11 April 2023

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 Protein-Sucrose in Sugar

In accordance with Title 21 of the Code of Federal Regulations (CFR) §170 Subpart E consisting of §170.203 through 170.285, Incredo Ltd. (formerly DouxMatok Ltd.), as the notifier, is submitting one hard copy and one electronic copy (on CD), of all data and information supporting the company's conclusion that protein-sucrose, as manufactured by Incredo, to be used in sugar, is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act based on Incredo's view that these notified uses of protein sucrose are Generally Recognized as Safe (GRAS). Information setting forth the basis for Incredo's GRAS conclusion, as well as a consensus opinion of an independent panel of experts, also are enclosed for review by the agency.

I certify that the enclosed electronic files were scanned for viruses prior to submission and are thus certified as being virus-free using Symantec Endpoint Protection 12.1.5.

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,

Anna Kiliman
Regulatory & QA Manager
Incredo Ltd.

Email: Anna.Kiliman@douxmatok.com
Tel: +972 50-4501655

GRAS NOTICE FOR PROTEIN-SUCROSE

04.12/2023

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:
Incredo Ltd. (formerly DouxMatok Ltd.)
9 Shimshon Street
Petach-Tikva 49517
Israel

DATE:
11 April 2023

GRAS Notice for Protein-Sucrose

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GRAS Notice for Protein-Sucrose

PART 1. §170.225 SIGNED STATEMENTS AND CERTIFICATION

In accordance with Title 21 of the *Code of Federal Regulations* (CFR) §170 Subpart E consisting of §170.203 through 170.285, Incredo Ltd. ("Incredo") hereby informs the United States (U.S.) Food and Drug Administration (FDA) that the intended uses of protein-sucrose, as manufactured by Incredo, in sugar, as described in Section 1.3 below, are not subject to the premarket approval requirements of the *Federal Food, Drug, and Cosmetic Act* based on Incredo's view that these notified uses of protein-sucrose are Generally Recognized as Safe (GRAS). In addition, as a responsible official of Incredo, the undersigned hereby certifies that all data and information presented in this GRAS Notice represent a complete and balanced submission that is representative of the generally available literature. Incredo considered all unfavorable as well as favorable information that is publicly available and/or known to Incredo and that is pertinent to the evaluation of the safety and GRAS status of protein-sucrose as a food ingredient for addition to sugar, as described herein.

Signed,

04/12/2023

Anna Kiliman
Regulatory & QA Manager
Incredo Ltd.
Anna.Kiliman@douxmatok.com

Date

1.1 Name and Address of Notifier

Incredo Ltd. (formerly DouxMatok Ltd.)
9 Shimson Street
Petach-Tikva 49517
Israel

1.2 Common Name of Notified Substance

The subject of this GRAS Notice is protein-sucrose, a substance composed of sucrose and 1 of 4 food-grade proteins (casein, calcium caseinate, pea protein, or rice protein). The trade name for protein-sucrose is "Incredo Sugar®."

1.3 Conditions of Use

Incredo intends to market protein-sucrose in sugar (sucrose). This is based on proprietary technology that has been developed by Incredo for flavor delivery, resulting in an increased perception of sweetness when consumed. The protein-sucrose produced using Incredo's proprietary technology is referred to as "Incredo Sugar®" throughout this GRAS Notice. The proposed use levels of protein-sucrose in sucrose are provided in Table 1.3-1 below. The food category is organized according to 21 CFR §170.3. Incredo notes that the ingredient is not intended for use in infant formula or infant food products, and the proposed food categories do not include food uses that are subject to the oversight by the United States Department of Agriculture and its Food Safety Inspection Service.

Table 1.3-1 Individual Proposed Food Use and Use Level for Protein-Sucrose in the United States

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Proposed Food Uses	Protein-Sucrose Use Levels (g/100 g)
Sugar, white, granulated	White sugar	0.01 to 0.8

CFR = *Code of Federal Regulations*.

1.4 Basis for GRAS

Pursuant to 21 CFR §170.30(a)(b) of the CFR (U.S. FDA, 2018b), Incredo has concluded that the intended uses of protein-sucrose 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 Notice 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:

Incredo Ltd.
9 Shimson Street
Petach-Tikva 49517
Israel

Should the U.S. FDA have any questions or additional information requests regarding this GRAS Notice, Incredo will supply these data and information upon request.

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

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

PART 2. §170.230 IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

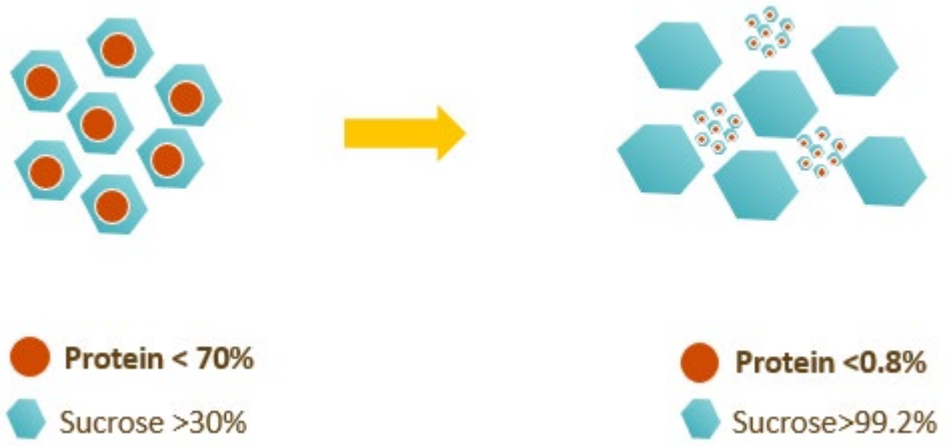
2.1 Identity

The purpose of embedding protein ingredients in sugar is to improve the delivery of sucrose and increase its rate of dissolution. The process is based on a proprietary technology that has been developed by Incredo for flavor delivery by coating/loading food-grade proteins with various nutritive and non-nutritive sweeteners to form a sweetener/protein composition through non-covalent interactions (hydrogen and van der Waals). This results in an increased perception of sweetness when consumed, thereby significantly reducing the amount of sugar needed to produce a desired level of sweetness in a food (Shimada *et al.*, 1990).

Protein-sucrose produced using Incredo's technology is referred to as "Incredo Sugar®" and contains not more than 70% protein content, while the protein content when Incredo Sugar® is added to sugar is 0.01 to 0.8% in the total dry sugar crystals. Figure 2.1-1 provides a pictorial representation of Incredo Sugar® (left) and its final inclusion in sugar (right).

The protein portion of protein-sucrose disrupts the normal crystal structure of the sucrose it binds but does not chemically alter it. The protein-sucrose (Incredo Sugar®) can then be added to sugar, or simply used in a recipe which contains sugar.

Figure 2.1-1 Schematic of Protein-Sucrose and its Incorporation in Sugar



Identity of Protein Ingredients

Incredo intends to use the following proteins in the manufacture of Incredo Sugar®: casein, calcium caseinate, pea protein, and rice protein. The amino acid profile for each protein is shown in Table 2.1-1. These data demonstrate that the amino acid composition of casein and calcium caseinate are very similar. Given caseinates are derived from casein, it can be presumed that the protein structure of both casein and caseinate is very similar. As such, for the purposes of this GRAS evaluation, they are treated the same. While the amino acid compositions of pea and rice proteins are similar, due to differences in structure they are evaluated separately.

Table 2.1-1 Amino Acid Profile of Casein, Calcium Caseinate, Pea Protein, and Rice Protein (g/100 g)

Amino Acid	Casein*	Calcium Caseinate*	Pea Protein (GRN 851 – U.S. FDA, 2020)	Rice Protein (GRN 609 – U.S. FDA, 2016a)
Aspartic acid	NM	NM	9.0	9.33
Glutamic acid	13.9	16.0	13.6	17.22
Alanine	2.0	2.6	3.3	5.67
Arginine	2.1	2.9	6.7	7.64
Cysteine	0.1	0.1	0.8	2.09
Glycine	1.2	1.5	3.2	4.33
Histidine	1.7	2.2	1.9	2.09
Isoleucine	2.3	3.0	3.6	4.40
Leucine	5.8	7.8	6.4	8.21
Lysine	4.6	5.9	5.8	3.21
Methionine	1.6	2.2	0.8	2.66
Phenylalanine	3.1	4.2	4.2	5.38
Proline	6.5	8.7	3.3	4.49
Serine	3.4	4.2	4.2	4.75
Threonine	2.6	3.5	3.0	3.57
Tyrosine	3.4	4.4	3.1	4.84
Valine	3.0	3.8	3.8	6.18
Tryptophan	NM	NM	0.7	1.12

GRN = Generally Recognized as Safe (GRAS) Notice; NM = not measured.
* As reported by Gorissen *et al.* (2018).

2.1.1 Casein and Calcium Caseinate

Casein is the principal protein present in bovine milk. Casein exists in milk as a stable suspension of calcium caseinate micelles, and its structure and properties have been studied extensively (Swaisgood, 1993). Isolated casein and caseinates are widely used as texturizing and stabilizing ingredients in the food industry (Fox and Mulvihill, 1982).

