CPKelco
A HUBER COMPANY

UNLOCKING NATURE-POWERED SUCCESS
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OFFICE OF FOOD
ADDITIVE SAFETY

5 May 2020

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

Dear Dr. Gaynor:

Re: GRAS Notice for NUTRAVA™ Citrus Fiber

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, CP Kelco ApS (CPK), as the notifier, is submitting all data and information supporting the company's conclusion that NUTRAVA™ Citrus Fiber is GRAS on the basis of scientific procedures, for use in specified conventional food and beverage products across multiple categories; these food uses of NUTRAVA™ Citrus Fiber are therefore not subject to the premarket approval requirements of the *Federal Food, Drug and Cosmetic Act*. Information setting forth the basis for CPK's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

Should you have any questions or concerns regarding this GRAS notice, please do not hesitate to contact me at any point during the review process so that we may provide a response in a timely manner.

Sincerely,

Kristina Elvebakken
SENIOR REGULATOPRY AFFAIRS MANAGER, EMEA

Email: kristina.elvebakken@cpkelco.com
Tel: +45 56 16 56 16 / +45 23 35 86 71

GRAS NOTICE FOR NUTRAVA™ CITRUS FIBER

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

SUBMITTED BY:
Kristina Elvebakken
Senior Regulatory Affairs Manager, EMEA
CP Kelco ApS
Ved Banen 16
4623 Lille Skensved
Denmark

DATE:
5 May 2020

GRAS Notice for NUTRAVA™ Citrus Fiber

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GRAS Notice for NUTRAVA™ Citrus Fiber

Part 1. § 170.225 Signed Statements and Certification

In accordance with 21 CFR §170 Subpart E consisting of §170.203 through 170.285, CP Kelco ApS (CPK) hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of Citrus Fiber (NUTRAVA™), as manufactured by CPK, in various conventional food and beverage products as described in Section 1.3 below, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on CPK's view that these notified uses of NUTRAVA™ Citrus Fiber are Generally Recognized as Safe (GRAS). In addition, as a responsible official of CPK, the undersigned hereby certifies that all data and information presented in this notice represent a complete and balanced submission that is representative of the generally available literature. CPK considered all unfavorable as well as favorable information that is publicly available and/or known to CPK and that is pertinent to the evaluation of the safety and GRAS status of NUTRAVA™ Citrus Fiber as a food ingredient for addition to food products, as described herein.

Signed,

5 May 2020

Kristina Elvebakken
Senior Regulatory Affairs Manager, EMEA
CP Kelco ApS
kristina.elvebakken@cpkelco.com

Date

1.1 Name and Address of Notifier

Kristina Elvebakken
Senior Regulatory Affairs Manager, EMEA
CP Kelco ApS
Ved Banen 16
4623 Lille Skensved
Denmark

1.2 Common Name of Notified Substance

The subject of this GRAS notification is citrus fiber from orange, lemon, or lime.

1.3 Conditions of Use

Citrus fiber manufactured by CPK is intended for use as a nutrient source of dietary fiber and multipurpose (*e.g.*, water binding, gelling aid, thickening aid, bulking aid, emulsion stabilizer, cloud stabilizer) food ingredient, and will be marketed under the brand name NUTRAVA™ Citrus Fiber. NUTRAVA™ Citrus Fiber is intended for addition to food and beverage products across multiple categories as described in Table 1.3-1 at use levels up to 3%. Food-uses are organized according to 21 CFR §170.3 (U.S. FDA, 2018a).

Table 1.3-1 Summary of the Individual Proposed Food-Uses and Use Levels for NUTRAVA™ Citrus Fiber in the U.S.

Food Category (21 CFR 170.3)	Proposed Food-Uses[a]	Citrus Fiber Use Level (g/100 g)
Baked Goods and Baking Mixes	Baked Goods and Baking Mixes	3
Beverages, Alcoholic	Cocktail Drinks	1
Beverages and Beverage Bases	Sport or Electrolyte Drinks, Fluid Replacement Drinks	2
	Non-Milk-Based Meal Replacement Beverages and Protein Drinks	1
Breakfast Cereals	Hot Breakfast Cereals (excluding instant)	1
	Ready-to-Eat Breakfast Cereals	3
Cheeses	Cream Cheese and Cheese-Based Spreads	2
	All Other Cheeses	1
Coffee and Tea	Specialty Coffee Drinks (Lattes, Cappuccinos, Mochas) and Ready-to-Drink Tea Beverages	1
Condiments and Relishes	Ketchup	2
	Relish	1
Confections and Frostings	Confections and Frostings (excluding raw sugars)	1
Dairy Product Analogs	Dairy Product Analogs	1
Egg Products	Egg Substitutes	1
Fats and Oils	Fat-Based Sauces; Salad Dressings; Margarine and Margarine-like Spreads; Mayonnaise and Mayonnaise-Type Dressings	2
Fish Products	Fish-Based Entrees[b]	2
Frozen Dairy Desserts	Frozen Dairy Desserts	1
Fruit and Water Ices	Fruit and Water Ices	1
Gelatins, Puddings, and Fillings	Gelatin, Puddings, and Fillings	2
Grain Products and Pastas	Grain Products and Pastas	3
Gravies and Sauces	Gravies and Sauces[c]	2
Jams and Jellies	Jams and Jellies	2
Milk Products	Milk-Based Meal Replacements	2
	All Other Milk Products (including cream, yogurt, dairy-based snack dips, and milk drinks, except fermented milks)	1
Nuts and Nut Products	Nuts and Nut Products	1
Plant Protein Products	Plant Protein Products	3
Processed Fruits and Fruit Juices	Fruit Juices, Drinks, Ades, Nectars, and Fruit Smoothies	1
	Fruit-Based Desserts	2
Processed Vegetables and Vegetable Juices	Processed Vegetables, Entrees, and Vegetable Juices	1
Soft Candy	Chocolate, Nougat, and Toffees	1
Soups and Soup Mixes	Soups and Soup Mixes[c]	2
Sweet Sauces, Toppings, and Syrups	Cocoa Syrups and Chocolate Toppings	1
	Fruit Sauces, Syrups, and Toppings	2

CFR = Code of Federal Regulations; U.S. = United States.

[a] NUTRAVA™ Citrus Fiber is intended for use in unstandardized products where standards of identity, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.

[b] Catfish excluded.

[c] Products containing <3% meat and poultry.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30 (a)(b) of the Code of Federal Regulations (CFR) (U.S. FDA, 2018a), CPK has concluded that the intended uses of NUTRAVA™ Citrus Fiber as described herein are GRAS on the basis of scientific procedures.

1.5 Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. FDA upon request, or will be available for review and copying at reasonable times at the offices of:

CP Kelco ApS
Ved Banen 16
4623 Lille Skensved
Denmark

Should the FDA have any questions or additional information requests regarding this Notification, CPK will supply these data and information upon request.

1.6 Freedom of Information Act, 5 U.S.C. 552

It is CPK's view that all data and information presented in Parts 2 through 7 of this Notice do not contain any trade secret, commercial, or financial information that is privileged or confidential, and therefore, all data and information presented herein are not exempted from the Freedom of Information Act, 5 U.S.C. 552.

Part 2. § 170.230 Identity, Method of Manufacture, Specifications, and Physical or Technical Effect

2.1 Identity

NUTRAVA™ Citrus Fiber is manufactured by CPK from citrus peel, which is a by-product of the juicing processes from oranges, lemons, and limes. Plant cell walls of citrus and other plants are comprised of structures consisting of cellulose fibrils enmeshed within a fibrillar network of pectin, hemicellulose, and lignin (Figure 2.1-1). The inner lamella of cells within fruit are rich in pectin and therefore have been a traditional source of food-grade pectin used as gelling aids in the food industry (*e.g.*, jam production). NUTRAVA™ Citrus Fiber is an off-white powder with a neutral to slight citrus odor and a suitable mesh size. NUTRAVA™ Citrus Fiber's main components are *ca*. 40 to 45% insoluble fiber and *ca*. 40 to 45% soluble fiber and may contain up to 12% moisture. The insoluble fiber is composed of mainly cellulose but also hemi-cellulose and, to a minor degree, lignin, and the soluble fiber consisting primarily of pectin (see Table 2.4.1-1).

Figure 2.1-1 Distribution of Fiber in Plant Cells (Maddon, 2000; Davidson and Florida State University, 2019; Liu, 2019)

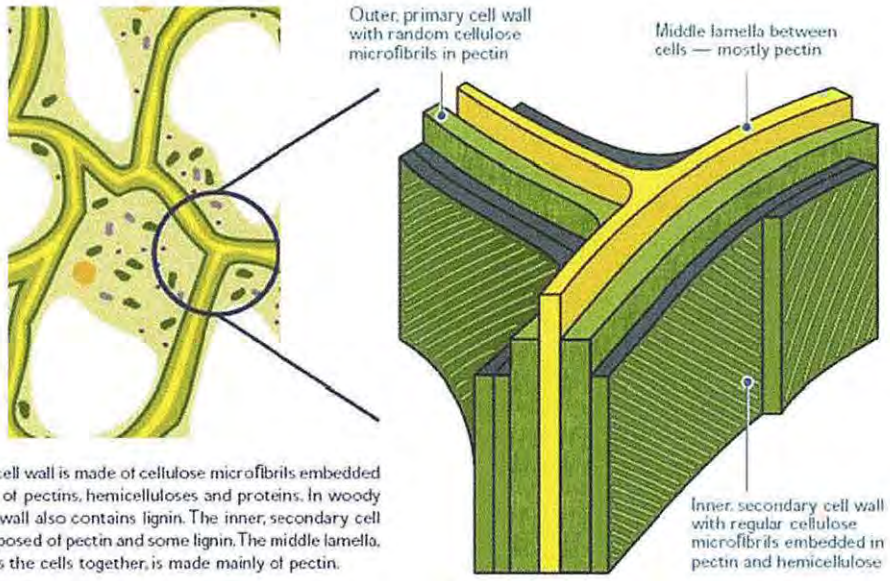

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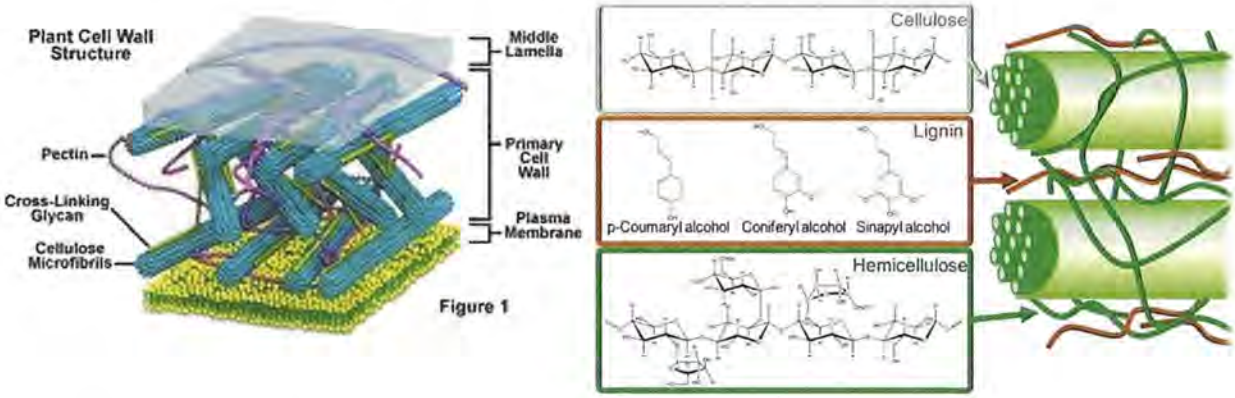
Figure 2.1-1 Distribution of Fiber in Plant Cells (Maddon, 2000; Davidson and Florida State University, 2019; Liu, 2019)



2.2 Manufacturing

2.2.1 Raw Material

The raw materials used for the manufacturing of NUTRAVA™ Citrus Fiber are by-products of citrus fruits (*i.e.,* oranges [*Citrus sinensis*], lemons [*Citrus limon*], and/or limes [*Citrus latifolia* or *Citrus aurantifolia*] after juicing and separation of citrus oils). The full citrus fruit by-product fraction can be used (*i.e.,* juice cells, peel, rag, and segment membranes). For convenience, the citrus fruit by-product is hereafter referred to as "citrus peel". The citrus peel may be fresh or dried. The citrus peel is chopped to reduce the particle size of the material to below 3 cm and then optionally washed with acidic aqueous ethanol and agitation to remove impurities and preserve the quality of the citrus peel.

2.2.2 Manufacturing Process

The manufacturing process of NUTRAVA™ Citrus Fiber is carried out according to current Good Manufacturing Practice (cGMP), and in compliance with the U.S. FDA Food Safety Modernization Act (FSMA). The manufacturing site is International Organization for Standardization (ISO) 9001:2015 and Food Safety System Certification (FSSC) 22000 certified.

Processing aids used by CPK for production of NUTRAVA™ Citrus Fiber include ethanol, an acid (sulfuric acid), and a base (*e.g.,* potassium hydroxide or sodium hydroxide). All processing aids used for the production of NUTRAVA™ Citrus Fiber are food-grade or equivalent and are used in accordance with appropriate federal regulations, have been previously determined to be GRAS for their respective uses, or have been the subject of an effective food contact notification (Table 2.2.2-1).

Table 2.2.2-1 Processing Aids for NUTRAVA™ Citrus Fiber

Processing Aids	Function	Regulatory Status (U.S. FDA, 2018a)
Ethanol	Washing	GRAS when used in accordance with cGMP (21 CFR §182.1 – U.S. FDA, 2018a)
Sulfuric acid	Acidification	Used in food at levels not to exceed GMP in accordance with 184.1(b)(1) (21 CFR §184.1095 – U.S. FDA, 2018a)
Potassium hydroxide	Neutralization	Used in food with no limitation other than cGMP (21 CFR §184.1631 – U.S. FDA, 2018a)

Table 2.2.2-1 Processing Aids for NUTRAVA™ Citrus Fiber

Processing Aids	Function	Regulatory Status (U.S. FDA, 2018a)
Sodium hydroxide	Neutralization	Used in foods at levels not to exceed cGMP (21 CFR §184.1763 – U.S. FDA, 2018a)

CFR = Code of Federal Regulations; cGMP = current Good Manufacturing Practice; GMP = Good Manufacturing Practice; GRAS = Generally Recognized as Safe.

The citrus peel raw material is inspected in accordance with CPK's quality control standards. The manufacturing process consists of (i) an acidification step, and (ii) a partial neutralization step. During the process, sugars, salts, residual oils, and flavonoids are washed out of the citrus peel, resulting in a citrus fiber material consisting mainly of pectin, cellulose, hemicellulose, and lignin.

During the acidification step, mechanical energy and heat are applied to the citrus peel in aqueous ethanol. To create the acidic conditions, the pH is adjusted to below 2.5 by a dilute acid. Mechanical energy is induced by passing the mixture several times through pumps to open the structure of the peel, creating a larger surface. The process of opening the structure is called defibrillation.

After the acidification step, the citrus fiber material is mechanically separated from the liquid phase. The citrus fiber material is then washed in aqueous ethanol and partially neutralized using a dilute base (potassium hydroxide or sodium hydroxide) to a pH of approximately 4.

The citrus fiber solution is then dried to below 12% moisture and milled to a suitable mesh size to produce the final NUTRAVA™ Citrus Fiber product. A schematic overview of the manufacturing process for NUTRAVA™ Citrus Fiber is presented in Figure 2.2.2-1.

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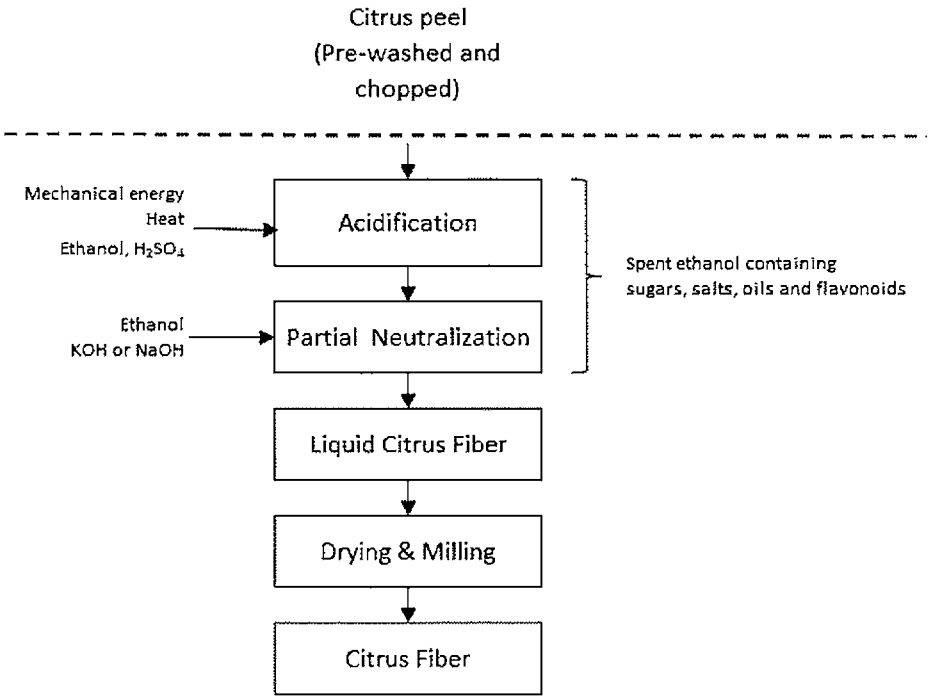
Figure 2.2.2-1 Schematic Overview of the Manufacturing Process for NUTRAVA™ Citrus Fiber



2.3 Specifications

Specifications for NUTRAVA™ Citrus Fiber are shown below in Table 2.3-1. The test methods used have been validated for their purpose. The ingredient is largely characterized by its dietary fiber content and water holding capacity. Additional analytical characterization of the ingredient is presented below in the Section 2.4.

Table 2.3-1 Specifications for NUTRAVA™ Citrus Fiber

Specification Parameter (wet weight basis)	Specification	Method**
Physical/Chemical Characteristics		
Dietary Fiber (%)*	More than 80	AOAC 985.29
Loss on Drying (%)	Not more than 12	CPK 0006042
Ash (%)*	Less than 5	BS 4401 Part 1 1998 Com. Reg. 152/2009
pH, 1%	3.0 to 5.0	CPK 0006041
Particle Size	Less than 3% on a 0.250 mm test sieve	CPK 0006013
Water Holding Capacity (g/g)	15 to 35	CPK 0901001
Ethanol (%)*	Not more than 0.5	MEBAK II, 4. Aufl., Kap. 2.10.6.3:2002
Microbiology		
Total Plate Counts (CFU/g)*	Not more than 5,000	NordVal 033
Yeast and Molds (CFU/g)*	Not more than 500	NordVal 043
Escherichia coli (CFU/g)*	Negative	CPK 0800004

Table 2.3-1 Specifications for NUTRAVA™ Citrus Fiber

Specification Parameter (wet weight basis)	Specification	Method**
Physical/Chemical Characteristics		
Dietary Fiber (%)*	More than 80	AOAC 985.29
Loss on Drying (%)	Not more than 12	CPK 0006042
Ash (%)*	Less than 5	BS 4401 Part 1 1998 Com. Reg. 152/2009
pH, 1%	3.0 to 5.0	CPK 0006041
Particle Size	Less than 3% on a 0.250 mm test sieve	CPK 0006013
Water Holding Capacity (g/g)	15 to 35	CPK 0901001
Salmonella (CFU/25 g)*	Negative	AFNOR: BRD 07/11-12/05 (Rapid)
Heavy Metals		
Lead (ppm)*	Less than 1 ppm	EPA 200.8 + CSN EN/ISO 17294-2
Arsenic (ppm)*	Less than 1 ppm	EPA 200.8 + CSN EN/ISO 17294-2

CFU = colony forming units; ppm = parts per million.

* Food safety parameters (microbiology and heavy metals) are in control by the Hazard Analysis and Critical Control Points setup of the manufacturing plant. Hence compliance with specification is verified by skip-lot testing. The frequency of skip-lot testing will be high in the start of the process, *e.g.*, 1 batch per day. In the case of eventual food safety parameter out-of-spec, batches skipped since previous compliant result will be quarantined and tested individually – and scrapped, if necessary. For quantitative parameters (all parameters marked with asterisks except but *E. coli* and *Salmonella*), test frequencies will be adjusted with increased knowledge of actual process variations relative to the specification limits. This setup is adapted from CPK's pectin producing plants, which also use citrus peel as raw material, and has for years been efficient and accepted by both regulating agencies and third-party certification bodies.

** Other validated methods with equal sensitivity as the ones listed may be applied, *e.g.*, for total plate count and yeast and molds where the NordVal methods may not be readily available in Brazil where CPK's production is located.

2.3.1 Batch Analysis

Analysis of 3 non-consecutive batches of NUTRAVA™ Citrus Fiber demonstrates that the manufacturing process as described in Section 2.2 produces a consistent product that meets specifications (see Table 2.3.1-1).

Table 2.3.1-1 Batch Analysis of NUTRAVA™ Citrus Fiber

Parameter (wet weight basis)	Specification	Manufacturing Batch		
		512-96-8	512-97-7	512-98-7
Physical/Chemical Characteristics				
Dietary Fiber (%)	More than 80	85.7	84.9	89.8
Loss on Drying (%)	Not more than 12	3.7	3.6	3.7
Ash (%)	Less than 5	3.6	4.2	3.8
pH, 1%	3.0 to 5.0	3.8	3.8	3.9
Particle Size	Less than 3% on a 0.250 mm test sieve	0.01	0.5	0.2
Water Holding Capacity (g/g)	15 to 35	22.2	21.2	23.8
Ethanol (%)	Not more than 0.5	0.0323	0.0814	0.0562
Microbiology				
Total Plate Counts (CFU/g)	Not more than 5,000	300	100	<100
Yeast and Molds (CFU/g)	Not more than 500	200	100	<10
Escherichia coli (CFU/g)	Negative	Negative	Negative	Negative
Salmonella (CFU/25 g)	Negative	Negative	Negative	Negative

Table 2.3.1-1 Batch Analysis of NUTRAVA™ Citrus Fiber

Parameter (wet weight basis)	Specification	Manufacturing Batch		
		512-96-8	512-97-7	512-98-7
Heavy metals				
Lead (ppm)	Less than 1 ppm	0.051	<0.050	0.112
Arsenic (ppm)	Less than 1 ppm	<0.10	<0.10	<0.10

CFU = colony forming units; ppm = parts per million.

