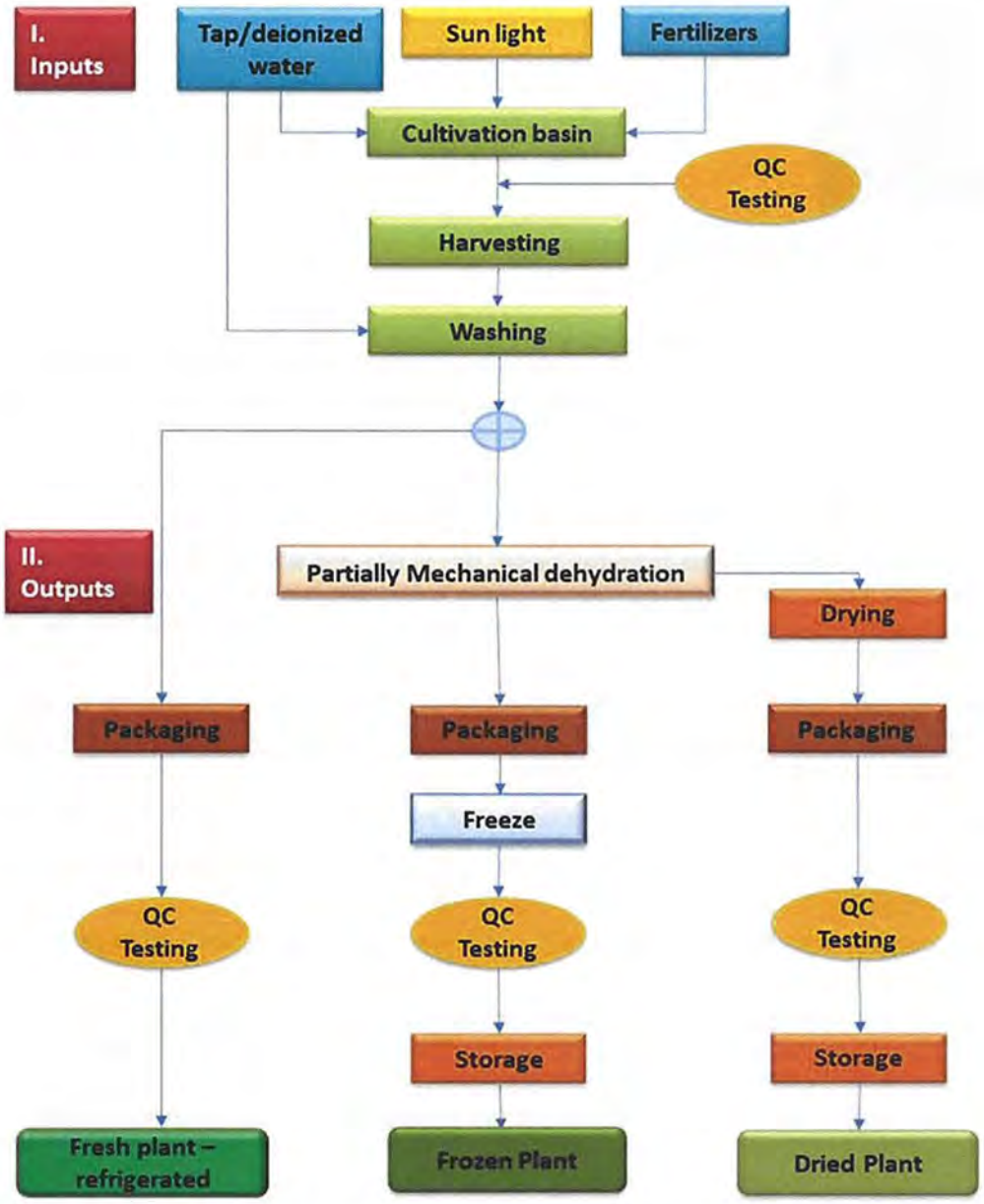
Mankai™ is produced and delivered in fresh, frozen, and dried forms. Biomass destined to be used in its fresh form passes a disinfected washing step, and is packed and placed in a refrigerator where it is chilled at a temperature of 4-8°C during storage and through the supply chain. Biomass to be used in a dried form is thermally treated, squeezed and then dried using methods such as: temperature-controlled dehydrator, spray dryer, fluid-bed dryer or a freeze-dryer. Frozen products pass a disinfection process (such as chemical wash or pasteurization step) followed by a partially mechanical dehydration step and then frozen and kept in that state throughout the supply chain.

After the plant material is processed to a product (fresh, frozen or dried), it is subject to QC analysis, which includes testing for specifications and nutritional composition as well as microbiological analysis.

For example, in Israel the fresh, frozen or dried Mankai™ products are produced under adherence to FSSC 22000 and ISO 9001 requirements at the company's certified manufacturing facilities (Figure 9 and Figure 10).

All of the procedures used in the growth, harvesting, and processing and packaging of Mankai™ comply with current Good Manufacturing Practice (cGMP) as laid out in 21 CFR 110, 112, and 117.

Figure 8. Mankai™ Production Flow Chart



IQC
Institute of Quality & Control

CERTIFICATE

NO. I17299

This is to certify that
the Quality Management System of

Hinoman Beeri Industries Ltd

Kibutz Beeri, Israel

Was audited by IQC and found to be
in compliance with the requirements of the standard:

ISO 9001:2015

This certificate is valid for
the following scope of activities:

Growing, processing and packaging of leaf vegetables

This certificate is valid until:	03.11.2021
Certification cycle will end on:	03.11.2021
Date of first approval:	11.11.2018

This certificate is subject to the continuing satisfactory operation
of the Management System and periodic auditing by IQC

11.11.2018
Issue date

Nir Halpern, CEO

IAF
MGMT. SYS
RvA C 560

Institute of Quality & Control Ltd.
6 Ravnitzky St., Petah Tikva 4900617, Israel
Tel: 03-9313535, Fax: 03-9044406
E-Mail: info@iqc.co.il, www.iqc.co.il

Figure 9. ISO 9001:2015 Certificate

IQC
Institute of Quality & Control

CERTIFICATE

NO. I17490U

This is to certify that
the food safety management system of

Hinoman Beeri Industries Ltd

Kibutz Beeri 8513500, Israel

has been audited by IQC and found to be
in compliance with the requirements of the standard:

Food Safety System Certification (FSSC) 22000
(version 4.1)

This certificate is valid for
the following scope of activities:

Processing and packaging of leaf vegetables

Food Chain (Sub) Category CII

Certification scheme for food safety management systems consisting of the following elements:
ISO 22000:2005, ISO/TS 22002-1:2009 and Additional FSSC requirements (version 4.1).

This certificate is valid until: ***09.12.2020***
Initial Certification date: ***09.12.2018***
Date of Certification decision: ***09.12.2018***

This certificate is subject to the continuing satisfactory operation
of the Management System and periodic auditing by IQC

10.12.2018
Date of Issue

Nir Halpern, CEO

FSSC 22000

MGMT. SYS.
RvA C 560

Validity of this certificate can be verified in the FSSC 22000 database of certified organizations available on www.fssc22000.com.

Institute of Quality & Control Ltd.
6 Ravnitzky St., Petah Tikva 4900617, Israel
Tel: 03-9313555, Fax: 03-9044406

Figure 10. Israeli Food Safety System Certification

2.6 Production Process and Batch Data

The growth medium consists of tap water and/or a combination of tap water and deionized water to which is added standard commercial fertilizers (meeting appropriate quality criteria for heavy metals, etc.) and nutrients appropriate for growing vegetables for human consumption in a precise and controlled process.

The growth medium is continuously monitored for several parameters including pH, EC, oxygen, and temperature to maintain the optimal growth parameters. Samples of the growth medium and biomass are routinely collected in order to assure high quality along with a consistent and stable composition.

The production process does not include any pesticides or fungicides being added to the growth medium.

Elemental analysis for heavy metal is done routinely. Analysis was completed by the Hebrew University Laboratory Faculty of Agriculture, Food and Environment in Rehovot, Israel. The results for selected elements are shown in Table 2.

The protocol used for the ICP-AES analysis is:

Water samples were thoroughly mixed, and 20 ml of sample aliquot was digested with HCl and HNO_3 according to EPA 200.2 method, revision 2.8, adopted for use with the Environmental Express Hotblock Digestion system.

Digestion was carried out in polypropylene digestion vessels. Element concentration was measured in the clear solutions using an End-On-Plasma ICP-AES model 'ARCOS' from Spectro GMBH, Germany. Measurements were calibrated with standards for ICP from Merck. The measurement was performed according to EPA standard method 6010c. Element concentrations that exceed the linear dynamic range were diluted and reanalyzed. Dilution was made using calibrated pipettes. The continuing calibration verification standard was measured to check the instrument stability.

Table 2. Heavy Metals in Water and Growth Medium (mg/L)

Sample	Sample #	Al	As	Cd	Cr	Hg	Ni	Pb
Water	483237*	<0.02	<0.01	<0.005	<0.05	<0.001	<0.005	<0.005
Growth Medium	1915**	0.01	<0.006	<0.001	<0.001	<0.004	<0.002	<0.005
	1916**	0.01	<0.006	<0.001	<0.001	<0.004	<0.002	<0.005
	1917**	0.01	<0.006	<0.001	<0.001	<0.004	<0.002	<0.005
	1918**	0.07	<0.006	<0.001	<0.001	<0.004	<0.002	<0.005

*Bactochem Laboratory, Israel – COA 483237; Note: Chromium as Cr^{6+}
**Hebrew University Laboratory Faculty of Agriculture, Food and Environment in Rehovot, Israel COA-18-11336

2.7 Plant Composition

Mankai™ has been analyzed for general nutritional content: namely protein, fat, carbohydrate, fiber and calories (typical values shown in Table 3). The percentage of dry matter in the fresh plant material is in the range of 4-6%. The percentage of dry matter in the frozen material may reach up to 35%. Typical values of the amino acid, vitamin, fat and elemental contents are depicted in Tables 4-7, based on dry matter. Polyphenol content is shown in Table 8.

Table 3. Nutritional Composition of Four Lots of Dried Mankai™ (g/100g) *

General Composition	Lot 911P[1]	Lot 110P[2]	Lot 1004P[3]	FDPR 10312017[4]
Protein (%)	45.62	43.17	44.92	45.00
Ash (%)	7.42	8.15	8.34	5.19
Carbohydrates, total (%)	36	37	35	37
Fat, total (%)	8.34	7.19	7.01	9.15
Dietary Fiber, total (%)	32.3	35.3	34.2	36.5
Calories (kcal/100g)	402	387	384	412

*Eurofins laboratory, USA:
[1]COA AR-17-QD-097687-01; [2]COA AR-17-QD-122301-01; [3]COA AR-17-QD-122302-01;[4]COA-AR-18-QD-002048-01

Table 4. Essential Amino Acid Content in Four Lots of Dried Mankai™ (g/100 g) *

Essential Amino acids	Lot 51.2PP[1]	Lot 911P[2]	Lot 1002P[3]	Lot 1004P[4]
Tryptophan	0.98	1.03	0.98	1.01
Methionine	0.93	0.87	0.84	0.86
Threonine	1.94	1.95	1.92	1.97
Valine	2.66	2.66	2.6	2.66
Isoleucine	2.08	2.07	2.04	2.06
Leucine	3.91	4.03	3.86	3.92
Phenylalanine	2.48	2.51	2.4	2.45
Total Lysine	3.31	3.21	2.94	2.98
Histidine	0.95	0.96	0.92	0.95

*Eurofins laboratory, USA:
[1]COA AR-17-QD-039535-01; [2]COA AR-17-QD-097687-01; [3]COA AR-17-QD-122301-01; [4]COA AR-17-QD-122302-01

Table 5. Vitamin Content in Four Lots of Dried Mankai™*

Vitamin	Lot 51.2PP[1]	Lot 911P[2]	Lot 1002P[3]	Lot 1004P[4]
Vitamin A (beta-carotene), mg/100g	46.19	20.095	31.85	28.98
Biotin (Vitamin B7), mg/100g	0.0487	0.0564	0.0518	0.0418
Vitamin B1 (Thiamine HCl), mg/100g	0.882	0.606	0.85	0.654
Vitamin B12, µg/100g	4.38	2.19	2.81	2.64
Vitamin B2 (Riboflavin), mg/100g	3.43	3.38	3.49	3.28
Vitamin B3 (Niacin), mg/100g	6.3	5.5	7.79	7.67
Vitamin B5 (Pantothenic Acid), mg/100g	0.851	0.281	0.36	0.3
Vitamin B6 (pyridoxine), mg/100g	1.25	1.25	1.27	1.1
Vitamin C, mg/100g	6.75	12.6	18.2	38
Vitamin E (Tocopherols), mg/100g	31.2	16.06	22.28	16.85
Vitamin K1, mg/100g	N/A	6.7	2.56	8.37

*Eurofins laboratory, USA:
[1]COA AR-17-QD-039535-01; [2]COA AR-17-QD-097687-01; [3]COA AR-17-QD- 122301-01; [4]COA AR-17-QD-122302-01. (N/A = not available)

Table 6. Lipid Content in Four Lots of Dried Mankai™ (g/100 g) *

Lipids	Lot 51.2PP[1]	Lot 911P[2]	Lot 1002P[3]	Lot 1004P[4]
Fat as Triglycerides (%)	7.48	8.34	7.19	7.01
Saturated Fat (%)	1.64	1.97	1.63	1.7
Trans Fat (%)	0.04	0.04	0.03	0.03
Polyunsaturated Fat (%)	5.08	5.51	4.76	4.56
Monounsaturated Fat (%)	0.38	0.45	0.44	0.4
Calories from Fat (kcal/100g)	67	75	65	63

*Eurofins laboratory, USA:
[1]COA AR-17-QD-039535-01; [2]COA AR-17-QD-097687-01; [3]COA AR-17-QD- 122301-01; [4]COA AR-17-QD-122302-01

Table 7. Elemental Composition of Four Lots of Dried Mankai™ *

Minerals	Lot 51.2PP[1]	Lot 911P[2]	Lot 1002P[3]	Lot 1004P[4]
Calcium (Ca), %	0.56	0.513	0.44	0.45
Sodium (Na), %	0.031	0.042	0.053	0.065
Iron (Fe), mg/kg	387	723	600	450
Aluminium (Al), mg/kg	3.3	12	4.6	4.2
Potassium (K), mg/kg	22,700	22,900	29,000	31,000
Magnesium (Mg), mg/kg	1,910	1,950	1,800	1,800
Selenium (Se), mg/kg	<0.23	<0.1	<0.2	<0.2
Strontium (Sr), mg/kg	49.3	54.1	50	50
Chromium (Cr), mg/kg	0.581	4	3.5	2.3
Barium (Ba), mg/kg	4.85	6.78	7.4	5.4
Titanium (Ti), mg/kg	0.83	2.6	0.9	0.6
Vanadium (V), mg/kg	0.213	<0.2	<0.2	<0.2
Nickel (Ni), mg/kg	0.273	1.3	1.4	1
Molybdenum (Mo), mg/kg	3.31	3.6	3.5	5.1
Phosphorus (P), mg/kg	8,440	8,300	9,800	9,800
Copper (Cu), mg/kg	20.3	17	14	18
Manganese (Mn), mg/kg	226	292	210	200
Boron (B), mg/kg	59.7	53.9	36	31
Cobalt (Co), mg/kg	0.145	<0.75	<0.2	<0.2
Zinc (Zn), mg/kg	363	264	110	190
Silicon (Si), mg/kg	10	11	22	33
*Eurofins laboratory, USA: [1]COA AR-17-QD-039535-01; [2]COA AR-17-QD-097687-01; [3]COA AR-17-QD- 122301-01; [4]COA AR-17-QD-122302-01. Conducted using ICP technology.				