Incredo uses food-grade casein in the production of Incredo Sugar®; the casein adheres to *Food Chemicals Codex* (FCC) monograph standards. Casein is manufactured from fresh, pasteurized skim milk using a low-heat membrane filtration process to ensure protein is undenatured. Casein undergoes additional microfiltration to slightly increase the casein-to-whey ratio from that which naturally occurs in milk. After membrane separation, casein is immediately spray dried and packaged in multi-wall paper bags with a polyethylene liner (net content of 20 kg), palletized, and wrapped to units of 800 kg. It is also available in polyethylene-lined totes (net unit weight of 500 kg).

Calcium caseinate is another milk protein used by Incredo in the production of Incredo Sugar®. Calcium caseinate is manufactured *via* acid preparation of casein from fresh skimmed milk. The casein is converted into its calcium salt by the addition of calcium hydroxide, and the resulting product is milled and dried.

FCC monograph standards for casein and calcium caseinate are presented in Table 2.1.1-1.

Table 2.1.1-1 *Food Chemicals Codex* Monograph Standards for Casein and Caseinate Salts

Specification Parameter	Unit	Specification
Protein	%	Acid casein: NLT 90.0 Rennet casein: NLT 86.0 Caseinate salts: NLT 84.0
Lead	mg/kg	NMT 1.0
Fat	%	NMT 2.25
Free acid (casein only)	mL of 0.1 N sodium hydroxide	NMT 2.7
Lactose	%	NMT 2.0
Loss on drying	%	NMT 12.0

NLT = not less than; NMT = not more than.

2.1.2 Pea Protein

Pea protein is purified from the dry common yellow pea *Pisum sativum.* Pea protein is a pure, free-flowing, beige powder that functions as a protein source in foods, and as a binder and extender in meat and poultry products.

Pea protein is used in the production of Incredo Sugar®. Manufacture of pea protein occurs *via* the processes described below, as reported in GRAS Notice (GRN) 851.

Peas are physically cleaned and ground to remove hulls to produce a pea flour, which is a mixture of protein, starch, fiber, sugar, and fat. Water is added to the pea flour, and the pea starch and fiber are then removed. The protein goes through separation flocculation steps to adjust the pea protein at the isoelectric point, which is where the proteins have the minimum solubility levels and are able to separate (isoelectric precipitation). The soluble pea protein (albumin) is then removed from the pea protein isolate. The pea protein is then coagulated, purified, and re-buffered to neutral pH. Following the extraction process, a heat treatment is used to effectuate microbial reduction and reduce moisture. Food-grade enzymes[1] are then used to enhance the pea protein isolate functionalities, such as by decreasing viscosity.

[1] The enzymes are described in GRN 851. The first enzyme is a concentrated food-grade enzyme preparation from the exopeptidase family with a lower activity endopeptidase. The second enzyme is a powdered food-grade enzyme from the exopeptidase family (aminopeptidase) and is derived from a highly concentrated fungal proteolytic food-grade enzyme, with low *alpha*-amylase activity and significant amino peptidase activity for debittering. The highly concentrated fungal proteolytic food grade enzyme is GRAS per

These added enzymes are destroyed with a thermal heat treatment before spray drying. The function of the enzymes is to split pea proteins *via* hydrolysis. This releases lower molecular weight peptides of shorter chain length, as well as amino acids. The final processing step includes drying the pea protein product in a spray dryer before it is packaged and stored.

The food-grade specifications for pea protein are presented in Table 2.1.2-1. Pea proteins purchased for production of Incredo Sugar® are GRAS as nutritional protein ingredients and adhere to food-grade specifications provided by the supplier. The pea protein purchased from Roquette for production of Incredo Sugar® adheres to its specifications as described in GRN 851.

Table 2.1.2-1 Specifications for Pea Protein per GRN 851

Specification Parameter	Unit	Specification
Appearance	Visual	Beige powder
Loss on drying	%	≤10
Ash content (on DS)	%	≤10
pH at 10% (w/w)	-	7.5
Bulk density	g/L	500
Aqueous solubility	%	50
Laser particle size	% >295 microns	≤5
Crude fiber (on DS)	%	≤10
Protein content	%	85
Contaminants		
Arsenic	mg/kg	≤0.2
Lead	mg/kg	≤0.2
Mercury	mg/kg	≤0.03
Cadmium	mg/kg	≤0.2
Ochratoxin A	µg/kg	≤20
Microbiological Data		
Total aerobic microbial count	CFU/g	≤5,000
Enterobacteriaceae	CFU/g	≤10
Total yeasts count	CFU/g	≤50
Total Molds Count	CFU/g	≤50
Escherichia coli	-	Not detected in 1 g
Salmonella	-	Not detected in 25 g
Staphylococcus aureus	-	Not detected in 1 g
Bacillus cereus	CFU/g	≤100

CFU = colony-forming units; DS = dry solids; GRN = Generally Recognized as Safe (GRAS) Notice.

GRN 90. Both enzymes are prepared from enzymes that have GRAS status and both enzymes are manufactured consistent with the FCC, Joint FAO/WHO Expert Committee on Food Additives, and World Health Organization/Food and Agriculture Organization of the United Nations recommendations for enzymes used in food processing.

2.1.3 Rice Protein

Rice protein is derived from non–genetically modified *Oryza sativa* whole-grain brown rice. Rice protein concentrate can be used as a substitute for, and/or in conjunction with, soy protein and whey protein in conventional food products.

Rice protein is used in the production of Incredo Sugar®. Rice protein is derived from the bran, germ, and endosperm extracted from whole-grain brown rice through a low-heat process. Briefly, whole-grain brown rice is received, tested, and approved for further processing. A hydrolysis process is performed to obtain whole brown rice protein (40 to 60% concentration). The enzyme amylase is used to separate protein from syrup solids, and only the whole brown rice protein concentrate is kept. A separation process is conducted to obtain whole brown rice protein concentrate. The concentrate is then washed, milled into the appropriate mesh, dried, and sterilized. It is packaged in 25-kg bags with inner polyethylene liners.

The food-grade specifications for rice protein are presented in Table 2.1.3-1. Rice proteins purchased for production of Incredo Sugar® are GRAS as nutritional protein ingredients and adhere to food-grade specifications provided by the supplier. Rice protein purchased from Axiom Foods for production of Incredo Sugar® adheres to its specifications as described in GRN 609.

Table 2.1.3-1 Specifications for Rice Protein per GRN 609

Specification Parameter	Unit	Specification
Appearance	Visual	Light-brown to beige powder
Protein (DMB)	%	≥90
Protein (as-is)	%	86.6
Moisture		≤5
Microbiological Data		
Total plate count	CFU/g	≤15,000
Coliform	CFU/g	≤30
Salmonella	CFU/375 g	Negative
Staphylococcus aureus	CFU/25 g	Negative
Escherichia coli	CFU/25 g	Negative
Yeast and Mold	CFU/g	≤100
Contaminants		
Arsenic	mg/kg	≤0.2
Cadmium	mg/kg	≤0.5
Lead	mg/kg	≤0.3
Mercury	mg/kg	≤0.045
Gluten	mg/kg	≤20

CFU = colony-forming units; DMB = dry matter basis; GRN = Generally Recognized as Safe (GRAS) Notice.

2.2 Manufacturing

Broadly, for the production of Incredo Sugar®, food-grade protein ingredients are mixed mechanically with sucrose in solution and then dried. Incredo notes that all ingredients and processing aids used in the manufacturing process are used in accordance with applicable U.S. regulations, have been concluded to be GRAS for their respective uses, or are the subject of effective food contact notifications. Additionally, Incredo Sugar® is manufactured in a facility under strict adherence to good manufacturing practices, and the process is monitored for potential microbial contamination.

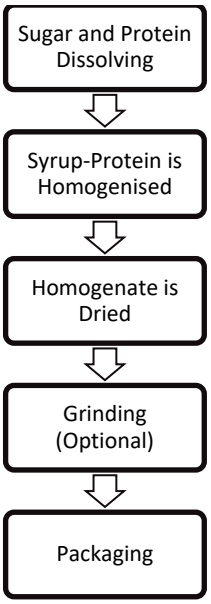
A sucrose syrup is prepared prior to the addition of the protein. The concentration of sucrose, with respect to water, is typically 20% w/w (may depend on the ratio between the protein and the sweetener). The protein is then added incrementally under constant mixing. Once the protein addition has been added, the mixing vessel continues to be stirred using a high-shear mixer until the protein is fully dispersed within the sweetener syrup. For proteins that are more difficult to disperse, the water fraction can optionally be pre-heated.

The protein-sweetener concentrate syrup is then dried. The concentrate is dried *via* vacuum drying (impeller mixed vacuum vessel or double-drum vacuum dryer) or spray drying. Typically, the concentrate is transferred to the heated double-jacketed vessel of the vacuum dryer. The vessel is heated (typically to 60 to 70°C), maintained under vacuum, and mixed constantly to evaporate the water, eventually producing a protein-sweetener concentrate powder that is typically fine and dry. The concentrate will have a ratio of up to 70:30 protein to sucrose.