2.4 Additional Product Characterization

2.4.1 Proximate Analysis

Three non-consecutive batches of NUTRAVA™ Citrus Fiber were analyzed for fat, protein, total carbohydrates, and soluble and insoluble fiber (Table 2.4.1-1). The fat and protein content is low, typically comprising <10% of the total composition. Analysis of the ingredient for soluble and insoluble fiber demonstrates that soluble and insoluble fiber are typically present in a ratio of 1:1 comprising 40 to 45% of the ingredient composition.

Table 2.4.1-1 Proximate Analysis of NUTRAVA™ Citrus Fiber

Parameter (wet weight basis)	Method	Manufacturing Batches		
		512-96-8	512-97-7	512-98-7
Fat (%)	NMR	0.7	1.3	1.1
Protein (%)	Dumas Leco Nitrogen	4.4	5.1	4.7
Total Carbohydrates (%)	Council Directive 90/496/EEC	87.8	85.7	87.1
Total Sugars as Glucose (%)	Commission Reg. (EC) 152/2009	<0.1	<0.1	<0.1
Soluble Fiber (%)	§ 64 LFGB L 00.00-18: 1997-01	45.5	43.6	41.9
Insoluble Fiber (%)	§ 64 LFGB L 00.00-18: 1997-01	39.8	40.8	42.2

NMR = nuclear magnetic resonance.

2.4.2 Analysis of Residual Processing Aids and Impurities

The same 3 non-consecutive batches of NUTRAVA™ Citrus Fiber were subjected to analysis for residual processing aids originating from the manufacturing process and the results are presented in Table 2.4.2-1. The content of sulfur dioxide, which notably is not added, has been monitored to verify that it is not created from sulfuric acid during the fiber process. As expected, sulfur dioxide has not been detected, which is in line with chemical literature describing that its formation from sulfuric acid requires very high temperature (>300°C) and presence of a suitable catalyst.

Table 2.4.2-1 Residual Processing Aids from the Manufacturing Process

Parameter (wet weight basis)	Method	Manufacturing Batch		
		512-96-8	512-97-7	512-98-7
Ethanol (ppm)	MEBAK II, 4. Aufl., Kap. 2.10.6.3:2002	323	814	562
Sulfur (ppm)[a]	CZ_SOP_D06_02_01	2,960	4,360	2,850
Sulfur Dioxide (ppm)	§ 64 LFGB L 00.00-46/I: 1999-01	<10	<10	<10
Potassium (ppm)	LC/UV	8,610	9,750	10,100

ppm = parts per million.
[a] Mainly present in the form of sulfate.

2.4.3 Mycotoxin and Pesticide Analysis

Analysis of mycotoxins including ochratoxin A, aflatoxin B1, B2, G1, and G2, in 3 batches of NUTRAVA™ Citrus Fiber demonstrated that residual content of each mycotoxin measured <0.5 µg/kg. Additionally, a GC-MS/MS Pesticide Screen (Method AM/R/1003) on 3 non-consecutive batches of NUTRAVA™ Citrus Fiber demonstrated all pesticides tested measured <0.01 mg/kg. All analyses for manufacturing by-products, impurities and contaminants were conducted using validated methods at accredited laboratories.

2.5 Stability

CPK provided microbial stability data demonstrating that NUTRAVA™ Citrus Fiber when stored in paper bags with a perforated polyethylene inner liner is stable for 18 months, as estimated by an accelerated shelf-life study (55°C and 80% relative humidity) performed for 13 weeks, corresponding to 84 weeks at ambient temperature (Figure 2.5-1). A real-time study (30°C and 80% relative humidity for 78 weeks) is currently ongoing. The initial bioburden was analyzed and determined to be low (Table 2.5-1) and storage of NUTRAVA™ Citrus Fiber at accelerated conditions demonstrated a water activity (a_w) below 0.6 over the 13-week storage period. Most microorganisms grow well at a_w between 0.91 and 0.99, while an a_w below 0.6 is regarded as safe from a microbial point of view. Therefore, the low bioburden in the freshly produced product combined with an a_w consistently below 0.6 indicates a relatively low microbiological risk.

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Figure 2.5-1 NUTRAVA™ Citrus Fiber Water Activity Development Over Time During an Accelerated Shelf-Life Study at 55°C and 80% Relative Humidity Corresponding to 84 weeks (real time) using a Q10 factor

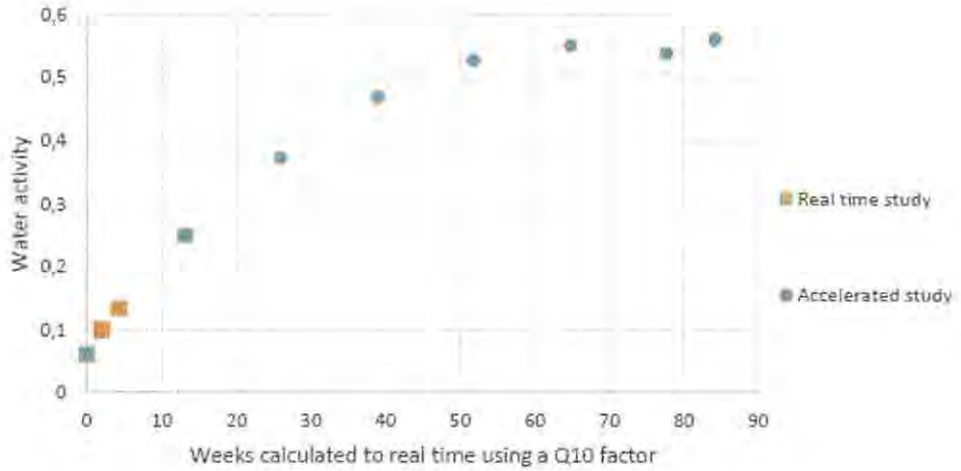

Table 2.5-1 Initial Bioburden of NUTRAVA™ Citrus Fiber

Parameter	Manufacturing Batch	
	512-521	**512-521**
Coliforms	<10 CFU/g	<10 CFU/g
Escherichia coli	<10 CFU/g	<10 CFU/g
Salmonella	Negative/25 g	Negative/25 g
Aerobic Spores	<10 CFU/g	<10 CFU/g
Aerobic Plate Count	<100 CFU/g	<100 CFU/g
Mold	<10 CFU/g	<10 CFU/g
Yeast	<10 CFU/g	<10 CFU/g
Anaerobic Spores	<10 CFU/g	20 CFU/g

CFU = colony forming units.

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Part 3. Dietary Exposure

3.1 History of Use of the GRAS Substance

3.1.1 History of Consumption of Citrus Fruit

Citrus fruits and products derived from these fruits have a long history of safe human consumption. Citrus fruits have been consumed for thousands of years, originating in Southeast Asia and India, introduced to Italy by the end of the Roman Empire, and spreading through the rest of Europe during the Middle Ages. Citrus fruits were first introduced to America by Spanish and Portuguese explorers, and citrus fruit orchards first appeared in Florida and California around 1655 and 1769, respectively (Liu *et al.*, 2012). Popular citrus fruits include oranges, lemons, grapefruit, and limes. Oranges account for the greatest value in terms of production, followed by grapefruit, lemons, and tangerines (U.S. DHHS & USDA, 2015). Systematic breeding of sweet oranges was initiated in Florida by the U.S. Department of Agriculture (USDA) in 1893 (Stover *et al.*, 2010). In the U.S., *per capita* citrus availability in 2016 totaled 75.9 lbs (fresh weight equivalent); this was led by oranges and followed by lemons, tangerines, limes, and grapefruits (USDA-ERS, 2017). Citrus fruits can be eaten fresh, juiced for use in food and beverages, and their peel can be used for garnishes or pickling. Citrus fruit peel is generally very bitter; however, it has been used in applications such as marmalade (when cooked and mixed with sugar). As described in several GRAS notifications of similar citrus fiber products, recipes for oranges in foods can be found in several historical cookbooks, including a recipe for orange marmalade published in *Culinary Chemistry* in 1821 by Fredrick Accum (Kansas State University Library, 2003; GRN 154 – U.S. FDA 2004; GRN 599 – U.S. FDA 2016a; and GRN 719 – U.S. FDA, 2017).

3.1.2 Regulatory Status

Several citrus fiber ingredients have GRAS status for use in food and beverages as sources of dietary fiber and general-purpose food ingredients (see Table 3.1.2-1). Food-uses of NUTRAVA™ Citrus Fiber will partially substitute for existing uses of citrus fiber in the U.S. marketplace.

Table 3.1.2-1 Citrus Fiber Ingredients with GRAS Status for Specified Food-Uses[a]

Regulatory Status	GRAS Substance	Food-Uses
GRN 154 (U.S. FDA, 2004)	Dried orange pulp	Use in baked goods, pastas, salad dressings, confectionery, processed cheese spreads, non-carbonated beverages and fruit drinks, frozen food entrees, such as frozen doughs, frozen meat products, frozen baked goods, frozen desserts, and frozen dairy products, and meat and poultry products at a maximum use level of 5 percent.
GRN 487 (U.S. FDA, 2014a)	Dried citrus pulp	As a moisture retention agent, a flavor enhancing agent, and a processing aid in foods.
GRN 541 (U.S. FDA, 2015a)	Insoluble fiber from citrus peel	Intended for use as a moisture retention agent, flavor enhancing agent, or processing aid in baked goods, pastas, salad dressings, confectionery, processed cheese spreads, frozen food entrees, and comminuted and whole muscle meat and poultry products at a maximum level of 5%; and as a flavor enhancer in non-carbonated beverages and fruit drinks; as a seasoning in brine and in comminuted and whole muscle meat and poultry products, and as an ingredient in salads, sauces, meats, fillings, dips, baked goods, dairy products, fruit- and vegetable based products, and pizza at a maximum level of 5%.
GRN 599 (U.S. FDA, 2016a)	Citrus fiber	Intended for use as a texturizer and moisture retention agent in yogurt, low fat, mayonnaise, ice cream, ice pops and sorbet (not to exceed 4%), and processed meat and poultry products (not to exceed 5%).

GRAS = Generally Recognized as Safe; GRN = GRAS Notice.

Table 3.1.2-1 Citrus Fiber Ingredients with GRAS Status for Specified Food-Uses[a]

Regulatory Status	GRAS Substance	Food-Uses

[a] https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=fiber

As discussed in Section 2.4, NUTRAVA™ Citrus Fiber is >85% dietary fiber with an approximate ratio of 1:1 insoluble fiber (cellulose, hemicellulose, and lignin) and soluble fiber (pectin). Several plant sources of dietary fiber comprised of cellulose, hemicellulose, and lignin also have GRAS status (see Table 3.1.2-2). NUTRAVA™ Citrus Fiber is compositionally similar to other plant-based fiber ingredients and will be partially substitutional to these ingredients in the diet. Pectins are affirmed as GRAS by the U.S. FDA under 21 CFR §184.1588 for use as stabilizers and thickeners at use levels limited to cGMP. It can be concluded that processed fiber ingredients containing cellulose, hemicellulose, lignin, and pectin are widely represented in the U.S. diet.

Table 3.1.2-2 Plant-Based Fiber Ingredients with GRAS Status for Specified Food-Uses[a]

GRN	GRAS Substance	Food-Uses
116 (U.S. FDA, 2003)	Carrot fiber	Use in baked goods as a texturizer at a maximum level of 5 percent by weight of flour; use in meat substitutes (*e.g.*, meatless sausages and meatless patties) at a maximum level of 5 percent; use in meat and poultry products as a binder/extender and to reduce water purging/gelling at a maximum level of 5 percent.
207 (U.S. FDA, 2006)	Barley fiber	Use in food in general, except for infant formula and meat and poultry products.
261 (U.S. FDA, 2009)	Out hull fiber	Use (1) as an ingredient in bread/pizza crust, cookies/crackers/bars, cereal (hot and cold), baby food cereal, and snacks (fried and baked) at levels ranging from 5 percent to 14 percent by weight; (2) as an ingredient in breaders and batters applied in coating onto meat and poultry at levels ranging from 2 percent by weight of the total food system (breaded or coated meats) to 5 percent by weight of the dry coating system (breader and batter); and (3) as an extender in meat products at levels ranging from 3 percent to 5 percent.
310 (U.S. FDA, 2010a)	Potato fiber	Use to improve the texture, stability, and oil/water binding capacity of whole muscle poultry products and comminuted meat and poultry products at a maximum level of 3.5 percent in the final product.
342 (U.S. FDA, 2010b)	Oat hull fiber	Ingredient in comminuted meat products and in both whole muscle and comminuted poultry products at levels consistent with current good manufacturing practice.
344 (U.S. FDA, 2011a)	Barley fiber	Ingredient in food generally, except infant formula and meat and poultry products.
373 (U.S. FDA, 2011b)	Rice bran fiber	As an ingredient in food, excluding meat and poultry, at levels consistent with current good manufacturing practice.
427 (U.S. FDA, 2012)	Corn hull fiber	Use as a formulation aid and/or source of fiber at levels ranging from 0.25 to 3.0 percent in: baked goods and cereal products; coatings and breadings; dairy products egg products; meat, poultry, and seafood products; dressings and dips; sauces, soups, and gravies; snacks; and fillings, toppings, and icings.
430 (U.S. FDA, 2013)	Sugar beet fiber	Use in various non-standardized meat and poultry products as a binder and texturizing agent, in bakery products as a binder and source of fiber, in cereals and muesli as a texturizing agent and source of fiber, in sauces as a thickening agent, and in cheese as an anti-caking and/or dispersing agent.
478 (U.S. FDA, 2015b)	Rice hull fiber	As a source of dietary fiber.
525 (U.S. FDA, 2014b)	Pea fiber	For use as a texturizer and humectant in baked goods (bread, cakes, and noodles), fruit juices, and milk (acidified) at levels up to 0.5 grams/serving.
646 (U.S. FDA, 2016b)	Pecan shell fiber	For use as a source of fiber and antioxidants in baked goods and baking mixes, breakfast cereals, confections and frostings, gelatins, puddings and fillings, grain

Table 3.1.2-2 Plant-Based Fiber Ingredients with GRAS Status for Specified Food-Uses[a]

GRN	GRAS Substance	Food-Uses
		products and pastas, meal replacements, snack food, soft candy, sweet sauces at levels ranging from 2 to 10 percent.

GRAS = Generally Recognized as Safe; GRN = GRAS Notice.
[a] https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=fiber

3.1.3 Nutritional and Technological Function

The main functionalities of NUTRAVA™ Citrus Fiber are (i) fiber enrichment (a combination of soluble and insoluble fiber, discussed in Section 3.1.3.1); (ii) water binding; (iii) rheology modifier; and (iv) stabilizer. The definition of dietary fiber in multiple jurisdictions is discussed in Sections 3.1.3.1.1 to 3.1.3.1.3.

3.1.3.1 Fiber Enrichment

NUTRAVA™ Citrus Fiber is a natural source of dietary fiber with a balanced ratio of soluble and insoluble fiber. NUTRAVA™ Citrus Fiber is *ca.* 40 to 45% insoluble fiber (*i.e.*, cellulose, hemicellulose, and lignin) and *ca.* 40 to 45% soluble dietary fiber (*i.e.*, pectin). Soluble fiber is readily fermented in the large intestine into gases and short-chain fatty acids. Insoluble fiber is fermented to a lesser degree; however, it possesses water-binding effects including fecal bulking.

Dietary fiber is an important component of a healthy diet. The Institute of Medicine of the National Academies (IOM) currently recommends an adequate intake level of 38 g total dietary fiber/day for males and 25 g total dietary fiber/day for females (ages 19 to 50 years) (IOM, 2005). For individuals greater than 50 years of age intakes of 30 g/day and 21 g/day for males and females respectively are recommended. The Nutrition Facts Panel required under 21 CFR §101.9 provides a good reference, stating as a goal, 28 g dietary fiber for a 2,000 calories/day diet for adults and children aged 4 years and older, and for pregnant women and lactating women (U.S. FDA, 2018a). Most people do not consume the recommended amount of fiber (U.S. DHHS & USDA, 2015), and according to the Reicks *et al.* (2014) and the National Health and Nutrition Examination Survey (NHANES) 2009-2010 data, mean fiber intake for U.S. children/adolescents aged 2 to 18 years was 13.6 g/day and for adults 19+ years was 17.0 g/day; far below the recommended levels of dietary fiber. The recommended intake of dietary fiber by adults in the European Union (EU) ranges from 25 to 38 g/day (European Commission, 2019).

Consistent evidence has demonstrated that consumption of dietary fiber leads to many nutritional benefits including lower risk of obesity, hypertension, coronary heart disease, stroke, diabetes, and various gastrointestinal disorders (constipation, hemorrhoids, diverticulitis, duodenal ulcer, and gastroesophageal reflux disease) (Anderson *et al.*, 2009). The European Commission (European Commission, 2019) describes the health benefits associated with dietary fiber to include gastrointestinal health and risk reduction of non-communicable diseases such as cardiovascular diseases, diabetes type 2, colorectal cancer and reduced risk of weight gain. Total fiber intake influences several metabolic functions, including fat metabolism and absorption of nutrients and carbohydrates. Soluble fibers have been reported to normalize glycemia and insulin sensitivity in non-diabetic and diabetic individuals, while insoluble fibers have been demonstrated to increase fecal mass and promote regularity (Anderson *et al.*, 2009).

3.1.3.1.1 United States Food and Drug Administration Definition of Dietary Fiber

According to the U.S. FDA, dietary fibers that can be declared on the Nutrition and Supplement Facts labels in food include *"naturally occurring fibers that are 'intrinsic and intact' in plants, and added isolated or synthetic non-digestible soluble and insoluble carbohydrates that FDA has determined have beneficial physiological effects to human health"*. These beneficial physiological effects include lowering blood glucose and cholesterol levels, lowering blood pressure, reducing calorie intake, increasing mineral absorption in the intestinal tract, and increasing the frequency of bowel movements (U.S. FDA, 2018b).

Naturally occurring dietary fibers are "intrinsic" to foods such as vegetables, whole grains, fruits, cereal bran, flaked cereal and flours and are considered to be "intact" because they have not been removed from the food. Added "isolated" or "synthetic" dietary fibers are a result of different types of processing methods (U.S. FDA, 2018b).

In a review of the scientific evidence on the physiological effects of certain non-digestible carbohydrates, the U.S. FDA has identified the following non-digestible carbohydrates, which are added to food, as meeting the dietary fiber definition (U.S. FDA, 2018b):

- Beta-glucan soluble fiber (form of hemicellulose);
- Psyllium husk (a form of hemicellulose);
- Cellulose;
- Guar gum;
- Pectin;
- Locust bean gum; and
- Hydroxypropylmethylcellulose.

Additionally, based on the scientific evaluation and the U.S. FDA criteria of dietary fiber, the FDA proposed the following non-digestible carbohydrates be added to the definition of dietary fiber (U.S. FDA, 2018b):

- Mixed plant cell wall fibers (a broad category that includes fibers like sugar cane fiber and apple fiber, among many others [contain 2 or more of the following plant cell wall fibers in varying proportions: cellulose; pectin; lignin[1]; beta-glucan; and arabinoxylan]);
- Arabinoxylan (a form of hemicellulose);
- Alginate;
- Inulin and inulin-type fructans;
- High amylose starch (resistant starch 2);
- Galactooligosaccharide;
- Polydextrose; and
- Resistant maltodextrin/dextrin.

NUTRAVA™ Citrus Fiber is a mixed plant cell wall fiber. Mixed plant cell wall fibers are considered isolated fibers because they are generally processed using methods that result in the plant cell being disrupted and/or various nutrients being removed from the plant, such that they are no longer intrinsic and intact. The U.S. FDA states in its review that, *"The interrelatedness of cellulose, pectin, ß-glucan, and/or arabinoxylan within the plant cell wall and the scientific evidence for each supports our decision to consider enforcement discretion for declaring these mixed plant cell wall fibers, as well as lignin, as dietary fiber if any of these plant cell wall fibers is present in a food"* (U.S. FDA, 2018b). The FDA also lists examples of mixed

[1] Although lignin is not a carbohydrate, it is covalently bound to hemicellulose within the plant cell matrix, and as such, is indicative of being intrinsic and intact in plants (IOM, 2001). Accordingly, the U.S. FDA considers lignin a dietary fiber when present in food.

plant cell wall fibers as including "*apple fibers, bamboo fibers, barley fibers, carrot fibers, **citrus fibers**, cocoa fibers, corn fibers (e.g., corn hull fiber), cotton seed fibers, oat fibers (e.g., oat hull fiber), pea fibers (e.g., pea hull fiber, pea seed coat fiber, inner cotyledon pea fiber), rice bran fibers, soy fibers (e.g., soy hull fiber, soy polysaccharide, soy cotyledon fiber), sugar beet fibers, sugar cane fibers, and wheat fibers*" (U.S. FDA, 2018b).

Under 21 CFR 101.9(c)(6)(i), cellulose is defined as a linear homopolymer of β-(1-4) linked glucose units that meets the dietary fiber definition based on improved laxation. 21 CFR §101.9(c)(6)(i)) also includes a definition of pectin, which is defined as polysaccharides with a backbone chain of α-(1→4)-linked D-galacturonic acid units interrupted by the insertion of (1→2)-linked L-rhamnopyranosyl residues in adjacent or alternate positions that meets the definition of dietary fiber based on its effect of attenuating blood cholesterol levels.

CPK has concluded that NUTRAVA™ Citrus Fiber is an isolated dietary fiber.

3.1.3.1.2 European Union Definition of Dietary Fiber

In the EU, fiber is defined as "*carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:*

- *edible carbohydrate polymers naturally occurring in the food as consumed,*
- *edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence,*
- *edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence*" (Regulation 1169/2011 [EU, 2011]).