Table 8. Polyphenol Composition of Five Lots of Dried Mankai™ (mg/100g)

Polyphenol	Lot 2004091701[1]	Lot 911P[2]	Lot 1002P[3]	Lot 1004P[4]	Lot FDPR-10312017[5]
Catechins (mg/100g)					
Epigallocatechin	<10	<10	<10	<10	<10
Catechin	<10	<10	<10	<10	<10
Epicatechin	16.5	17.4	<10	<10	20.5
Epigallocatechin Gallate	<10	<10	<10	<10	<10
Gallocatechin Gallate	<10	104	67.8	67	<10
Epicatechin Gallate	<10	<10	<10	<10	37.8
Catechin Gallate	<10	<10	<10	<10	<10
Gallocatechin	<10	<10	<10	<10	<10
Total Catechins	16.5	121	67.8	67	58.3
Phenolic acids (mg/100g)					
p-Coumaric acid	6.02	<3.33	5.54	7.73	ND
Ferulic acid	<3.33	<3.33	3.97	3.81	ND
Caffeic acid	110	56.2	91	55.9	ND
Sinapic acid	<3.33	<3.33	11.9	11.6	ND
Total Polyphenols (mg/100g)	700	477	493	382	393
Covance Laboratories, USA: COA [1]1803617-0; [2]1859912-0; [3]1896585-0; [4]1896586-0; [5]2016842-0					

2.7.1 Oxalic Acid

Wolffia species contain oxalic acid but do not accumulate calcium oxalate crystals (Landolt and Kandeler 1987). The amount of oxalic acid in dried Mankai™ analyzed by Covance is reported in Table 9., and the amount in two lots of fresh Mankai™ is reported in Table 10. Levels of oxalic acid in Mankai™ are comparable to, or lower, than those that may be ingested from common vegetables, such as spinach, which contains 970 mg/100g, based on fresh weight (USDA 1984), or 5,300-11,600 mg/100g dry weight (Mou 2008) (see Section 6.1.5).

Table 9. Oxalic Acid Content in processed and Dried Mankai™

Sample	Oxalic acid (mg/100g)
Lot 51.2PP	458
Lot 2004091701	318
Lot 911P	489
Lot 2006111701	277
Lot 1002P	637
Lot 1004P	629
Lot 2237P	230
Lot 2241P	279
Lot D20190114	424

Table 10. Oxalic Acid Content in Fresh Mankai™

Sample	Analysis COA*	Oxalic Acid (mg/100g fresh) **
Lot 175P	1755361-0	47.3
Lot 602P	1755362-0	50
*Covance Laboratory, USA (Official Methods of Analysis of AOAC International (2005) 18th Ed., AOAC International, Gaithersburg, MD, USA, Official Method 986.13. (Modified). ** Test is done on dried material (Air oven dried), calculation is based on moisture content of 95% in fresh Mankai™.		

2.7.2 Microcystins and Cylindrospermopsin

Hinoman tests for the presence of Microcystins and Cylindrospermopsin in its growing ponds. Table 11 shows the test results sampled on July 26, 2017. The analyzes were carried out by Israel Oceanographic & Limnological Research Ltd. using the HPLC-DAD system and calibrated after appropriate treatment of the samples.

Table 11. Total Microcystins and Cylindrospermopsin in Dried Mankai™

Sample	Total Microcystin (µg/g)	Cylindrospermopsin (µg/g)
LB1-1 26.7.17	bdl	bdl
LB1-2 26.7.17	bdl	bdl
LB1-3 26.7.17	bdl	bdl
SB7 26.7.17	bdl	bdl
bdl = below detection limit (10 ng)		

2.7.3 Heavy metals

Hinoman routinely monitors the Mankai™ for heavy metal content using inductively coupled plasma mass spectrometry (ICP-MS) analysis (Table 12).

Table 12. ICP Analysis of Heavy Metal Content of Six Lots of Dried Mankai™

Metal, mg/kg	Lot 51.2P[1]	Lot 911P[2]	Lot 1002P[3]	Lot 1004P[4]	SO18077922[5]	SO18077924[5]
Cadmium (Cd)	0.058	0.042	0.02	0.04	0.014	0.005
Lead (Pb)	0.234	0.495	0.33	0.92	0.112	0.222
Mercury (Hg)	<0.012	<0.011	<0.005	<0.005	<0.005	<0.005
Arsenic (As)	0.031	0.064	<0.1	0.3	0.019	0.04
Eurofins laboratory, USA: [1]COA AR-17-QD-039535-01; [2]COA AR-17-QD-097687-01; [3]COA AR-17-QD-122301-01; [4]COA AR-17-QD- 122302-01 ; [5]Analysis done by Milouda & Migal Merieux Nutrisciences, Israel						

2.7.4 Microbiological Content

Measures at Hinoman's growth facilities to control microbiological content of Mankai™ include fully controlled cultivation which are free of extraneous vegetation and organic materials, the use of latex gloves when sampling and handling pool contents, and regular sanitation of the harvesting equipment prior to harvesting.

In addition, the water used for filling and maintaining the pools and for post-harvest rinsing is tap water or a combination of tap water and deionized water subjected to regular QC testing.

Results of microbiological analysis of water and growth medium are shown in Table 13, of fresh Mankai™ in Table 14, of frozen Mankai™ in Table 15, and of dried Mankai™ in Table 16.

Table 13. Microbiological Analysis of Water and Growth Medium

	Tap Water[1]	Growth medium[2]
Total count (1 ml)	2	63000
Yeast (100 ml)		2
Mold (100 ml)		4
Coliforms (100 ml)	<1	<1
E. coli (100 ml)	<1	<1
Clostridium sulfite-reducing bacteria (50 ml)		1
Bacillus cereus (1 ml)		<50
Listeria monocytogenes (100 ml)		Negative
Salmonella (20 ml)		Negative
Staph. aureus (coag+, 100 ml)		<1
[1] Bactochem, Israel – COA 483237; [2] Bactochem, Israel – COA 482300		

The test results are based on the following methods of analysis for water and growth medium:

Microbial Characteristics	Method	Reference
Total count (1 ml)	SM 9215 (A+B)	Online version of Standard Methods for the Examination of Water and Wastewater. Approval year by Standard Methods, 2009
Yeast (100 ml)	SI 885 PART 7	Israel Standard for microbiology test methods for foodstuffs
Mold (100 ml)	SI 885 PART 8	Israel Standard for microbiology test methods for foodstuffs
Coliforms (100 ml)	SM 9222B	Standard Methods for the Examination of Water and Wastewater, 21st Edition 2005
E. coli (100 ml)	SM 9222G	Standard Methods 9222G: MF Partition procedures *Escherichia coli* Partition Methods
Clostridium sulfite reducing bacteria (100 ml)	SI 885 PART 9	The standard Institution of Israel, 1987
Bacillus cereus (1 ml)	FDA, 2003	Bacteriological Analytical Manual Chapter 14, 2001
Listeria monocytogenes (100 ml)	USDA/FSIS Chapter 8	Laboratory Guidebook revised 2017
Salmonella (20 ml)	SM 9260B	Standard Methods for the Examination of Water and Wastewater, 21st Edition 2005
Staph. aureus coag+, (100 ml)	SI 885 PART 6	Israel Standard for microbiology test methods for foodstuffs

Table 14. Microbiological Analysis of Fresh Mankai™*

Microbial Characteristics (SI 2202 part 2)	Lot LB1	Lot LB2	Lot XLB
Mold (1 g)	60	<10	30
E. coli (1 g)	<10	<10	<10
E. coli 0157 (25g)	Negative	Negative	Negative
Listeria monocytogenes (25g)	Negative	Negative	Negative
Salmonella (25g)	Negative	Negative	Negative
*In conformity with SI 2202 part 2 – Standard Israel; Analysis done by Milouda & Migal Merieux Nutrisciences, Israel: COA 17043027; 17043028; 17043030			

Table 15. Microbiological Analysis of Frozen Mankai™*

Microbial Characteristics (SI877)	Lot 2008061801/1	Lot 2008061801/2	Lot 2008091801/1	Lot 2008091801/2	Lot 2008131801
Enterobacteriaceae	100	30	350	110	130
Molds	<10	<10	60	<10	<10
E. coli	<10	<10	<10	<10	<10
E. coli O157	Negative	Negative	Negative	Negative	Negative
Salmonella	Negative	Negative	Negative	Negative	Negative
Bacillus cereus	<50	<50	<50	<50	<50
Listeria monocytogenes	Negative	Negative	Negative	Negative	Negative
* In conformity with SI 877 - Standard Israel; Analysis done by Bactochem, Israel: COA 480815					

Table 16. Microbiological Analysis of Dried Mankai™

Microbiology Analysis	Lot-911P[1]	Lot-68.7.1[2]	Test 2A (Lot 1002P)[3]	Test 3 (Lot 1003P)[3]	Test 4 (Lot 1004 P)[3]
Total count/1g	<10	75	90	40	55
Coliforms/1g	<10	<10	<10	<10	<10
Enterobacteriaceae/1g	<10	<10	<10	<10	<10
Molds/1g	25	<10	<10	<10	<10
Yeast/1g	<10	<10	<10	<10	<10
E. coli/1g	<10	<10	<10	<10	<10
Bacillus cereus/1g	<50	<50	<50	<50	<50
L. monocytogenes/25g	Negative	Negative	Negative	Negative	Negative
Salmonella/25g	Negative	Negative	Negative	Negative	Negative
Institute for Food Microbiology and Consumer Goods: [1]COA 17-123179; [2]COA 17-131895; [3]COA 17-130978					

2.7.5 Pesticides

No pesticides are used in the cultivation of Mankai ™, and since it is cultivated in off greenhouses away from other farms, using high-quality pesticide-free materials, no pesticide residues are expected in Mankai ™. To confirm this, dried samples of Mankai™ were tested for the possible presence of pesticide residues from the types, Organophosphorus, Organochlorine, Organic Nitrogen and Pyrethroid. Analyses were performed at Eurofins laboratory, USA (COA AR-14-QD-143943-01; COA AR-18-QD-146225-01; COA AR-18-QD-146226-01) using GC-MS and LC-MS and covered more than 240 pesticides and pesticide metabolites/degradation products. The results for all analytes were below the limits of detection.

2.7.6 Aflatoxin Screens

Dried samples of Mankai™ were tested by Milouda & Migal Laboratories - Merieux Nutrisciences: (COA SO17085614) for Aflatoxins B1, B2, G1, and G2 by HPLC. None was found at a detection limit of 0.25 µg/kg. Two additional lots were tested by Eurofins Laboratories. None was found at a detection limit of 5 µg/kg (Table 17).

Table 17. Aflatoxin Analysis of Dried Mankai™

Analyte	Lot 1003P[1]	Lot 2237P[2]	Lot 2241P[3]
Aflatoxin Profile	<1 µg/kg	<5 µg/kg	<5 µg/kg
Aflatoxin B1	<0.25 µg/kg	<5 µg/kg	<5 µg/kg
Aflatoxin B2	<0.25 µg/kg	<5 µg/kg	<5 µg/kg
Aflatoxin G1	<0.25 µg/kg	<5 µg/kg	<5 µg/kg
Aflatoxin G2	<0.25 µg/kg	<5 µg/kg	<5 µg/kg
[1] Milouda & Migal Laboratories - Merieux Nutrisciences: (COA SO17085614); [2] Eurofins laboratories, USA – COA AR-18QD-154688-01; [3] Eurofins laboratories, USA – COA AR-18QD-154689-01;			

2.7.7 Residual Culture Media Components

Hinoman analyzed four batches of dried Mankai™ for residual culture media components, intentionally added or not. No residuals were detected for either nitrite or EDTA. The amounts reported for nitrate are lower than those reported for other vegetables like spinach or lettuce (EFSA 2008). The results are shown in Table 18.

Table 18. Residual Culture Media Components in Two Batches of Dried Mankai™ (mg/kg) *

Analyte	Lot 4B456082014	Lot 4c14122014	Lot 2237P	Lot 2241P
Nitrite	<50	<20	<1(as N)	<1(as N)
EDTA	<500	Not done	<380	<380
*Eurofins laboratory, USA: COA AR-14-QD-143943-01& AR-15-QD-030442-01; AR-18-QD-154688-01; AR-18-QD-154689-01				

Analyte	Lot 2237P	Lot 2241P	Lot D20190114	Lot D20190408
Nitrate	85	200	870	1300
*Eurofins laboratory, USA: AR-18-QD-154688-01; AR-18-QD-154689-01; AR-19-QD-049820-01; AR-19-QD-099344-01				

2.7.8 Protein Digestibility Corrected Amino Acid Score (PDCAAS)

A sample of dried Mankai™ was evaluated for protein quality by PDCAAS, which is a method of evaluating the protein quality of foods based upon both the amino acid requirements of humans and their ability to digest it.[2] The test was performed *in vivo* at Product Safety Lab, USA on a sample called in the report, "Asian water meal" (Lot 4C14122014). The control used in the test was casein. The results are presented in Table 19.

Table 19. AAS and PDCAAS for dried Mankai™

Amino Acids (mg/g protein)	Reference pattern-children (6 mo-3 yr) [1]	Mankai™ Amino Acid Profile [2]
Histidine	20.00	20.03
Isoleucine	32.00	37.61
Leucine	66.00	72.32
Lysine	57.00	59.86
Methionine + Cystine	27.00	28.26
Phenylalanine + Tyrosine	52.00	81.00
Threonine	31.00	40.50
Tryptophan	8.50	20.92
Valine	43.00	49.18
AAS		1.002
True Digestibility %		89
PDCAAS %		89
[1] FAO (2013) Dietary protein quality evaluation in human nutrition: FAO Food and Nutrition Paper 92 [2] Eurofins COA AR-15-QD-026803-01/2015		

[2] Protein quality evaluation. Report of Joint FAO/WHO Expert Consultation, Bethesda, MD, 4-8 December 1989. FAO Food and Nutrition Paper 51. FAO, Rome, 1991. Available at http://apps.who.int/iris/bitstream/handle/10665/38133/9251030979_eng.pdf;jsessionid=D7EF384D9EA1E156B5F740389B116939?sequence=1

2.8 Product Varieties and Specifications

All Hinoman products are produced from Hinoman *Wolffia globosa* 'Mankai'- a registered strain US Patent No. PP29977 (Patent application No. 15/330,249).

Fresh Mankai™ – fresh *Wolffia globosa* Mankai. The product has a green to light green color with a natural characteristic odor and has up to 96% water content. Specifications for Fresh Mankai™ (US Plant Patent PP29977) are shown in Figure 11.

Figure 11. Specifications for Fresh Mankai™:

MANKAI™ M310

Product Specifications

Description	Fresh Mankai™
Ingredients	100% fresh Mankai™
Source	*Wolffia globosa* 'Mankai' – a registered strain

Analysis	**Specification**	**Method**
Description	Fresh green biomass	Visual Analysis
Flavor	Fresh Mankai flavor with no off-flavor	Sensory Analysis
Protein	Min 2%	Kjeldahl method
Fibers	Min 1.2%	AOAC 991.43
Fat	Min 0.35%	AOAC 996.06
Ash	Min 0.7%	Residue on Ignition
Water	Max 95%	Moisture Analyzer
Arsenic	Max 0.1 ppm	ICP-MS
Lead	Max 0.3 ppm	ICP-MS
Mercury	Max 0.1 ppm	ICP-MS
Cadmium	Max 0.2 ppm	ICP-MS
Molds	Max 10^2 cfu*/g	SI 885 part 8/FDA-BAM
E. coli	Max10 cfu/g	SI 885 part 12/ISO 16649-2
E. coli O157:H7	Negative/25g	FDA-BAM Chapter 4A
Salmonella	Negative/25 g	SI 885 part 7/ISO 6579
Listeria monocytogenes	Negative/25g	ISO11290-1/PCR-BAX-RT

*Colony forming unit

Packaging and storage: 250g and 500g in food grade sealed box. Keep refrigerated.