Optionally, the powder can be transferred to an oven (typically operating at 65°C) for further drying or can be milled, for example using a classifying mill, pin mill, or hammer mill, to a specific particle size distribution.

No chemical bonds are formed between sugar and proteins; instead, sugar and protein molecules are held together *via* hydrogen and van der Waals interactions. A schematic of the manufacturing process is provided in Figure 2.2-1 below.

Figure 2.2-1 Schematic Overview of the Manufacturing Process for Incredo Sugar®



2.3 Product Specifications and Batch Analyses

The specifications and methods of analysis for Incredo Sugar® are presented in Table 2.3-1.

Table 2.3-1 Specifications and Methods of Analysis for Incredo Sugar®

Parameter	Specification	Method*
Appearance	Characteristic	Appearance
Sucrose (%)	>30	AM/C/1014 by ion exchange chromatography
Protein (%)	<70	AM/C/224 by Dumas method
Ash	<2.5	AM/C/803 based on BS 4401: Part 1:1998
Loss on drying (%)	≤10	AM/C/801 based on Feeding Stuff Regulations 2000
Total fat	<1.5	AM/C/1015 by oven drying and pulsed NMR
Microbial Contaminants (SI 885/3)		
Total count (CFU/g)	<10,000	ESGMM300 using PCA pour plate technique
Yeast count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Mold count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Escherichia coli (CFU/g)	<10	ESGMM561 based on ISO 16649-3:2015
Salmonella (negative in 25 g)	Negative	ESGMM515 Solus ELISA Kit method and DYNEX equipment

BS = British Standard; CFU = colony-forming units; ELISA = enzyme-linked immunosorbent assay; ISO = International Organization for Standardization; NMR = nuclear magnetic resonance; PCA = plate count agar; SI = Israeli Standard.
* Testing conducted at an ISO 17025:2005 accredited laboratory.

A summary of chemical analysis for 7 lots of Incredo Sugar® is provided in Table 2.3-2. Results of these analyses indicate that Incredo Sugar® meets specifications of less than 70% protein and meets the specifications of microorganisms.

Table 2.3-2 Summary of the Chemical Product Analysis for 7 Lots of Incredo Sugar®

Parameter	Specification	Manufacturing Lot						
		S6SU122	S6SU126	S6SU180	S6SU183	S6SU200	S6SU201	S6SU202
Appearance	Characteristic	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms	Conforms
Sucrose (%)	>30	51.3	49.7	73.6	71.6	58.9	59.1	59.2
Protein (%)	<70	40.9	45.3	24.6	24.8	35.1	36.4	35.4
Ash (%)	<2.5	1.8	1.9	1.9	1	0.4	0.6	0.5
Loss on drying (%)	≤10	5.1	6.2	2.6	5.2	4.7	8.3	4.4
Total fat (%)	<1.5	0.4	0.5	0.2	0.3	0.8	0.7	0.8
Microbial Contaminants								
Total count (CFU/g)	<10,000	260	210	20	370	60	50	70
Yeast count (CFU/g)	<100	<20	<20	<20	<20	<20	<20	<20
Mold count (CFU/g)	<100	<20	<20	<20	<20	<20	<20	<20
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10	<10	<10	<10
Salmonella (negative in 25 g)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

CFU = colony-forming units.

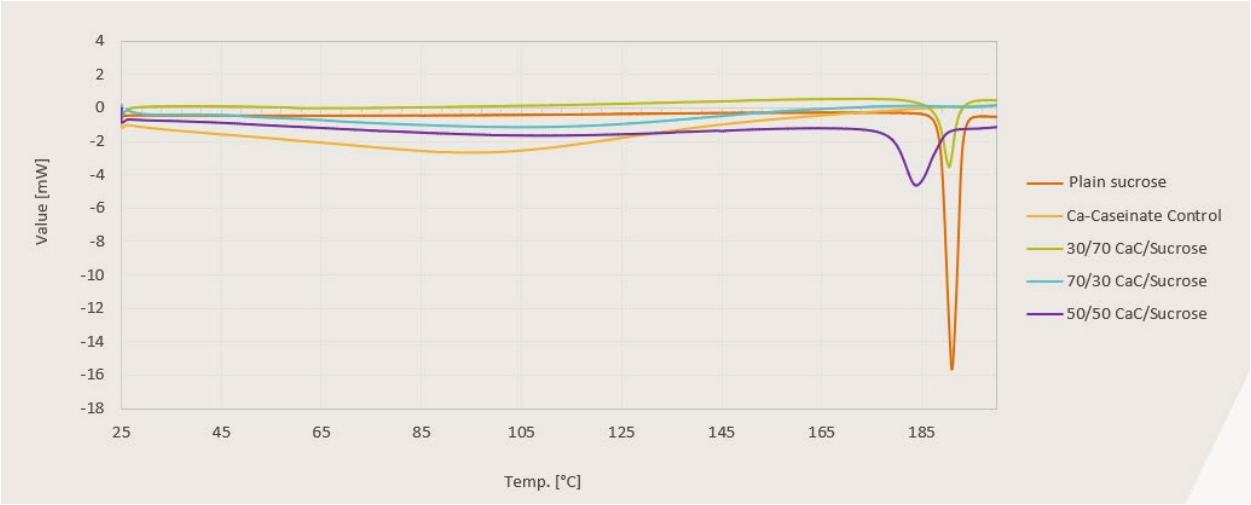
2.4 Additional Characterization

2.4.1 Alteration of Sucrose Crystal Structure

Incredo has conducted differential scanning calorimetry (DSC)[2] on Incredo Sugar® to demonstrate the difference in crystal structure of sucrose. DSC can be used to detect phase transitions, which are dependent on the substance's heat capacity. Generally, well-organized crystal structures require higher temperatures to undergo a phase transition from solid to liquid. Therefore, DSC can compare the phase transitions of sugar and protein to that of Incredo Sugar®. Figure 2.4.1-1 demonstrates this principle, as the peak for the melting point of sugar (186°C) is shown to diminish with increased incorporation of calcium caseinate. Therefore, more heat flow (mW)[3] is needed to melt sugar than Incredo Sugar®, demonstrating the disruption of sucrose's crystal structure.

The resultant disruption in crystal structure is expected to elicit an increased perception of sweetness when consumed, thereby significantly reducing the amount of sugar needed to produce a desired level of sweetness in a food.

Figure 2.4.1-1 Differential Scanning Calorimetry of Sugar and Calcium Caseinate Controls to Different Ratios of Protein in Incredo Sugar®



Ca = calcium; CaC = calcium caseinate; mW = milliwatts.

[2] DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature.

[3] Heat flow values are negative, as melting is an endothermic process.

Incredo also conducted analysis of the sugar present in Incredo Sugar® *via* ion chromatography with electrochemical detection (CSN EN 12630). Twelve batches of Incredo Sugar® from various proteins, composed of 43.2 to 81.4% (w/w) sugars, were measured for their sugars profile (see Table 2.4.1-1). The results of this assay indicate that, within the limit of reporting (0.05%), sucrose is the sole sugar present in Incredo Sugar® manufactured using calcium caseinate, rice protein, and pea protein. For batches manufactured from micellar casein, sugars other than sucrose (*i.e.*, lactose and glucose) could be present. Glucose was determined to be present at values ranging from 0.137 to 0.151%. Lactose was determined to be present at levels ranging from 0.352 to 0.368%. These sugars may be endogenous to the raw material (sucrose) or produced from hydrolysis. In either event, the presence of these sugars does not pose a safety concern.

Table 2.4.1-1 Detection of Sugars *via* Ion Chromatography with Electrochemical Detection

Batch	Protein Type	Sucrose (%)	Lactose (%)	Glucose (%)	Limit of Reporting
S6SU122	Calcium caseinate	62.9	-	-	0.05
S6SU126		43.2	-	-	
S6SU114		55.5	-	-	
S6-SU-170	Rice	64.1	-	-	
S6-SU-171		65.1	-	-	
S6-SU-172		67.3	-	-	
S6-195	Pea	70.3	-	-	
S6-255		65.7	-	-	
S6-256		71.8	-	-	
S6-263	Micellar casein	81.4	0.357	0.142	
S6-269		79	0.352	0.151	
S6-270		78.8	0.368	0.137	

Nuclear magnetic resonance (NMR)[4] was also conducted and demonstrated that the sample only contains sucrose (see Appendix A). However, it is possible that some samples will contain minor inversion products (hydrolysis of sucrose to fructose and glucose). This minor inversion is within the limit of normal occurrence in commercial sucrose and typically under the limit of reporting based on the sugar analysis by ion chromatography.