The specific non-digestible carbohydrates in NUTRAVA™ Citrus Fiber (cellulose, hemicellulose, and pectin) are all non-starch polysaccharides that fall under this definition (see Section 3.1.3.1.1 for scientific justification of beneficial physiological effect)

3.1.3.1.3 Food and Agriculture Organization Definition of Dietary Fiber

The Food and Agriculture Organization (FAO) defines dietary fiber as "*carbohydrate polymers with ten or more monomeric units, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories:*

- *Edible carbohydrate polymers naturally occurring in the food as consumed,*
- *carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities,*
- *synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities*" (Codex Alimentarius, 2009).

The specific non-digestible carbohydrates in NUTRAVA™ Citrus Fiber (cellulose, hemicellulose, and pectin) are all non-starch polysaccharides that fall under this definition (see Section 3.1.3.1.1 for scientific justification of beneficial physiological effect).

3.2 Estimated Intake of NUTRAVA™ Citrus Fiber from Proposed Food and Beverage Uses

3.2.1 Methodology

An assessment of the anticipated intake of NUTRAVA™ Citrus Fiber as an ingredient under the intended conditions of use (see Table 1.3-1) was conducted using data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics (NCHS)'s NHANES (CDC, 2018a,b; USDA, 2018).

The NHANES data are collected and released in 2-year cycles with the most recent cycle containing data collected in 2015-2016. Information on food consumption was collected from individuals *via* 24-hour dietary recalls administered on 2 non-consecutive days (Day 1 and Day 2). Sample weights were incorporated with NHANES data to compensate for the potential under-representation of intakes from specific populations and allow the data to be considered nationally representative (CDC, 2018a,b; USDA, 2018). The NHANES data were employed to assess the mean and 90th percentile intake of NUTRAVA™ Citrus Fiber for each of the following population groups:

- Infants and young children, up to and including 2 years;
- Children, ages 3 to 11;
- Female teenagers, ages 12 to 19;
- Male teenagers, ages 12 to 19;
- Female adults, ages 20 and up;
- Male adults, ages 20 and up; and
- Total population (all age and gender groups combined).

Consumption data from individual dietary records, detailing food items ingested by each survey participant, were collated by computer and used to generate estimates for the intake of NUTRAVA™ Citrus Fiber by the U.S. population[2]. Estimates for the daily intake of NUTRAVA™ Citrus Fiber represent projected 2-day averages for each individual from Day 1 and Day 2 of NHANES 2015-2016; these average amounts comprised the distribution from which mean and percentile intake estimates were determined. Mean and percentile estimates were generated incorporating survey weights in order to provide representative intakes for the entire U.S. population. *"Per capita"* intake refers to the estimated intake of NUTRAVA™ Citrus Fiber averaged over all individuals surveyed, regardless of whether they consumed food products in which NUTRAVA™ Citrus Fiber is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing NUTRAVA™ Citrus Fiber during the 2 survey days). "Consumer-only" intake refers to the estimated intake of NUTRAVA™ Citrus Fiber by those individuals who reported consuming food products in which the use of NUTRAVA™ Citrus Fiber is currently under consideration. Individuals were considered "consumers" if they reported consumption of

[2] Statistical analysis and data management were conducted in DaDiet Software (Dazult Ltd., 2018). DaDiet Software is a web-based software tool that allows accurate estimate of exposure to nutrients and to substances added to foods, including contaminants, food additives and novel ingredients. The main input components are concentration (use level) data and food consumption data. Data sets are combined in the software to provide accurate and efficient exposure assessments.

1 or more food products in which NUTRAVA™ Citrus Fiber is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of NUTRAVA™ Citrus Fiber were generated using the maximum use level indicated for each intended food-use, as presented in Table 1.3-1, together with food consumption data available from the 2015-2016 NHANES datasets. The results for these assessments are presented in Section 3.2.2.

3.2.2 Results of Intake Estimates for NUTRAVA™ Citrus Fiber

A summary of the estimated daily intake of NUTRAVA™ Citrus Fiber from proposed food-uses is provided in Table 3.2.2-1 on an absolute basis (g/person/day), and in Table 3.2.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was high among all age groups evaluated in the current intake assessment; greater than 99% of the population groups consisted of consumers of food products in which NUTRAVA™ Citrus Fiber is currently proposed for use (Table 3.2.2-1). Children, female teenagers, and female adults had the greatest proportion of consumers at 100%. The consumer-only estimates are more relevant to risk assessments as they represent exposures in the target population; consequently, only the consumer-only intake results are discussed in detail herein.

Among the total population (all ages), the mean and 90th percentile consumer-only intakes of NUTRAVA™ Citrus Fiber were determined to be 11.4 and 20.0 g/person/day, respectively. Of the individual population groups, male teenagers were determined to have the greatest mean and 90th percentile consumer-only intakes of NUTRAVA™ Citrus Fiber on an absolute basis, at 13.1 and 24.1 g/person/day, respectively, while infants and young children had the lowest mean and 90th percentile consumer-only intakes of 6.9 and 13.4 g/person/day, respectively (Table 3.2.2-1).

Table 3.2.2-1 Summary of the Estimated Daily Intake of NUTRAVA™ Citrus Fiber from Proposed Food-Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (g/day)		Consumer-Only Intake (g/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	5.6	12.7	82.2	512	6.9	13.4
Children	3 to 11	11.2	18.0	100.0	1,177	11.2	18.0
Female Teenagers	12 to 19	10.0	16.6	100.0	473	10.0	16.6
Male Teenagers	12 to 19	13.1	24.1	99.8	493	13.1	24.1
Female Adults	20 and up	10.5	17.8	100.0	2,205	10.5	17.8
Male Adults	20 and up	12.9	23.3	99.6	1,995	12.9	23.3
Total Population	All ages	11.3	20.0	99.2	6,855	11.4	20.0

n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

On a body weight basis, the total population (all ages) mean and 90th percentile consumer-only intakes of NUTRAVA™ Citrus Fiber were determined to be 199 and 402 mg/kg body weight/day, respectively. Among the individual population groups, infants and young children were identified as having the highest mean and 90th percentile consumer-only intakes of any population group, of 562 and 1,065 mg/kg body weight/day, respectively. Female adults had the lowest mean and 90th percentile consumer-only intakes of 145 and 256 mg/kg body weight/day, respectively (Table 3.2.2-2).

Table 3.2.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of NUTRAVA™ Citrus Fiber from Proposed Food-Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/kg bw/day)		Consumer-Only Intake (mg/kg bw/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Young Children	0 to 2	461	1,012	82.1	507	562	1,065
Children	3 to 11	436	726	100.0	1,172	436	726
Female Teenagers	12 to 19	172	306	100.0	465	172	306
Male Teenagers	12 to 19	203	415	99.8	492	203	415
Female Adults	20 and up	145	256	100.0	2,191	145	256
Male Adults	20 and up	151	290	99.6	1,969	151	291
Total Population	All ages	197	401	99.2	6,796	199	402

bw = body weight; n = sample size; NHANES = National Health and Nutrition Examination Survey; U.S. = United States.

3.2.3 Summary and Conclusions

Consumption data and information pertaining to the intended food-uses of NUTRAVA™ Citrus Fiber were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment which render exposure estimates highly conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain NUTRAVA™ Citrus Fiber at the maximum specified level of use. In reality, the levels added to specific foods will vary depending on the nature of the food product and it is unlikely that NUTRAVA™ Citrus Fiber will have 100% market penetration in all identified food categories.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of NUTRAVA™ Citrus Fiber by the total U.S. population from proposed food-uses in the U.S., were estimated to be 11.4 g/person/day (199 mg/kg body weight/day) and 20.0 g/person/day (402 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of NUTRAVA™ Citrus Fiber were determined to be 13.1 g/person/day (203 mg/kg body weight/day) and 24.1 g/person/day (415 mg/kg body weight/day), as identified among male teenagers. These intake estimates are similar to dietary intake values previously estimated for other citrus fiber ingredients that have been concluded to be GRAS. For example, as reported in GRN 487, GRAS uses of dried citrus pulp were estimated to result in daily intakes of 23.4 g/day (0.4 g/kg body weight/day) and 39.5 g/day (0.8 g/kg body weight /day) at the mean and 90th percentile, respectively for the general population of U.S. consumers >2 years of age. As reported in GRN 541, the estimated per user daily intake of citrus fiber from all proposed food-uses was 20.24 g/day and 34.17 g/day at the mean and 90th percentile, respectively for the general population of U.S. consumers >2 years of age. Since the majority of proposed food-uses of NUTRAVA™ Citrus Fiber will be largely substitutional to existing food-uses of citrus fiber in the diet, no appreciable change in the dietary intake of citrus fiber is expected from the introduction NUTRAVA™ Citrus Fiber to the U.S. marketplace.

Part 4. Self-Limiting Levels of Use

The proposed food and beverage uses of NUTRAVA™ Citrus Fiber are considered self-limiting due to the viscosity and high water-retention capacity of the ingredient, which at high levels impair processability and eating qualities of the food.

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Part 5. Experience Based on Common Use in Food Before 1958

Not applicable.

Part 6. Narrative

6.1 Introduction

CPK intends to market NUTRAVA™ Citrus Fiber, as a dietary fiber and general-purpose food ingredient in a variety of food and beverage products in the U.S. NUTRAVA™ Citrus Fiber is a food ingredient made using washed and mechanically treated citrus peel from oranges, lemons, or limes as raw material. NUTRAVA™ Citrus Fiber is manufactured in accordance with cGMP using acidic aqueous ethanol to produce a finished product. The composition of NUTRAVA™ Citrus Fiber is characterized by analytical testing and consists of carbohydrates, moisture, ash, protein, and fat (see Table 2.4.1-1). The carbohydrate component consists of 40 to 45% insoluble fiber (cellulose, hemicellulose, and lignin) and 40 to 45% soluble fiber (pectin). Analytical characterization demonstrated that adequate manufacturing controls are in-place to ensure that no undesirable constituents, processing aids, or by-products of the manufacturing process (*e.g.*, residual minerals, heavy metals, solvents, microbial contaminants; and pesticides) are present in the ingredient at levels of toxicological concern. CPK recognizes that citrus peel contains flavonoids with biological activity; however, these compounds are removed in the manufacturing process. Based on the identity of the source material (*i.e.*, safe plant sources with a history of consumption), the nature of the processes applied to the source material, the citrus fiber production process (*i.e.*, processing with acidic aqueous ethanol; washing out sugars, salts, residual oils, and flavonoids) and the compositional identity of the ingredient (*i.e.*, plant-based cellulose, hemicellulose, lignin, and pectin), CPK can conclude that NUTRAVA™ Citrus Fiber meets the definition of plant-based dietary fiber.

Authoritative opinions (*e.g.*, IOM, European Food Safety Authority) on the nutritional value, and therefore inherent safety, of plant-based sources of dietary fiber are applicable to the safety of NUTRAVA™ Citrus Fiber as a source of dietary fiber and general-purpose food ingredient. No upper limit has been established for dietary fiber intake and untoward effects of consuming dietary fiber in excess are generally recognized to be self-limiting effects produced by osmotic mechanisms from the presence and fermentation of the dietary fiber in the large intestine. A discussion on the safety of dietary fiber is presented in Section 6.2.1. CPK also notes that NUTRAVA™ Citrus Fiber is comprised of cellulose, hemicellulose, and pectin fibers and therefore is compositionally representative of other citrus fiber ingredients produced from citrus peel that have been previously concluded to be GRAS (*i.e.*, GRN 154/487, 541, and 599) (U.S. FDA, 2004, 2014a, 2015a, 2016a). Data and information forming the basis for these GRAS conclusions are reviewed as they are considered relevant to the GRAS status of NUTRAVA™ Citrus Fiber.

Based on the identity of NUTRAVA™ Citrus Fiber and the applicable long-history of consumption of plant-based fiber in the diet, toxicological evaluations of NUTRAVA™ Citrus Fiber were not considered necessary for the safety evaluation. CPK considers this conclusion to be consistent with international efforts to reduce unnecessary testing of animals. Comprehensive searches of the literature for studies in animals and humans administered citrus fiber have been concluded during previous GRAS evaluations; summaries of this information are presented in Section 6.2.2. An updated literature search was conducted for the period of 01 January 2015 (date of citrus fiber literature search conducted in GRN 599 – U.S. FDA, 2016a) and 30 May 2019 using the electronic search tool ProQuest Dialog™ including the following databases: Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, CAB ABSTRACTS,

Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, and ToxFile® (Appendix A). A summary of pertinent information is included in this dossier.

The data and information summarized in this dossier demonstrate that NUTRAVA™ Citrus Fiber, produced using cGMP and meeting appropriate food-grade specifications, is GRAS, based on scientific procedures, under its conditions of intended use in foods.

6.2 Safety Data

6.2.1 Dietary Fiber

No tolerable upper intake level has been set for dietary fiber; however, the IOM suggested that there may be a need for a tolerable upper intake level in the future if supplements or foods with added functional fiber were to become ubiquitous. Fibers such as guar gum, inulin and oligofructose, fructooligosaccharides, polydextrose, resistant starch, and psyllium have been reported to cause gastrointestinal distress, including abdominal cramping, bloating, gas, and diarrhea (IOM, 2005). The IOM concluded that no serious chronic adverse effects have been reported for these fibers and excess consumption of these fibers is self-limiting due to their bulky nature (IOM, 2005). The adequate intake level set by the IOM for dietary fiber is 38 g total fiber/day for males and 25 g total fiber/day for females (ages 19 to 50 years) based on the intake level reported to protect against coronary heart disease (CHD) (IOM, 2005; see Section 3.1.3.1).

The IOM reviewed multiple epidemiological and intervention studies evaluating the correlation or effect of fiber intake on the risk of CHD. Based on the epidemiological studies evaluated, the IOM reported *"reduced CHD rates in individuals consuming high amounts of Dietary Fiber and fiber-rich foods (Bolton-Smith et al., 1992; Fraser et al., 1992; Humble et al., 1993; Jacobs et al., 1998; Khaw and Barrett-Connor, 1987; Kushi et al., 1985; Morris et al., 1977; Pietinen et al., 1996; Rimm et al., 1996; Todd et al., 1999; Wolk et al., 1999)"* (IOM, 2005). Additionally, based on the intervention studies evaluated, the IOM concluded that *"Viscous Functional Fibers and foods sources of viscous Dietary Fiber reduce both total and LDL* [low-density lipoprotein] *cholesterol concentrations, and may also reduce serum triglycerides"* (IOM, 2005). Therefore, the IOM was able to conclude that *"these data suggest an intake of 14 g of Dietary Fiber/1,000 kcal, particularly from cereals, to promote heart health"* (IOM, 2005).

Due to the ability of certain dietary fibers to delay absorption of triacylglycerols, it has been proposed that fiber intake may alter absorption of fat-soluble vitamins and minerals by binding or entrapping them in the small intestine. The results from studies evaluating the effect of dietary fiber on fat-soluble vitamin absorption are inconsistent, and there is little evidence that individuals consuming nutritionally adequate diets rich in high fiber foods have any problems with vitamin or mineral deficiencies (Williams and Bollella, 1995; FAO/WHO, 1998). Williams and Bollella (1995) reviewed published studies on dietary fiber intake in childhood for safety concerns and concluded that *"it is estimated that even with a doubling of current dietary fiber, there is unlikely to be an adverse effect on serum vitamin and mineral concentrations in healthy US children consuming a balanced diet containing adequate levels of nutrients"*. The IOM concluded that fiber as part of an overall healthy diet has not been reported to adversely affect the calcium, magnesium, iron, or zinc status of healthy people at recommended intake levels (IOM, 2005). Therefore, it seems unlikely that there will be any significant effects on vitamin and mineral absorption from the estimated increase in fiber intakes from foods containing NUTRAVA™ Citrus Fiber.

6.2.2 Citrus Fiber

There are limited studies in the scientific literature on the toxic effects of citrus fiber, particularly due to the long history of safe consumption of citrus fruit. Previous GRAS notifications of similar citrus fiber products to NUTRAVA™ Citrus Fiber have reviewed published information and data on citrus fiber prior to 2015. The studies evaluated in previous GRAS notifications of citrus fiber prior to 2015 are summarized below in Table 6.2.2-1; there are no effects reported in these studies to suggest that citrus fiber or other constituents derived from citrus peel are unsafe for food-use at any dietary level.

Table 6.2.2-1 Studies on Citrus Fiber Products Prior to 2015

Experiment	Results[a]	Adverse Effects/Toxicity	Reference
In vitro Studies			
Cellulose, beet pulp, citrus pulp, and citrus pectin were incubated for 6, 12, 24, and 48 hours with ruminal fluid from cattle or feces from dogs, cats, pigs, horses, or humans.	• Digestibility (organic matter disappearance and short chain fatty acids production) ranked cellulose < beet pulp < citrus pulp < citrus pectin.	-	Sunvold *et al.* (1995)
16 dietary fibers fermented using canine fecal inoculum for 8 and 72 hours.	• Citrus pectin and pea fiber showed a low R_{max} (maximal rate of gas production) occurring later compared with sugar beet fiber, sugar beet pulp, soy fiber, and wheat middlings.	-	Bosch *et al.* (2008)
Mikan (Japanese mandarin orange: Citrus unshiu) albedo incubated with pancreatic lipase.	• Inhibited enzyme degradation of triolein by pancreatic lipase.	-	Iwata *et al.* (2012)
1-methyl-1,2,3,4-tetrahydro-p-carboline-3-carboxylic acid (MTCCA), nitrite, ethanol, and citrus fruits lemon (*Citrus limon*), yuzu (*C.junos*), and sudachi (*C. sudachi*), in a mutation test with *Salmonella typhimurium* strain TA100.	• Citrus fruits reduced the mutagenic activity of MTCCA. • Greater reduction seen by the addition of citrus peel compared to citrus juice.	-	Higashimoto *et al.* (1998)
Isolated fiber rich fractions (FRFs; insoluble dietary fiber, alcohol-insoluble solid, and water-insoluble solid) from the peel of *Citrus sinensis* L. cv. Liucheng (Liucheng sweet orange).	• Three FRFs were able to slow glucose diffusion, adsorb glucose, and inhibit α-amylase activity. • Potential to decrease the rate of glucose absorption and control the concentration of postprandial serum glucose.	-	Chau *et al.* (2003)
Animal Studies			
Male weanling F344 (N=12 to 94/group) Fed semipurified diets containing 5% alphacel, 5% alphacel + 15% wheat bran or 5% alphacel + 15% citrus fiber. At 7 weeks of age, given subcutaneous injections of vehicle or 3,2'-dimethyl-4aminobiphenyl (DMAB; 50 mg/kg bw weekly) for 20 weeks. The experiment was terminated 20 weeks after the last injection of DMAB.	• ↓ incidence small intestine tumors. • ↓ number of adenomas but not adenocarcinomas.	No effect on body weights. ↑ food intake (not statistically significant) and stool output.	Reddy and Mori (1981)

Table 6.2.2-1 Studies on Citrus Fiber Products Prior to 2015

Experiment	Results[a]	Adverse Effects/Toxicity	Reference
Male weanling F344 (N=12 to 96/group) Fed semipurified diets containing 5% alphacel, 5% alphacel + 15% wheat bran or 5% alphacel + 15% citrus fiber. At 7 weeks of age, given subcutaneous injections of vehicle or azoxymethane (AOM; 8 mg/kg bw weekly) for 10 weeks. The experiment was terminated 20 weeks after the last injection of AOM.	• ↓ percent of animals with adenoma and adenocarcinoma in the colon or small intestine. • ↓ number of small intestinal tumors (adenocarcinomas).	No effect on body weights. ↑ food intake (not statistically significant) and stool output.	Reddy *et al.* (1981)
Male New Zealand rabbits (10/group) Controls: Rats given a control diet or a hypercholesterolemic diet for 2 months Experimental: Rats given a hypercholesterolemic diet and either 5 ml/day lime juice or 1 g/day dried lime peel powder for 2 months	• ↑ serum total antioxidant capacity; lime juice was more effective than lime peel. • ↓ presence of fatty streaks in coronary arteries and aorta; lime peel was more effective than lime juice.	Not reported.	Boshtam *et al.* (2013)
Male Wistar-Hannover GALAS rats (6/group) Control: Control diet (5% cellulose) for 4 weeks Experimental: Experimental diet (4% cellulose and 1% albedo dietary fiber) for 4 weeks	• ↑ fecal lipid excretion. • ↓ serum triacylglycerol concentrations. • ↑ number of beneficial bifidobacterial in the cecum.	Not reported.	Iwata *et al.* (2012)
Ramboullet/Suffolk Sheep (8/group) Sheep given orange peel products in diet (0, 10, 20% orange product) and inoculated with *Salmonella typhimurium* (10^{10} colony forming units) *via* oral gavage every 24 hours for 96 hours	• ↓ *S. typhimurium* populations in the cecum of sheep consuming 10% orange product diet. • Palatability issues at higher than 20% citrus peel product in diet.	Not reported.	Callaway *et al.* (2011a)
Ramboullet/Suffolk Sheep (8/group) Sheep given orange peel products in diet (0, 10, 20% orange product) and inoculated with *Escherichia coli* O157:H7 (10^{10} colony forming units) *via* oral gavage every 24 hours for 96 hours	• ↓ *E. coli* O157:H7 shedding in sheep consuming 10% orange product diet. • ↓ *E. coli* O157:H7 populations in the cecum and rectum of sheep consuming 5 and 10% orange product diet, and in the rumen of sheep consuming 10% orange product diet.	Not reported.	Callaway *et al.* (2011b)
Clinical Studies			
Case-control study on an older Southwestern population in Arizona (N=470; ≥30 y) Relationship between citrus consumption (source of d-*limonene*) and squamous cell carcinoma (SCC) of the skin.	• 64.3% and 74.5% of the respondents reported weekly consumption of citrus fruits and citrus juices, respectively. • Peel consumption was not uncommon (34.7%). • No association between the overall citrus consumption and skin SCC. • ↑ peel consumption was associated with ↓ degree of skin SCC risk.	Not reported.	Hakim *et al.* (2000)

Table 6.2.2-1 Studies on Citrus Fiber Products Prior to 2015

Experiment	Results[a]	Adverse Effects/Toxicity	Reference
Case-control study on an older Southwestern population in Arizona (N=450; ≥30 y) Relationship between citrus peel or black tea consumption and SCC of the skin.	• Controls were more likely than were SCC cases to report citrus peel consumption (OR = 0.67). • Peel consumption was reported by 34.5% of subjects. • Citrus peel and strong, hot black tea intake potentially have independent protective effects against SCC of the skin.	Not reported.	Hakim and Harris (2001)
Men (N=8; 20 to 27 y) Effect of citrus pectin on vitamin B6 bioavailability Group 1: Administered pectin in diet, no pectin and then pectin for 18 days per treatment period. Group 2: No pectin, pectin, then on pectin for 18 days per treatment period.	• ↑ fecal vitamin B6 in 5 subjects.	Not reported.	Miller *et al.* (1980)

bw = body weight; OR = odds ratio.
[a] Statistically significant result compared to respective control group, unless otherwise stated.