Dried Mankai™ – made from *Wolffia globosa* 'Mankai' is a green powder with a natural characteristic odor, free of additives or excipients. Specifications for Dried Mankai™ are shown in Figure 12.

Figure 12. Specifications for Dried Mankai™:

MANKAI™ D110

Product Specifications

Description: Free flowing Mankai™ powder

Ingredients: 100% Mankai™

Source: *Wolffia globosa* 'Mankai' – a registered strain

Analysis	Specification	Method
Description	Dried green powder	Visual Analysis
Flavor	Fresh dry Mankai flavor with no off-flavor	Sensory Analysis
pH	6.5±1	pH-meter (glass electrode)
Moisture	Max 5%	Moisture Analyzer
Arsenic	Max 1 ppm	ICP-MS
Lead	Max 1.5 ppm	ICP-MS
Mercury	Max 0.15 ppm	ICP-MS
Cadmium	Max 0.6 ppm	ICP-MS
Total Aflatoxins	Max. 10 mcg/kg	HPLC
Total Plate Count	Max 10^4 cfu* /g	SI 885 part 3/FDA-BAM
E. coli	Max 10 cfu/g	SI 885 part 12/ISO 16649-2
Coliforms	Max 10^2 cfu/g	SI 885 part 4/AOAC
Mold	Max 10^2 cfu/g	SI 885 part 8/FDA-BAM
Yeast	Max 10^2 cfu/g	SI 885 part 8/FDA-BAM
Salmonella	Negative/25g	SI 885 part 7/ISO 6579

* Colony Forming Unit

Packaging and storage: 5, 10 kg in Food Grade Laminated Modified Atmosphere. Keep closed under dark conditions at ambient temperature.

Frozen Mankai™ – made from partially mechanically dehydrated *Wolffia globosa* 'Mankai' up to 35% dried matter, frozen at approximately -18°C. Specifications for Frozen Mankai™ are shown in Figure 13.

Figure 13. Specifications for Frozen Mankai™:

MANKAI™ F210

Product Specifications

Description Frozen Mankai™

Ingredient: 100% Mankai™

Source: *Wolffia globosa* 'Mankai' – a registered strain

Analysis	Specification	Method
Description	Frozen green plant	Visual Analysis
Flavor	Mankai flavor with no off-flavor	Sensory Analysis
Water	Max 90%	Moisture Analyzer
Arsenic	Max 0.1 ppm	ICP-MS
Lead	Max 0.3 ppm	ICP-MS
Mercury	Max 0.1 ppm	ICP-MS
Cadmium	Max 0.2 ppm	ICP-MS
Enterobacteriaceae	Max 10^3 cfu*/g	ISO 21528-2:2004
Molds	Max 10^3 cfu/g	SI 885 part 8/FDA-BAM
E. coli	Max 10 cfu/g	FDA BAM Chapter 4
E. coli O157	Negative/25g	ISO 16654:2001
Salmonella	Negative/25	SI 885 part 7/ISO 6579
Listeria monocytogenes	Negative/25g	ISO 11290
Bacillus cereus	Max 10^3 cfu/g	FDA BAM Chapter 14

*Colony Forming Unit

Packaging: Mankai™ F210 is packaged in food grade plastic box.

Storage Keep frozen in the original unopened package at 0°F.

3. DIETARY EXPOSURE

3.1 Introduction

Mankai™ is intended to be marketed for human consumption as a food ingredient in fresh, dried and frozen forms. As demonstrated in Section 2.3.3, it is high in protein, carbohydrate, dietary fiber and several vitamins, and low in fat, making it a desirable source of nutrition.

3.2 Fresh Product

It is intended that fresh Mankai™ will be added in a fresh state to shakes, juices, salads, veggie patties, bakery goods, beverages, meal replacements, pasta, noodles and other foods. It is also intended that the fresh plant material will be supplied to bakeries which will integrate it into baked goods and by other food makers into other dough products including spaghetti, grissini, pizza dough and the like.

3.3 Frozen Product

The frozen state is targeted as a base for vegetable and fruit Ready-to-Drink (RDT) shakes, or for the preparation of veggie patties and other foods as listed for the fresh product category above.

3.4 Dried Powder

It is intended that dried Mankai™ will be available in powder form to be added by food manufacturers and by the consumer to foods and beverages such as baked goods, shakes, powder beverages and powder shakes, meal replacement, veggie patties, or to other foods as desired, as a food ingredient. Dried Mankai™ is intended also as a water-dispersible Mankai™ and will be available in powder form to be added by the consumer to foods and beverages such as nutritional and juice-based shakes, DBB, powder beverages, powder shakes and fruit drinks.

3.5 Estimate of Dietary Exposure to the Notified Substance

Exposure to Mankai™ may come from consumption of the fresh, frozen, or dried product and addition of the product to food, as described above, or may result from consumption of food products into which Mankai™ has been incorporated by food manufacturers

To estimate dietary exposure to Mankai™, information on the addition level of Mankai™ (normalized to the dried form) planned for inclusion in various food categories (as defined in 21 CFR 170.3(n)) in which Mankai™ may be used (Table 20) was combined with data from the most recent National Health and Nutrition Examination Survey (NHANES) data (from surveys conducted in 2013-2014 and 2015-2016) of consumption of these various food categories. To avoid underestimation, consumption was calculated for each subcategory for consumers of that subcategory only. The overall total consumption from all categories was calculated by summing over all categories for each individual who consumed any one or more of the individual subcategories and taking the mean. Hence, the NHANES sample size for total consumption (15,979) is larger than for any of the individual subcategories, as some individuals consumed some of the subcategories, but not others. Based on this information, the mean intake of Mankai™ (expressed as the dried form) is presented in Table 21. As shown there, the mean Mankai™ consumption by consumers from all food categories is estimated as 58.8 g/day of dried Mankai™ (95% solids), equivalent to 1117 g/day of fresh Mankai™ (5% solids) or 466 g/day of frozen Mankai™ (typically 12% solids). The vast majority of this (43.8 g/day) is estimated to come from noncarbonated beverages. This is likely to be an overestimate, since it assumes that individuals will consume more than 1 liter (or 33.8 fluid oz.) of such beverages containing Mankai™ every day. It is more likely that consumers will consume just one such beverage (12 oz, or 360 ml) per day, thus reducing the likely intake of dried Mankai™ to about 14.4 g/day, equivalent to 114 g/day frozen, or 274 g/day fresh Mankai™. By comparison, consumption of Mankai™ in the 1.5-year clinical study, described in Section 6.3.2, was 100 g/day of frozen Mankai™ (having 20% solids) with no health hazards reported.

While the calculation of the estimated intake presented below is based on commercial foods into which the Mankai™ is incorporated, exposure of consumers who add the Mankai™ directly into their own foods will be similar, based on labeling.

Table 20. Proposed Food Category Uses for Mankai™

Baked goods & baking mixes	Baked goods and baking mixes, including all ready-to-eat and ready-to-bake products, flours, and mixes requiring preparation before serving.
Beverages & Beverage Bases	Non-alcoholic, including special or spiced teas, soft drinks, coffee substitutes, and fruit- and vegetable-flavored gelatin drinks
Breakfast Cereals	Ready-to-eat and instant and regular hot cereals
Condiments and relishes	including plain seasoning sauces and spreads, olives, pickles, and relishes, but not spices or herbs
Dairy product analogs	including nondairy milk, frozen or liquid creamers, coffee whiteners, toppings, and other nondairy products
Fresh Fruit & Fruit Juices	Raw fruits, citrus, melons, and berries, and home-prepared "ades" and punches made therefrom.
Frozen Dairy Desserts & Mixes	Ice cream, ice milks, sherbets, and other frozen dairy desserts & specialties.
Grain Products & Pasta	Macaroni and noodle products, rice dishes, and frozen multicourse meals, without meat or vegetables.
Processed Vegetable & Vegetable Juices	Commercially processed vegetables, vegetable dishes, frozen multicourse vegetable meals, and vegetable juices and blends
Snack Foods	Chips, pretzels, and other novelty snacks.
Soups & Soup Mixes	Commercially prepared meat, fish, poultry, vegetable, and combination soups and soup mixes.

Table 21. Dietary Intake Calculation for Hinoman's Mankai™

Food Category (21 CFR 170.3)	USDA Product Sub-Category	Mankai ™ Use Level (% as dried)	NHANES Sample Size	USDA Mean Grams of food consumed (consumers only)	Mean Grams Mankai™ consumed (consumers only)
Baked goods & baking mixes			14278	101.7	3.1
	Biscuits, croissants, tortillas	3%	2932	72.3	2.2
	Breads	3%	12772	87.6	2.6
	Cookies	3%	5065	53.2	1.6
	Grain-based bars	3%	1353	45.9	1.4
Beverages & Beverage Bases	Noncarbonated beverages	4%	12754	1094.9	43.8
Breakfast Cereals	Breakfast cereals	3%	6625	93.2	2.8
Condiments and relishes	Minor main entree sauces (e.g., pizza sauce, pesto sauce, Alfredo sauce), other sauces used as toppings	3%	10607	58.4	1.8
Dairy product analogs			9956	325.0	9.8
	Milk, milk substitute, and fruit juice concentrates (without alcohol) (e.g., drink mixers, frozen fruit juice concentrate, sweetened cocoa powder)	3%	9703	313.1	9.4
	Shakes or shake substitutes, e.g., dairy shake mixes, fruit frost mixes	3%	646	393.7	11.8
Fresh Fruit & Fruit Juices			13293	325.5	13.1
	Shakes	5%	593	431.6	21.6
	Juices, nectars, fruit drinks	4%	8383	341.8	13.7
	Fruits used primarily as ingredients, others	4%	10702	184.7	7.4
Frozen Dairy Desserts & Mixes	Ice cream, frozen yogurt, sherbet, frozen flavored and sweetened ice and pops, frozen fruit juices	5%	3874	130.3	6.5
Grain Products & Pasta	Pasta Ready to eat	3%	4768	138.7	4.2
Processed Vegetable & Vegetable Juices			10472	137.8	4.1
	All other vegetables without sauce: Fresh, canned, or frozen	3%	10377	130.6	3.9
	Vegetable juice	3%	378	292.4	8.8
Snack Foods	All varieties, chips, pretzels, popcorn, extruded snacks, fruit and vegetable-based snacks (e.g., fruit chips), grain-based snack mixes	3%	9654	43.6	1.3
Soups & Soup Mixes	All varieties	4%	3641	378.4	15.1
Dressings (Added Category)	All varieties	3%	4497	34.2	1.0
Total (calculated on the individual level)			15979	1591.7	**58.8**

4. SELF-LIMITING LEVELS OF USE

Not Relevant.

5. HISTORY OF COMMON USE IN FOOD

Wolffia grown in Burma, Laos and northern Thailand has been used as a vegetable "for many generations" (Bhanthumnavin and McGarry 1971). The local Thai name for the plant is "Khai-nam" which literally translates as "eggs of the water" (Figure 14).

The species in this region was originally described as *Wolffia arrhiza* but is now thought to be *Wolffia globosa;* Professor Armstrong reported after examining a sample of "khai-nam" from Thailand that it appeared to be *Wollfia globosa* rather than *Wolffia arrhiza* (Armstrong; Landolt 1980) although there is often confusion between the two as also reported by Landolt (2014b).

Khai-nam grows on the surface of the water forming a thick mass of yellowish-green. The plant is cultivated locally in rain-fed open ponds which are shaded by bamboo groves. The plant is also grown commercially in Thailand. In northern Thailand, the edible vegetable is harvested between November and July. During this time the plant reproduces via budding, with a generation time of approximately 4 days.

Figure 14. Harvesting *Khai-nam* in Thailand

A survey conducted by Siripahanakul and colleagues on *Wolffia* consumption in the Loei province of Thailand found that the locals still consume *Wolffia* in traditional ways, i.e. as an ingredient in spicy soup and spicy salad (Siripahanakul et al. 2013). The survey indicated that there was a high demand of *Wolffia* incorporated in other local foods, especially *Wolffia*-pork ball and fermented *Wolffia*-pork sausage. Other dishes mentioned in the survey were *Wolffia* rice noodle, *Wolffia* crisps, *Wolffia* cookies and *Wolffia* bread.

Shirai & Rambo (2014) presented findings of a survey from 2006 of wild species of plants, fungi, and animals sold in the main urban market in Khon Kaen Municipality in Thailand. *Wolffia globosa* was one of the species listed in the inventory of edible species sold at the market.

Wolffia globosa known locally in northeastern Thailand as "pum," is the main ingredient in "kaeng pum", a popular vegetable dish in that area (Tangkanakul et al. 2006).

A treatise on the edible aquatic plants of northern Thailand by Buri (1978) lists *Wolffia* as a food source for this region. He notes that fish farmers who have not been entirely successful with farming Tilapia have turned to growing *Wolffia* in their fishponds. The author offers two recipes: "Fried *Wolffia* with curry paste" and "Spirogyra (a freshwater alga) and *Wolffia* salad."

The Japan International Research Center for Agricultural Sciences includes a listing for *Wolffia globosa* on their web site under the category of Local Vegetables of Thailand (JIRKAS 2010). The listing says that the plant is normally collected from natural waterways for home consumption and is occasionally seen in local markets throughout Thailand and Laos. In Thailand, *Wolffia* is cooked in

curries with minced pork or chicken. *Wolffia* species are also listed as perennial vegetable, which is defined as a vegetable not destroyed by harvesting, in a web site, CD and book dedicated to permaculture (Toensmeier 2013). In addition, an encyclopedia of edible plants published in California includes *Wolffia globosa* (Facciola 1998).

The previously described USDA (2019) GRIN database also lists *Wolffia globosa* as human and animal food.

A database dedicated to tropical species lists *Wolffia globosa* as having an excellent flavor, tasting somewhat like a sweet cabbage (Toensmeier 2013). It was rated as category 4 for taste on an international scale of 1-5, with 5 being excellent. In addition, it was described as very nutritious, containing about 20% protein, 44% carbohydrate, 5% fat and are rich in vitamins A, B2, B6, C and nicotinic acid.

Wayne Armstrong, on his comprehensive and authoritative website, shows pictures of potential menu items: *Wolffia columbiana* and *W. borealis* baked into muffins, as a spread in a sandwich with tomato, as a dip for potato chips and as a topping for apple pie.[3]

The latest publication to support prior use of Mankai™ in SouthEast Asia was published in 2017 by Prof. Appenroth and colleagues (2017), where it is reported that:

> "For thousands of years, duckweed species have been on the menu in Asian countries such as Thailand, Cambodia and Laos."

In particular, Prof. Appenroth singles out the species, *Wolffia globosa*, which is served in Asia in the form of soup, as a vegetable or in omelets. In the latest tests by his research group, *Wolffia globosa* showed itself to be the most promising as a human food source.

[3] https://www2.palomar.edu/users/warmstrong/1wayindx.htm and associated web pages.

6. NARRATIVE

6.1 Safety Based on Composition

As described in detail in Section 2.4, above, the composition of Mankai™ reveals a highly nutritious product with no significant deficiencies. On a dry weight basis, it contains about 43% protein, 37% carbohydrate, less than 10% fat, with most of the fat in the form of polyunsaturated fat (Table 6), and about 35% dietary fiber (Table 3). Notably, the amino acid composition of the protein is of high quality, with adequate levels of the essential amino acids, lysine, methionine, and threonine (Table 4), which are sometimes limited in proteins of plant origin. Leng (1995) stated that "duckweed protein has a better array of essential amino acids than most vegetable proteins and more closely resembles animal protein."