2.4.2 Absence of Chemical Bonds Between Protein Ingredients and Sugar in Incredo Sugar®

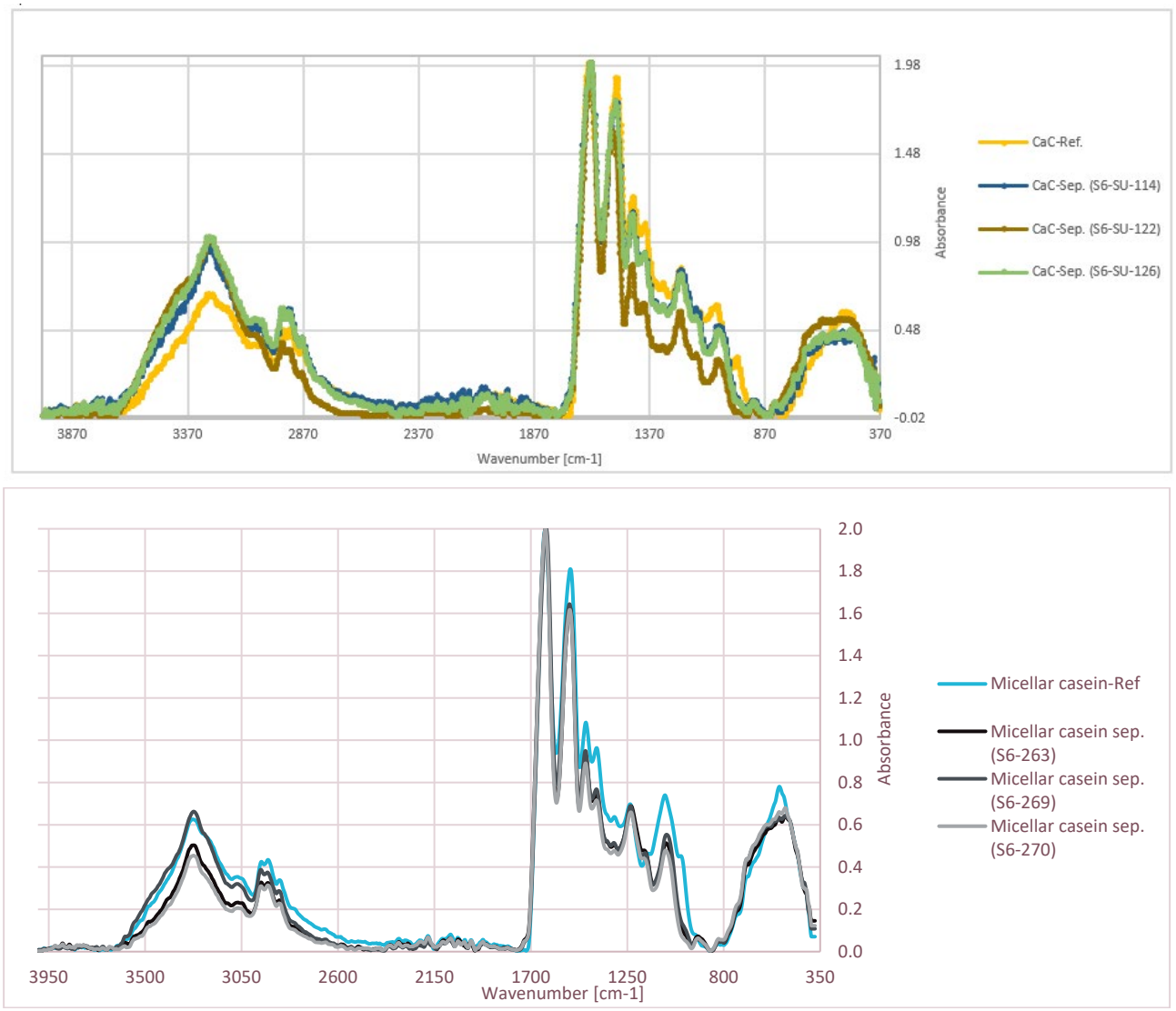
2.4.2.1 Attenuated Total Reflection with Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

In Incredo Sugar®, no chemical bonds are formed between the sugar and protein ingredients. Instead, sugar and proteins are held together *via* hydrogen bonding and van der Waals interactions. Using attenuated total reflection with Fourier-transform infrared spectroscopy (ATR-FTIR), Incredo demonstrated that once in water, sugar and proteins are completely dissociated. Briefly, Incredo Sugar® was dissolved in water and separated using sedimentation, dialysis, and filtration. After separation, the 2 materials were dried and analyzed. The sample was analyzed using ATR-FTIR and compared to protein, sucrose, and protein sample separated from Incredo Sugar® through centrifugation. The ATR-FTIR used a diamond ATR crystal as the internal reflection element, and the incident angle was set at 45°. The powders were scanned 24 times at a 4 cm^{-1} resolution. This comparison was conducted for each protein ingredient used for production of

[4] NMR allows the molecular structure of a material to be analyzed by observing and measuring the interaction of nuclear spins when placed in a powerful magnetic field.

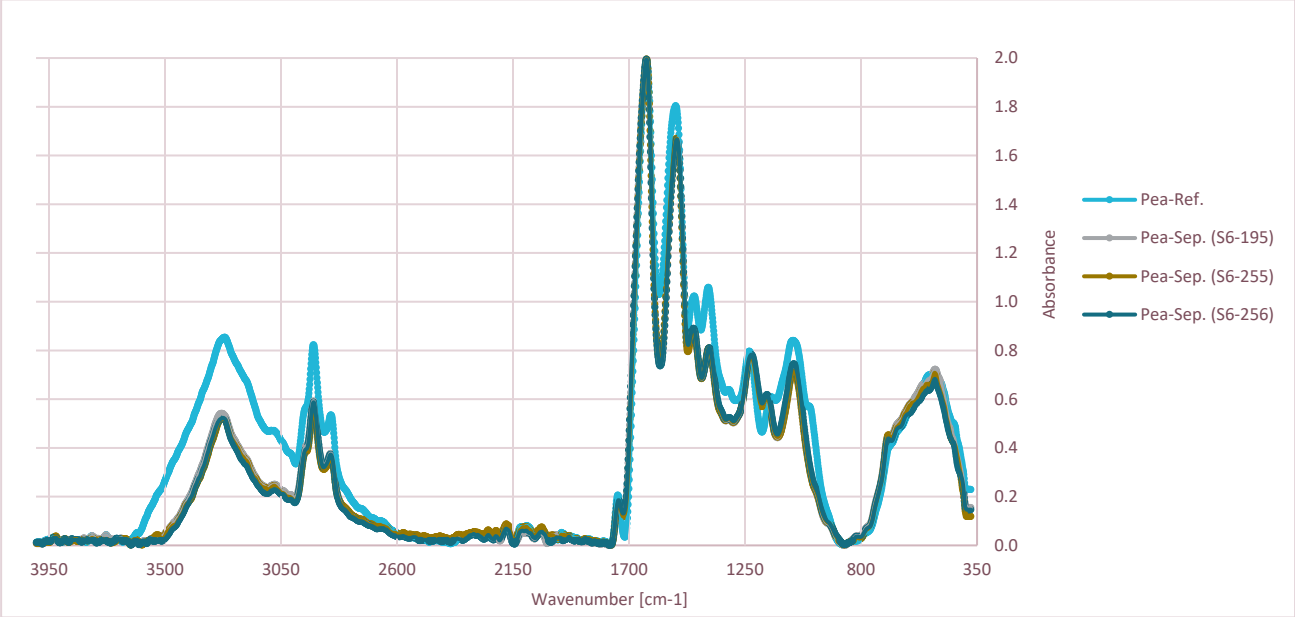
Incredo Sugar® (see Figures 2.4.2.1-1 to 2.4.2.1-3). Incredo also analyzed the sucrose component of each type of Incredo Sugar®. Data from these analyses indicate that the ATR-FTIR spectrum of reference sucrose and sucrose isolated from Incredo Sugar® are identical, such that no new substances are formed during the manufacture of Incredo Sugar® (see Appendix A).