A thorough search and review of the published scientific literature from 2015 to present identified 2 toxicology studies conducted using test articles derived from citrus peel (Shikishima *et al.*, 2016; Li *et al.*, 2019). Li *et al.* (2019) reported on the effects of high doses of hesperidin isolated from orange peel; Shikishima *et al.* (2016) reported on the toxicity of a citrus fruit extract powder. The test articles used in these studies are not representative of NUTRAVA™ Citrus Fiber and are not relevant to the safety assessment. There were no notable findings reported in these studies to suggest that use of citrus peel to produce NUTRAVA™ Citrus Fiber would be unsafe (Table 6.2.2-2). The abstracts of additional identified studies that primarily focus on efficacy are provided in Appendix A for further supporting information.

Table 6.2.2-2 Toxicity Studies on Citrus Fiber Products from 2015 to Present

Experiment	Results[a]	Adverse Effects/Toxicity	Reference
In Vitro Studies			
Ames' bacterial reverse mutation assay (OECD 471) (OECD, 1997) *Salmonella typhimurium* TA1535, TA97a, TA98, TA100, and TA102; +/- S9 50, 150, 500, 1,500, and 5,000 µg *Citrus sudachi* Extract Powder/plate	• No biologically relevant increases in revertant colony numbers of any of the 5 tester strains at any concentration level, in the presence or absence of metabolic activation.	Non-mutagenic	Shikishima *et al.* (2016)
In vitro mammalian cell gene mutation test (OECD 490) (OECD, 2016) L5178Y/TK+/- 3.7.2C Mouse Lymphoma Cell Line; +/- S9	• Mutant Frequencies (MF) were similar to the average MF of their concurrent vehicle control cultures.	Non-mutagenic	Shikishima *et al.* (2016)

Table 6.2.2-2 Toxicity Studies on Citrus Fiber Products from 2015 to Present

Experiment	Results[a]	Adverse Effects/Toxicity	Reference
312.5, 625, 1,250, and 2,500 µg *Citrus sudachi* Extract Powder/ml			
Animal Studies			
Sprague-Dawley rats (3F) 5,000 mg *Citrus sudachi* Extract Powder/kg bw by oral gavage; then monitored once daily for 14 days; followed by necropsy Acute oral toxicity (Up and down procedure; OECD 425) (OECD, 2008)	• No mortality or any signs of gross toxicity, adverse pharmacological effects, or abnormal behavior.	LD_{50} >5,000 mg/kg bw	Shikishima *et al.* (2016)
Sprague-Dawley rats (5/sex/group) 55, 175, 550, 1,750, and 5,000 mg hesperidin/kg bw by oral gavage; then monitored once daily for 14 days Acute toxicity evaluation of hesperidin (73%) isolated from a methanolic extract of *Citrus sinensis* peel (OECD 425) (OECD, 2008)	• No toxicity or deaths at doses below 5,000 mg/kg. • 10% mortality in the 5,000 mg/kg group. • ↑ absolute liver and spleen weights in the 5,000 mg/kg group. • ↑ relative liver weights in both sexes in the 5,000 mg/kg group. • ↑ relative spleen weights in females in the 5,000 mg/kg group.	LD_{50} of 4,837.5 mg/kg	Li *et al.* (2019)
Sprague-Dawley rats (15/sex/group) 0, 250, 500, and 1,000 mg hesperidin/kg bw/d by oral gavage Sub-chronic 13-week toxicity evaluation of hesperidin (73%) isolated from a methanolic extract of *Citrus sinensis* peel (OECD 408) (OECD, 1998)	• All animals survived the 13-week dosing period and recovery period. • No adverse effects in body weight, food consumption, clinical signs, ophthalmological and neurological observations, urine analysis, hematology, clinical chemistry, organ weights, and gross pathology in the 250 and 500 mg/kg bw/d group. • Significant alterations in body and organ weights, hematology, clinical chemistry, and tissue histopathology in the 1,000 mg/kg bw/d group.	LOAEL of 1,000 mg/kg	Li *et al.* (2019)

bw = body weight; d = day; F = female; LD_{50} = median lethal dose; LOAEL = lowest-observed-adverse-effect level; OECD = Organisation for Economic Co-operation and Development; S9 = rat liver metabolic activation system.
[a] Statistically significant result compared to respective control group, unless otherwise stated.

6.2.2.1 Other Related Citrus Fiber Ingredients with GRAS Status

Three citrus fiber ingredients have GRAS status in the U.S. (see Table 6.2.2.1-1). These ingredients are produced from similar raw materials and are manufactured using comparable methods that principally involve acid and solvent washes followed by mechanical grinding.

Table 6.2.2.1-1 Nutrient Composition of NUTRAVA™ Citrus Fiber and Comparison to GRAS Sources of Citrus Fiber

Specification Parameter	NUTRAVA™ Citrus Fiber	GRN 154/487	GRN 541	GRN 599
Physical and Chemical				
Total dietary fiber	>80	73.4%	86.5%	≥50%

Table 6.2.2.1-1 Nutrient Composition of NUTRAVA™ Citrus Fiber and Comparison to GRAS Sources of Citrus Fiber

Specification Parameter	NUTRAVA™ Citrus Fiber	GRN 154/487	GRN 541	GRN 599
Insoluble dietary fiber	39.8 to 42.4%[a]	37.4%	85.0%	48.3 to 54.5%[a]
Soluble dietary fiber	41.9 to 45.5%[a]	36%	1.5%	10.0 to 13.4%[a]
Protein	4.4 to 5.1%[a]	7.47%	2.0%	2.92 to 3.66%[a]
Fat	0.7 to 1.3%[a]	1.04%	0.5%	≤0.5%
Glucose	<0.1%	--	--	--
Sucrose	--	--	--	25 to 40%
Total Carbohydrates	85.7 to 87.8%[a]	82.2%	--	--
Residual Ethanol	≤0.5%	--	--	<0.0015%[b]
Ash	<5%	2.44%	2.0%	≤5.0%
Moisture	≤12%	6.84%	9.0%	≤10%
pH	3.0 to 5.0	5.5 to 7.5	7.0 to 9.0	≤5.0

[a] Range of 3 nonconsecutive batches.
[b] Based on batch analysis.
[c] Converted % to mg/kg.

Citri-Fi™ Citrus Fiber 100 (dried orange pulp; GRN 154 – U.S. FDA, 2004) is an orange product consisting of washed orange juice pulp cells that have been dewatered, sheared, stabilized/dried, and ground to 10 to <200 mesh. This product has achieved GRAS status as a moisture retention, shelf life extension, and flavor enhancing agent in various food products at levels not to exceed 5%, and at levels ranging from 0.1 to 0.3% in meat and poultry products. Estimated intake levels based on Citri-Fi™ Citrus Fiber 100's intended uses and use levels were not discussed. Citri-Fi™ Citrus Fiber 100's GRAS notification consisted of a thorough review of the current publicly available scientific literature and revealed no evidence of any adverse effects associated with citrus fiber. Therefore, the data and information presented in Citri-Fi™ Citrus Fiber 100's GRAS notification are considered to support the current GRAS notification of NUTRAVA™ Citrus Fiber.

Citri-Fi™ Citrus flour (GRN 487 – U.S. FDA, 2014a) is a citrus product made from juice cells, peels, cores, rags, or segment membranes from oranges (including mandarins and tangerines; excluding bitter oranges), lemons, limes, and grapefruits that have been dried, mechanically sheared, and ground. This product has achieved GRAS status as a moisture retention, flavor enhancing, and processing aid agent in a variety of food products at a maximum level of 5%, and at levels up to 3% in meat and poultry products. The mean and 90th percentile cumulative exposure to Citri-Fi™ Citrus flour was calculated to be 23.4 g/day (0.4 g/kg body weight/day) and 39.5 g/day (0.8 g/kg body weight/day), respectively. Citri-Fi™ Citrus flour's GRAS notification consisted of a thorough review of the current publicly available scientific literature and revealed no evidence of any adverse effects associated with citrus fiber. As flavonoids are prominently found in citrus fruits, the safety of flavonoids, particularly quercetin, were evaluated and reported as safe. Therefore, the data and information presented in Citri-Fi™ Citrus fiber's GRAS notification are considered to support the current GRAS notification of NUTRAVA™ Citrus Fiber.

Ceamfiber™ Citrus fiber (GRN 541 – U.S. FDA, 2015a) is a citrus product made from the citrus peel, including orange, lemon, and lime peels, that have been mixed with water and food-grade acid, partially de-pectinized, neutralized, activated with sodium hydroxide, dried, ground, and then milled and sieved to the desired particle size. The ingredient has GRAS status as a moisture retention, flavor enhancing, and processing aid agent in a variety of foods products at levels not to exceed 5%, and at levels up to 3% in meat and poultry products. The mean and 90th percentile exposure to Ceamfiber™ Citrus fiber was calculated to be 23.4 g/day (0.4 g/kg body weight/day) and 39.5 g/day (0.8 g/kg body weight/day), respectively.

Ceamfiber™ Citrus fiber's GRAS notification consisted of common knowledge of safe use of citrus fruits, evaluation of similar GRAS products, a thorough review of the current publicly available scientific literature on citrus and other fibers (*e.g.*, cellulose, modified celluloses, vegetable/grain-based fibers), and revealed no evidence of any adverse effects associated with citrus fiber. Therefore, the data and information presented in Ceamfiber™ Citrus fiber's GRAS notification are considered to support the current GRAS notification of NUTRAVA™ Citrus Fiber.

CitriTex® Citrus fiber (GRN 599 – U.S. FDA, 2016a) is a citrus product made from the citrus peel, including partially de-pectinized orange, lemon and lime peels, that have been diluted with water, optionally decolorized, activated by wet milling and high-pressure homogenization, treated with isopropylalcohol, and then dissolved with sucrose, dried, and milled. This ingredient has GRAS status for use as a moisture retention and texturizing agent in a variety of food products at levels not to exceed 5%. The mean and 90th percentile cumulative exposure to CitriTex® Citrus fiber was calculated to be 7.1 g/day (0.12 g/kg body weight/day) and 11.36 g/day (0.21 g/kg body weight/day), respectively. CitriTex® Citrus fiber's GRAS notification consisted of common knowledge of safe use of citrus fruits, evaluation of similar GRAS products, a thorough review of the current publicly available scientific literature on citrus and other fibers (*i.e.*, cellulose, modified celluloses, vegetable/grain-based fibers), and revealed no evidence of any adverse effects associated with citrus fiber. Therefore, the data and information presented in CitriTex® Citrus fiber's GRAS notification are considered to support the current GRAS notification of NUTRAVA™ Citrus Fiber.

6.3 Allergenicity

Allergic reactions to citrus fruits are rare and typically mild. Citrus fruits are not listed as one of the major food allergens under the U.S. Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). A letter from Steve L. Taylor, Ph.D., Professor and Co-Director at University of Nebraska Lincoln, is provided in GRN 487, stating that in his expert opinion, *"Citrus fiber products present essentially no allergenic risk to consumers"* (GRN 487 – U.S. FDA, 2014a). GRN 541 and GRN 599 review literature on orange allergy and report that the allergy is rare and scarcely investigated, but has potential cross reactivity with pollen, and may be caused by the orange seeds or preservatives present in some juices (GRN 541 – U.S. FDA 2015a; and GRN 599 – U.S. FDA, 2016a). Therefore, it seems unlikely that there will be any significant allergic reactions associated with intakes of foods containing NUTRAVA™ Citrus Fiber.

6.4 Expert Panel Evaluation

CPK has concluded that NUTRAVA™ Citrus Fiber is GRAS for use in traditional food products, as described in Section 1.3, on the basis of scientific procedures. This GRAS conclusion is based on data generally available in the public domain pertaining to the safety of NUTRAVA™ Citrus Fiber, as discussed herein, and on consensus among a panel of experts (the GRAS Panel) who are qualified by scientific training and experience to evaluate the safety of food ingredients. The Expert Panel consisted of the following qualified scientific experts: Dr. Joseph F. Borzelleca (Virginia Commonwealth University); Dr. George Fahey (University of Illinois at Urbana); Dr. Robert Nicolosi (University of Massachusetts Lowell).

The GRAS Panel, convened by CPK, independently and critically evaluated all data and information presented herein, and also concluded that NUTRAVA™ Citrus Fiber is GRAS for use in traditional food products as described in Section 1.3, based on scientific procedures and corroborated by a long history of safe consumption by humans. A summary of data and information reviewed by the Expert Panel, and

evaluation of such data as it pertains to the proposed GRAS uses of NUTRAVA™ Citrus Fiber is presented in Appendix B[3].

6.5 Conclusion

Based on the above data and information presented herein, CPK has concluded that NUTRAVA™ Citrus Fiber is GRAS, on the basis of scientific procedures, for use in traditional food products as described in Section 1.3. General recognition of CPK's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts (the GRAS Panel), qualified by experience and scientific training, to evaluate the use of NUTRAVA™ Citrus Fiber in food, who similarly concluded that the proposed uses of NUTRAVA™ Citrus Fiber are GRAS on the basis of scientific procedures.

NUTRAVA™ Citrus Fiber may therefore be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under Title 21, Section 170.3 of the Code of Federal Regulations.

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[3] The proposed food-uses and use-levels for NUTRAVA™ Citrus Fiber described in the GRAS Panel Consensus Statement included meat and poultry uses. Meat suitability testing for NUTRAVA™ Citrus Fiber is currently ongoing, with data to be submitted to the USDA when available and an amendment or supplement will be submitted to this GRAS notice in accordance with 21 CFR §170.260 and §170.280 respectively.

Part 7. §170.255 List of Supporting Data and Information

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

Part	Section §	Section Title
101—Food labeling	101.9	Nutrition labeling of food
170—Food Additives	170.3	Definitions
	170.30	Eligibility for classification as generally recognized as safe (GRAS)
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe
184—Direct food substances affirmed as generally recognized as safe	184.1095	Sulfuric acid
	184.1588	Pectin
	184.1631	Potassium hydroxide
	184.1763	Sodium hydroxide

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APPENIX A

IDENTIFICATION OF PERTINENT SCIENTIFIC LITERATURE REGARDING THE METABOLISM AND PRE-CLINICAL AND CLINICAL SAFETY OF CITRUS FIBER

Identification of Pertinent Scientific Literature Regarding the Metabolism and Pre-clinical and Clinical Safety of Citrus Fiber

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Identification of Pertinent Scientific Literature Regarding the Metabolism and Pre-clinical and Clinical Safety of Citrus Fiber

1.0 INTRODUCTION

To identify pertinent scientific literature regarding the metabolism and pre-clinical and clinical safety of citrus fiber, a comprehensive search of the scientific literature was conducted using the electronic search tool ProQuest Dialog™.

The methods used to identify the scientific literature and the results of the literature search are provided in Section 2.0 and 3.0, respectively.

2.0 IDENTIFICATION OF PERTINENT STUDIES

2.1 Literature Search Strategy

To retrieve relevant literature on the metabolism, allergenicity, and pre-clinical and clinical safety of citrus fiber, 13 literature databases were searched in 30 May 2019 using the electronic search tool ProQuest Dialog™. The databases that were searched, as well as the search terms that were used, are listed in Table 2.1-1 and Tables 2.1-2 to 2.1.4, respectively. To increase the relevance and specificity of the literature search, the search terms were selected to reflect the exposure to citrus fiber in combination with metabolism, allergenicity, and pre-clinical/clinical parameters. The search was also limited to articles with full texts in English language and publication dates after 1 January 2015[1]. Studies published as abstracts, commentaries, *etc.* were also excluded.

Table 2.1-1 Electronic Databases Used to Retrieve Literature

Electronic Database	Date Range	Update Frequency
Adis Clinical Trials Insight	1990 to present	Weekly
AGRICOLA	1970 to present	Monthly
AGRIS	1975 to present	Monthly
Allied & Complimentary Medicine™	1985 to present	Monthly
BIOSIS® Toxicology	1969 to present	Weekly
BIOSIS Previews®	1926 to present	Weekly
CAB ABSTRACTS	1910 to present	Weekly
EMBASE®	1947 to present	Daily
Foodline®: SCIENCE	1972 to 2016	Stopped updating April 2016; previously twice weekly
FSTA®	1969 to present	Weekly
MEDLINE®	1946 to present	Daily with annual refresh
NTIS: National Technical Information Service	1964 to present	Weekly
ToxFile®	1946 to present	Daily with annual refresh

[1] At the time of this dossier preparation, GRN 599 was the most recent GRAS notification relevant to citrus fiber to receive a "no questions" letter from the U.S. FDA with a clearly defined literature search term as prior to 1 January 2015.

The literature search strategy to identify pre-clinical safety data related to citrus fiber is provided below in Table 2.1-2.

Table 2.1-2 Keywords Used to Retrieve Pre-clinical Safety Literature During the Updated Literature Search (01 January 2015 to 30 May 2019)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonyms within the publication: • "citrus fiber" or "citrus fibre" or "citrus pulp" or "citrus flour" or "citrus fruit" or "citrus pectin"
Set 2: Animal study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet
Set 3: Route of administration terms	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or gavage or feed or feeding or diet or dietary or intub* or "drinking water" or intragastric or administ* or provid*
Set 4: Safety terms	Within the results from Set 3, searched for records containing the following terms within the publication: • toxic* or mortal* or lethal* or adverse* or safe* or risk* or hazard*
Set 5: Acute/repeat dose study terms	Within the results from Set 4, searched for records containing the following terms within the publication: • acute* or subacute or "sub acute" or "single dose" or "short term" or subchronic* or "sub chronic*" or chronic* or "long term" or day or week or month or year
Set 6: Safety factors terms	Within the results from Set 3, searched for records containing the following terms within the publication: • LD50 or NOAEL or LOAEL or "no observed adverse effect*" or "low* observed adverse effect*" or NOEL or LOEL or "no observed effect level" or "low* observed effect level" or "maximum tolerated dose" or safety NEAR/2 assess* or risk NEAR/2 assess*
Set 7: Carcinogenicity terms	Within the results from Set 3, searched for records containing the following terms within the publication: • carcino* or tumor* or tumour* or neoplas* or oncogen* or cancer*
Set 8: Reproductive toxicity terms	Within the results from Set 3, searched for records containing the following terms within the publication: • teratol* or teratogen* or reproduct* NEAR/5 toxic* or development* NEAR/5 toxic* or reproduct* NEAR/5 effect* or development* NEAR/5 effect* or fetus or foetus or fetal or foetal or prenatal* or postnatal* or perinatal* or litter or litters or "2 generation*" or "two generation*" or "multi generation*"
Set 9: Genotoxicity terms	Within the results from Set 1, searched for records containing the following terms within the publication: • genotox* or genetox*or mutagen* or mutat* or Ames or "dna repair" or "dna lesion*" or micronucle* or clastogen* or "DNA adduct*" or "comet assay*"

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation; NEAR/5 = search terms may appear within 5 words of each other with either term appearing first within the record

The literature search strategy to identify clinical safety data related to citrus fiber is provided below in Table 2.1-3.

Table 2.1-3 Keywords Used to Retrieve Clinical Safety Literature During the Updated Literature Search (01 January 2015 to 30 May 2019)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonyms within the publication: • "citrus fiber" or "citrus fibre" or "citrus pulp" or "citrus flour" or "citrus fruit" or "citrus pectin"
Set 2: Keywords used to identify human studies	Within the results from Set 1, searched for records containing the following terms within the publication: • human or humans or subject or subjects or patient* or clinical* or volunteer* or men or women or male or female or "double blind*" or "single blind*" or "open label*" or "cross over" or crossover or cohort or randomiz* or randomis* or "placebo control*"
Set 3: Keywords used to identify route of administration	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or diet or dietary or ingest* or capsule or tablet or supplement* or consum* or provid* or administ*
Set 4: Safety terms	Within the results from Set 3, searched for records containing the following terms within the publication: • safe* or risk or "adverse effect*" or "adverse event*" or "adverse reaction*" or "maximum tolerated dose" or "permissible dose level" or "maximum dose level" or threshold or tolerability or tolera* or "side effect*" or toxic*

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation; and NEAR/5 = search terms may appear within 5 words of each other with either term appearing first within the record.

The literature search strategy to identify nutritive safety and metabolism data related to citrus fiber is provided below in Table 2.1-4.

Table 2.1-4 Keywords Used to Retrieve Metabolism and Allergenicity Literature During the Updated Literature Search (01 January 2015 to 30 May 2019)

Strategy[a]	
Set 1: Substance terms	Searched for records containing the following chemical names and synonyms within the publication: • "citrus fiber" or "citrus fibre" or "citrus pulp" or "citrus flour" or "citrus fruit" or "citrus pectin"
Set 2: Animal study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • animal or rat or mouse or mice or dog or rabbit or pig or hamster or monkey or rodent or pig or piglet
Set 3: Animal route of administration terms	Within the results from Set 2, searched for records containing the following terms within the publication: • oral* or gavage or feed or feeding or diet or dietary or intub* or "drinking water" or intragastric or administ* or provid*
Set 4: Clinical study terms	Within the results from Set 1, searched for records containing the following terms within the publication: • human or humans or subject or subjects or patient* or clinical* or volunteer* or men or women or male or female or "double blind*" or "single blind*" or "open label*" or "cross over" or crossover or cohort or randomiz* or randomis* or "placebo control*"
Set 5: Clinical route of administration terms	Within the results from Set 4, searched for records containing the following terms within the publication: • oral* or diet or dietary or ingest* or capsule or tablet or supplement* or consum* or provid* or administ*
Set 6: Metabolism terms	Within the results from Set 3 and 5, searched for records containing the following terms within the titles: • metabolis* or metaboliz* or "metabolic* fate*" or "metabolic* path*" or hydroly* or absorb* or absorp* or excret* or eliminat* or pharmacokinetic* or pharmacodynamic* or toxicokinetic* or toxicodynamic* or bioavailab* or biotransform* or ferment* or digest*
Set 7: Allergenicity terms	Within the results from Set 1, searched for records containing the following terms within the publication: • allerg* or hypersensitiv* or hypersensitiz* or hypersensitis*

[a] Syntax for the search strategy is as follows: " " = search terms must appear directly beside each other in the exact order; * = truncation.