6.1.1 Safety of Protein Content of Mankai™

Human dietary proteins are typically derived from animal, vegetable, and to a lesser extent microbialsources. The Institute of Medicine (IOM 2005) has derived a Recommended Dietary Allowance (RDA) for protein for men and women of "0.80 g of good quality protein/kg body weight/d." IOM (2005) also notes that the recommended protein digestibility corrected amino acid scoring pattern (PDCAAS) for proteins for children 1 year of age and older and all older age groups is as follows (in mg/g of protein): isoleucine, 25; leucine, 55; lysine, 51, methionine + cysteine (SAA), 25; phenylalanine + tyrosine, 47; threonine, 27; tryptophan, 7; valine, 32; and histidine, 18. As shown in Table 19, the amino acid profile of Mankai exceeds these values for all of these amino acids, and shows a very high digestibility (89%).

IOM (2005) also recommended an upper range for total protein in the diet as a percent of total energy intake of no more than 35 percent to decrease risk of chronic disease, but noted that there were insufficient data to provide dose–response relationships to establish a Tolerable Upper Intake Level (UL) for total protein or for any of the amino acids. IOM did, however, report that when more than 45% of calories were provided by protein, subjects developed weakness, nausea, and diarrhea, which was resolved when the dietary protein content was reduced to 20 to 25 percent of calories. This is likely related to the condition known as "rabbit starvation" by early American explorers, as rabbit meat contains very little fat, which can result in death after several weeks of consumption of a diet containing more than 45% of calories from protein, with very little fat (IOM 2005). In addition, some investigators such as Eisenstein et al. (2002) consider that the consumption of protein more than two to three times the US RDA (i.e., more than 20-30% of energy intake) contributes to bone loss and urinary calcium loss, and individuals who are predisposed to kidney stones or kidney disease should use caution with high protein intake.

While dried Mankai™ does contain a high level of protein, it would not represent the sole source of nutrients. As discussed in Section 3, its intended uses are as components, added at 1-3% by weight to various food products. This would not result in excessive protein intake.

The safety of products containing high levels of proteins is also documented by the issuance by FDA of "no questions" letters for 28 GRAS notices regarding a wide variety of protein preparations listed in Table 22. These notices document the safety of proteins from a wide variety of sources.

Table 22. FDA "No Questions" GRAS Notices for Protein Preparations

GRAS Notice	Product
GRN 026	Isolated wheat protein
GRN 037	Whey protein isolate and dairy product solids
GRN 086	Coagulated potato protein, hydrolyzed potato protein, or clarified hydrolyzed potato protein
GRN 091	Mycoprotein
GRN 134	Soy protein hydrolyzate with enzyme-modified lecithin
GRN 147	Extracted "*seafood species*" protein

GRN 168	Poultry protein
GRN 182	Hydrolyzed wheat gluten isolate; pea protein isolate
GRN 196	Bovine milk basic protein fraction
GRN 199	Concentrated hydrolyzed milk protein
GRN 284	Baker's yeast mannoprotein
GRN 313	Beef protein
GRN 314	Pork protein
GRN 327	Cruciferin-rich canola/rapeseed protein isolate and napin-rich canola/rapeseed protein isolate
GRN 360	Hydrolyzed sardine protein
GRN 386	Canola protein isolate and hydrolyzed canola protein isolate
GRN 447	Potato protein isolates
GRN 504	Milk protein concentrate and milk protein isolate
GRN 519	*Chlorella protothecoides* strain S106 flour with 40-75% protein
GRN 575	Oat protein
GRN 581	Pea protein
GRN 608	Pea protein concentrate (80-90% protein)
GRN 609	Rice protein
GRN 633	Concentrated milk protein
GRN 644	Non-fat dry goat milk and goat whey protein
GRN 683	Canola protein isolate
GRN 684	Mung bean protein isolate
GRN 742	Duckweed (subfamily Lemnoideae) powder
GRN 771	Hemp seed protein
GRN 788	Pea protein concentrate

Similarly, FDA has issued "no questions" letters for GRNs 127, 351, 394, 417, 469, 519, and 773 related to several aquatic microalgae products (*Spirulina* and various green algae) and for GRN 742 for Duckweed (subfamily *Lemnoideae*) powder, that contain high levels of protein. The protein content of these GRAS substances, derived from aquatic plants and algae, is summarized in Table 23.

Table 23. Protein Content of GRAS Substances Derived from Aquatic Food Sources

GRN #	Substance	Protein Content (%)
127	Sprulina (*Arthrospira platensis*) powder	53-62%
351	*Dunaliella bardawil* powder	22-24%
394	Sprulina (*Arthrospira platensis*) powder	≥ 60%
417	Sprulina (*Arthrospira platensis*) powder	56-69%
469	*Chlorella prothecoides* flour	4.4-7.1%
519	*Chlorella prothecoides* algal protein	50.0-56.4%
773	*Chlamydomonas rheinhardtii* dried powder	30-70%
742	Duckweed (subfamily *Lemnoideae*) powder	40-50%

All of these GRAS notices support the safety of products containing levels of protein as high as, or higher than Mankai™. In particular, GRN 742, which covers a mixture of species of duckweed, including *Wolffia globosa,* supports the safety of the protein content of Mankai™.

6.1.2 Safety of Dietary Fiber Content of Mankai™

As noted in Table 3 (Section 2.4), Mankai™ contains about 35% dietary fiber (on a dry-weight basis). This material conforms to the Institute of Medicine's definition of Dietary Fiber (IOM 2005) – "nondigestible carbohydrates and lignin that are intrinsic and intact in plants." The dietary fiber content of Mankai™ is comparable, on a dry-weight basis, to that of a variety of commonly eaten fruits and vegetables. Table 24 shows data on components of dietary fiber in a variety of commonly consumed vegetables, fruits, and grains that are all clearly safe to consume (Holtzapple 2003). While the proportions of cellulose, hemicellulose, and lignin vary from species to species, these variations do not affect their safety.

Table 24. Dietary Fiber Content of Vegetables, Fruits, and Grains (g/100 g of dry matter)

Food	Cellulose	Lignin	Hemicellulose	Pectin	Total Fiber
Vegetables, leafy					
Broccoli	7.2	0.26	24	–	31.5
Brussels sprouts	9.04	2.1	26	–	37
Cabbage	8.9	4.3	26	–	39
Cauliflower	13.4	Tr*	13	–	26
Lettuce	20.6	Tr	9.2	–	30
Vegetables, legumes					
Beans, haricot	5.3	0.9	22.0	–	28
Beans, runner	17	3	21	–	41
Peas	14	2	36	–	52
Vegetables, root					
Carrot	12.9	Tr	19	–	32
Turnip	11	Tr	23	–	34
Fruiting vegetables					
Pepper	3.5	Tr	10	–	14
Tomato	9.1	5.3	11	–	25
Vegetables, Tuber					
Potato	1.2	Tr	9.2	–	10
Fruits					
Apples	2.9	Tr	5.8	2.3	11
Apricots	—15—			3.3	18
Banana	1.3	0.93	3.83	–	6
Blackberries	—44—			1.9	46
Cherries, sweet	1.2	0.3	4.5	0.4	6
Grapefruit	0.6	0.9	4.9	–	6
Lemons	—35—			3.4	38
Oranges	—14—			3.3	17
Peaches	1.8	5.1	12.2	3.3	22
Pears	4.2	2.7	8.2	–	15
Pineapples	—7.64—			0.25	8
Strawberries	3.6	8.4	10	3.5	25
Seeds					
Barley	—5.3—			–	5
Corn	—2.4—			–	2
Grain sorghum	—2.7—		2.5	–	5
Oats	—11.9—			–	12
Peanut	—2.8—		2.5	–	5
Wheat	—2.1—		–	–	2
*Tr = trace					

The safety of dietary fiber of this sort, particularly cellulose and hemicellulose, is well documented (IOM 2005; Anderson et al. 1992; LSRO 1973). Moreover, FDA (2018b) has recently reviewed the safety and beneficial effects of a variety of dietary fibers, including cellulose and hemicellulose. The fiber component of Mankai™ is likely to be similar in safety to those materials.

6.1.3 Safety of Heavy Metal Content, Microbiological Content and Other Potential Contaminants

As noted in Section 2.3, for Mankai™ production, Hinoman uses high-quality tap water and deionized water that is subjected to regular QC testing to ensure the absence of potentially hazardous contaminants. Hinoman also routinely monitors the Mankai™ for heavy metal content using inductively coupled plasma mass spectrometry (ICP-MS) analysis (Table 12). All analyses show low levels of heavy metals, well below levels that might be of concern. The average concentrations of cadmium, lead, mercury, and arsenic found in these samples were 0.03 mg/kg cadmium, 0.386 mg/kg lead, <0.012 mg/kg mercury, and 0.091 mg/kg arsenic, all on a dry-weight basis. Based on the water content of the fresh product, these concentrations correspond to concentrations of 0.0015, 0.019, <0.0006, and 0.0045 mg/kg, respectively, on a whole-commodity basis. By comparison, Codex Alimentarius (2015) standards for these metals in whole-commodity vegetables (where they exist) are orders of magnitude higher. For example, the Codex standard for cadmium in whole-commodity leafy vegetables is 0.2 mg/kg, more than 100-times higher than the average in Mankai™, while that for lead is 0.3 mg/kg, more than 15-times higher than in Mankai™.

Microbiological analysis of tap water and growth medium, and of samples of fresh and frozen Mankai™ demonstrate an absence of significant levels of potentially harmful microorganisms (Tables 13, 14, 15, and 16).

No pesticides are used in the cultivation of Mankai™, and since it is cultivated in closed controlled greenhouses away from other farms, using high-quality pesticide-free materials, no pesticide residues are expected in Mankai™. To confirm this, dried samples of Mankai™ were tested for the possible presence of pesticide residues from the types: Organophosphorus, Organochlorine, Organic Nitrogen, and Pyrethroid. Analyses were performed at Eurofins laboratory, USA (COA AR-14-QD-143943-01; COA AR-18-QD-146225-01; COA AR-18-QD-146226-01) using GC-MS and LC-MS and covered more than 240 pesticides and pesticide metabolites/degradation products. The results for all analytes were below the limits of detection.

Testing of dried samples of Mankai™ for aflatoxins B1, B2, G1, and G2 similarly revealed no detectable aflatoxins (Table 17). Similarly, testing for Microcystins and Cylindrospermopsin showed none were present above the detection limit of 10 ng (Table 11)

6.1.4 Potential Allergenicity Issues

Food allergies affect a small but significant portion of the population. Food allergens are generally naturally occurring proteins present in the food (Taylor & Baumert 2012). Eight foods or food groups – milk, eggs, fish, crustacea (shrimp, crab, lobster, etc.), peanuts, soybeans, tree nuts, and wheat – are responsible for the vast majority of food allergies worldwide (Taylor & Baumert 2012). Leafy green vegetables, like duckweed, are not generally allergenic.

It is important to clarify that Wolffia globosa (erroneously identified in 1971 as Wolffia arrhiza [Bhanthumnavin and McGarry 1971] [see Landolt & Kandler 1987, page 382]) has served as a traditional food called khai-nam for many generations in Burma, Laos and northern Thailand. Moreover, due to its neutral taste and high concentration of protein in the dried plant, *Wolffia globosa* makes up a major part of the final product in a number of popular spicy dishes; for example, in kaeng pum, a Thai curry dish, 42% (Tangkanakul et al. 2006), and in wolffia-pork sausages, 16% (Siripahanakul et al. 2013). In these publications, which analyzed several different parameters such as color, antioxidant capacity, phenolic content (gallic acid equivalent), protein, fat, total dietary fiber and energy content, and in all other local Southeast Asian publications that we are aware of dealing with duckweed, there is no mention of allergenicity issues.

Equally important, a word search in Elias Landolt's comprehensive 2-volume monograph on duckweeds (Landolt 1986, Landolt & Kandler 1987) did not reveal any hits for the terms "allerg.." or "allogen.." in the more than 1000 pages of the monograph's text. Landolt writes in his Introduction to volume 1: "The aim of this monograph is to give a survey of the present status of knowledge on

Lemnaceae. All characteristics and all possible aspects should be presented and discussed... morphology, ecology, geographical distribution, taxonomy, nomenclature... phytochemistry, physiology, and application." Along with this, the monograph's bibliography has been acclaimed for its completeness (Edelman 2015).

Finally, in this vein, van der Spiegel et al. (2013) in a recent, well-received review on the safety of novel protein sources, including duckweed, and legislative aspects for their application in food and feed production, conspicuously omit duckweed from their discussion of allergenicity issues. The lack of any mention of allergenicity concerns for duckweed protein in this review mirrors the lack of reports of allergy issues concerning duckweeds in the scientific literature.

Overlaid on all the above, production of *Wolffia globosa* 'Mankai' by Hinoman is exclusively vegetative and genetically stable. Mankai™ is grown solely in designated basins, isolated from any other crop from the growth stage, through processing up to the final dried product.

Furthermore, we would like to state that we are aware of the fact that while the experience in southeast Asia offers some confidence that duckweed meal or duckweed protein is not a potent allergenic food, the fact remains that almost all foods might have some allergenic potential. Therefore, Hinoman intends to use the Mankai name on the label of foods containing this ingredient to assure that if any allergy issue arises in the future that consumers will be able to recognize the source of the protein to avoid any consumption. This labeling approach is consistent with regulation such as the FDA stance on using the ingredient label of foods to inform allergic consumers to potential hazards.

In summary, we find no mention in the scientific literature of allergenicity issues with duckweeds and specifically with *Wolffia globosa*, while vegetative growth of *Wolffia globosa var. Mankai*, the procedure employed exclusively by Hinoman, further separates the commercial product from any allogenic concerns. Based on this, we believe that there is no evidence to suspect an allergenicity issue with Mankai.

6.1.5 Safety of Oxalic Acid Content

Like many vegetables commonly eaten in the United States, duckweed can contain oxalic acid (Table 9), which is the only identified compound produced by duckweed species that might be toxic to animals at high levels (Stomp 2005). Excessive intake of oxalic acid, mainly in the form of calcium oxalate, is associated with the formation of kidney stones (OHF. No Date). As shown in Table 9, analysis of nine lots of dried Mankai™ showed an average concentration of 0.42 g/100 g oxalic acid, while fresh Mankai™ contained an average of 0.05 g/100 g oxalic acid.

Some foods have more oxalic acid than others and several have oxalic acid levels higher than Mankai™ (on an edible portion (fresh) basis), including commonly eaten vegetables such as spinach (0.97 g/100 g), chives (1.48 g/100 g), beet leaves (0.61g/100 g), carrots (0.5 g/100 g) and parsley (1.70 g/100 g) (USDA 1984). Even potatoes contain similar amounts of oxalic acid as fresh Mankai™ (0.05 g/100 g) (USDA 1984). More recently, Mou (2008) analyzed oxalate concentrations in 64 spinach genotype samples, reporting concentrations ranging from 5.3 to 11.6 g/100 g dry weight, more than ten-times higher than in Mankai™.

Additionally, the oxalic acid in Mankai appears as a soluble acid form with no evidence for the presence of calcium oxalate crystals. As noted by Landolt and Kandeler (1987), while in *Lemna* and *Spirodela* genera the calcium oxalate crystals form is dominant, in *Wolffia* genus a soluble form is produced, making *Wolffia* more suited for human consumption (Landolt and Kandeler 1987). Nevertheless, even if present, calcium oxalate crystals can easily be broken down by thoroughly cooking or drying the plant (Tropical Plants Database).