Figure 2.4.2.1-1 Attenuated Total Reflection with Fourier-Transform Infrared Spectroscopy Spectrum of Pure Calcium Caseinate and Separated Calcium Caseinate from Incredo Sugar® Concentrate (top) and Pure Micellar Casein and Separated Micellar Casein from Incredo Sugar® Concentrate (bottom)



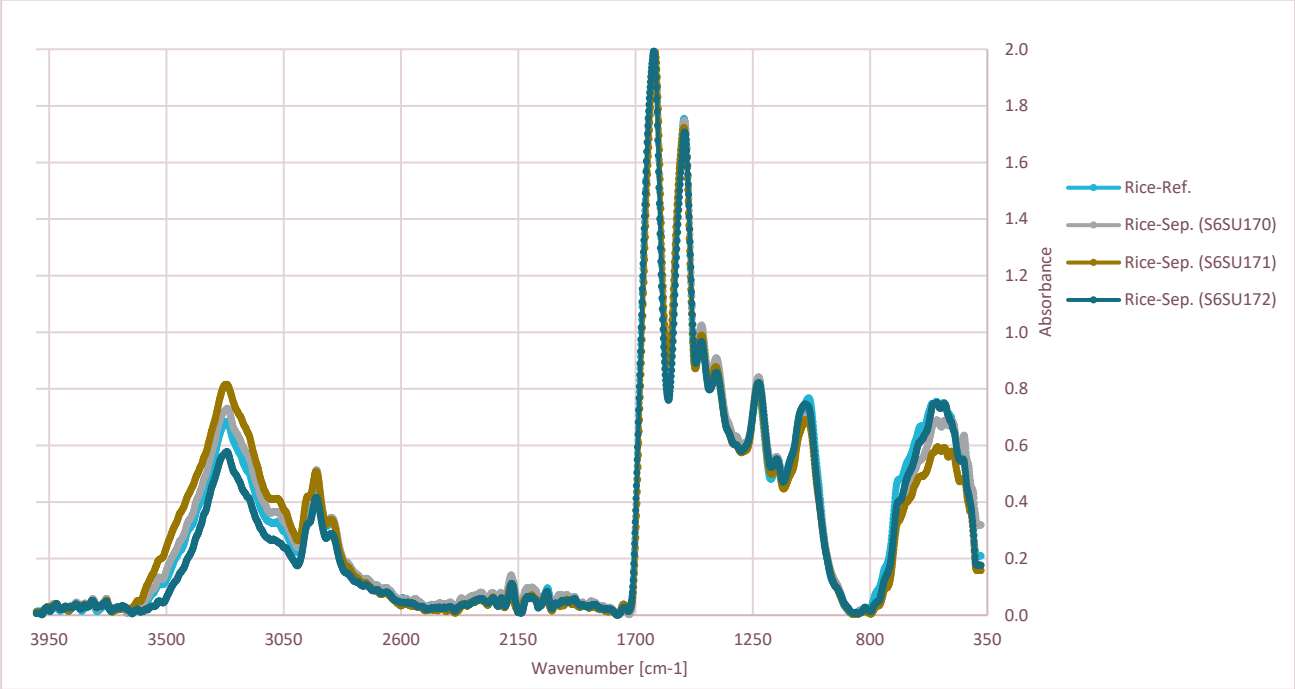
Ca = calcium; CaC = calcium caseinate; sep. = separated.

Figure 2.4.2.1-2 Attenuated Total Reflection with Fourier-Transform Infrared Spectroscopy Spectrum of Pure Pea Protein and Separated Pea Protein from Incredo Sugar® Concentrate Second Generation



Sep. = separated.

Figure 2.4.2.1-3 Attenuated Total Reflection with Fourier-Transform Infrared Spectroscopy Spectrum of Pure Rice Protein and Separated Rice Protein from Incredo Sugar® Concentrate Second Generation

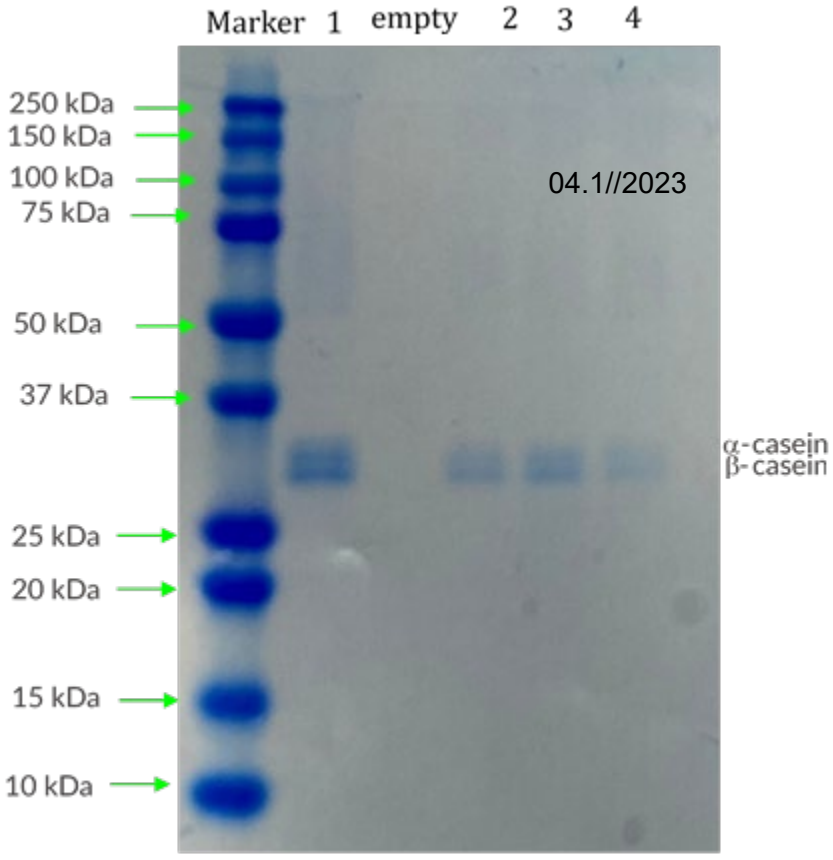


Sep. = separated.

2.4.2.2 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

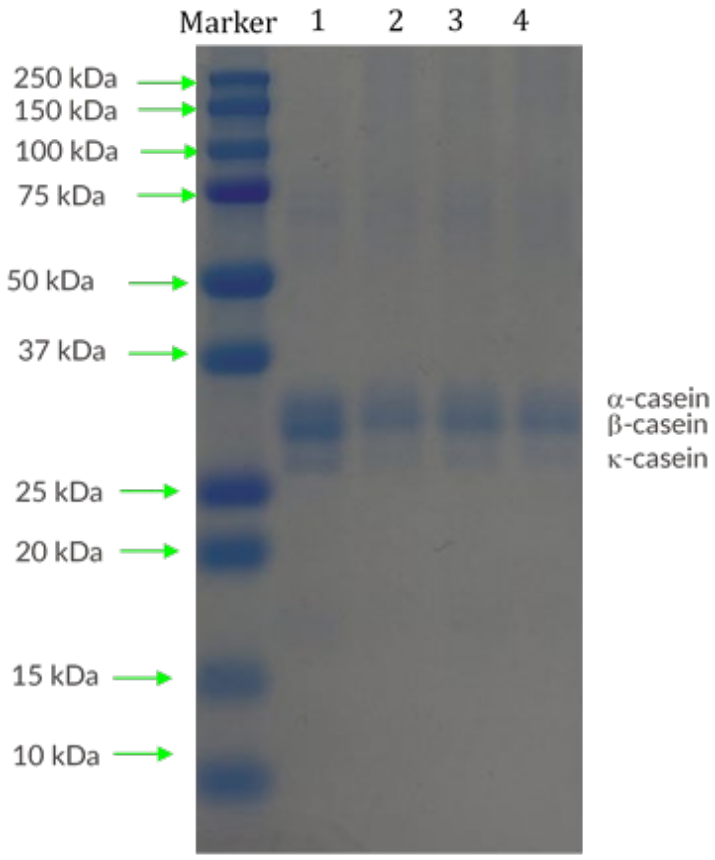
Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was also conducted on Incredo Sugar® and its respective protein ingredients to demonstrate that there are identical protein fractions before and after separation (see Figures 2.4.2.2-1 to 2.4.2.2-4). Protein solutions of 0.1 to 0.3 mg/mL were prepared in 0.0625 M Tris-HCl [(hydroxymethyl)aminomethane hydrochloride] buffer (pH 6.8), Lamelli sample buffer, and 0.04 M DTT (DL-1,4-dithiothreitol). For rice and pea protein, the solution was heated to 37°C before the addition of Lamelli and DTT. The samples were heated at 95°C for 5 minutes prior to loading on the gel (8 to 16% Mini-PROTEAN® TGX™ Gel). Electrophoresis was carried out at a constant voltage (160 V) for 1 hour using a tris-glycine buffer (pH 8.3) containing 0.1%, w/w sodium dodecyl sulfate. The gel was removed from the electrophoresis unit and stained with Bio-Safe™ Coomassie Premixed Staining Solution for 60 to 120 minutes, then washed with water.