2.2 Literature Filtration

Once the search strategy was implemented and the publication titles were retrieved, the relevance of the publications was determined at 3 stages using the titles, abstracts, and the full-text of publications. At each stage, the output was manually reviewed against the inclusion/exclusion criteria listed in Table 2.2-1 were applied to determine literature relevance. The 3 stages are outlined below in greater detail.

- Stage 1: Titles of articles were reviewed, and abstracts of titles determined to be potentially relevant were retrieved.
- Stage 2: Abstracts were reviewed, and full-length articles of abstracts determined to be potentially relevant were retrieved.
- Stage 3: Full-length articles were reviewed, and those determined not to meet all the inclusion criteria specified in Table 2.2-1 were excluded.

Table 2.2-1 Inclusion and Exclusion Criteria Used to Filter the Identified Literature

Inclusion Criteria
• The food/food constituent studied was citrus fiber • A full-length article published in a peer-reviewed journal • The report analyzed safety-related parameters or metabolism parameters
Exclusion Criteria
• The food/food constituent studied was not citrus fiber • A full-length article published in a non-peer-reviewed source (*e.g.*, website, magazine, *etc.*) • Published in abstract form only or as a short communication (*e.g.*, conference abstract, letter to the editor, commentary, *etc.*) • A research synthesis study (*e.g.*, narrative review, systematic review, meta-analysis, *etc.*) • The report did not consider safety-related parameters or metabolism parameters • The study was a duplicate record in the literature search • Full publication study report not in English language

3.0 LITERATURE SEARCH RESULTS

The literature searches resulted in the identification of 519 potentially relevant titles, and abstracts were retrieved for 87 records. Following review of the 87 abstracts and recalling the potentially relevant full texts, 2 relevant toxicity studies were identified for citrus fiber. These studies are summarized in the GRAS dossier. The remaining relevant abstracts describe, 2 clinical studies, 4 reviews, 7 *in vitro* studies, 13 mouse studies, 8 rat studies, and 2 pig studies that primarily analyzed the efficacious effects of citrus fiber. The results from relevant studies identified during the literature search did not present findings that are inconsistent with the GRAS status of citrus fiber for use as a food ingredient. The abstracts for these articles are provided below in Table 3-1.

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Clinical Studies	
Effects of Lemon and Seville Orange Juices on the Pharmacokinetic Properties of Sildenafil in Healthy Subjects **Authors:** Abdelkawy, Khaled S; Donia, Ahmed M; Turner, R Brigg; Elbarbry, Fawzy **Journal:** Drugs R D. 2016 Sep;16(3):271-278. PURPOSE Several severe drug interactions have been reported when sildenafil, a potent drug for the treatment of erectile dysfunction, is co-administered with drugs or herbal remedies that inhibit cytochrome P450 (CYP) 3A4. This study evaluates the effects of two citrus fruit juices, lemon and Seville orange, on the pharmacokinetics of sildenafil in male healthy subjects following a single oral dose. METHODS We conducted an open-label, three-way crossover study in nine healthy male volunteers. Participants received a single oral dose of sildenafil (50 mg) after pretreatment with 250 mL of either water (control), undiluted lemon juice, or Seville orange juice for 3 consecutive days. All subjects were monitored for adverse effects during the study period. Plasma samples were collected for 12 h after dosing and analyzed for sildenafil concentration. RESULTS Compared with pretreatment with water, Seville orange juice significantly increased the area under the plasma concentration-time curve from time zero to infinity and the peak plasma concentration of sildenafil by 44 % (90 % confidence interval [CI] 30-60) and 18 % (90 % CI 108-129), respectively, without affecting the time to reach peak plasma concentration. Additionally, Seville orange juice significantly reduced the apparent oral clearance of sildenafil by 30 % (90 % CI 63-75) without affecting its elimination half-life. In contrast, lemon juice did not cause any significant alterations in the pharmacokinetics of sildenafil. There was no significant treatment-related adverse effects reported during the study. CONCLUSIONS Although it is considered as a moderate CYP3A4 inhibitor, Seville orange only caused a mild increase in exposure to sildenafil after a single oral dose, without manifestation of any adverse effects. The enhanced bioavailability of sildenafil by Seville orange may be attributed to inhibition of its intestinal first-pass effect (CYP3A4 and or p-glycoprotein). Lemon juice, in contrast, had no effects on the pharmacokinetics of sildenafil.	Full text not reviewed

[2] It should be noted that the reference and abstracts for the potentially relevant full texts have been copied directly from the published abstract.

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Citrus consumption and risk of basal cell carcinoma and squamous cell carcinoma of the skin **Authors:** Wu, Shaowei; Cho, Eunyoung; Feskanich, Diane; Li, Wen-Qing; Sun, Qi; Han, Jiali; Qureshi, Abrar A **Journal:** Carcinogenesis. 2015 Oct;36(10):1162-8. doi: 10.1093/carcin/bgv109. Epub 2015 Jul 29. Animal experiments have demonstrated the photocarcinogenic properties of furocoumarins, a group of naturally occurring chemicals that are rich in citrus products. We conducted a prospective study for citrus consumption and risk of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin based on data from 41530 men in the Health Professionals Follow-up Study (1986-2010) and 63759 women in the Nurses' Health Study (1984-2010) who were free of cancers at baseline. Over 24-26 years of follow-up, we documented 20840 incident BCCs and 3544 incident SCCs. Compared to those who consumed citrus products less than twice per week, the pooled multivariable-adjusted hazard ratios were 1.03 [95% confidence interval (95% CI): 0.99-1.08] for BCC and 1.14 (95% CI: 1.00-1.30) for SCC for those who consumed two to four times per week, 1.06 (95% CI: 1.01-1.11) for BCC and 1.15 (95% CI: 1.02-1.28) for SCC for five to six times per week, 1.11 (95% CI: 1.06-1.16) for BCC and 1.22 (95% CI: 1.08-1.37) for SCC for once to 1.4 times per day and 1.16 (95% CI: 1.09-1.23) for BCC and 1.21 (95% CI: 1.06-1.38) for SCC for 1.5 times per day or more (P trend = 0.001 for BCC and 0.04 for SCC). In contrast, consumption of non-citrus fruit and juice appeared to be inversely associated with risk of BCC and SCC. Our findings support positive associations between citrus consumption and risk of cutaneous BCC and SCC in two cohorts of men and women, and call for further investigations to better understand the potential photocarcinogenesis associated with dietary intakes.	Full text not reviewed
Reviews	
Chemopreventive agents and inhibitors of cancer hallmarks: May citrus offer new perspectives? **Authors:** Cirmi, Santa; Ferlazzo, Nadia; Lombardo, Giovanni E; Maugeri, Alessandro; Calapai, Gioacchino; Gangemi, Sebastiano; Navarra, Michele **Publication info:** Nutrients. 2016 Nov 4;8(11). pii: E698. Fruits and vegetables have long been recognized as potentially important in the prevention of cancer risk. Thus, scientific interest in nutrition and cancer has grown over time, as shown by increasing number of experimental studies about the relationship between diet and cancer development. This review attempts to provide an insight into the anti-cancer effects of Citrus fruits, with a focus on their bioactive compounds, elucidating the main cellular and molecular mechanisms through which they may protect against cancer. Scientific literature was selected for this review with the aim of collecting the relevant experimental evidence for the anti-cancer effects of Citrus fruits and their flavonoids. The findings discussed in this review strongly support their potential as anti-cancer agents, and may represent a scientific basis to develop nutraceuticals, food supplements, or complementary and alternative drugs in a context of a multi-target pharmacological strategy in the oncology.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Phytochemistry and bioactivity of Citrus flavonoids: a focus on antioxidant, anti-inflammatory, anticancer and cardiovascular protection activities **Authors:** Yi, Lunzhao; Ma, Shasha; Ren, Dabing **Journal:** Phytochemistry Reviews. 2017 Jun. 16(3):479-511. doi: http://dx.doi.org/10.1007/s11101-017-9497-1 Epidemiological studies have suggested an inverse relationship between increased consumption of fruits and reduced risk of chronic diseases, such as cardiovascular diseases, cancer, and diabetes. Citrus fruit is one of the mostly consumed fruits worldwide, and numerous studies have revealed its remarkable health-promoting activities, such as antioxidant, anticancer, anti-inflammatory, and cardiovascular protection activities. These activities largely depend upon the diverse chemical constituents of Citrus fruits, including vitamins, minerals, terpenoids, and flavonoids. Notably, dietary flavonoids occurring in Citrus fruits have attracted growing interest due to their distinct beneficial effects on human health. In this review, we outlined the main health-related properties of Citrus flavonoids, with a focus on antioxidant, anticancer, anti-inflammation, and cardiovascular protection activities. Also the bioavailability, a critical factor that influences the biological efficacy, of Citrus flavonoids was discussed. It was believed that insights about these advances may encourage researchers to discover new phytochemical components and further study specific bioactivities from Citrus fruits.	Full text not reviewed
A recent review of citrus flavanone naringenin on metabolic diseases and its potential sources for high yield-production **Authors:** Karim, Naymul; Cui, Sunliang; Chen, Wei; Zheng, Xiaodong; Jia, Zhenquan **Journal:** Trends in food science & technology. 2018 Se; 79: 35-54. doi: http://dx.doi.org/10.1016/j.tifs.2018.06.012 Metabolic syndromes are the multi-metabolic abnormality characterized by hyperlipidemia, obesity, hyperglycemia, diabetes, hypertension, cardiovascular disease, and neuro-dysfunction. Naringenin, a naturally occurring flavanone compound, abundantly found in citrus fruit, has demonstrated diverse biological activities. In this context, the role of naringenin in the treatment of metabolic disease and alternative sources for high-yield production of naringenin have recently drawn full scientific attention and become an important issue in research.This review focuses on recent findings of naringenin against metabolic disorders including oxidative stress, hyperlipidemia, obesity, diabetes, inflammation, and organ toxicity. Also, this review highlights the potential sources of naringenin production.Naringenin exerts its protective effect against metabolic diseases through multiple mechanisms including its antioxidant activity by scavenging free radicals, inducing antioxidant enzymes and targeting on phosphoinositide 3-kinase/protein Kinase B/nuclear factor (erythroid-derived 2)-like 2 (PI3K/Akt/Nrf2), nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (NRf2/ARE), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), mitogen-activated protein kinase (MAPK), 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, peroxisome proliferator-activated receptor (PPAR), and nitric oxide-cGMP-protein kinase G-induced KATP channel (NO-cGMP-PKG-KATP). Moreover, microbial production is recommended as a promising alternative method for large-scale production of naringenin. In conclusion, naringenin is a promising compound for the prevention and management of metabolic diseases. Further clinical studies and trials are needed to prove its protective effects on metabolic syndrome in the human population.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Anti-diabetic and anti-cancerous potential of citrus flavonoid naringin: a review. **Authors:** Sehrawat, Nirmala; Devi, Sunita; Devi, Ashwanti; Yadav, Mukesh **Journal:** International Journal of Phytomedicines and Related Industries. 2018; 10(3):171-175. doi: http://dx.doi.org/10.5958/0975-6892.2018.00028.x Flavonoids are naturally occurring phenolic compounds with a diverse range of bioactivities. Naringin "$C_{27}H_{32}O_{14}$" is a flavonoid compound present in citrus fruits. It contributes a characteristic bitter flavor to citrus fruit juice. Naringin and its aglycone naringenin exhibit various pharmacological actions. Investigations on naringin also revealed that its supplementation is beneficial for the treatment of obesity, diabetes and hypertension. Effect of naringin on cancer, diabetes, osteogenesis, osteoporosis, bone regeneration, cardioprotective, renoprotective, hepatoprotective and other systems has been reported. Diabetes and cancer are common diseases with great impact on health worldwide. Effect of naringin on diabetes and cancer has also been investigated. Present review focuses on anti-diabetic and anti-cancerous effect of naringin and its potential to be used as phytomedicine for possible cure of these diseases.	Full text not reviewed
In vitro studies	
Identification of a cytotoxic molecule in heat-modified citrus pectin **Authors:** Leclere, Lionel; Fransolet, Maude; Cambier, Pierre; El Bkassiny, Sandy; Tikad, Abdellatif; Dieu, Marc; Vincent, Stephane P.; Van Cutsem, Pierre; Michiels, Carine **Journal:** Carbohydr Polym. 2016 Feb 10;137:39-51. doi: 10.1016/j.carbpol.2015.10.055. Epub 2015 Oct 19. Modified forms of citrus pectin possess anticancer properties. However, their mechanism of action and the structural features involved remain unclear. Here, we showed that citrus pectin modified by heat treatment displayed cytotoxic effects in cancer cells. A fractionation approach was used aiming to identify active molecules. Dialysis and ethanol precipitation followed by HPLC analysis evidenced that most of the activity was related to molecules with molecular weight corresponding to low degree of polymerization oligogalacturonic acid. Heat-treatment of galacturonic acid also generated cytotoxic molecules. Furthermore, heat-modified galacturonic acid and heat-fragmented pectin contained the same molecule that induced cell death when isolated by HPLC separation. Mass spectrometry analyses revealed that 4,5-dihydroxy-2-cyclopenten-1-one was one cytotoxic molecule present in heat-treated pectin. Finally, we synthesized the enantiopure (4R,5R)-4,5-dihydroxy-2-cyclopenten-1-one and demonstrated that this molecule was cytotoxic and induced a similar pattern of apoptotic-like features than heat-modified pectin.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Fermented Extraction of Citrus unshiu Peel Inhibits Viability and Migration of Human Pancreatic Cancers **Authors:** Lee, Jungwhoi; Lee, Jungsul; Kim, Myungseung; Kim, Jae Hoon **Journal:** J Med Food. 2018 Jan;21(1):5-12. doi: 10.1089/jmf.2017.3984. Pancreatic cancer is one of the most dangerous cancers with high mortality rates. Despite continuous efforts, there has been limited improvement in its prognosis. In this study, we prepared fermented extract of Citrus unshiu peel (fCUP) from the by-product after juice processing and then examined the anticancer effects of fCUP on human pancreatic cancer cells. Treatment with fCUP inhibited the growth of human pancreatic cancer cells through induction of caspase-3 cleavage both in vitro and in vivo. Treatment with fCUP also blocked the migration of human pancreatic cancer cells through activation of intracellular signaling pathways such as MKK3/6 and P38. In contrast, treatment with fCUP did not inhibit growth and migration of human umbilical vein endothelial cells. In addition, we found that fCUP mainly consisted of aboriginal compounds, narirutin and hesperidin, as well as newly generated compounds, naringenin and hesperetin. In silico analysis showed that naringenin and hesperetin were the unique modules related to anticancer effect. Furthermore, fCUP exhibited the anticancer effects in in vivo xenograft models. Collectively, these results suggest that fCUP might have the potential to be developed into an effective anticancer drug for pancreatic cancers without causing adverse side-effects.	Full text not reviewed
Modulation of gut microbiota from obese individuals by in vitro fermentation of citrus pectin in combination with Bifidobacterium longum BB-46 **Authors:** Bianchi, Fernanda; Larsen, Nadja; de Mello Tieghi, Thatiana; Adorno, Maria Angela Tallarico; Kot, Witold; Saad, Susana Marta Isay; Jespersen, Lene; Sivieri, Katia **Journal:** Appl Microbiol Biotechnol. 2018 Oct;102(20):8827-8840. doi: 10.1007/s00253-018-9234-8. Epub 2018 Aug 18. This study aimed to evaluate the effects of three treatments, i.e., Bifidobacterium longum BB-46 (T1), B. longum BB-46 combined with the pectin (T2), and harsh extracted pectin from lemon (T3) on obesity-related microbiota using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The effects of the treatments were assessed by the analysis of the intestinal microbial composition (using 16S rRNA gene amplicon sequencing) and the levels of short-chain fatty acids (SCFAs) and ammonium ions (NH4+). Treatments T2 and T3 stimulated members of the Ruminococcaceae and Succinivibrionaceae families, which were positively correlated with an increase in butyric and acetic acids. Proteolytic bacteria were reduced by the two treatments, concurrently with a decrease in NH4+. Treatment T1 stimulated the production of butyric acid in the simulated transverse and descending colon, reduction of NH4+ as well as the growth of genera Lactobacillus, Megamonas, and members of Lachnospiracea. The results indicate that both B. longum BB-46 and pectin can modulate the obesity-related microbiota; however, when the pectin is combined with B. longum BB-46, the predominant effect of the pectin can be observed. This study showed that the citric pectin is able to stimulate butyrate-producing bacteria as well as genera related with anti-inflammatory effects. However, prospective clinical studies are necessary to evaluate the anti/pro-obesogenic and inflammatory effects of this pectin for future prevention of obesity.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Migration and proliferation of cancer cells in culture are differentially affected by molecular size of modified citrus pectin **Authors:** Ramos do Prado, Samira Bernardino; Shiga, Tania Misuzu; Harazono, Yosuke; Hogan, Victor A.; Raz, Avraham; Carpita, Nicholas C.; Fabi, Joao Paulo **Journal:** Carbohydr Polym. 2019 May 1;211:141-151. doi: 10.1016/j.carbpol.2019.02.010. Epub 2019 Feb 4. While chemically and thermally modified citrus pectin (MCP) has already been studied for health benefits, it is unknown how size-fractionated oligo-and polysaccharides differentially affect cancer cell behavior. We produced thermally MCP and fractionated it by molecular size to evaluate the effect these polymers have on cancer cells. MCP30/10 (between 30 and 10 kDa) had more esterified homogalacturonans (HG) and fewer rhamnogalacturonans (RG-I) than MCP and MCP30 (higher than 30 kDa), while MCP10/3 (between 10 and 3 kDa) showed higher amounts of type I arabinogalactans (AGI) and lower amounts of RG-I. MCP3 (smaller than 3 kDa) presented less esterified HG and the lowest amount of AGI and RG-I. Our data indicate that the enrichment of deesterified HG oligomers and the AGI and RG-I depletions in MCP3, or the increase of AGI and loss of RGI in MCP30/10, enhance the anticancer behaviors by inhibiting migration, aggregation, and proliferation of cancer cells.	Full text not reviewed
Hesperidin regresses cardiac hypertrophy by virtue of PPAR-γ agonistic, anti-inflammatory, antiapoptotic, and antioxidant properties **Authors:** Bhargava, Poorva; Verma, Vipin Kumar; Malik, Salma; Khan, Sana Irfan; Bhatia, Jagriti; Arya, Dharamvir Singh **Journal:** Journal of Biochemical and Molecular Toxicology 33.5 (May 2019). Hesperidin (HES), a flavanone glycoside, predominant in citrus fruits, has an agonistic activity on peroxisome proliferator-activated receptor gamma (PPAR-γ). PPAR-γ is an inhibitor of cardiac hypertrophy (CH) signaling pathways. In this study, we investigated the cardioprotective effect of HES in isoproterenol (ISO)-induced CH through PPAR-γ agonistic activity. For this, male albino Wistar rats were divided into six groups (n = 6), that is, normal, ISO-control, HES treatment group (200 mg kg^{-1}; p.o.), HES per se (200 mg kg^{-1}; p.o.), enalapril treatment group (30 mg kg^{-1}; p.o.), and combination group (HES 200 mg kg^{-1}; p.o.+enalapril 30 mg kg^{-1}; p.o.). ISO (3 mg kg^{-1}; s.c.) was administered to all groups except normal and per se to induce CH. HES or enalapril treatment of 28 days significantly attenuated pathological changes, improved cardiac hemodynamics, suppressed oxidative stress, and apoptosis along with an increased PPAR-γ expression. The combination of enalapril with HES exhibited an effect similar to that of HES or enalapril alone on all the aforementioned parameters. Therefore, HES may be further evaluated as a promising molecule for the alleviation of CH.	Full text not reviewed
Antidiabetic Properties of Naringenin: A Citrus Fruit Polyphenol **Authors:** Den Hartogh, Danja J.; Tsiani, Evangelia **Journal:** Biomolecules. 2019 Mar 12;9(3). pii: E99. doi: 10.3390/biom9030099. Type 2 diabetes mellitus (T2DM) is a metabolic disease characterized by insulin resistance and hyperglycemia and is associated with personal health and global economic burdens. Current strategies/approaches of insulin resistance and T2DM prevention and treatment are lacking in efficacy resulting in the need for new preventative and targeted therapies. In recent years, epidemiological studies have suggested that diets rich in vegetables and fruits are associated with health benefits including protection against insulin resistance and T2DM. Naringenin, a citrus flavanone, has been reported to have antioxidant, anti-inflammatory, hepatoprotective, nephroprotective, immunomodulatory and antidiabetic properties. The current review summarizes the existing in vitro and in vivo animal studies examining the anti-diabetic effects of naringenin.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Behaviour of citrus pectin during its gastrointestinal digestion and fermentation in a dynamic simulator (simgi®) **Author:** Ferreira-Lazarte, Alvaro; Moreno, F Javier; Cueva, Carolina; Gil-Sánchez, Irene; Villamiel, Mar **Journal:** Carbohydr Polym. 2019 Mar 1;207:382-390. doi: 10.1016/j.carbpol.2018.11.088. Epub 2018 Nov 28. The behaviour of citrus pectin during digestion and its potential prebiotic properties were examined using a Dynamic Gastrointestinal Simulator (simgi®) model for the human gut, which simulates processes in the stomach, small intestine, ascending, transverse and descending colon. A remarkable non-digestibility of pectin in the upper gastrointestinal tract was observed by HPLC-ELSD analysis, where ~88% of citrus pectin remained intact during its transit through the stomach and small intestine. Fermentation of pectin stimulated the growth of beneficial bacteria such as Bifidobacterium spp, Bacteroides spp and Faecalobacterium prausnitzii. High increases of short-chain fatty acids (SCFA) were observed, especially in acetate and butyrate concentration due to direct fermentation of pectin or by cross-feeding interaction between bacteria. This is the first study on the digestibility and fermentation of pectin carried out in a complex dynamic gastrointestinal simulator, being of special relevance the results obtained for F. prausnitzii.	Full text not reviewed
Mouse studies	
A polymethoxy flavonoids-rich Citrus aurantium extract ameliorates ethanol-induced liver injury through modulation of AMPK and Nrf2-related signals in a binge drinking mouse model **Authors:** Choi, Bong-Keun; Kim, Tae-Won; Lee, Dong-Ryung; Jung, Woon-Ha; Lim, Jong-Hwan; Jung, Ju-Young; Yang, Seung Hwan; Suh, Joo-Won **Journal:** Phytother Res. 2015 Oct;29(10):1577-84. doi: 10.1002/ptr.5415. Epub 2015 Jul 14. Nobiletin and tangeretin are polymethoxy flavonoids (PMFs), found in rich quantities in the peel of citrus fruits. In the present study, we assessed the biological effect of the PMFs on liver damage using a mouse model of binge drinking. First, we extracted PMFs from the peels of Citrus aurantium to make Citrus aurantium extract (CAE). Male C57BL/6 mice were orally treated with silymarin and CAE (50, 100, and 200-mg/kg) for 3-days prior to ethanol (5-g/kg, total of 3 doses) oral gavage. Liver injury was observed in the ethanol alone group, as evidenced by increases in serum hepatic enzymes and histopathologic alteration, as well as by hepatic oxidative status disruption. CAE improved serum marker and hepatic structure and restored oxidative status by enhancing antioxidant enzyme levels and by reducing lipid peroxidation levels. In addition, CAE evidently suppressed inflammation and apoptosis in the livers of mice administered with ethanol, by 85% (tumor necrosis factor-α) and 44% compared to the control group, respectively. Furthermore, CAE activated lipid metabolism related signals and enhanced phosphorylation of AMP-activated protein kinase (AMPK) and nuclear factor E2-related factor 2 (Nrf2) with several cytoprotective proteins including heme oxygenase-1, NAD(P)H quinone oxidoreductase 1, and γ-glutamylcysteine synthetase. Taken together, the present study demonstrated that, CAE possesses antioxidant, anti-inflammatory, and antiapoptotic activity against ethanol-induced liver injury.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Green tea, red wine and lemon extracts reduce experimental tumor growth and cancer drug toxicity **Authors:** Zaletok, S; Gulua, L; Wicker, L; Shlyakhovenko, V; Gogol, S; Orlovsky, O; Karnaushenko, O; Verbinenko, A; Milinevska, V; Samoylenko, O; Todor, I; Turmanidze, T **Journal:** Exp Oncol. 2015 Dec;37(4):262-71. Aim: To evaluate antitumor effect of plant polyphenol extracts from green tea, red wine lees and/or lemon peel alone and in combination with antitumor drugs on the growth of different transplanted tumors in experimental animals. Materials and Methods: Green tea extract (GTE) was prepared from green tea infusion. GTE-based composites of red wine (GTRW), lemon peel (GTRWL) and/or NanoGTE as well as corresponding nanocomposites were prepared. The total polyphenolics of the different GTE-based extracts ranged from 18.0% to 21.3%. The effects of GTE-based extracts were studied in sarcoma 180, Ehrlich carcinoma, B16 melanoma, Ca755 mammary carcinoma, P388 leukemia, L1210 leukemia, and Guerin carcinoma (original, cisplatin-resistant and doxorubicinresistant variants). The extracts were administered as 0.1% solution in drinking water (0.6-1.0 mg by total polyphenolics per mouse per day and 4.0-6.3 mg per rat per day). Results: Tumor growth inhibition (TGI) in mice treated with NanoGTE, cisplatin or cisplatin + NanoGTE was 27%, 55% and 78%, respectively, in Sarcoma 180%, 21%, 45% and 59%, respectively, in Ehrlich carcinoma; and 8%, 13% and 38%, respectively in B16 melanoma. Composites of NanoGTE, red wine, and lemon peel (NanoGTRWL) enhanced the antitumor effects of cyclophosphamide in mice with Ca755 mammary carcinoma. The treatment with combination of NanoGTE and inhibitors of polyamines (PA) synthesis (DFMO + MGBG) resulted in significant TGI of P388 leukemia (up to 71%) and L1210 leukemia. In rats transplanted with Guerin carcinoma (parental strain), treatment with GTRW or GTE alone resulted in 25-28% TGI vs. 55-68% TGI in cisplatin-treated animals. The inhibition observed in the case of combination of GTE or GTRW with cisplatin was additive giving 81-88% TGI. Similar effects were observed when combinations of the cytostatics with GTE (or NanoGTE) were tested against cisplatin- or doxorubicin-resistant Guerin carcinoma. Moreover, the plant extracts lowered side toxicity of the drugs. Treatment with GTE, NanoGTE, and NanoGTRW decreased the levels of malondialdehyde in heart, kidney and liver tissue of experimental animals, as well as the levels of urea and creatinine in blood serum, increased erythrocyte and platelet counts, hemoglobin content, and decreased leucocyte counts. Conclusion: The obtained data indicate the prospects for further deve lopment of GTE and corresponding nanocomposites as auxiliary agents in cancer chemotherapy.	Full text not reviewed
The accelerating action of lipid excretion of immature Citrus fruits **Authors:** Kim, Ki Jung; Ko, Sung Kwon **Journal:** Korean Journal of Pharmacognosy. 2017 Jan. 48(2):134-140. In a series of investigations to develop a potential anti-obese agent, we prepared a immature Citrus fruits (IMF) and compared its lipid excretion effects to those of Citrus fruits (MF, CP, JP) in an ICR mouse model of obesity induced by a high fat diet. The body weights of IMF fed mice were found to be significantly lower from 2 weeks oral administration, despite the fact that their food intakes were similar to that of the HFD control mice. The fecal cholesterol content showed a significant increase in IMF (52.0 mg/g) at 7 weeks oral administration, and mature Citrus fruits (MF, 41.3 mg/g), immature Citrus peel (CP, 32.2 mg/g), mature Citrus peel (JP, 30.0 mg/g) respectively. And also, the triglyceride content in the feces showed a significant increase in MF (14.6 mg/g), and IMF (14.2 mg/g), CP (10.5 mg/g) and JP (10.0 mg/g), respectively. On the other hand, in the inhibition activity of pancreatic lipase, MF (0.78 nmol/min/ml) showed the greatest decrease in glycerol, CP (0.79 nmol/ min/ml), JP, (0.93 nmol/min/ml) and IMF (1.42 nmol/min/ml).	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Nobiletin as a therapeutic for gestational diabetes mellitus (GDM) **Authors:** Liong, Stella; Lim, Ratana; Nguyen-Ngo, Caitlyn; Quak, Stephanie; Barker, Gillian; Lappas, Martha **Journal:** Reproductive Sciences. 2017 Mar; 24(1):185-186. doi: http://dx.doi.org/10.1177/1933719117699773 INTRODUCTION: Maternal insulin resistance, a central feature of GDM, contributes to increased nutrient supply to the fetus, predisposing the offspring to childhood obesity and later adult disease. Proinflammatory cytokines, secreted by placenta and adipose tissue, can induce maternal peripheral resistance. There are no treatments that can prevent GDM and its adverse outcomes. Nobiletin, a citrus fruit flavonoid, is a promising therapeutic agent for type 2 diabetes; however, its effects on GDM are not unknown. The aims were to determine the effect of nobiletin on inflammation in human placenta and adipose tissue; and the insulin signalling pathway in human skeletal muscle in vitro. A genetic mouse model of GDM (heterozygous leptin receptor-deficient; db/+) was also used to determine if nobiletin can prevent the development of GDM and improve neonatal outcomes. METHODS: Human placenta, adipose tissue and skeletal muscle explants were stimulated with bacterial (LPS) and viral (poly(I: C)) products, and pro-inflammatory cytokines (TNF-α, IL-1β) to generate a GDM-like model in vitro. The pro-inflammatory profile in placenta and adipose tissue was determined by qRT-PCR and ELISA. In skeletal muscle, expression of insulin signalling pathway intermediates was determined by Western blotting, and glucose uptake was assessed using a radiolabelled assay. In vivo, nobiletin was administered daily to pregnant GDM mice (day 1-17 of pregnancy), an oral glucose tolerance test was performed on d17 and tissues were collected at d18. RESULTS: Nobiletin (i) decreased IL-6, IL-8 and MCP-1 mRNA expression and release from human placenta and adipose tissue, and (ii) restored the defects in insulin-mediated glucose uptake induced by inflammation or infection. Excitingly, in GDM mice, nobiletin (i) increased maternal glucose tolerance, (ii) decreased inflammation in placenta and adipose tissue; and (iii) improved offspring outcomes (littersize and fetal weights). CONCLUSIONS: Nobiletin can reduce inflammation and improve insulin resistance in both in vitro and in vivo models of GDM. These findings have significant implications for nobiletin as a therapeutic for GDM, given a safe commercial preparation exists. The effects of nobiletin on the health of offspring of GDM mice up to 6 months of age are being assessed to determine if nobiletin can improve long-term adverse fetal outcomes.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Modified citrus pectin inhibits galectin-3 function to reduce atherosclerotic lesions in apoE-deficient mice **Authors:** Lu, Yonggang ; Zhang, Mingming; Zhao, Pei; Jia, Min; Liu, Bing; Jia, Qian; Guo, Jun; Dou, Lin; Li, Jian **Journal:** Mol Med Rep. 2017 Jul;16(1):647-653. doi: 10.3892/mmr.2017.6646. Epub 2017 May 29. Galectin-3 is a carbohydrate-binding lectin, which has been implicated in the modulation of atherosclerotic pathophysiology, and is highly expressed in monocytes, macrophages and endothelial cells within atherosclerotic plaques. Modified citrus pectin (MCP) is produced from citrus pectin via pH and temperature modifications, which break it into shorter, non-branched, galactose-rich carbohydrate chains. MCP is able to tightly bind with galectin-3, via recognition of its carbohydrate recognition domain, and facilitates the modulation of galectin-3-induced bioactivity. The present study explored the effects of MCP on the initiation of atherosclerosis. Eight-week-old apolipoprotein E-deficient mice were treated with 1% MCP and fed an atherogenic diet for 4 weeks. The effects of MCP on atherosclerotic initiation were determined by pathological analysis and scanning electron microscope (SEM) imaging. MCP treatment reduced the size of atherosclerotic lesion areas, which was accompanied by decreased numbers of macrophages and smooth muscle cells (SMCs). Furthermore, SEM examination of the surface of the atheroma-prone vessel wall indicated that MCP treatment reduced endothelial injury. To analyze the effects of MCP on monocyte adhesion, firstly, oxidized-low density lipoprotein and various concentrations of MCP (0.025, 0.05, 0.1 and 0.25%) were incubated with the human umbilical vein endothelial cells (HUVECs) for stimulation and following this, the U937 cells were plated onto the HUVECs. The results revealed that MCP reduced the adhesion of U937 monocytes to HUVECs, indicating the adhesion-inhibiting effects of MCP. In conclusion, the present study revealed that MCP, a galectin-3 inhibitor, reduced the size of atherosclerotic lesions by inhibiting the adhesion of leucocytes to endothelial cells. Inhibition of galectin-3 function may be a therapeutic strategy for the treatment of atherosclerosis.	Full text not reviewed