Oxalate synthesis in duckweed is amenable to some control by manipulating physical (temperature, light and CO_2 (Landolt and Kandeler 1987)) and chemical (low manganese and calcium (Landolt and Kandeler 1987; Franceschi 1989)) growth conditions.

A recent publication by Lapidot et al (2020) discusses the chemical nature and levels of oxalic acid in various plant species, particularly duckweeds. In some species of duckweed, specifically *Lemna minor* and *Spirodela polyrhiza*, oxalic acid is found in the form of needle-shaped crystals (raphides)

of calcium oxalate (Mazen et al. 2003; Franceschi 1989; Landolt 1986; Saito 1996). Lapidot et al. (2020) conducted scanning electron microscopy (SEM) combined with analytical electron microscopy analysis (Raman, 2014) to evaluate the presence of oxalate crystals in powdered duckweed species, examining a mixed duckweed species (mainly *Lemna sp.* and *Spirodela sp.*) and in the Mankai™ strain (*Wolffia globosa* 'Mankai').

Analysis of a mix of duckweed species, rather than *Wolffia globosa* by itself, revealed the presence of many raphide crystals, and elemental composition analysis of these crystals showed that they were composed of calcium oxalate (Figure 15). In contrast, a pure culture of *Wolffia globosa* 'Mankai' examined for raphides revealed no such phenomena.

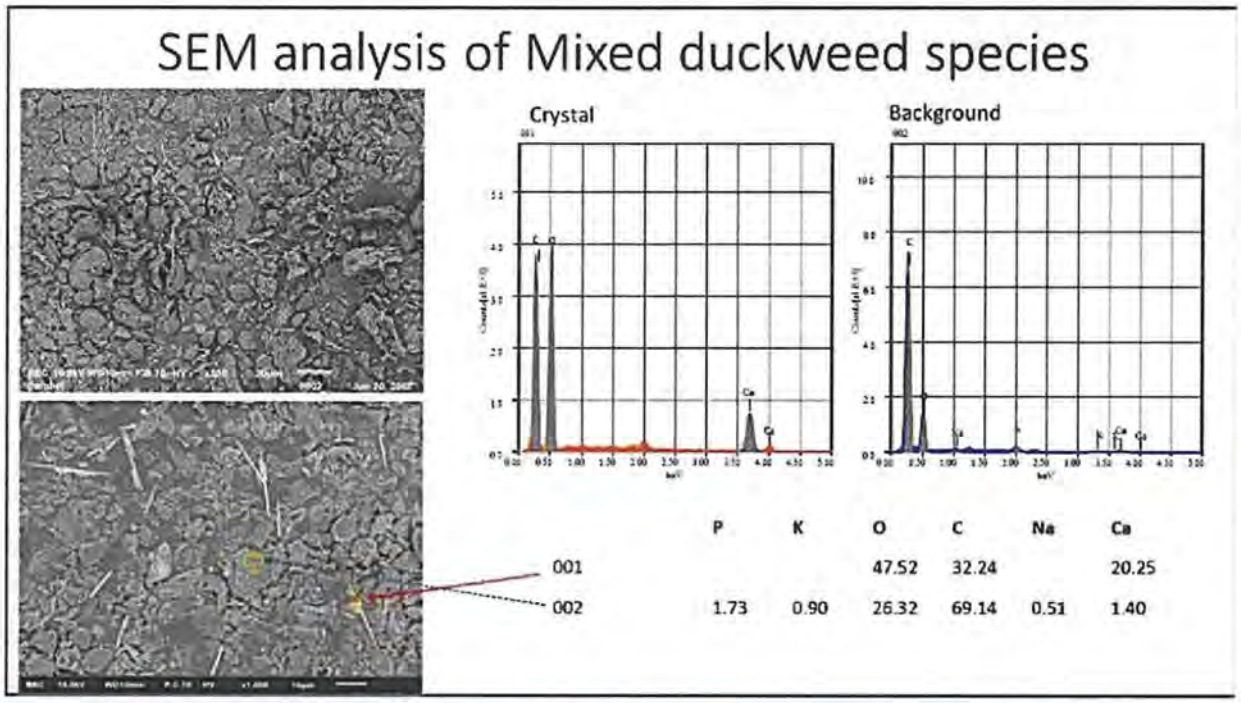


Figure 15. Scanning Electron Micrograph of Mixed Duckweed Species Powder and Elemental Analysis

When Mankai™ powder was similarly examined, however, there was no evidence of calcium oxalate crystals (Figure 16),

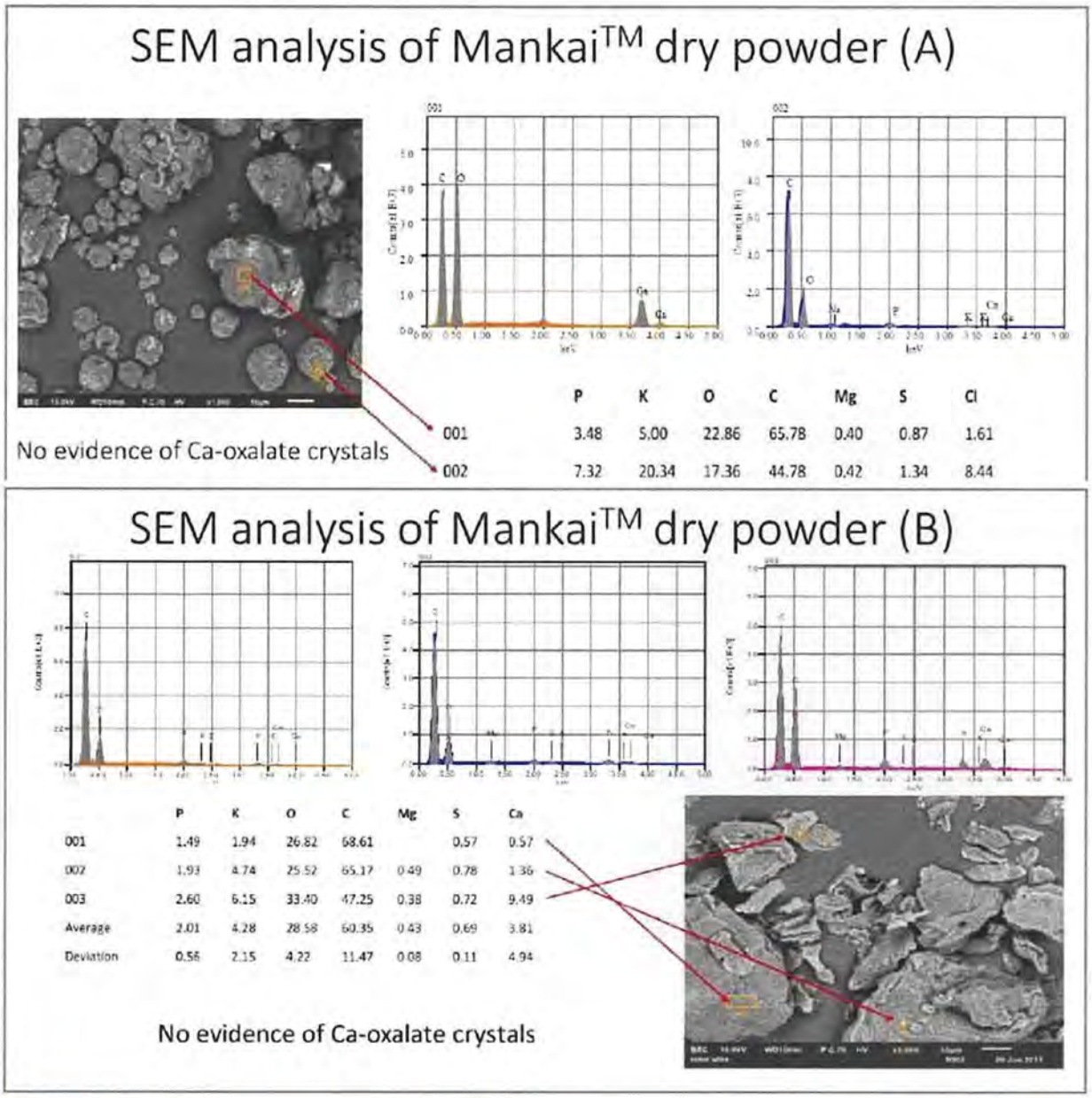


Figure 16. Scanning Electron Micrographs of Two Samples, (A) and (B), of Mankai™ Dried Powder.

Oxalic acid levels were measured in Mankai™ biomass grown in the closed controlled conditions of Hinoman's greenhouse facility using a HPLC (LC-DAD) method (AOAC 986.13 modified). Average amounts of 50mg/100g of oxalic acid in the fresh plants and 416 mg/100g of oxalic acid were found in the dried product. Comparison with other crops shows that the level found in Mankai™ is relatively low compared to most common vegetable used in a Western or Mediterranean diet (Table 27).

Table 25. Comparison of Oxalate Content in Fresh Mankai and Other Green Vegetables

Vegetable	Oxalate (mg/100 g, Fresh Weight)*
Parsley	1700
Chives	1480
Amaranth	1090
Spinach	970
Lettuce	330
Broccoli	190
Celery	190
Asparagus	130
Cabbage	100
Pea	50
Mankai™	**50**
Cucumber	20
Kale	20
Squash	20
*Values, except for Mankai™, are from USDA (1984)	

While some of these vegetables (e.g., parsley and chives) are consumed in relatively small amounts, others, such as lettuce and spinach, are consumed in amounts comparable to those proposed to for Mankai™. Hence, based on an anticipated intake of the equivalent of 58.8 g/day of dried Mankai™ (Part 3), and an average oxalic acid content in dried Mankai of 416 mg/100g, the anticipated daily intake of oxalic acid from Mankai is about 244 mg, which is approximately the amount in 25 grams of fresh spinach, or 74 grams of fresh lettuce (Table 27).

The oxalic acid content in dry powder of Mankai™ as a final food product revealed values in the range of 230-489 mg/100g. This range is much lower than the range of 5,300 -11,600mg/100g in a germplasm collection of dried spinach (Mou 2008).

6.2 Primary Animal Study Supporting Safety - 90-Day Dietary Toxicity Study in Rats

In an OECD (Guideline 408) 90-day oral toxicity study conducted in compliance with OECD Principles of Good Laboratory Practice, the toxicity profile of Mankai™ was assessed in Sprague-Dawley rats [Crl:CD(SD), 10 animals/sex/group, 6 weeks of age at the start of administration] by dietary administration for 91 days (Kawamata et al. 2020). Male and female rats were fed diets containing dry Mankai™ at concentrations of 0, 5, 10, and 20% (w/w) in powdered basal diet (CR-LPF). The following parameters were evaluated for evidence of toxicity: mortality, clinical signs, detailed clinical observations, manipulative tests, measurement of grip strength and motor activity, body weights, food consumption, ophthalmology, urinalysis, hematology, blood chemistry, hormone analysis (T3, T4, and TSH), organ weights, gross pathology, and histopathology.

There were no test-article-related changes in mortality, clinical observations, detailed clinical observations, manipulative tests, measurement of grip strength and motor activity, body weight (Figure 17), food consumption, ophthalmology, hormone analysis (T3, T4, and TSH) (Table 28), organ weight (Table 29), gross pathology, and histopathology. The mean test article intake in the 5, 10, and 20% groups was 3,176, 6,491, and 13,165 mg/kg/day for males and 3,583, 7,423, and 15,027 mg/kg/day for females, respectively.

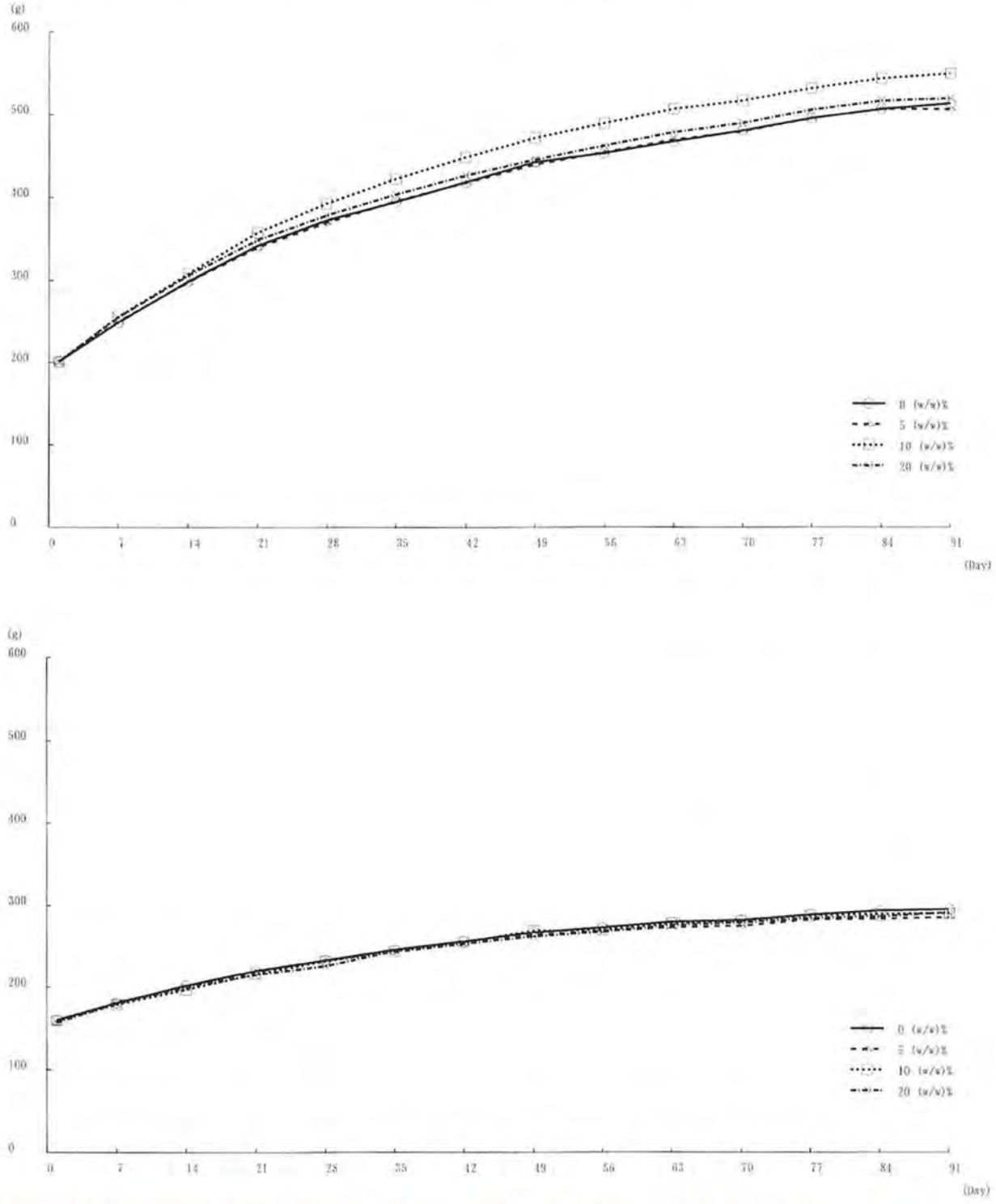


Figure 17. Change in Body Weight of Males (top) and Females (bottom) Receiving Mankai™

Table 26. Summary of Mean Serum Thyroid Hormone Concentrations in Rats Receiving Mankai™

Sex	Male				Female			
Dose (w/w)%	0	5	10	20	0	5	10	20
No. of animals	10	10	10	10	10	10	10	10
T3 (ng/mL)	0.56	0.52	0.5	0.54	0.75	0.78	0.68	0.73
T4 (ng/mL)	40.1	39.8	36	36.5	28.6	26.4	25.1	25.9
TSH (ng/mL)	6.5	5.5	5.9	6	3.8	3.2	3.9	3.2

Table 27. Summary of Mean Organ Weights in Rats Receiving Mankai™

Sex	Male				Female			
Dose (w/w)%	0	5	10	20	0	5	10	20
No. of animals	10	10	10	10	10	10	1	10
Body weight at necropsy (g)	495	492	530	503	283	274	280	279
Heart (g)	1.49	1.45	1.57	1.47	0.94	0.84*	0.91	0.93
(g/100 g bw)	0.3	0.3	0.3	0.29	0.33	0.31*	0.33	0.33
Testis (g)	3.73	3.34	3.4	3.42	--	--	--	--
(g/100 g bw)	0.76	0.68	0.64*	0.69	--	--	--	--
Prostate (g)	1.38	1.26	1.48	1.19	--	--	--	--
(g/100 g bw)	0.28	0.26	0.28	0.24*	--	--	--	--
*: p≤0.05 (significantly different from the control group)								

The following changes were recorded; however, they were judged to have no toxicological signification because the changes were minimal, the values were generally within the historical control range in the test facility, or there were no related histopathological changes in any organs/tissues: in the urinalysis, an increase in water intake and a decrease in one day's excretion of sodium in males in the 20% group, decreases in one day's excretion of sodium and potassium in females in the 10 and 20% groups, and a decrease in one day's excretion of chlorine in females in all dose groups (Table 30).