Figure 2.4.2.2-1 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Pure Calcium Caseinate Compared to the Separated Calcium Caseinate from 3 Batches of Incredo Sugar® Concentrate Second Generation (50% calcium caseinate/50% sucrose)



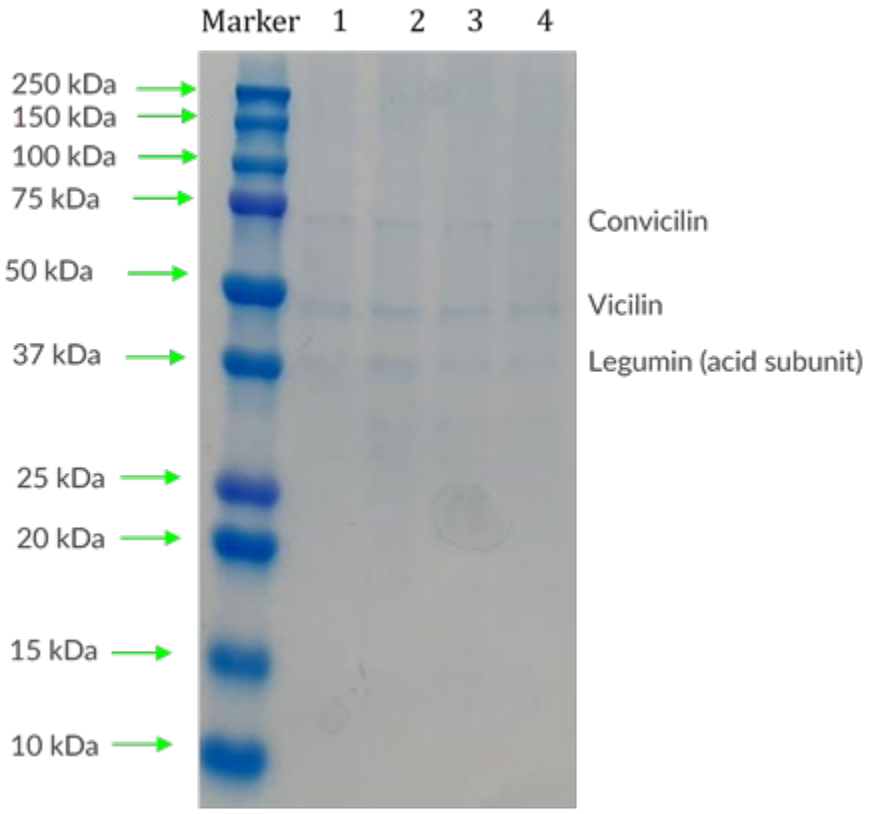
Lane 1 = pure Ca-caseinate; Lane 2 = SSU122; Lane 3 = SSU114; Lane 4 = S6SU126.

Figure 2.4.2.2-2 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Pure Micellar Casein Compared to the Separated Micellar Casein from 3 Batches of Incredo Sugar® Concentrate Second Generation (30% micellar casein/70% sucrose)



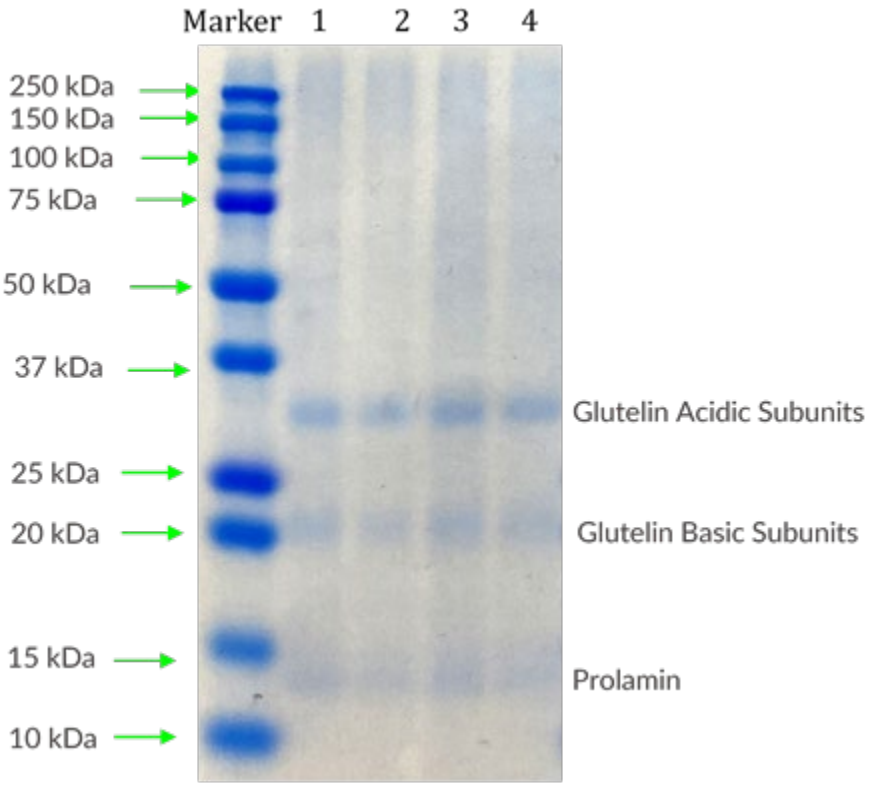
Lane 1 = pure micellar casein; Lane 2 = S6-269; Lane 3 = S6-270; Lane 4 = S6-263.

Figure 2.4.2.2-3 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Pure Pea Protein Compared to the Separated Pea Proteins from 3 Batches of Incredo Sugar® Concentrate Second Generation (30% pea protein/70% sucrose)



Lane 1 = pure pea protein; Lane 2 = S6-195; Lane 3 = S6-255; Lane 4 = S6-256.

Figure 2.4.2.2-4 Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis of Pure Rice Protein Compared to the Separated Rice Proteins from 3 Batches of Incredo Sugar® Concentrate Second Generation (30% rice protein/70% sucrose)



Lane 1 = pure rice protein; Lane 2 = S6SU170; Lane 3 = S6SU171; Lane 4 = S6SU172.

As shown in these figures, there is no evidence for sugar-protein interactions following Incredo Sugar®'s dissolution in water, indicating that, once dissolved, Incredo Sugar® is completely dissociated into its components: protein and sugar.

2.5 Stability

Incredo conducted stability studies according to Israeli Standard (SI) 885/3, 855/8, 885/12, and 885/7, and International Organization for Standardization (ISO) 6579. Samples were tested in accelerated microbiology (*i.e.*, 40° ± 2 C and 75% ± 5% relative humidity) in 4-time intervals to be equivalent to 24 months (*e.g.*, T1 = 8 months, T2 = 16 months). Data from these studies demonstrate that the shelf-life of Incredo Sugar® is at least 24 months (see Table 2.5-1).

Table 2.5-1 Stability of 5 Lots of Incredo Sugar® Manufactured with 50% Calcium Caseinate

Specification Parameter	Specification	Manufacturing Lot				
		S6-SU-163	S6-SU-164	S6-SU-165	S6-SU-166	S6-SU-167
Time 0						
Total count (CFU/g)	<10,000	505	110	220	200	380
Yeast count (CFU/g)	<100	<10	<10	<10	<10	<10
Mold count (CFU/g)	<100	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10	<10
Time 8 Months						
Total count (CFU/g)	<10,000	95	120	130	95	245
Yeast count (CFU/g)	<100	<10	<10	<10	<10	<10
Mold count (CFU/g)	<100	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10	<10
Time 16 Months						
Total count (CFU/g)	<10,000	95	80	115	85	120
Yeast count (CFU/g)	<100	<10	<10	<10	<10	<10
Mold count (CFU/g)	<100	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10	<10
Time 24 Months						
Total count (CFU/g)	<10,000	95	80	115	150	105
Yeast count (CFU/g)	<100	<10	<10	<10	<10	<10
Mold count (CFU/g)	<100	<10	<10	<10	<10	<10
Escherichia coli (CFU/g)	<10	<10	<10	<10	<10	<10

CFU = colony-forming units.

PART 3. §170.235 DIETARY EXPOSURE

3.1 Current Regulatory Status

3.1.1 Casein

Casein is listed in the U.S. FDA's *Substances Added to Food Database* (formerly EAFUS),[5] with technical effects ranging from emulsifier or emulsifier salt, flavor enhancer, flavoring agent or adjuvant, formulation aid, nutrient supplement, processing aid, stabilizer or thickener, surface-finishing agent, and texturizer.

Casein is GRAS as a substance migrating to food from paper and paperboard products used in food packaging (21 CFR §182.90 – U.S. FDA, 2021a). Casein is also a substance that may be added to ice cream mix containing not less than 20% total milk solids [21 CFR §135.110(c) – U.S. FDA, 2021a], to sherbet mix [21 CFR §135.140(c) – U.S. FDA, 2021a], and to margarine [21 CFR §166.110(a)(2)(ii) – U.S. FDA, 2021a]. Additionally, peptones derived from the partial hydrolysis of casein are direct food substances affirmed as GRAS for use as nutrient supplements, processing aids, and as surface active agents (21 CFR §184.1553 – U.S. FDA, 2021a). Casein is also used as a protein quality reference standard as measured by protein efficiency ratio for various other food ingredients [21 CFR §101.9(c)(7) – U.S. FDA, 2021a].

The U.S. FDA issued a "no questions" letter to the notification of the GRAS status of the use of concentrated milk protein with a ≥60:40 whey:casein ratio for use as an emulsifier, flavoring agent, formulation aid, humectant, stabilizer, thickener, texturizer, and protein source in the following food categories at varying use levels depending on technical effect: meal replacements and meal supplements; powdered nutritional beverages; nutritional bars; acidified sports beverages; milk products; yogurt and fermented milk products; non-standardized cheese products; spreads, dips and cream substitutes; frozen dairy desserts and mixes; desserts and mousses; confections; snack foods; coatings and fillings; salad dressings; soups, soup mixes, and sauces (GRN 633 – U.S. FDA, 2016b).