Table 3-1 **Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)**

Reference[2]	Electronic Copy of Publication
Fenugreek galactomannan and citrus pectin improve several parameters associated with glucose metabolism and modulate gut microbiota in mice **Authors:** Shtriker, Miriam G; Hahn, Michal; Taieb, Elise; Nyska, Abraham; Moallem, Uzi; Tirosh, Oren; Madar, Zecharia **Journal:** Nutrition. 2018 Feb; 46:134-142.e3. doi: 10.1016/j.nut.2017.07.012. Epub 2017 Aug 3. OBJECTIVE Galactomannans derived from fenugreek confer known health benefits; however, there is little information regarding health benefits of citrus pectin (CP) and its association with gut microbiome metabolites. The aim of this study was to examine links between galactomannan and CP consumption, microbiota development, and glucose metabolism. DESIGN Male C57 BL/6 J mice ages 7 to 8 wk were fed ad libitum with a normal diet or one supplemented with 15% of either galactomannan or CP. At 3 wk, an oral glucose tolerance test was performed. Animals were sacrificed at 4 wk and relevant organs were harvested. RESULTS Fiber enrichment led to reductions in weight gain, fasting glucose levels, and total serum cholesterol ($P < 0.05$). Compared with mice fed the normal diet, microbiota populations were altered in both fiber groups and were found to be richer in Bacteroidetes rather than Firmicutes ($P < 0.05$). The modification was significantly greater in galactomannan-fed than in CP-fed mice ($P < 0.0001$). Also, enhanced levels of the short-chain fatty acid (SCFA) propionate were found in the cecal contents of CP-fed animals ($P < 0.05$). Protein expression levels of monocarboxylate transporter 1, which may promote transport of SCFA, were measured in the large intestines after fiber consumption. Enhanced adenosine monophosphate-activated protein kinase (AMPK) activation was observed in livers of galactomannan-fed mice ($P < 0.05$). CONCLUSION Consumption of diets containing soluble fibers, as used in this study, resulted in gut microbiota comprising a healthier flora, and led to positive effects on weight, glycemic control, and liver β oxidation via AMPK.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Yuzu (Citrus junos Tanaka) Peel Attenuates Dextran Sulfate Sodium-induced Murine Experimental Colitis **Authors:** Hiroko Abe, Mika Ishioka, Yasuko Fujita, Aya Umeno, Mayu Yasunaga, Akihiko Sato, Shigehiko Ohnishi, Shingo Suzuki, Noriko Ishida, Mototada Shichiri, Yasukazu Yoshida, Yoshihiro Nakajima **Journal:** Journal of Oleo Science. 2018 Mar 1;67(3):335-344. doi: 10.5650/jos.ess17184. Epub 2018 Feb 19. Ulcerative colitis is a well-known inflammatory bowel disease. Although there are drugs that are effective against this disease, the prevention and attenuation of ulcerative colitis by food rich in functional ingredients without side effects is desired because some drugs have side effects. In this study, we investigated the effects of yuzu (Citrus junos Tanaka), a citrus fruit native to northeast Asia, on a mouse dextran sulfate sodium (DSS)-induced colitis model. Mice given drinking water containing DSS showed significant weight loss, colon shortening, diarrhea, and visible fecal blood. In contrast, mice fed a diet containing 5% yuzu peel for 14 d before receiving DSS showed significant attenuation of these phenotypes. To clarify the mechanism underlying the attenuation, we investigated the anti-inflammatory and antioxidant effects of yuzu peel. We found that yuzu peel extract suppressed tumor necrosis factor-α (TNF-α) production in lipopolysaccharide (LPS)-stimulated mice and murine macrophage cell line through suppression of nuclear factor-κB (NF-κB) activation. In addition, we confirmed that yuzu peel extract had a moderate antioxidant effect. These results suggest that yuzu peel attenuates the pathologies of DSS-induced colitis by coordinately suppressing inflammation and oxidative stress against lipids in vivo.	Full text not reviewed
Beneficial Effects of Hesperetin in a Mouse Model of Temporal Lobe Epilepsy **Authors:** Jae Young Kwon, Un Ju Jung, Dong Woon Kim, Sehwan Kim, Gyeong Joon Moon, Jungwan Hong, Min-Tae Jeon, Minsang Shin, Jeong Ho Chang, Sang Ryong Kim **Journal:** Journal of Medicinal Food. 2018 Aug 23. doi: 10.1089/jmf.2018.4183. [Epub ahead of print] Abnormal reorganization of the dentate gyrus and neuroinflammation in the hippocampus represent characteristic phenotypes of patients suffering from temporal lobe epilepsy. Hesperetin, a flavanone abundant in citrus fruit, is known to have protective effects by preventing inflammation and oxidative stress in neuronal cultures and in the adult murine brain. However, the protective effects of hesperetin against epileptic seizures in vivo remain unclear, despite one study reporting anticonvulsant effects in vitro. In this study, we report that oral administration of hesperetin not only delays the onset of seizures triggered by kainic acid (KA) but also contributes to the attenuation of granule cell dispersion in the KA-treated hippocampus. Moreover, we observed that hesperetin administration inhibited the expression of pro-inflammatory molecules produced by activated microglia in the hippocampus. Thus, administration of hesperetin might be beneficial for preventing epileptic seizures.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Hesperidin in citrus fruit juice plays a role in preventing cognitive impairment induced by ischemic brain damage **Authors:** Moe Kawakami, Jun Iwanami, Kana Tsukuda, Akinori Higaki, Li-Juan Min, Masaki Mogi, Masatsugu Horiuchi **Journal:** Hypertension. 2018 Sep 1. doi: https://doi.org/10.1161/hyp.72.suppl_1.P332. Recently we reported that drinking Citrus iyo juice (CI) inhibited more effectively vascular remodeling in the inflammation-induced vascular injury mouse model than Citrus unshiu juice (CU). These results led us to explore the possibility that citrus fruits juice drinking could attenuate cognitive decline in transient cerebral ischemia mouse model, focusing on the effects of flavanone, hesperidin, which is more abundantly contained in CI compared with CU and has antioxidant activity. Eight-week-old male C; 57BL/6 mice were administrated 10 % CI or CU in drinking water or 100 mg/kg/day hesperidin orally by gavage. Two weeks after administration, brain ischemia was induced by bilateral common carotid artery occlusion (BCCAO) for 18 minutes. The Y maze task was performed 2 weeks after BCCAO operation. After cognitive task, cerebral blood flow (CBF) was measured by a laser speckle flowmetry. Morphological changes in the hippocampus were examined. Administration of CI, CU or hesperidin did not influence systolic blood pressure, body weight and brain weight. Cognitive function was significantly impaired (sham, 71% (16 of 23) vs BCCAO, 55% (14 of 27) in Y maze) with the increase in superoxide anion production after BCCAO. The cognitive impairment was more effectively attenuated by the administration of CI than CU (CI, 66% (13 of 23); CU, 61% (17 of 27)) with the significant increase in CBF. Interestingly, we also observed that the treatment with hesperidin significantly prevented cognitive decline (67% (13 of 21)) after BCCAO. The increase in superoxide anion production 24 hours after BCCAO (expressed as fold-increase compared to sham) was attenuated by CI or hesperidin, not by CU (BCCAO, 4.0; CI, 1.2; hesperidin, 1.1; CU, 3.0). The fold-increase in TNF-α mRNA in the hippocampus 24 hours after BCCAO was prevented by hesperidin (BCCAO 5.0; hesperidin, 2.8). Cell number in CA1 region of hippocampus decreased in BCCAO-operated mice. Hesperidin treatment attenuated this decrease (sham, 163±5; BCCAO, 136±4; hesperidin, 154±4). These results suggest that the intake of hesperidin in citrus fruits juice should prevent cognitive decline after brain ischemia at least in part due to reduction of oxidative stress, inflammatory cytokine and an increase in CBF.	Full text not reviewed
Anti-inflammatory bowel effect of industrial orange by-products in DSS-treated mice **Authors:** Teresa M Pacheco, Javier F Moreno, Villamiel Mar, Patricia Diez-Echave, Pilar Utrilla, Teresa Vezza **Journal:** Food & Function. 2018 Sep 19;9(9):4888-4896. doi: 10.1039/c8fo01060a. This work addresses the role of different by-products derived from the industrial extraction of orange juice in a possible anti-inflammatory effect in mice with colitis induced by dextran sulfate sodium (DSS). Fresh orange residue (FOR), dry orange residue (DOR), orange liqueur (OL) and animal feed (AF), as well as commercial citrus pectin (CP), were administered to C57BL/6J mice for 15 days before starting the DSS treatment. Analysis of macroscopic parameters such as the Disease Activity Index (DAI) and the colonic weight/length ratio revealed an anti-inflammatory effect following intake of FOR, AF or CP. Moreover, q-PCR of RNA from colonic tissue indicated measurable changes in the expression of TNF-α, IL-1β, iNOS, and intercellular adhesion molecules ICAM I, as well as in intestinal barrier proteins such as MUC-3, occludin, and ZO-1. Pectin, phenolic compounds and/or Maillard reaction products formed at initial steps were identified as relevant components exerting the ascribed beneficial effects. Our findings could open up the further application of a variety of orange by-products as food supplements in the potential amelioration of inflammatory bowel diseases.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Immunomodulatory effect of natural and modified Citrus pectin on cytokine levels in the spleen of BALB/c mice **Author:** Rihab Merheb, Roula M Abdel-Massih, Marc C Karam **Journal:** International Journal of Biological Macromolecules. 2019 Jan;121:1-5. doi: 10.1016/j.ijbiomac.2018.09.189. Epub 2018 Oct 3. Pectin is present in the cell wall of different vegetables and fruits. Beside its importance in the plant cell wall, pectin has enticed great attention for its beneficial effects on human health. It was shown to decrease cholesterol levels, to possess anti-oxidative, anti-bacterial and anti-cancer activity. The immunomodulatory activity of pectin and its mechanism of action is recently being investigated. In this study, the differential immunomodulatory activities of both CP (citrus pectin) and MCP (modified citrus pectin) were investigated. Females BALB/c mice (20-25 g) were randomly divided into 7 groups and different concentrations of CP and MCP (0%, 1.5%, 3% and 5%) were added to their drinking water for 21 days. Then, the splenic level of IL-1β, IL-4, IL-10, IL-17, IFN-γ and TNF-α were evaluated using ELISA. Both CP and MCP exhibited immunomodulatory activities by increasing the levels of the pro-inflammatory cytokines IL-17, IFN-γ and TNF-α levels. This tendency seems to be regulated by the up-regulation of IL-4 levels but with no major effect on those of IL-10. Therefore, CP and especially MCP have potential immunomodulatory effects which might be highly beneficial in immunotherapy.	Full text not reviewed
Citrus peels prevent cancer **Authors:** Ajikumaran S Nair, Rajani Kurup SR, Akhila S Nair, Sabulal Baby **Journal:** Phytomedicine. 2018 Nov 15;50:231-237. doi: 10.1016/j.phymed.2017.08.011. Epub 2017 Aug 17. Background: Citrus comprises the largest fruit sector worldwide, and its fruit peels are the dominant 'residue' of the industry. Though not profitable, Citrus peels are industrially used for making some byproducts (cattle feed, molasses, ethanol, fiber) and for the extraction of bioactives (flavonoids, essential oils, D-limonene). Still huge amounts of peels are wasted by Citrus industries, juice and other vending sectors. Purpose: The biological potentials of these unutilized or 'wasted' Citrus peels are least exploited. Here we tested the anticancer potentials of Citrus medica (2 morphotypes), C. sinensis, C. maxima, C. limon and C. reticulata peels by in vitro assays and in vivo cancer models. Methods: Chemical profiles of Citrus peel oils and peel extracts were analyzed by gas chromatographic techniques (GC-FID, GC–MS) and HPTLC-densitometry, respectively. Anticancer potentials of Citrus peels (Citrus medica 2 morphotypes, C. sinensis, C. maxima, C. limon and C. reticulata) were evaluated by various in vitro assays (MTT assay, morphological observations, fast halo assay, flow cytometric analysis) and in vivo cancer models. Results: C. reticulata peels (extracts, essential oils) showed significant activity against DLA cell line in MTT assay. We found C. reticulata peel water extract inducing cell cycle arrest of DLA in G0/G1 phase followed by nuclear condensation, membrane blebbing, formation of apoptotic bodies and DNA damage leading to apoptosis. In in vivo experiments, C. reticulata peel extract pre-treated mice were significantly (50%) protected from DLA compared to post-treated mice (33%), without any conspicuous toxic symptoms. Citrus peels have volatiles (essential oils, limonoids) and non-volatiles (mainly polymethoxy flavones) as their bioactive/anticancer constituents. Conclusion: Our results encourage the use of Citrus peels, which is wasted in huge amounts, as cancer preventive food additives and as anticancer agents.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Modified citrus pectin inhibited bladder tumor growth through downregulation of galectin-3 **Authors:** Tian Fang, Dan-Dan Liu, He-Ming Ning, Liu Dan, Jing-Ya Sun, Xiao-Jing Huang, Yu Dong, Mei-Yu Geng, Shi-Feng Yun, Jun Yan, Rui-Min Huang **Journal:** Acta Pharmacologica Sinica. 2018 Dec;39(12):1885-1893. doi: 10.1038/s41401-018-0004-z. Epub 2018 May 16. Modified citrus pectin (MCP) is a carbohydrate enriched complex, which has been implicated in cancer treatment and prevention. However, the effects of MCP on urinary bladder cancer (UBC) are unknown. In this study, MCP was first tested in T24 and J82 human UBC cells and showed the inhibition of cell viability by the sulforhodamine B (SRB) assay. The MCP-treated UBC cells exhibited G2/M phase arrest with the decrease of Cyclin B1 and phosphorylated Cdc2. Caspase-3 was also activated, leading to the cleavage of Caspase-3 and PARP. We further explored the possible molecular mechanisms upon MCP treatment in UBC cells. Reduction of galectin-3 was observed and followed with the inactivation of Akt signaling pathway. Of note, galectin-3 knockdown by RNA interference recapitulated the MCP-mediated anti-proliferation, cell cycle arrest and apoptosis. Moreover, oral administration of MCP to the T24 xenograft-bearing nude mice inhibited the tumor growth significantly ($P < 0.05$). Quantification analysis of immunohistochemistry staining for Ki67 and cleaved Caspase-3 confirmed the decrease of proliferation index ($P < 0.05$) and the increase of apoptosis index ($P < 0.01$) in 700 mg/kg MCP-fed UBC xenografts. Using the information from TCGA database, we revealed that the overexpression of galectin-3 was associated with high tumor grade with lymph node metastasis, poor overall survival in UBC patients. Considering the remarkable inhibitory effects of MCP on UBC cell proliferation and survival in vitro and in vivo mainly through galectin-3, which is upregulated in UBCs, MCP may become an attractive agent, as a natural dietary fiber, for prevention and therapy of UBCs.	Full text not reviewed