Table 28. Summary of Urinalysis in Rats Receiving Mankai™

Sex	Male				Female			
Dose (w/w)%	0	5	10	20	0	5	10	20
No. of animals	10	10	10	10	10	10	10	10
Urine volume (mL/24h)	10.8	9.9	11.8	13.5	14.7	8.5	8	7
Water intake (mL/24h)	36	37	35	45**	39	31	32	37
Urine-Na (mmol/24h)	1.9	1.5	1.7	1.3*	1.5	1.3	1.0**	0.7**
Urine-K (mmol/24h)	3.9	3.6	3.8	3.5	3.4	2.7	2.4*	2.0**
Urine-Cl (mmol/24h)	2.8	2.5	2.7	2.3	2.5	1.9*	1.5**	1.2**
*: p≤0.05, **: p≤0.01 (significantly different from the control group)								

In hematology, there was a decrease in fibrinogen in females in the 10 and 20% groups (Table 31) and in the blood chemistry, decreases in total cholesterol, phospholipid, calcium, and inorganic phosphorus in males in the 20% group and increases in glucose, blood urea nitrogen, and urea in females in the 20% group (Table 32).

Table 29. Summary of Hematology in Rats Receiving Mankai™

Sex	Male				Female			
Dose (w/w)%	0	5	10	20	0	5	10	20
No. of animals	10	10	10	10	10	10	10	10
MCHC (g/dL)	34.4	34.1	34.2	34.6	35	35.5*	35.3	34.7
Reticulocyte (10^9/L)	161.8	150.7	169.9	150.8	157	123.7*	149	148.3
MONO (10^2/μL)	2.2	1.3*	1.7	1.8	1.2	1.2	1.2	0.9
FIB (mg/dL)	290	295	300	289	221	206	183**	180**
*: p≤0.05, **: p≤0.01 (significantly different from the control group)								

Table 30. Summary of Mean Blood Chemistry Values in Rats Receiving Mankai™

Sex	Male				Female			
Dose (w/w)%	0	5	10	20	0	5	10	20
No. of animals	10	10	10	10	10	10	10	10
T-CHO (mg/dL)	71	65	61	53**	81	75	72	72
PL (mg/dL)	106	98	93	80**	151	148	140	140
GLU (mg/dL)	151	169	166	157	112	113	124	131*
BUN (mg/dL)	19	19	17	18	17	20	18	21*
Urea (mg/dL)	40	42	36	39	37	44	39	44*
Ca (mg/dL)	10.5	10.1*	10.3	10.0**	10.6	10.7	10.6	10.4
P (mg/dL)	6	5.7	5.7	5.3*	5	4.5	4.5	4.4
*: p≤0.05, **: p≤0.01 (significantly different from the control group)								

In conclusion, it was judged that the no observed adverse effect level (NOAEL) of Mankai™ was 20% (w/w) in the diet (13,164 mg/kg/day for males and 15,027 mg/kg/day for females).

6.3 Genotoxicity/Mutagenicity

Kawamata et al. (2020) also reported the results of an OECD GLP-compliant Bacterial Reverse Mutation Test (OECD Guideline 471) and an OECD GLP-compliant *in vitro* micronucleus test in human lymphoblastoid (TK6) cells, both with and without metabolic activation by rat liver S9 fraction.

In the bacterial reverse mutation test, Mankai™ D110 (Dry powder Mankai™) was tested in *Salmonella* typhimurium TA100, TA 1535, TA98, and TA 1537 and in *Escherichia* coli WP2 uvrA, with and without metabolic activation by the pre-incubation method. Mankai™ was tested in a dose range-finding test at 19.5 to 5,000 μg/plate with no sign of growth inhibition, though precipitation was noted at all dose levels; therefore, the main test, which was performed twice with the same dose levels, used 5 dose levels ranging from 313 to 5,000 μg/plate. 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2), sodium azide (SAZ), 2-methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino]acridine-2HCl (ICR-191), 2-aminoanthracene (2AA), and benzo[*a*]pyrene (BaP) were used as positive controls. While the positive controls all showed the expected substantial increase in mutation frequency in the appropriate tester strains, Mankai™ showed no indication of a dose-related increase in mutation frequency in any strain, with or without metabolic activation. The

authors concluded that Mankai™ had no potential to induce gene mutations under the conditions of this study (Kawamata et al. 2020).

In the *in vitro* micronucleus test in human lymphoblastoid (TK6) cells, Mankai™ D110 was tested in a preliminary range-finding toxicity test at concentrations between 1 and 2,000 µg/ml, with signs of toxicity at 2,000 µg/ml, and abundant precipitate that interfered with slide preparation at 667-2,000 µg/ml. For the main study, seven concentrations from 15.6 to 1,000 µg/ml were used. Cells were exposed to Mankai™ at these concentrations in RPMI-10 culture medium for 4 hours with and without metabolic activation for the short-term treatment, followed by 20 hours in test material-free medium, and separate culture were exposed to these concentrations for 24 hours (continuous treatment) without metabolic activation. No increase in the frequency of micronuclei was seen in any of the cultures at any concentration of Mankai™, with or without metabolic activation, while the positive control materials (cyclophosphamide and colchicine) produced the expected increases in micronuclei. The authors concluded that Mankai™ had no potential to induce chromosome aberrations under the conditions of this study (Kawamata et al. 2020).

6.4 Supporting and Unpublished Mankai™ Studies

6.4.1 4-Day Toxicity Study (Pharmaseed 2016)

Four groups of female SD rats were orally administered for four consecutive days a daily dose of Mankai™ at dose levels of 0, 1,700, 2,500 and 3,400 mg/kg body weight, equivalent to 20, 40 and 60 g/day human dose. No signs of morbidity or distress were seen. Body weight gain was normal, with no differences observed among the treatment groups. Clinical chemistry and hematology results revealed some biochemical differences between groups. However, in all cases: Liver /renal status biomarkers, stress indicators and minerals, did not show adverse effects. Beyond the safety indicators above: there was a *significant decrease* in fasting glucose and the stress marker enzymes LDH and CPK (although within the normal range). No gross pathology abnormalities were observed in all animals from all groups.

6.4.2 28-Day Oral Toxicity, GLP Study (American Preclinical Services 2017)

In an OECD (Protocol 407) Repeated Dose 28-Day Oral Toxicity Study in Rodents, conducted in compliance to the FDA GLP Regulations (21 CFR Part 58), groups of six Wistar rats of each sex (8-9 weeks old at the start of the study) were given diets containing 0, 5, 10, or 20 g Mankai™ powder per kg diet (providing average daily doses of Mankai™ of 0, 0.34, 0.70, and 1.39 g/kg body weight in males and 0. 0.36, 0.70, and 1.43 g/kg body weight in females). No effects on feed consumption or body weight gain were observed. No toxic effects were observed. There were no trends observed for any coagulation, hematology or serum chemistry parameter that might be attributed to control or test treatment. No evidence of neurotoxic effects was seen in the weekly functional observational battery (FOB) assessments. Organ weights and organ weight to body weight ratios were not affected by treatment.

There were no notable gross observations in organs or tissues from test or control animals of either sex. There were no notable histopathology findings suggestive of systemic toxicity within the tissue sections examined from either test groups or control groups. All histopathology findings of the organs and tissues examined, were within normal limits or were incidental findings common for animals of this species, strain and age and there were no notable observations from vaginal cytology findings. In conclusion, there was no evidence of systemic toxicity in rats fed dried Mankai™ at a daily dose of up to 1.4 g/kg body weight/day, equivalent to about 100g/person on a body-weight basis for a 70 kg adult. These results are consistent with those of the published 90-day study described above (Kawamata et al. 2020).

6.4.3 Preliminary Findings from Clinical DIRECT PLUS RCT Trial (https://clinicaltrials.gov/ct2/show/results/NCT03020186)

In the 18-month DIRECT PLUS clinical trial (sponsored by Ben Gurion University, Israel in collaboration with Leipzig University, Germany; Harvard School of Public Health, USA; and Edmund Mach Foundation Institute, Italy), preliminary results are available from 294 participants enrolled

since May 2017, randomly divided among three treatment groups. The Reference Group (physical activity (PA) with healthy dietary guidelines) is compared to PA+ Mediterranean (MED) diet (+28g/day walnuts, increment of 440mg/day of polyphenols) and to PA+ green-MED diet (Med diet plus daily intake of 28g/day walnuts, 800mL/day green tea and Mankai™ (100g frozen cubes/day, equivalent to 20g/day of dry Mankai™), increment of 1320 mg/day of polyphenols), all with free, monitored gym membership.

The 6-month mid-term analysis (adherence rate = 98%) of the DIRECT PLUS RCT suggests that the green-MED diet is beneficial in terms of adiposity, blood pressure, lipid, glycemic control and inflammation, with results as good or better than the MED diet alone, compared to the Reference Group. Several scientific papers based on these findings are already published or are under review (see below).

After 18 months, the DIRECT PLUS Study's retention rate was 89.8%. No significant symptoms, adverse effects, or health issues were related to the consumption of the green-MED diet, which provided 100 g/day of frozen Mankai™, among the 98 participants who were randomized to receive it. Overall, the 18-month dropout rate was similar between all the intervention groups ($p=0.263$ between groups). Liver function biomarkers (ALT, AST and AlkP remained improved in all the intervention groups with significantly greater decrease in the Mankai consumers (Green-Med group) (Figure 16). At 18-months, the decrease of the adipokines: leptin, chemerin, FGF21 and fetuin continued to be greater in the green-MED/ Mankai consumer group, although the statistically significant difference remained only for leptin ($P<0.05$ VS both MED diet and PA group) and FGF21 ($P<0.05$ VS PA group) PA (Figure 17).

After 18 months of lifestyle intervention, weight loss and waist circumference (WC) in both the PA+green-MED diet (-3.7±6.3kg, 6.1±6.2cm; $p<0.001$ vs. baseline for both) and the PA+MED diet (-2.7±5.6kg, -5.3±5.7cm; $p<0.001$ vs. baseline for both) were similar, and were greater than the effects achieved in the PA group (-0.4±4.7kg, $p=0.348$ vs. baseline; -4.0±5.6cm, $p<0.001$ vs. baseline; $p<0.05$ for PA between MED groups for weight, $p=0.014$ for PA+green-MED vs. PA) (Figure 15).

The mean of MRI assessed intrahepatic fat (IHF%) loss among the PA+green-MED/ Mankai consumer group (-4.0±9.1% absolute units, -37.7% relative change) was significantly greater than the reduction achieved in the PA+MED (-1.7±8.6% absolute units, -16.8% relative change; $p=0.026$ vs. PA+green-MED) and in the PA group (-1.2±6.9% absolute units, -12.2% relative change; $p=0.004$ vs. PA+green-MED). Within the green-MED diet group, increased actual consumption of Mankai™ shake was significantly associated with higher IHF% loss, even after controlling for weight loss.

Overall, preliminary results of the 18-month analysis of the DIRECT PLUS RCT suggest that the green-MED diet, based on daily Mankai™ consumption, is safe and exerts beneficial effects on adiposity, cardiometabolic risk parameters and inflammation, although some of the significant achievements demonstrated after the 6-month rapid weight loss phase were attenuated with the weight regain phase. Nevertheless, the superior effect of the green-MED diet remained with the same positive trend.

No adverse health effects were reported to be associated with consumption of 100 g of frozen Mankai™ every day for 18 months. The lack of reports of adverse effect in subjects receiving Mankai™, together with the objective clinical chemistry findings (Figures 18 & 19) show that ingestion of 100 g of frozen Mankai™ daily for 18 months was safe for human consumption.

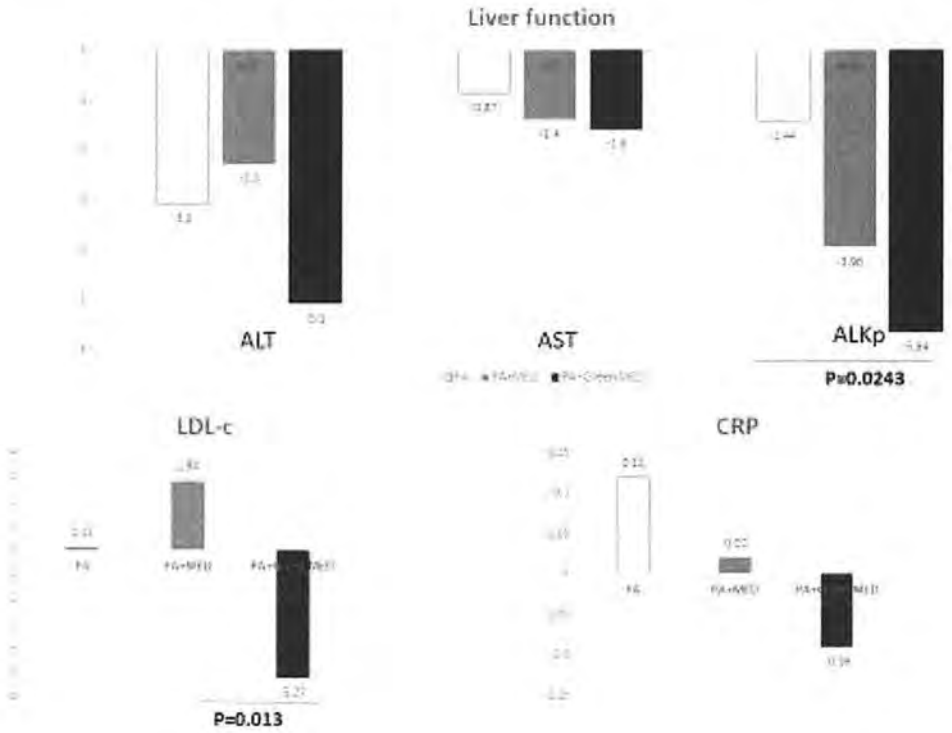


Figure 18. Changes of liver function biomarkers, CRP and LDL-c during 18 m of DIRECT PLUS intervention (n=294)-preliminary analysis