Currently, there are no regulatory provisions that would allow use of casein in sugar (sucrose).

3.1.2 Calcium Caseinate

Calcium caseinate is present in the U.S. FDA's *Substances Added to Food Database* (formerly EAFUS),[6] with technical effects including color or coloring adjunct, formulation aid, nutrient supplement, stabilizer or thickener, and texturizer.

Calcium caseinate is described as an ingredient in vitamin-mineral preparations, in regulations pertaining to polysorbate 80 (polysorbate 80 as a food additive permitted for direct addition to food for human consumption) (21 CFR §172.840 – U.S. FDA, 2021a). Calcium caseinate is also a substance that may be added to ice cream mix containing not less than 20% total milk solids [21 CFR §135.110(c) – U.S. FDA, 2021a], and to sherbet mix [21 CFR §135.140(c) – U.S. FDA, 2021a].

[5] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances&id=CASEIN (last updated: 05/17/2022) (U.S. FDA, 2022a).
[6] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/?set=FoodSubstances&id=CALCIUMCASEINATE (last updated: 05/17/2022) (U.S. FDA, 2022b).

The U.S. FDA issued a “no questions” letter to the notification of the GRAS status of the use of calcium casein peptone-calcium phosphate for use in chewing gum as a texturizer at a use level of up to 5% w/w (GRN 011 – U.S. FDA, 1999).

Currently, there are no regulatory provisions that would allow use of calcium caseinate in sugar (sucrose).

3.1.3 Pea Protein

The U.S. FDA issued a “no questions” letter to the notification of the GRAS status of the use of pea protein for use as a source of protein in foods at levels ranging from 1 to 90% in a variety of food categories and as a binder and extender in meat and poultry applications. It is also intended for use in specialty foods intended to meet the protein requirements for sports activity or for weight control (GRN 851 – U.S. FDA, 2020). This ingredient is identical to the pea protein sourced by Incredo for production of Incredo Sugar®. Various other pea protein ingredients have been notified to the FDA and received a “no questions” response, but they have been omitted for brevity. A summary of these ingredients is presented in Table 3.1.3-1.

Currently, there are no regulatory provisions that would allow use of pea protein in sugar (sucrose).

Table 3.1.3-1 GRAS Notices for Pea Protein Ingredients

Company and GRN No.	Ingredient Description	Intended Conditions of Use	Maximum Intakes
GRN No. 851 – Roquette Freres (U.S. FDA, 2020)	**Pea protein**	**For use as a source of protein in foods at levels ranging from 1 to 90% in a variety of food categories, and as a binder and extender in meat and poultry applications.**	**Consumption of foods containing the pea protein isolate would not reasonably result in a daily consumption greater than the DRV of 50 g/day of protein for adults and children 4 or more years of age.**
GRN No. 948 – Yantai Oriental Protein Tech Co., Ltd. (U.S. FDA, 2021c)	Enzyme-treated pea protein	For use as a food ingredient, formulation aid, nutrient supplement, stabilizer and thickener, and texturizer in baked goods and baking mixes, beverages and beverage bases, breakfast cereals, dairy product analogs, fats and oils, grain products and pastas, milk products, plant protein products, processed fruits and fruit juices, processed vegetables and vegetable juices, soups and soup mixes at levels from 0.96 to 34.3%.	On a consumer-only basis, the mean and 90th percentile intakes were estimated to be 28.42 g/person/day (0.47 g/kg body weight/day) and 51.62 g/person/day (0.97 g/kg body weight/day), respectively.
GRN No. 804 – Burcon NutraScience Corporation (U.S. FDA, 2019a)	Pea protein	For use as a source of protein in foods at use levels ranging from 1 to 35 g pea protein per 100 g of food in a variety of food categories.	On a consumer-only basis, the mean and 90th percentile intakes were estimated to be 28.42 g/person/day (0.47 g/kg body weight/day) and 51.62 g/person/day (0.97 g/kg body weight/day), respectively.

Table 3.1.3-1 GRAS Notices for Pea Protein Ingredients

Company and GRN No.	Ingredient Description	Intended Conditions of Use	Maximum Intakes
GRN No. 803 – Ingredion Inc. and Shandong Jianyuan Bioengineering (U.S. FDA, 2019b)	Pea protein	For use as a formulation aid, nutrient supplement, stabilizer and thickener, and texturizer in conventional food products including meat and poultry products at a maximum exposure of 30 g/person/day.	Consumption of foods containing the pea protein isolate would not reasonably result in a daily consumption greater than the DRV of 50 g/day of protein for adults and children 4 or more years of age.
GRN No. 788 – Yantai Oriental Protein Tech Co., Ltd. (U.S. FDA, 2018)	Pea protein concentrate	Ingredient, formulation aid, source of protein, stabilizer, thickener, and texturizer in conventional foods at levels ranging from 0.96 to 34.3%.	The 90th percentile intake of pea protein from the intended uses of the pea protein in different food categories is 17.3 g/person/day; the maximum intake of pea protein from its uses in sports nutrition is 30 g/person/day.
GRN No. 608 – Axiom Foods (U.S. FDA, 2016c)	Pea protein concentrate	Ingredient, formulation aid, and texturizer in conventional foods at levels ranging from 0.96 to 34.3%.	The 90th percentile all-person and all-user intakes of pea protein concentrate from the proposed food uses by the total population were 17.2 g/person/day (385 mg/kg body weight/day) and 17.3 g/person/day (388 mg/kg body weight/day), respectively.
GRN No. 581 – Word Food Processing, LLC (U.S. FDA, 2016d)	Un-hydrolyzed and hydrolyzed pea protein	For use as an ingredient in a variety of food categories at levels ranging from 2 to 90% of the finished food.	The mean daily protein intake from pea protein *per capita* is 0.0688 g/person/day.
GRN No. 182 – Martin Vialatte (U.S. FDA, 2006)	Pea protein isolate	Fining agent in wine making.	The use of plant protein in winemaking results in the protein reacting with the tannin in the wine forming an insoluble protein-tannin complex which precipitates; therefore, there is no increase in dietary exposure to consumers of wine processed with plant protein.

DRV = daily reference value; GRN = Generally Recognized as Safe (GRAS) Notice; No. = Number.

3.1.4 Rice Protein

The U.S. FDA issued a "no questions" letter to the notification of the GRAS status of the use of rice protein for use as a source of protein in foods at levels ranging from 0.96 to 34.3% in a variety of food categories (GRN 609 – U.S. FDA, 2016a). This ingredient is identical to the rice protein sourced by Incredo for production of Incredo Sugar®. A notification of GRAS status for rice protein hydrolysate has also been submitted by BASF Corporation to the FDA and received a "no questions" response (GRN 944 – U.S. FDA, 2021b). The rice protein hydrolysate is intended for use as a protein source in a variety of food categories at levels ranging from 1.0 to 83%. Additionally, a notification of GRAS status for barley rice protein containing up to 60% rice protein hydrolysate (derived from *Oryza sativa*) has been submitted by EverGrain, LLC to the FDA and received a "no questions" response (GRN 1031 – U.S. FDA, 2022c). The barley rice protein is intended for use as a protein source in a variety of food categories at levels ranging from 0.5 to 90%.

Currently, there are no regulatory provisions that would allow use of rice protein in sugar (sucrose).

3.1.5 Sucrose

Sucrose is universally recognized as a safe food ingredient, is GRAS under 21 CFR §184.1854, and its use in food and beverage production is limited only by current Good Manufacturing Practices (cGMP).

3.2 Estimated Intake of Protein-Sucrose

Incredo intends to market Incredo Sugar® as an ingredient in sugar at levels ranging from 0.01 to 0.8% protein, as shown in Table 3.2-1.

Table 3.2-1 Individual Proposed Food Use and Use Level for Protein-Sucrose in the United States

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Proposed Food Uses	Protein-Sucrose Use Levels (g/100 g)
Sugar, white, granulated	White sugar	0.01 to 0.8

CFR = Code of Federal Regulations.

The intake of additional sucrose from the proposed uses of Incredo Sugar® is not necessary to evaluate due to the long history of safe consumption of sucrose and to the universal recognition of this by regulatory agencies world-wide. Therefore, solely intake of protein is considered as part of the dietary exposure assessment.

3.3 Estimated Dietary Consumption of Protein-Sucrose

3.3.1 Methodology

An assessment of the anticipated intake of proteins under the intended conditions of use (see Table 3.3.2-1) was conducted using data available in the 2015-2016 cycle of the U.S. National Center for Health Statistics' National Health and Nutrition Examination Survey (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 protein for each of the following population groups:

- Infants and toddlers, less than 2 years of age;
- Young children, ages 2 to 5 years;
- Children, ages 6 to 11 years;
- Female teenagers, ages 12 to 19 years;
- Male teenagers, ages 12 to 19 years;
- Female adults, ages 20 years and up;
- Male adults, ages 20 years and up; and
- Total population (ages 2 years and older, and both 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 protein by the U.S. population.[7] Estimates for the daily intake of protein 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 protein averaged over all individuals surveyed, regardless of whether they consumed food products in which protein is proposed for use, and therefore includes individuals with "zero" intakes (*i.e.*, those who reported no intake of food products containing protein during the 2 survey days). "Consumer-only" intake refers to the estimated intake of protein by those individuals who reported consuming food products in which the use of protein is currently under consideration. Individuals were considered "consumers" if they reported consumption of 1 or more food products in which protein is proposed for use on either Day 1 or Day 2 of the survey.