Table 3-1 Full-Text and/or Abstracts for Potentially Relevant Studies Identified in the Updated Literature Search (01 January 2015 to 31 May 2019)

Reference[2]	Electronic Copy of Publication
Rat Studies	
Safety and toxicological evaluation of a novel *Citrus sudachi* extract powder. **Authors:** Shikishima, Y; Yoshinari, O; Shiojima, Y; Moriyama, H; Bagchi, M; Deshmukh, Narendra; Bagchi, D **Journal:** Functional Foods in Health and Disease. 2016; 6(10):677-690. Background: *Citrus sudachi*, an evergreen tree primarily found in the prefecture of Tokushima, Japan, is a widely used popular citrus fruit used in cooking and consumed as a juice. *Citrus sudachi* peels are rich in flavonoids including sudachitin (5,7,4′-trihydroxy-6,8,3′-trimethoxyflavone), and exhibit potent antioxidant, antimicrobial and anti-diabetic properties; additionally, several limonoids and their glucosides are found in its seeds. We examined the broad spectrum safety of a novel light yellow to golden yellow *Citrus sudachi* Extract Powder (CSEP), organic, nutritive from the dried fruit rind (25:1 herbs to extract ratio) containing no less than 1% sudachitin in various toxicology models in GLP-approved laboratories. Methods: The acute oral toxicity study was conducted in female Sprague-Dawley rats with an up and down procedure. The single dose acute dermal LD_{50} of CSEP was assessed in both male and female rats. The primary skin irritation toxicity of CSEP was assessed in female New Zealand Albino rabbits in order to determine the potential for CSEP to produce irritation after a single topical application, while primary eye irritation index of CSEP was conducted in female New Zealand Albino rabbits. Ames' bacterial reverse mutation assay was conducted to determine the ability of CSEP to induce reverse mutation at selected histidine loci in five tester strains of *Salmonella typhimurium* viz. TA1535, TA97a, TA98, TA100, and TA102 in the presence and absence of a metabolic activation system (S9) at the doses of 5000, 1500, 500, 150 and 50 mg/plate. The mutagenic potential of CSEP was also evaluated in an *in vitro* mammalian cell gene mutation test using the thymidine kinase gene of L5178 Tk+/-3.7.2C mouse lymphoma cell line. Results: The acute oral LD50 of CSEP was found to be greater than 5000 mg/kg body weight. The single dose acute dermal LD_{50} of CSEP was found to be greater than 2000 mg/kg body weight in both male and female rats. In the primary skin irritation test, CSEP was found to be slightly irritating to the skin. The primary dermal irritation index (PDII) calculated for CSEP was found to be 0.8. In the primary eye irritation test, the maximum mean total score (MMTS) of CSEP was found to be 2.7. Thus, CSEP was classified as minimally irritating to the eye. In both Ames' bacterial reverse mutation assay and *in vitro* mammalian cell gene mutation test, no mutagenicity was observed. Conclusion: Overall, these toxicological evaluations demonstrate the broad spectrum safety of CSEP.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Modified citrus pectin stops progression of liver fibrosis by inhibiting galectin-3 and inducing apoptosis of stellate cells **Authors:** Abu-Elsaad, Nashwa M; Elkashef, Wagdi Fawzi **Journal:** Can J Physiol Pharmacol. 2016 May;94(5):554-62. doi: 10.1139/cjpp-2015-0284. Epub 2015 Dec 16. Modified citrus pectin (MCP) is a pH modified form of the dietary soluble citrus peel fiber known as pectin. The current study aims at testing its effect on liver fibrosis progression. Rats were injected with CCl4 (1 mL/kg, 40% v/v, i.p., twice a week for 8 weeks). Concurrently, MCP (400 or 1200 mg/kg) was administered daily in drinking water from the first week in groups I and II (prophylactic model) and in the beginning of week 5 in groups III and IV (therapeutic model). Liver function biomarkers (ATL, AST, and ALP), fibrosis markers (laminin and hyaluronic acid), and antioxidant biomarkers (reduced glutathione (GSH) and superoxide dismutase (SOD)) were measured. Stained liver sections were scored for fibrosis and necroinflammation. Additionally, expression of galectin-3 (Gal-3), α-smooth muscle actin (SMA), tissue inhibitor metalloproteinase (TIMP)-1, collagen (Col)1A1, caspase (Cas)-3, and apoptosis related factor (FAS) were assigned. Modified pectin late administration significantly ($p < 0.05$) decreased malondialdehyde (MDA), TIMP-1, Col1A1, α-SMA, and Gal-3 levels and increased levels of FAS, Cas-3, GSH, and SOD. It also decreased percentage of fibrosis and necroinflammation significantly ($p < 0.05$). It can be concluded that MCP can attenuate liver fibrosis through an antioxidant effect, inhibition of Gal-3 mediated hepatic stellate cells activation, and induction of apoptosis.	Full text not reviewed
Effect of pectin feeding on obesity development and duodenal alkaline phosphatase activity in Sprague-Dawley rats fed with high-fat/high-energy diet **Authors:** Šefčíková, Z; Raček, L **Journal:** Physiol Int. 2016 Jun 1;103(2):183-190. doi: 10.1556/036.103.2016.2.5. Purpose The objective of this study was to evaluate whether pectin feeding would affect the small intestinal function and whether these changes would lead to obesity prevention in rats fed with high-fat diet. Three groups of weaned male rats (ad lib. fed; rats fed with diet containing 15% w/w of citrus pectin; restrictedly pair-fed rats) were fed with either a standard diet (9.5% fat) or a high-fat diet (30% fat) for 10 days. Results Our results revealed that pectin feeding led to significant decreases in body weight, energy intake and fat pad weight in rats fed with the standard as well as high-fat diet. Moreover, compared to the restrictedly pair-fed rats, in both groups of rats fed with the diet containing pectin, significant decrease in duodenal alkaline phosphatase (AP) activity was observed in histochemically stained cryostat sections. In contrast, despite their lower energy intake, restrictedly pair-fed rats showed similar fat pad deposition accompanied by unchanged values of AP activity in comparison to the controls. Conclusions Our findings indicate that daily pectin consumption could be beneficial in suppressing body weight gain and reducing probability of obesity risk in rats fed with a high-fat diet.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Anti-diabetic effect of citrus pectin in diabetic rats and potential mechanism via PI3K/Akt signaling pathway **Authors:** Liu, Yanlong; Dong, Man; Yang, Ziyu; Pan, Siyi **Journal:** Int J Biol Macromol. 2016 Aug;89:484-8. doi: 10.1016/j.ijbiomac.2016.05.015. Epub 2016 May 6. This study was performed to investigate the anti-diabetic effect of citrus pectin in type 2 diabetic rats and its potential mechanism of action. The results showed that fasting blood glucose levels were significantly decreased after 4 weeks of citrus pectin administration. Citrus pectin improved glucose tolerance, hepatic glycogen content and blood lipid levels (TG, TC, LDL-c and HDL-c) in diabetic rats. Citrus pectin also significantly reduced insulin resistance, which played an important role in the resulting anti-diabetic effect. Moreover, after the pectin treatment, phosphorylated Akt expression was upregulated and GSK3β expression was downregulated, indicating that the potential anti-diabetic mechanism of citrus pectin might occur through regulation of the PI3K/Akt signaling pathway. Together, these results suggested that citrus pectin could ameliorate type 2 diabetes and potentially be used as an adjuvant treatment.	Full text not reviewed
An anti-diabetic poly-herbal medicine prepared from extracts of Annona Stenophylla, Citrus Limon and Zingiber Officinale **Authors:** Verengai, Walter; Chagonda, Lameck; Chitindingu, Kudakwashe; Marume, Amos; Taderera, Tafadzwa **Journal:** International Journal of Pharmaceutical Sciences and Research 8(3):1048-1055. 2017 March. doi: 10.13040/IJPSR.0975-8232.8(3).1048-55. Background: Diabetes poses a burden on the limited health care delivery system. Current pharmacological anti-diabetic agents cause undesirable side effects and are very expensive. Herbal remedies offer the potential for alternative treatment strategies. This study was therefore conducted in an effort to formulate a poly herbal medicine for management of diabetes. Methods: A stenophylla root bark was extracted with aqueous ethanol (70%) and quantitative analysis for total phenolic content, total alkaloids and flavonoids was carried out on the dried extract. The hypoglycaemic effects of aqueous A. stenophylla root bark extract, powdered Zingiber officinale and powdered Citrus Limon peels was determined on alloxan-induced diabetic rats singly and in combinations over 14 days. Glibenclamide administered at 0.2 mg/kg b. wt was used as the positive control. Results: All the plants decreased plasma glucose levels in a dose-dependent manner. The glucose levels of diabetic rats treated with all the plants combined showed the greatest reduction (59.2%). A. stenophylla and Z. officinale showed a glucose level reduction of 47.7%, A. stenophylla and C. limon (42.9%) whilst Z. officinale and C. limon (42.7%). The reductions in glucose levels were comparable to glibenclamide positive control which showed a 61% reduction. Conclusion: Combining the three plants (A. stenophylla, Z. officinale and C. limon) led to the greatest reduction in blood glucose levels of alloxan induced diabetic rats. The formulation demonstrates more potential in combinational herbal medicines therapy in diabetes management.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
The Effect of Consumption of Citrus Fruit and Olive Leaf Extract on Lipid Metabolism **Authors:** Merola, Nicola; Castillo, Julián; Benavente-García, Obdulio; Ros, Gaspar; Nieto, Gema **Publication info:** Nutrients. 2017 Sep 26;9(10). pii: E1062. doi: 10.3390/nu9101062. Citrus fruit and olive leaves are a source of bioactive compounds such as biophenols which have been shown to ameliorate obesity-related conditions through their anti-hyperlipidemic and anti-inflammatory effect, and by regulating lipoproteins and cholesterol body levels. Citrolive™ is a commercial extract which is obtained from the combination of both citrus fruit and olive leaf extracts; hence, it is hypothesised that Citrolive™ may moderate metabolic disorders that are related to obesity and their complications. Initially, an in vitro study of the inhibition of pancreatic lipase activity was made, however, no effect was found. Both preliminary and long-term evaluations of Citrolive™ on lipid metabolism were conducted in an animal model using Wistar rats. In the preliminary in vivo screening, Citrolive™ was tested on postprandial plasma triglyceride level after the administration of an oil emulsion, and a significant reduction in postprandial triacylglycerol (TAG) levels was observed. In the long-term study, Citrolive™ was administered for 60 days on Wistar rats that were fed a high-fat diet. During the study, several associated lipid metabolism indicators were analysed in blood and faeces. At the end of the experiment, the livers were removed and weighed for group comparison. Citrolive™ treatment significantly reduced the liver-to-body-weight ratio, as supported by reduced plasma transaminases compared with control, but insignificantly reduced plasma low density lipoprotein (LDL) and postprandial TAG plasma levels. In addition, faecal analysis showed that the treatment significantly increased total cholesterol excretion. On the other hand, no effect was found on faecal TAG and pancreatic lipase in vitro. In conclusion, treatment ameliorates liver inflammation symptoms that are worsened by the effects of high fat diet.	Full text not reviewed
Naringin ameliorates endothelial dysfunction in fructose-fed rats **Authors:** Wachirawadee Malakul, Sirinat Pengnet, Chanon Kumchoom, Sakara Tunsophon **Journal:** Experimental and Therapeutic Medicine. 2018 Mar;15(3):3140-3146. doi: 10.3892/etm.2018.5759. Epub 2018 Jan 17. High fructose consumption is associated with metabolic disorders including hyperglycemia and dyslipidemia, in addition to endothelial dysfunction. Naringin, a flavonoid present in citrus fruit, has been reported to exhibit lipid lowering, antioxidant, and cardiovascular protective properties. Therefore, the present study investigated the effect of naringin on fructose-induced endothelial dysfunction in rats and its underlying mechanisms. Male Sprague-Dawley rats were given 10% fructose in drinking water for 12 weeks, whereas control rats were fed drinking water alone. Naringin (100 mg/kg) was orally administered to fructose fed rats during the last 4 weeks of the study. Following 12 weeks, blood samples were collected for measurement of blood glucose, serum lipid profile and total nitrate/nitrite (NOx). Vascular function was assessed by isometric tension recording. Aortic expression of endothelial nitric oxide synthase (eNOS), phosphorylated eNOS (p-eNOS), and nitrotyrosine were evaluated by western blot analysis. Fructose feeding induced increased levels of blood glucose, total cholesterol, triglyceride, and low density lipoprotein. In rat aortae, fructose reduced acethycholine-induced vasorelaxation, without affecting sodium nitroprusside-induced vasorelaxation. Treatment of fructose-fed rats with naringin restored fructose-induced metabolic alterations and endothelial dysfunction. Fructose-fed rats also exhibited decreased serum NOx level, reduced eNOS and p-eNOS protein expression, and enhanced nitrotyrosine expression in aortae. These alterations were improved by naringin treatment. The results of the present study suggested that naringin treatment preserves endothelium-dependent relaxation in aortae from fructose fed rats. This effect is primarily mediated through an enhanced NO bioavailability via increased eNOS activity and decreased NO inactivated to peroxynitrite in aortae.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Naringenin (4,5,7-trihydroxyflavanone) suppresses the development of precancerous lesions via controlling hyperproliferation and inflammation in the colon of Wistar rats **Authors:** Muneeb U Rehman, Manzoor Ur Rahman Mir, Adil Farooq, Shahzada Mudasir Rashid, Bilal Ahmad, Sheikh Bilal Ahmad, Rayeesa Ali, Ishraq Hussain, Mubashir Masoodi, Showkeen Muzamil, Hassan Madkhali, Majid Ahmad Ganaie **Journal:** Environmental Toxicology. 2018 Apr;33(4):422-435. doi: 10.1002/tox.22528. Epub 2018 Jan 18. Colon cancer is a world-wide health problem and one of the most dangerous type of cancer, affecting both men and women. Naringenin (4, 5, 7-trihydroxyflavanone) is one of the major flavone glycoside present in citrus fruits. Naringenin has long been used in Chinese's traditional medicine because of its exceptional pharmacological properties and non-toxic nature. In the present study, we investigated the chemopreventive potential of Naringenin against 1,2-dimethyhydrazine (DMH)-induced precancerous lesions, that is, aberrant crypt foci (ACF) and mucin depleted foci (MDF), and its role in regulating the oxidative stress, inflammation and hyperproliferation, in the colon of Wistar rats. Animals were divided into five groups. In groups 3-5, Naringenin was administered at the dose of 50mg/kg b. wt. orally while in groups 2-4, DMH was administered subcutaneously in the groin at the dose of 20mg/kg b. wt. once a week for first 5weeks and animals were euthanized after 10weeks. Administration of Naringenin ameliorated the development of DMH-induced lipid peroxidation, ROS formation, precancerous lesions (ACF and MDF) and it also reduced the infiltration of mast cells, suppressed the immunostaining of NF-B-p65, COX-2, i-NOS PCNA and Ki 67 Naringenin treatment significantly attenuated the level of TNF- and it also prevented the depletion of the mucous layer. Our findings suggest that Naringenin has strong chemopreventive potential against DMH-induced colon carcinogenesis but further studies are warranted to elucidate the precise mechanism of action of Naringenin.	Full text not reviewed

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Reference[2]	Electronic Copy of Publication
Pig Studies	
Interactive effect of dietary protein and dried citrus pulp levels on growth performance, small intestinal morphology, and hindgut fermentation of weanling pigs **Authors:** Almeida, V V; Nuñez, A J C; Schinckel, A P; Alvarenga, P V A; Castelini, F R; Silva-Guillen, Y V; Thomaz, M C. Weanling pigs (= 108, 21 d of age, 5.82 ± 0.16 kg initial BW) were assigned to a 2 × 2 factorial arrangement of treatments to evaluate the effects of dietary levels of CP (high- and low-CP diets) and dried citrus pulp (DCP; 0% and 7.5%) on growth performance, small intestinal morphology, and hindgut fermentation. Pigs were blocked by initial BW and allotted to 1 of 9 pens, each containing 3 pigs. The high-CP diets consisted of feeding 20% and 21% CP levels throughout phase 1 (0 to 14 d) and phase 2 (14 to 28 d), respectively. For the low-CP diets, CP levels were reduced by 4% units as compared with the high-CP diets in both phases. Crystalline AA were supplied to maintain an ideal AA pattern. Pig BW and pen feed disappearance were recorded weekly. On d 7 and 28 postweaning, 1 pig from each pen was euthanized for collection of small intestinal tissues and digesta from cecum and colon. There were no CP × DCP interactions for growth performance and gut morphology. Although the low-CP diet decreased ADG (= 0.03) and G:F (= 0.02) from d 21 to 28 postweaning, overall performance was unaffected by the treatments. On d 7 postweaning, pigs fed the low-CP diet tended to have increased (= 0.09) crypt depth in the duodenum. Low-CP diets tended to increase (= 0.06) crypt depth and reduce (= 0.08) villus:crypt ratio in the jejunum on d 7. Dietary treatments did not affect ileal morphology. On d 7 postweaning, low-CP diets tended to reduce (= 0.09) cecal total VFA, whereas dietary DCP inclusion tended to decrease (= 0.07) colonic propionate. Including 7.5% DCP to the diet decreased (<0.05) colonic isovalerate and ammonia N concentrations on d 7 only for pigs fed the low-CP diet. On d 28 postweaning, DCP inclusion in low-CP diets decreased (<0.05) butyrate, isovalerate, and valerate concentrations in the cecum, as well as isovalerate, valerate, and ammonia N concentrations in the colon. Including 7.5% DCP to the diet increased (<0.05) acetate:propionate ratio in the hindgut on both d 7 and 28 postweaning only for pigs fed the high-CP diet. Lactate concentration was unaffected by the treatments. These results indicate that feeding low-CP AA-supplemented diets did not compromise overall growth performance, but slightly increased damage in the gut morphology of weanling pigs. Moreover, adding 7.5% DCP to low-CP AA-supplemented diets shifted the fermentation pattern in the hindgut of weanling pigs by decreasing protein fermentation metabolites.	Full text not reviewed
Effects of dried citrus pulp and fermented medicinal plants on growth performance, nutrient digestibility, blood characteristics, and meat quality in growing-finishing pigs **Authors:** Xin Jian Lei, Yong Mm Kim, In Ho Kim **Journal:** Canadian Journal of Animal Science. 2018, 98(4): 875-883, https://doi.org/10.1139/cjas-2017-0170 The present experiment was conducted to determine the effects of dried citrus pulp and fermented medicinal plants in growing-finishing pigs. A total of 96 pigs (62.34 +/- 1.96 kg body weight) were randomly allotted into three dietary treatments: (1) control, basal diet (CON); (2) diet containing 10% dried citrus pulp (DCP); (3) diet containing 10% dried citrus pulp supplemented with 0.1% fermented medicinal plants (DCPFMP). From weeks 0 to 5 and 0 to 10, pigs fed the DCPFMP diet had significantly decreased (P <0.05) average daily feed intake and increased (P <0.05) gain:feed ratio compared with those fed the CON diet. The apparent total tract digestibility of gross energy was greater (P <0.05), and serum total cholesterol concentration was decreased (P <0.05) for pigs fed the DCPFMP diet compared with those fed the DCP diet in week 10. In addition, an increase (P <0.05) in Longissimus muscle area was observed for pigs fed the DCPFMP diet compared with those fed the CON diet. In conclusion, supplementation with fermented medicinal plants in a diet containing 10% dried citrus pulp improved growth performance and Longissimus muscle area and lowered serum low-density lipoprotein cholesterol and total cholesterol concentrations.	Full text not reviewed

APPENIX B

GRAS Panel Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of NUTRAVA™ Citrus Fiber for Use in Foods

4 November 2019

INTRODUCTION

At the request of CP Kelco ApS (CPK), a panel of independent scientists (the "GRAS Panel"), qualified by their relevant experience and scientific training to evaluate the safety of food ingredients, was specially convened to conduct a critical and comprehensive evaluation of the available pertinent data and information, and to determine whether, under the conditions of intended use as an ingredient in traditional foods, NUTRAVA™ Citrus Fiber would be Generally Recognized as Safe (GRAS), based on scientific procedures. The GRAS Panel consisted of the below-signed qualified scientific experts: Dr. Joseph Borzelleca (Virginia Commonwealth University), Dr. George Fahey (University of Illinois at Urbana), and Dr. Robert Nicolosi (University of Massachusetts Lowell).