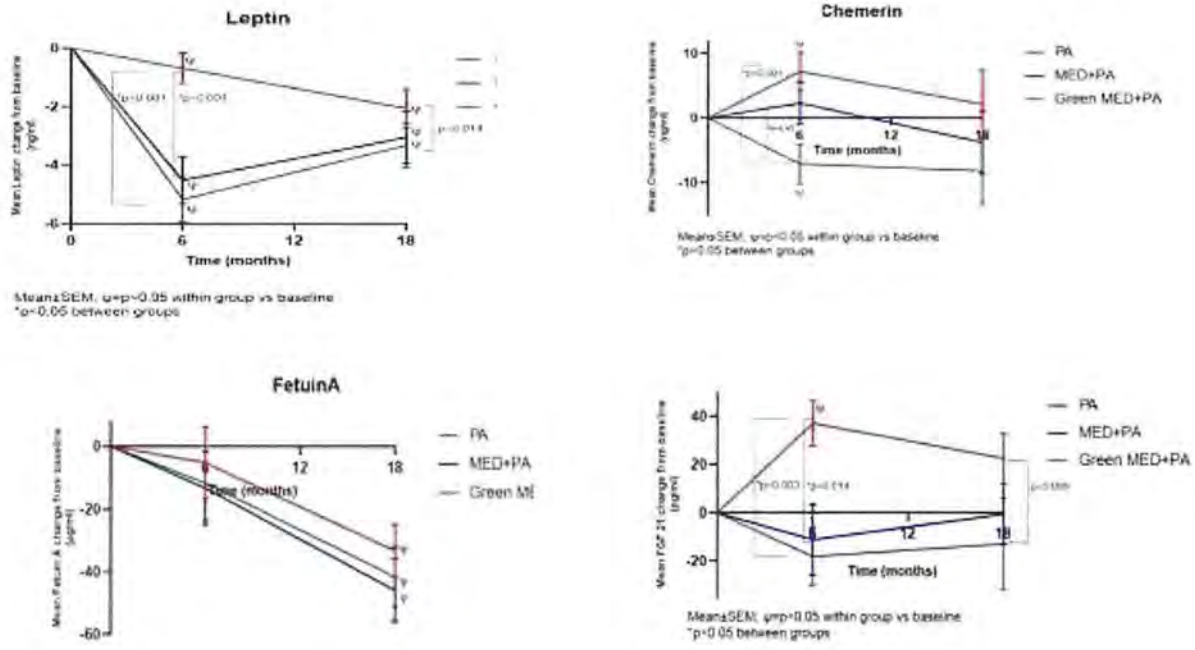


Figure 19. Changes of adipokines during 18 m of DIRECT PLUS intervention (n=294)-preliminary analysis

6.4.4 Protein Bioavailability Human Clinical Trial (https://clinicaltrials.gov/ct2/show/NCT03020225)

In a randomized controlled clinical trial (Kaplan et al. 2019), 36 men, subjected for 3 days to a stable diet and subsequent overnight (12h) fast, were randomized to consume one of three test meals, each containing 30 grams of protein (from 333 g soft cheese, 600 g green peas, or 410 g Mankai™). Blood samples were collected at 0, 30, 90 and 180 minutes, and analyzed for essential amino acids (see Figure 20). The 3-hour blood concentrations of the essential amino acids, histidine, phenylalanine, threonine, lysine, and tryptophan, triggered by intake of Mankai™, was increased significant as compared to baseline ($p<0.05$) and was similar to that seen with soft cheese and pea intake ($p>0.05$ between groups) (Figure 16). Although branched-chain-amino-acids (leucine/isoleucine, valine) increased significantly by Mankai™ within 3 hours ($p<0.05$ vs. baseline), the change was relatively higher for cheese as compared to Mankai™ or peas ($p<0.05$ between groups). These results indicate that Mankai™ may provide a high-quality substitute source for animal protein. Although this trial was not designed as a safety assessment, the fact that no adverse health effects were reported is consistent with the results of other studies in humans and animals and supports the safety of Mankai™.

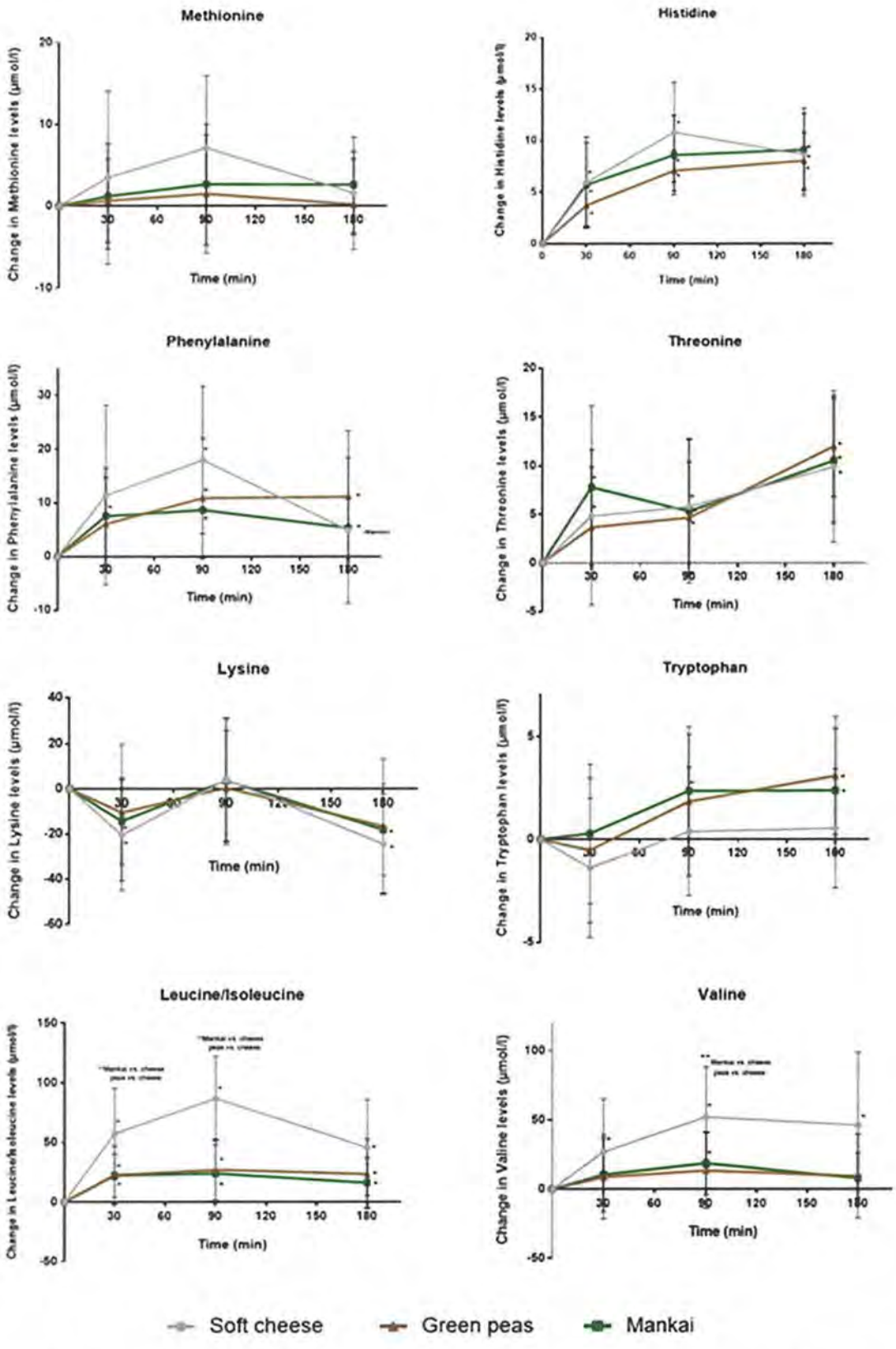


Figure 20. Change in Blood Essential Amino Acid Concentration Following Meal with Different Sources of Protein

6.4.5 Iron Bioavailability (Human and Animal Experiments)

In a report in the *Journal of Nutrition*, Yaskolka Meir et al. (2019) report on studies examining the bioavailability of iron from Mankai™ in humans and rats. In the human study, 294 abdominally-obese/dyslipidemic non-anemic participants (age = 51.1yrs; body-mass-index =31.3 kg/m^2; 88% men, triglycerides >150mg/dL, high-density lipoprotein-cholesterol ≤40 mg/dL for men, ≤50 mg/dL for women) were randomized to physical activity (PA, moderate-intensity with free gym membership), PA+MED diet, or PA+green-MED diet. Both iso-caloric MED groups consumed 28g/day walnuts and the low-meat Green-MED further consumed green tea (800 mL/day) and Mankai™ (100 g frozen Mankai™/day). After 6 months of intervention, the iron-status trajectory did not differ between PA and PA+MED groups. Hemoglobin modestly increased in the PA+green-MED group (0.23g/dL) as compared to PA (-0.1g/dL; $p<0.001$) and PA+MED (-0.1g/dL; $p<0.001$). Serum-iron and serum-transferrin saturation increased in the PA+green-MED group as compared to the PA-group (8.21µg/dL vs. -5.23µg/dL and 2.39% vs. -1.15%; $p<0.05$ for both comparisons), as did folic acid ($p=0.011$). Thus, the Green-MED, low-meat diet containing Mankai™ did not impair iron-homeostasis in humans and did not cause any adverse health effects.

In the rat study (Yaskolka Meir et al. 2019), following 44-days of feeding an iron-deficient anemia-inducing diet, 50 female Sprague-Dawley rats (3 weeks of age) were randomized into: iron-deficient diet (vehicle), or vehicle+iso-iron: ferrous-gluconate (FG)14, Mankai™-50 and Mankai™-80 versions (1.7mg·kg^{-1}·day^{-1} elemental iron), or FG9.5 and Mankai™ 50-C version (1.15mg·kg^{-1}·day^{-1} elemental iron). In these rats, hemoglobin decreased from 15.7mg/dL to 9.4mg/dL after 44 days of diet-induced anemia. During the 3-week post-depletion treatment, during which the basal diet continued to be iron-deficient, the vehicle-treated group had a further decrease of 1.3mg/dL, whereas both FG and Mankai™ iso-iron treatments similarly rebounded hemoglobin levels (FG14:+10.8mg/dL, Mankai™ 50:+6.4mg/dL, Mankai™ 80:+7.3mg/dL; FG9.5:+5.1mg/dL, Mankai™ 50-C:+7.1mg/dL; $p<0.05$ for all vs. vehicle group). These results indicate that iron derived from Mankai™ is bioavailable and effective in reversal of anemia due to an iron-deficient diet. These studies also demonstrated an absence of any adverse health effects attributable to consumption of Mankai™.

6.4.6 Other Animal Feeding Studies

Duckweeds, including *Wolffia* species, are safely consumed by wild and domestic fish, birds and herbivorous animals. A number of experiments have been conducted exploring the use of *Wolffia* species as feed for fish, quail and chickens and these studies are summarized below. Many of the studies involve the use of *Wolffia* meal as an ingredient to replace soybean meal in the feed. There were no histological examinations in these studies or analysis of blood chemistry or composition, as the focus of the studies were on performance and quality characteristics of the animals for food quality purposes, however they do demonstrate that *Wolffia* is safe for consumption by animals.

6.4.6.1 Fish

According to an FAO report of 2009, fresh duckweed was fed to fish as the sole food as well as being included in the dried form as an ingredient in food pellets. Feeding trial studies have been carried out in laboratories and in field conditions (FAO 2009).

An Indian study reported that six species of fingerling carp grown in a cement cistern with fresh *Wolffia arrhiza* (L) Horkel ex Wimmer as the only added food successfully gained weight and were harvested after 155 days (Naskar et al. 1986).

Wolffia meal (*Wolffia arrhiza*) was evaluated as a replacement for soybean meal in the diet of Nile Tilapia (*Oreochromis niloticus L*) raised in a laboratory in Khon Kaen University in Thailand (Chareontesprasit and Jiwayam 2001). Using a randomized block design, the effects of 4 feeds were evaluated in fingerlings (2.5 g). The four formulated food rations included dried *Wolffia* as 0%, 15%, 30% and 45% of the total feed. The proportional quantity of soybean meal in the feed decreased in the 4 formulations (20%, 14%, 7% and 0%) and the quantity of rice also decreased (29%, 20%, 12% and 4%). The amount of crude protein remained constant, as did the gross energy supplied by the food. Twenty fish were included in each experimental group. They were fed solely with the experimental rations which were supplied twice a day for 8 weeks. Body live weights and length were recorded at 2-week intervals. The digestibility of the meal was measured by analyzing

hydrolysis-resistant matter in the fish excrement. The digestibility of the meal and growth of the fish did not differ between the groups. Measurements of weight, length, feed intake, feed conversion ratio, protein efficiency ratio and net protein utilization and growth rate were not significantly different between groups. However, the highest survival rate for the fish was obtained with the feed containing 15% *Wolffia*. That group had a survival rate of 72.5% compared to 58.8% for the control feed, 35% for the feed with 30% *Wolffia* and 27.5% for the fish given feed containing 45% *Wolffia*. The authors concluded that "The results indicated the appropriate level of *Wolffia* meal for growth and production of the experimental fish. 15% of *Wolffia* meal should be the highest rate for use."

6.4.6.2 Japanese quail

Dried *Wolffia globosa* meal was added to feed for Japanese quail in quantities of 0, 8.92%, 17.84% and 26.76%, replacing 0, 25%, 50% and 75% of the soybean meal in the feed (Chantiratikul et al. 2010a). This research was conducted in Thailand and used 288 quail at 7 days of age which were randomly assigned to 4 groups, 6 replicates of 12 birds each, fed with the respective diets for 42 days. Feed containing *Wolffia*, at any of the amounts added to the feed, did not alter average weight gain (g/day), feed conversion ratio (feed/gain), protein efficiency ratio or mortality rate. However, there were differences on feed intake at the highest intake level when *Wolffia* made up 26.76% of the food (replacing 75% of the soybean meal). Carcass dressing and characteristics were not altered but the color of the breast skin was increased proportionally with the increase in *Wolffia* in the diet. The authors concluded that "The optimal replacement level from *Wolffia* meal for soybean meal was 50% in the diets of quail."

Wolffia meal *(Wolffia arrhiza)* was evaluated as a protein replacement for soybean meal in the diet of laying Japanese quails (Coturnix japonica) domesticated in Thailand (Suppadit et al. 2012). A total of 480 laying hens (4 weeks old) were randomly allocated to 6 groups. *Wolffia* meal was incorporate into the diet at levels from 0 to 20% in the 6 groups (control, 4%, 8%, 12%, 16% and 20%). The *Wolffia* meal replaced soybean meal in the diet which was included at 27.3% of the feed for the control group and at decreasing levels in the other groups: 23.3%, 18.8%, 14.1%, 9.34% and 4.60%. The feed in all groups supplied a consistent source of metabolic energy to the animals, which were evaluated for a period of 35 days. The quantities of feed intake per bird per day were reduced when the *Wolffia* meal reached the quantity of 20%, but no effect was observed with a quantity of 16%. Daily egg laying was also reduced in the group given feed containing 20% *Wolffia* meal but was unaffected by lower amounts. The quality of the eggs (size, weight, thickness of shell and fracture data) were reduced for the group provided with feed containing *Wolffia* meal at 20%, but not for any of the other groups.

The color of the yolks increased proportionally with the increase in *Wolffia* meal. The authors speculated that the color was due to beta-carotene content in the *Wolffia* meal. Mortality of the animals over the time period ranged from 2.60% to 3.00% and was unaffected by any of the test quantities of *Wolffia* meal. The study authors concluded that, "The use of *Wolffia arrhiza* meal to replace soybean meal could be possible only up to a level of 16% in the ration."