The GRAS Panel, independently and collectively, critically examined a comprehensive package of publicly available scientific information and data compiled from the literature and other published sources based on previously GRAS-notified citrus fiber ingredients (GRN 154/487, 541, and 599 – U.S. FDA, 2004, 2014a, 2015a, 2016a) and an updated search of the published scientific literature conducted for literature available between 01 January 2015 (date of citrus fiber literature search conducted in GRN 599 – U.S. FDA, 2016a) and 30 May 2019. In addition, the GRAS Panel evaluated other information deemed appropriate or necessary, including data and information provided by CPK. The data evaluated by the GRAS Panel included information pertaining to the method of manufacture and product specifications, analytical data, intended use levels in specified food products, consumption estimates for all intended uses, and comprehensive literature on the safety of NUTRAVA™ Citrus Fiber and its individual components.

Following independent, critical evaluation of such data and information, the GRAS Panel unanimously concluded that under the conditions of intended use in traditional foods described herein, NUTRAVA™ Citrus Fiber, meeting appropriate food-grade specifications, and manufactured and used in accordance with current good manufacturing practice, is GRAS based on scientific procedures. A summary of the basis for the GRAS Panel's conclusion, excluding confidential data and information, is provided below.

COMPOSITION, MANUFACTURING, AND SPECIFICATIONS

NUTRAVA™ Citrus Fiber is manufactured by CPK from citrus peel, which is a by-product of the juicing processes from oranges, lemons, and limes. NUTRAVA™ Citrus Fiber is a white, yellowish, light greyish, or light brownish powder, with a neutral to slight citrus odor and a suitable mesh size. NUTRAVA™ Citrus Fiber's main components are *ca.* 40 to 45% insoluble fibers and *ca.* 40 to 45% soluble fibers and may contain up to 12% moisture. The insoluble fiber is composed of mainly cellulose but also hemi-cellulose and, to a minor degree, lignin, and the soluble fiber consisting primarily of pectin.

The manufacturing process of NUTRAVA™ Citrus Fiber is carried out according to current Good Manufacturing Practice (cGMP) and in compliance with the United States (U.S.) Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA). The manufacturing site is International Organization for Standardization (ISO) 9001:2015 and Food Safety System Certification (FSSC) 22000 certified. The full citrus fruit by-product fraction can be used (*i.e.*, juice cells, peel, rag, and segment membranes). For convenience, the citrus fruit by-product is hereafter referred to as 'citrus peel'. All processing aids used for production of NUTRAVA™ Citrus Fiber are food-grade or equivalent and are used in accordance with appropriate federal regulations; they have previously been determined to be GRAS and, where applicable, have been the subject of an effective food contact notification.

The citrus peel raw material is inspected in accordance with CPK's quality control standards. The manufacturing process consists of 2 overall steps: (i) an acidification step; and (ii) a partial neutralization step. During the process, sugars, salts, residual oils, and flavonoids are washed out of the citrus peel, resulting in a citrus fiber material consisting mainly of pectin, cellulose, hemicellulose, and to a minor extent lignin. During the acidification step, mechanical energy and heat are applied to the citrus peel in aqueous ethanol. To create the acidic conditions, the pH is adjusted to below 2.5 by a dilute acid. Mechanical energy is induced by passing the mixture several times through pumps to open the structure of the peel, creating a larger surface. The process of opening the structure is called defibrillation. After the acidification step, the citrus fiber material is mechanically separated from the liquid phase. The citrus fiber material is then washed in aqueous ethanol and partially neutralized using a dilute base (potassium hydroxide or sodium hydroxide) to a pH of approximately 4. Finally, the citrus fiber solution is dried to below 12% moisture and milled to a suitable mesh size to produce the final NUTRAVA™ Citrus Fiber product.

The final specifications for NUTRAVA™ Citrus Fiber are presented in Table 1. Apart from being a dietary fiber, the ingredient is largely characterized by its water holding capacity. Analysis of 3 lots of NUTRAVA™ Citrus Fiber demonstrates that the manufacturing process produces a consistent product that meets the ingredient's specifications. Additional product characterization demonstrates that NUTRAVA™ Citrus Fiber is composed largely of dietary fiber (>85%) and contains low amounts or is negative for general hygiene indicators, relevant food-borne pathogens, residual processing aids, heavy metals, mycotoxins, and pesticides.

Table 1 Specifications for NUTRAVA™ Citrus Fiber

Specification Parameter (wet weight basis)	Specification	Method
Water holding capacity (g/g)	15 to 35	CPK 0901001
pH, 1%	3.0 to 5.0	CPK 0006041
Loss on drying (%)	Not more than 12%	CPK 0006042
Particle size	Less than 3% on a 0.250 mm test sieve	CPK 0006013

NUTRAVA™ Citrus Fiber, when stored in paper bags with a perforated PE inner liner, was demonstrated to have low microbiological risk across 18 months, as estimated by an accelerated shelf-life study (55°C and 80% relative humidity) performed for 13 weeks, corresponding to 84 weeks at ambient temperature.

INTENDED USE AND ESTIMATED EXPOSURE

NUTRAVA™ Citrus Fiber is intended for use as a nutrient source of dietary fiber and multipurpose (*e.g.*, water binding, gelling aid, thickening aid, bulking aid, emulsion stabilizer, cloud stabilizer) food ingredient. NUTRAVA™ Citrus Fiber is intended for addition to food and beverage products across multiple categories as described in Table 2 at use levels up to 3%. Food-uses are organized according to 21 CFR §170.3 (U.S. FDA, 2018a). In addition to the food and beverage uses listed in Table 2, NUTRAVA™ Citrus Fiber is also intended to be used at up to 5% in the same meat and poultry products described for other citrus fiber ingredients in GRN 154, 487, 541, and 599 (U.S. FDA 2004, 2014a, 2015a, 2016a). NUTRAVA™ Citrus Fiber will be fully substitutional to current uses and is intended to be added to meat and poultry products in place of other similar citrus fiber ingredients present in meat and poultry products currently on the market and therefore will not change the current exposure to citrus fiber in the diet from these food uses.

Table 2 Summary of the Individual Proposed Food-Uses and Use-Levels for NUTRAVA™ Citrus Fiber in the U.S.

Food Category (21 CFR 170.3)	Proposed Food-Uses[a]	Citrus Fiber Use Level (g/100 g)
Baked Goods and Baking Mixes	Baked Goods and Baking Mixes	3
Beverages, Alcoholic	Cocktail Drinks	1
Beverages and Beverage Bases	Sport or Electrolyte Drinks, Fluid Replacement Drinks	2
	Non-Milk-Based Meal Replacement Beverages and Protein Drinks	1
Breakfast Cereals	Hot Breakfast Cereals (excluding instant)	1
	Ready-to-Eat Breakfast Cereals	3
Cheeses	Cream Cheese and Cheese-Based Spreads	2
	All Other Cheeses	1
Coffee and Tea	Specialty Coffee Drinks (Lattes, Cappuccinos, Mochas) and Ready-to-Drink Tea Beverages	1
Condiments and Relishes	Ketchup	2
	Relish	1
Confections and Frostings	Confections and Frostings (excluding raw sugars)	1
Dairy Product Analogs	Dairy Product Analogs	1
Egg Products	Egg Substitutes	1
Fats and Oils	Fat-Based Sauces; Salad Dressings; Margarine and Margarine-like Spreads; Mayonnaise and Mayonnaise-Type Dressings	2
Fish Products	Fish-Based Entrees	2
Frozen Dairy Desserts	Frozen Dairy Desserts	1
Fruit and Water Ices	Fruit and Water Ices	1
Gelatins, Puddings, and Fillings	Gelatin, Puddings, and Fillings	2
Grain Products and Pastas	Grain Products and Pastas	3
Gravies and Sauces	Gravies and Sauces	2
Jams and Jellies	Jams and Jellies	2
Meat Products	Meat Products	5
Milk Products	Milk-Based Meal Replacements	2
	All Other Milk Products (including cream, yogurt, dairy-based snack dips, and milk drinks, except fermented milks)	1

Table 2 Summary of the Individual Proposed Food-Uses and Use-Levels for NUTRAVA™ Citrus Fiber in the U.S.

Food Category (21 CFR 170.3)	Proposed Food-Uses[a]	Citrus Fiber Use Level (g/100 g)
Nuts and Nut Products	Nuts and Nut Products	1
Plant Protein Products	Plant Protein Products	3
Poultry Products	Poultry Products	5
Processed Fruits and Fruit Juices	Fruit Juices, Drinks, Ades, Nectars, and Fruit Smoothies	1
	Fruit-Based Desserts	2
Processed Vegetables and Vegetable Juices	Processed Vegetables, Entrees, and Vegetable Juices	1
Soft Candy	Chocolate, Nougat, and Toffees	1
Soups and Soup Mixes	Soups and Soup Mixes	2
Sweet Sauces, Toppings, and Syrups	Cocoa Syrups and Chocolate Toppings	1
	Fruit Sauces, Syrups, and Toppings	2

CFR = Code of Federal Regulations; U.S. = United States.
[a] NUTRAVA™ Citrus Fiber is intended for use in unstandardized products where standards of identity, as established under 21 CFR §130 to 169, do not permit its addition in standardized products.

Consumption data and information pertaining to the intended food-uses of NUTRAVA™ Citrus Fiber were used to estimate the *per capita* and consumer-only intakes of this ingredient for specific demographic groups and for the total U.S. population. In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of NUTRAVA™ Citrus Fiber by the total U.S. population from proposed food-uses in the U.S. were estimated to be 11.4 g/person/day (199 mg/kg body weight/day) and 20.0 g/person/day (402 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of NUTRAVA™ Citrus Fiber were determined to be 13.1 g/person/day (203 mg/kg body weight/day) and 24.1 g/person/day (415 mg/kg body weight/day), as identified among male teenagers. These intake estimates are similar to dietary intake values previously estimated for other citrus fiber ingredients that have been concluded to be GRAS (GRN 154/487, 541, and 599 – U.S. FDA, 2004, 2014a, 2015a, 2016a). For example, as reported in GRN 487, GRAS uses of dried citrus pulp were estimated to result in daily intakes of 23.4 g/day (0.4 g/kg body weight/day) and 39.5 g/day (0.8 g/kg body weight/day) at the mean and 90th percentile, respectively for the general population of U.S. consumers >2 years of age. As reported in GRN 541, the estimated per user daily intake of citrus fiber from all proposed food-uses was 20.24 g/day and 34.17 g/day at the mean and 90th percentile, respectively for the general population of U.S. consumers >2 years of age. Since the majority of proposed food-uses of NUTRAVA™ Citrus Fiber will be largely substitutional to existing food-uses of citrus fiber in the diet (including uses in meat and poultry and meat and poultry containing foods), no appreciable change in the dietary intake of citrus fiber is expected from the introduction NUTRAVA™ Citrus Fiber to the U.S. marketplace.

DATA PERTAINING TO SAFETY

History of Use

Citrus fruits and products derived from these fruits have a long history of human consumption. As described in several GRAS notifications of similar citrus fiber products, recipes for oranges in foods can be found in several historical cookbooks, including a recipe for orange marmalade published in *Culinary Chemistry* in 1821 by Fredrick Accum (Kansas State University Library, 2003; GRN 154 – U.S. FDA 2004; GRN 599 – U.S. FDA 2016a; and GRN 719 – U.S. FDA, 2017).

Several citrus fiber ingredients have GRAS status for use in food and beverages as sources of dietary fiber and general-purpose food ingredients (see Table 3). Food-uses of NUTRAVA™ Citrus Fiber will partially substitute for existing uses of citrus fiber in the U.S. marketplace.

Table 3 Citrus Fiber Ingredients with GRAS Status for Specified Food-Uses[a]

Regulatory Status	GRAS Substance	Food-Uses
GRN 154 (U.S. FDA, 2004)	Dried orange pulp	Use in baked goods, pastas, salad dressings, confectionery, processed cheese spreads, non-carbonated beverages and fruit drinks, frozen food entrees, such as frozen doughs, frozen meat products, frozen baked goods, frozen desserts, and frozen dairy products, and meat and poultry products at a maximum use level of 5 percent.
GRN 487 (U.S. FDA, 2014a)	Dried citrus pulp	As a moisture retention agent, a flavor enhancing agent, and a processing aid in foods.
GRN 541 (U.S. FDA, 2015a)	Insoluble fiber from citrus peel	Intended for use as a moisture retention agent, flavor enhancing agent, or processing aid in baked goods, pastas, salad dressings, confectionery, processed cheese spreads, frozen food entrees, and comminuted and whole muscle meat and poultry products at a maximum level of 5%; and as a flavor enhancer in non-carbonated beverages and fruit drinks; as a seasoning in brine and in comminuted and whole muscle meat and poultry products, and as an ingredient in salads, sauces, meats, fillings, dips, baked goods, dairy products, fruit- and vegetable based products, and pizza at a maximum level of 5%.
GRN 599 (U.S. FDA, 2016a)	Citrus fiber	Intended for use as a texturizer and moisture retention agent in yogurt, low fat, mayonnaise, ice cream, ice pops and sorbet (not to exceed 4%), and processed meat and poultry products (not to exceed 5%).

GRAS = Generally Recognized as Safe; GRN = GRAS Notice.
[a] https://www.accessdata.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=fiber

Safety Data

Based on the identity of NUTRAVA™ Citrus Fiber and the applicable long-history of consumption of plant-based fiber in the diet, toxicological evaluations of NUTRAVA™ Citrus Fiber were not considered necessary for the safety evaluation. CPK considers this conclusion to be consistent with international efforts to reduce unnecessary testing of animals.

Dietary Fiber

No tolerable upper intake level has been set for dietary fiber; however, the Institute of Medicine of the National Academies (IOM) suggested that there may be a need for a tolerable upper intake level in the future if supplements or foods with added functional fiber were to become ubiquitous. Fibers such as guar gum, inulin and oligofructose, fructooligosaccharides, polydextrose, resistant starch, and psyllium have

been found to cause gastrointestinal distress, including abdominal cramping, bloating, gas, and diarrhea (IOM, 2005). However, the IOM concluded that no serious chronic adverse effects have been observed for these fibers and excess consumption of these fibers is self-limiting due to their bulky nature (IOM, 2005). The adequate intake level set by the IOM for dietary fiber is 38 g total fiber/day for males and 25 g total fiber/day for females (ages 19 to 50 years) based on the intake level observed to protect against coronary heart disease (IOM, 2005).

Due to the ability of certain dietary fibers to delay absorption of triacylglycerols, it has been proposed that fiber intake may alter absorption of fat-soluble vitamins and minerals by binding or entrapping them in the small intestine. However, the results from studies evaluating the effect of dietary fiber on fat-soluble vitamin absorption are inconsistent, and there is little evidence that individuals consuming nutritionally adequate diets rich in high fiber foods have any problems with vitamin or mineral deficiencies (Williams and Bollella, 1995; FAO/WHO, 1998). Williams and Bollella (1995) reviewed published studies on dietary fiber intake in childhood for safety concerns and concluded that *"it is estimated that even with a doubling of current dietary fiber, there is unlikely to be an adverse effect on serum vitamin and mineral concentrations in healthy US children consuming a balanced diet containing adequate levels of nutrients"*. In addition, the IOM concluded that fiber as part of an overall healthy diet has not been found to adversely affect the calcium, magnesium, iron, or zinc status of healthy people at recommended intake levels (IOM, 2005). Therefore, it seems unlikely that there will be any significant effects on vitamin and mineral absorption from the estimated increase in fiber intakes from foods containing NUTRAVA™ Citrus Fiber.

Citrus Fiber

There are limited studies in the scientific literature on the toxic effects of citrus fiber, particularly due to the long history of safe consumption of citrus fruit. Previous GRAS notifications of similar citrus fiber products to NUTRAVA™ Citrus Fiber have reviewed extensive published information and data on citrus fiber prior to 2015. There are no effects reported in these studies to suggest that citrus fiber or other constituents derived from citrus peels are unsafe for food-use at any dietary level. The GRAS sources of citrus fiber are produced from similar raw materials and are manufactured using comparable methods that principally may involve acid and solvent washes followed by mechanical grinding; resulting in a nutrient composition similar to NUTRAVA™ Citrus Fiber (Table 4). Therefore, the data and information presented in these citrus fiber GRAS notifications (GRN 154/487, 541, and 599 – U.S. FDA, 2004, 2014a, 2015a, 2016a) are considered to support the GRAS status of NUTRAVA™ Citrus Fiber.

Table 4 Nutrient Composition of NUTRAVA™ Citrus Fiber and Comparison to GRAS Sources of Citrus Fiber Produced from Citrus Peel

Specification Parameter	NUTRAVA™ Citrus Fiber	GRN 154/487	GRN 541	GRN 599
Physical and Chemical				
Total dietary fiber	84.9 to 89.8%[a]	73.4%	86.5%	≥50%
Insoluble dietary fiber	39.8 to 42.4%[a]	37.4%	85.0%	48.3 to 54.5%[a]
Soluble dietary fiber	41.9 to 45.5%[a]	36%	1.5%	10.0 to 13.4%[a]
Protein	4.4 to 5.14%[a]	7.47%	2.0%	2.92 to 3.66%[a]
Fat	0.7 to 1.3%[a]	1.04%	0.5%	≤0.5%
Glucose	<0.1%	--	--	--
Sucrose	--	--	--	25 to 40%
Total Carbohydrates	85.7 to 87.8%[a]	82.2%	--	--
Residual Ethanol	323 to 814 mg/kg[a]	--	--	<15 mg/kg[b]
Ash	3.6 to 4.2%[a]	2.44%	2.0%	≤5.0%

Table 4 **Nutrient Composition of NUTRAVA™ Citrus Fiber and Comparison to GRAS Sources of Citrus Fiber Produced from Citrus Peel**

Specification Parameter	NUTRAVA™ Citrus Fiber	GRN 154/487	GRN 541	GRN 599
Moisture	3.3 to 3.7%[a]	6.84%	9.0%	≤10%
pH	3.0 to 5.0	5.5 to 7.5	7.0 to 9.0	≤5.0

[a] Range of 3 nonconsecutive batches.
[b] Based on batch analysis.
[c] Converted % to mg/kg.

A thorough search and review of the published scientific literature from 2015 to present identified 2 toxicology studies conducted using test articles derived from citrus peels (Shikishima *et al.*, 2016; Li *et al.*, 2019). The study by Li *et al.* (2019) evaluated high doses of hesperidin isolated from orange peels, and the study by Shikishima *et al.* (2016) evaluated the toxicity of a citrus fruit extract powder. The test articles used in these studies are not representative of NUTRAVA™ Citrus Fiber and are therefore not relevant to the safety assessment. Nevertheless, there were no notable findings reported in these studies to suggest that use of citrus peel to produce NUTRAVA™ Citrus Fiber would be unsafe.

Allergenicity

In terms of allergenicity, allergic reactions to citrus fruits are both rare and typically mild. Citrus fruits are not listed as one of the major food allergens under the U.S. Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). A letter from Steve L. Taylor, Ph.D., Professor and Co-Director at University of Nebraska Lincoln, is provided in GRN 487, stating that in his expert opinion, "*citrus fiber products present essentially no allergenic risk to consumers*" (GRN 487 – U.S. FDA, 2014a). GRN 541 and GRN 599 review literature on orange allergy and report that the allergy is rare and scarcely investigated, but has potential cross reactivity with pollen, and may be caused by the orange seeds or preservatives present in some juices (GRN 541 – U.S. FDA 2015a; and GRN 599 – U.S. FDA, 2016a). Therefore, it seems unlikely that there will be any significant allergic reactions associated with intakes of foods containing NUTRAVA™ Citrus Fiber.

CONCLUSION

We, the undersigned independent qualified members of the Generally Recognized as Safe (GRAS) Panel, have independently and collectively, critically evaluated the data and information summarized above that is pertinent to the safety of the proposed use of NUTRAVA™ Citrus Fiber. We unanimously conclude that NUTRAVA™ Citrus Fiber, produced in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food grade specifications, is GRAS based on scientific procedures under the conditions of intended use in foods specified herein.

It is our professional opinion that other qualified experts would also concur with this conclusion.

[redacted]

15 November 2019

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Professor Emeritus
Virginia Commonwealth University School of Medicine

Date

[redacted]

11/14/19

Dr. George C. Fahey, Jr, Ph.D.
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Date

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13 November 2019

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Date