6.4.6.3 Chickens

In a study examining the use of two species of duckweed (*Lemna gibba* and *Wolffia arrhiza)*, one hundred and fifty Topaz layers (43-weeks old) were divided into three treatment groups (Haustein et al. 1990). The birds were acclimated for 2 weeks and then started on one of three test diets: control (0% duckweed), 15% *Lemna gibba* or 15% *Wolffia arrhizal* (providing 5% crude protein to replace that provided by soybean meal in the control diet). The duckweed was manually harvested, sundried to approximately 40% moisture, dried to 10% total moisture in a forced air oven and then stored in bags until used. The dried duckweed was ground prior to preparation of the diets. All diets used in the study were isonitrogenous (17% crude protein) and isocaloric (2,800 metabolizable energy (ME) kcal/kg). The base diets contained ground yellow corn, wheat middling, fishmeal, fish oil, limestone, dicalcium phosphate and premix. The control diet also contained soybean meal (providing 5% crude protein) and iodized salt. The birds were on the experimental diets for a total of 90 days following a 14-day adaptation period, during which time all birds received the control diet and no experimental data were collected. The group receiving *Wolffia* had a shorter period due to limited supply of this duckweed species. Feed and water were supplied ad libitum. Egg production, feed consumption, feed conversion, mean egg weight, mean weight gain, number of eggs per hen per week, and yolk

pigmentation were measured. There was no significant difference in egg production, feed conversion, egg weight, egg number or mean egg weights between the experimental groups and the control. No adverse effects were reported in the birds receiving either species of duckweed

Dried *Wolffia globosa* meal was considered as a replacement for soybean meal in the diets of laying hens (Chantiratikul et al. 2010b). A total of 180 Rohman laying hens (71 weeks old) were randomly allocated to 5 groups, with each group containing 4 replicates with 9 hens per replicate. The dietary treatments included a control diet and diets containing 3.04%, 6.07%, 9.10% and 12.14% of the total feed, replacing 25%, 50%, 75% and 100% of soybean meal. All of the diets contain an equivalent amount of metabolizable energy (1,302 kcal/kg). Feed consumption and egg production were recorded daily, and the animals were weighed at the beginning and end of the 8-week period of experimental feeding. When *Wolffia* meal was present in the feed at 12.14%, completely replacing the soybean meal, daily feed intake, intake of metabolizable energy, body weight and egg production were significantly reduced compared to the control group. These variables were not significantly different from control when *Wolffia* was present in the feed at lesser amounts. Egg yolk color increased proportionally with the increase in *Wolffia* content in the feed. The results indicated that *Wolffia* meal could be used in the feed at a concentration of 9.10%, replacing 75% of the soybean meal. In the author's words, "75% of crude protein from soybean meal can be replaced by crude protein from *Wolffia* meal in the diet of laying hens."

A similar experiment with diet was conducted by the same group of researchers in male broiler chickens (Arbor Acres) that were 10 days old (Chantiratikul et al. 2010c). A total of 384 broilers were randomly assigned to 4 groups, with 6 replicates of 16 animals each. The dietary treatments were composed of starter diets and grower diets. In each diet the *Wolffia globosa* meal replaced 25%, 50%, 75% and 100% of soybean meal in the feed, but the actual amounts of *Wolffia* differed. The starter diets (10-21 days) contained 0%, 6.34%, 12.65% and 18.95, while the grower diets (22-42 days) contained 0%, 4.85%, 9.65%, and 14.50% *Wolffia* meal. Both the feed intake (g/bird/day) and average weight gain (g/bird/day) from 10 to 42 days was significantly reduced for all quantities of *Wolffia* in the diet compared to controls. Body live weight and body weight after plucking were also significantly decreased. On the other hand, liver weight as a percentage of body weight was increased for all quantities of *Wolffia* in the diet compared to controls. The mortality rate of the animals was not affected. The skin color of the animals increased proportionally with the increase in *Wolffia* content in the feed. The authors speculated that the negative effect of *Wolffia* meal on body weight and liver weight might be due to changes in amino acid balance compared to soybean meal. *Wolffia* meal is reported to be lower in lysine, isoleucine, arginine and methionine compared to soybean meal. The authors concluded that "The results indicated that less than 25% of crude protein from soybean meal could be replaced by protein from *Wolffia* meal in broiler diet."

6.4.6.4 Synopsis of Fish and Animal Feeding Experiments using Wolffia Meal

The data from the feeding experiments is summarized in Table 33. Suitable amounts of *Wolffia* meal in feed for Nile Tilapia and Japanese quail was fairly consistent as 15 to 18% of the total feed, while a smaller percentage was suitable for Rohman laying hens (9%) and the suitable amount for broiler chickens was less than the amounts tested. The mortality rate in tested birds was unaffected by the amount of *Wolffia* used as replacement feed. *Wolffia* improved the survival rate of Tilapia up to 15% replacement.

Table 31. Synopsis of Fish and Animal Feeding Experiments Using *Wolffia* Meal

Bibliographic Reference	Subjects	*Wolffia* meal in feed (maximum, %)	Soybean meal replacement (%)
Chareontesprasit & Jiwyam (2001)	Nile Tilapia	15%	25%
Chantriatikul et al. (2010a)	Japanese quail	18%	50%
Suppadit et al. (2012)	Japanese quail	16%	34%
Chantiratikul et al. (2010b)	Rohman laying hens	9.1%	75%
Chantiratikul et al. (2010c)	Broiler chickens	<4.9%	<25%

Haustein et al. (1990)	Topaz laying hens	15%	100%

6.5 Other Relevant Information on Safety

As previously noted, duckweed (including *Wolffia globosa*) is edible by humans and many animal species. Along with its long history as a food source in Southeast Asia, it is recognized as an edible vegetable for humans in several databases, including the USDA (2014) GRIN database.

One potential concern with respect to the safety of duckweed depends on the quality of the media in which it is grown. Duckweed can accumulate heavy metals, phenols, pesticides, dioxins, and pathogens from contaminated water (Spiegel 2013). However, growing under GAP conditions, i.e careful control and rapid monitoring of the growth media during the cultivation of duckweed, as occurs in the Hinoman production process for Mankai™, (as described in Section 2, above) prevents the concentration of these toxins in the plants. This is confirmed by the absence of such substances of concern in the chemical analyses performed on samples of the product (see Section 2.4).

6.6 Summary and Conclusions

The subject of this assessment is Mankai™, a cultivated strain of *Wolffia globosa*, which is an aquatic plant commonly known as duckweed. Duckweeds are flowering aquatic plants that float on or just beneath the surface of still or slow-moving bodies of water. There is a long history of the safe use of *Wolffia globosa* as food, especially in Southeast Asia.

Mankai™ is grown in closed, controlled cultivation basins covered by food-grade PVC foil in greenhouse construction. The cultivation basins are supplied with tap water and/or deionized water as well as fertilizer. The cultivation process is fully controlled and monitored while harvesting is completed semi/fully automatically. All processes in the cultivation, harvesting, processing, and packaging of Mankai™ comply with current Good Manufacturing Practice (21 CFR 110).

The nutritional composition of Mankai™ has been determined and it is found to be high in protein (40-50% dry weight) and carbohydrate (35-45% dry weight) and low in fat (7-9% dry weight). Analysis of the amino acid composition reveals that the protein is of high quality with abundant levels of all essential amino acids.

Inductively coupled plasma mass spectrometry (ICP-MS) analysis has repeatedly determined that the heavy metal composition in Hinoman's production is low and was found to be below inadmissible levels in all tests performed. Quality test results for microbiological content, pesticides, and aflatoxin are compatible with common safety requirements for vegetables in the US. No Microcystin or Cylindrospermopsin was detected in four samples of dried Mankai™. Levels of oxalic acid are comparable to or lower than those that may be ingested from common vegetables, such as spinach.

Based on the composition of Mankai™, it is safe for human consumption at levels at least as high as those proposed.

This composition-based safety assessment is also supported by the results of feeding studies in various animal species, lasting 5 to 22 weeks, in which *Wolffia* meal was used to replace the typical protein source (soybean meal) and the animals showed normal growth at expected consumption rates. In addition, results of clinical and non-clinical studies, including a 28-day feeding study in rats receiving diets containing Mankai™ powder at up to 20 g/kg diet, and 100 g/day of frozen Mankai™ (equivalent to 20 g/day dry Mankai™) in humans for up to 18 months demonstrate that Mankai™ is safe in humans. Intake levels in these clinical and animal studies where no adverse effects were seen were as high as or higher than the estimated intake levels shown in Section 3.5. No genotoxic activity of Mankai™ was detected in an Ames test or in an *in vitro* micronucleus test.

Most importantly, no adverse health effects of any kind were seen in an OECD (Guideline 408) 90-day oral toxicity study in rodents when dried Mankai™ was incorporated in the diet at up to 20% (w/w), resulting in average daily doses of up to 13,164 mg/kg/day for males and 15,027 mg/kg/day for females (Kawamata et al. 2020). These amounts are equivalent, on average, to an intake of approximately 920 g/day in 70 kg humans.

Having independently and critically evaluated the data and information summarized above on the history of safe use, the detailed results of analysis of the composition of Mankai™, and the results of the available animal and human feeding studies, we conclude that:

> The proposed use of Mankai™, at the proposed levels of use of the forms identified in Section 3.2, is safe and Generally Recognized as Safe (GRAS) based on scientific procedures, and a history of safe use in food.

7. LIST OF SUPPORTING DATA AND INFORMATION

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From: Gavin P Thompson
To: Bonnette, Richard
Cc: Joseph V Rodricks; Duncan Turnbull; Carlson, Susan
Subject: RE: [EXTERNAL] RE: Hinoman Mankai GRN
Date: Friday, March 12, 2021 9:59:33 AM

Dear Mr. Bonnette,

Thank you for the update and confirmation.

Kind regards,
Gavin

Gavin P Thompson, PhD
Principal Consultant
Ramboll US Consulting
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gthompson@ramboll.com

From: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Sent: Friday, 12 March, 2021 5:47 AM
To: Gavin P Thompson <GThompson@ramboll.com>
Cc: Joseph V Rodricks <JRodricks@ramboll.com>; Duncan Turnbull <DTurnbull@ramboll.com>; Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Subject: RE: [EXTERNAL] RE: Hinoman Mankai GRN

Hello Gavin,
We have the submission, though it was received by our office Dec. 1. It has completed our pre-filing evaluation and will be assigned to a review team soon. Hope this is helpful.
Regards,
Richard

From: Gavin P Thompson <GThompson@ramboll.com>
Sent: Thursday, March 11, 2021 6:15 PM
To: Bonnette, Richard <Richard.Bonnette@fda.hhs.gov>
Cc: Joseph V Rodricks <JRodricks@ramboll.com>; Duncan Turnbull <DTurnbull@ramboll.com>; Carlson, Susan <Susan.Carlson@fda.hhs.gov>
Subject: [EXTERNAL] RE: Hinoman Mankai GRN
Importance: High

Dear Mr. Bonnette,

My colleague, Duncan Turnbull, shipped a GRAS Notice (GRN) to FDA/CFSAN/OFAS on October 21, 2020 by Priority Mail, and according to the USPS tracking web site, it was delivered to FDA/CFSAN/OFAS, COLLEGE PARK, MD 20740 on October 22, 2020 at 10:18 am. Dr. Turnbull, now retired, has reported that he has not received any notification from FDA that the GRN was received and that it was filed.

The GRN concerns the proposed food ingredient (referred to as “Mankai”) derived from a strain of *Wolffia globosa*, one of several species of small flowering aquatic plants commonly known as duckweed.

Ramboll US Consulting has been providing technical and scientific support to the Notifier, **Hinoman Ltd.**, and submitted the GRN on Hinoman’s behalf.

We are aware of logistical issues due to COVID-19 that have slowed the normal filing process for notifications. Please advise us 1) if the hard copy document has been located, when is a filing status likely to be determined; or 2) if the hard copy document cannot be located, how we may deliver the GRN via other means, e.g., electronically.

Kind regards,
Gavin

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From:	Gavin P Thompson
To:	Gaynor, Paulette M
Subject:	[EXTERNAL] RE: FSIS clarifying question concerning GRN 000984
Date:	Wednesday, May 12, 2021 1:34:58 AM
Attachments:	image001.png image002.png image003.png image004.png image005.png image006.png

Dr. Gaynor,

Hinoman mankai ingredient is intended to be added to foods as a plant-based source of high-quality protein.
If you have any additional questions or requests, please contact us.

Kind regards,
Gavin

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From: Gaynor, Paulette M <Paulette.Gaynor@fda.hhs.gov>
Sent: Tuesday, 4 May, 2021 6:54 AM
To: Gavin P Thompson <GThompson@ramboll.com>
Subject: FSIS clarifying question concerning GRN 000984
Importance: High

Dear Dr. Thompson,

FSIS has informed FDA that some additional information is needed before FSIS can enter into the system to be assigned. Please see below.

> Based on the information provided, FSIS cannot determine the intended use of the substance. What is the purpose of the substance being added as an ingredient? Without that, FSIS can't determine how to identify it in the system and determine if the proper efficacy data has been provided.

Please send the response to me, and I will convey to FSIS. If you have any questions or would like to discuss before responding, please let me know.

Sincerely,
Paulette Gaynor

Paulette M. Gaynor, Ph.D.
Senior Policy Advisor

Center for Food Safety and Applied Nutrition
Office of Food Additive Safety, Division of Food Ingredients
U.S. Food and Drug Administration
Tel: 240-402-1192
Paulette.Gaynor@fda.hhs.gov

From:	Gavin P Thompson
To:	Gaynor, Paulette M
Cc:	Ephi Eyal; Miri Lapidot; Monica Colt; Joseph V Rodricks; Duncan Turnbull
Subject:	[EXTERNAL] RE: GRN 984 - response requested
Date:	Thursday, September 23, 2021 11:51:39 AM
Attachments:	210923 GRN000984-CTE_Request.pdf

Dear Dr. Gaynor:

Hinoman's request to FDA to cease-to-evaluate GRN 984 is attached (letter).

Regards,
Gavin

Gavin P Thompson, PhD
Principal Consultant
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September 22, 2021

Paulette M. Gaynor, Ph.D.
Division of Food Ingredients
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Center for Food Safety & Applied Nutrition
5001 Campus Drive
College Park, MD 20740

Subject: GRAS Notice No. GRN 000984 (*Wolffia globosa*)
Cease-to-Evaluate Request

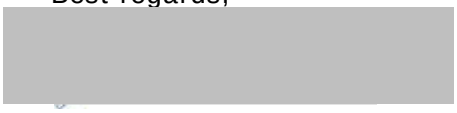
Dear Dr. Gaynor:

In accordance with 21 CFR § 170.260(b), Hinoman, Ltd. requests that the Food and Drug Administration (FDA) cease to evaluate the generally recognized as safe (GRAS) notice GRN 000984 which Hinoman submitted to FDA and was filed by FDA on April 23, 2021.

We are making this request after our discussions with you and your team of technical reviewers on September 2nd, 2021 and your email on September 9th, 2021. Based on these discussions and communications, we understand that the FDA prefers that we submit a revised GRAS Notice after certain ingredient uses of the *Wolffia globosa* "Mankai" are clarified and other modifications to the GRN. To that end, we thank you for the FDA review team's discussions with us.

Therefore, we intend to submit a new GRN to better align with the input the FDA provided. Once again, we sincerely thank you for your time. Looking forward to speaking with you again shortly regarding the resubmission.

Best regards,



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On behalf of:

Miri Lapidot, PhD
Chief Scientific Officer
Hinoman Ltd.
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Rishon LeZion 7546302
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