The estimates for the intake of protein were generated using the maximum use level indicated for the intended food uses, as presented in Table 3.2-1, together with food consumption data available from the 2015-2016 NHANES datasets. The results for these assessments are presented in Section 3.3.2.

3.3.2 Results of Intake Estimates for Protein Ingredients

A summary of the estimated daily intake of protein from the proposed food use of white sugar is provided in Table 3.3.2-1 on an absolute basis (mg/person/day), and in Table 3.3.2-2 on a body weight basis (mg/kg body weight/day).

The percentage of consumers was evaluated among the total population (*i.e.*, 2 years and older) and among individual population groups in the current intake assessment; greater than 20.6% of the individual population groups consisted of consumers of food products in which protein ingredients are currently proposed for use. Female adults had the greatest proportion of consumers at 53.5%. 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 (2 years and older), the mean and 90th percentile consumer-only intakes of protein ingredients were determined to be 122.66 and 274.66 mg/person/day, respectively. Of the individual population groups, male adults were determined to have the greatest mean and 90th percentile consumer-only intakes of protein ingredients on an absolute basis, at 141.34 and 368 mg/person/day, respectively, while infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 32 and 48 mg/person/day, respectively.

[7] 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.

Table 3.3.2-1 Summary of the Estimated Daily Intake of Protein Ingredients from Proposed Food Uses in the United States by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and toddlers	0 to <2	5.34	8.00	20.6	87	32.00	48.00
Young children	2 to 5	16.00	53.34	37.8	212	45.34	93.34
Children	6 to 11	26.66	72.00	48.5	398	56.00	133.34
Female teenagers	12 to 19	53.34	152.00	49.7	210	109.34	224.00
Male teenagers	12 to 19	40.00	120.00	38.2	189	106.66	240.00
Female adults	20 and older	66.66	184.00	53.5	1,302	125.34	282.66
Male adults	20 and older	72.00	192.00	51.3	1,083	141.34	368.00
Total population	2 and older	61.34	157.34	50.4	3,394	122.66	274.66

n = sample size; NHANES = National Health and Nutrition Examination Survey.

On a body weight basis, the total population (2 years and older) mean and 90th percentile consumer-only intakes of protein ingredients were determined to be 1.74 and 4.06 mg/kg body weight/day, respectively. Among the individual population groups, infants and toddlers were identified as having the highest mean consumer-only intake of any population group, of 2.70 mg/kg body weight/day, while young children had the highest 90th percentile intake estimate of 5.50 mg/kg body weight/day. Male teenagers had the lowest mean and 90th percentile consumer-only intakes of 1.58 and 3.76 mg/kg body weight/day, respectively.

Table 3.3.2-2 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Protein Ingredients from Proposed Food Uses in the United States 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 toddlers	0 to <2	0.56	0.78	20.6	87	2.70	4.90
Young children	2 to 5	0.98	3.54	37.9	210	2.64	5.50
Children	6 to 11	0.86	2.30	48.5	397	1.76	4.18
Female teenagers	12 to 19	0.88	2.86	49.8	206	1.76	4.38
Male teenagers	12 to 19	0.62	1.90	38.4	189	1.58	3.76
Female adults	20 and older	0.90	2.42	53.5	1,293	1.70	3.98
Male adults	20 and older	0.88	2.16	51.4	1,072	1.70	4.16
Total population	2 and older	0.88	2.42	50.5	3,367	1.74	4.06

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

3.3.3 Summary and Conclusions

Consumption data and information pertaining to the intended food uses of protein ingredients were used to estimate the *per capita* and consumer-only intakes of protein ingredients 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 suitably conservative. For example, it has been assumed in this exposure assessment that all food products within a food category contain protein ingredients 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 protein ingredients will have 100% market penetration in the food category of white sugar.

In summary, on a consumer-only basis, the resulting mean and 90th percentile intakes of protein ingredients by the total U.S. population from proposed food uses in the U.S. were estimated to be 122.66 mg/person/day (1.74 mg/kg body weight/day) and 274.66 mg/person/day (4.06 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of protein ingredients were determined to be 141.34 mg/person/day (1.70 mg/kg body weight/day) and 368 mg/person/day (4.16 mg/kg body weight/day), respectively, as identified among male adults. While infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 32 and 48 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest mean daily intake of 2.70 mg/kg body weight/day while young children had the highest 90th percentile intake estimate of 5.50 mg/kg body weight/day.

PART 4. §170.240 SELF-LIMITING LEVELS OF USE

As Incredo Sugar® is intended to be used (together with sugar) as a sweetener, there are self-limiting levels of use due to alterations in flavor profile. Incredo Sugar® is intended to be used at a level of 0.01 to 0.8% in the finished food product. A higher use level results in diminished sweetness, and thus the use is self-limiting.

PART 5. §170.245 EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

Not applicable.

PART 6. §170.250 NARRATIVE AND SAFETY INFORMATION

6.1 Safety Narrative

The food-grade proteins intended for use in Incredo Sugar® are mixed mechanically with sucrose and then crystalized or dried. Throughout this process, no chemical bonds are formed between sugar and proteins (as demonstrated in Section 2.4.2); instead, sugar and proteins are held together *via* hydrogen and van der Waals interactions. Considering the absence of any chemical interactions between proteins and sucrose, and given that the proteins discussed herein are chemically representative of the protein ingredients that were either (i) concluded as a GRAS substance by the Select Committee on GRAS Substances (SCOGS) or (ii) previously concluded to be GRAS by an Expert Panel with notification to the U.S. FDA (*i.e*., GRNs 609 – U.S. FDA, 2016b, 851 – U.S. FDA, 2020, and 948 – U.S. FDA, 2021c), a discussion of publicly available data and information relevant to the safety of protein ingredients is incorporated by reference to pivotal studies discussed in GRNs 609 and 851. The safety of sucrose is not discussed as part of this narrative because it is universally recognized as a safe food ingredient and is GRAS under 21 CFR §184.1854.

To identify new data pertinent to the safety of each selected protein published since its GRAS status was last evaluated, a comprehensive search of the published scientific literature was conducted. The search was conducted using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. Based on this updated search of the literature, Incredo is not aware of any newly published studies that suggest that the selected proteins would be unsafe when used as a food ingredient.

A summary of the pertinent toxicological studies from prior GRAS Notices and newly identified studies or publicly available scientific evaluation relevant to the safety of each selected protein is provided in the sections that follow. Based on conclusions from previous expert panels on the GRAS status of each protein, corresponding “no questions” letters issued by the U.S. FDA, the widespread history of use of such proteins and sucrose as food ingredients globally, and conclusions from other authoritative and scientific bodies on the safety of selected proteins (*e.g*., SCOGS) and sucrose, an Expert Panel concluded that the current GRAS status of each protein and sucrose can be extended to their use in the manufacture of Incredo Sugar®. Incredo therefore concluded that sucrose, casein, calcium caseinate, pea protein, and rice protein, as described herein, are GRAS, for use in the production of Incredo Sugar®, based on scientific procedures.

The available data related to the safety of each protein and sucrose are summarized below.

6.2 Metabolic Fate

The metabolic pathway of all protein ingredients is expected to imitate any other protein ingredient in the human diet.

6.3 Safety Evaluations of Ingredients Used in the Manufacture of Incredo Sugar®

The safety of various protein ingredients has been extensively reviewed by a number of authoritative and scientific bodies. Pivotal studies from these evaluations are summarized in the sections below as they relate to the use of the protein ingredients in sucrose. A brief statement on the safety profile of sucrose is also provided.

6.3.1 Casein and Calcium Caseinate

The published scientific literature regarding the safety of casein and calcium caseinate was previously reviewed by SCOGS in 1979. Since the SCOGS review, several GRAS conclusions were notified to the U.S. FDA and received "no questions" responses (GRN 011 – U.S. FDA, 1999, GRN 633 – U.S. FDA, 2016b). An updated search of the published scientific literature conducted from late-2015 did not reveal new toxicological studies relevant to the safety of casein or calcium caseinate. However, several studies administering casein in the diet to laboratory animals as part of efficacy studies, or as controls in safety studies of unrelated test articles, were identified (Jones *et al.*, 2015; Singh *et al.*, 2016; Fuc *et al.*, 2019; Shahkhalili *et al.*, 2020; de Gaudry *et al*., 2021; Roman *et al.*, 2021; AL Tamimi *et al*., 2022; Menikdiwela *et al*., 2022; Zhang *et al*., 2022; Zhao *et al*., 2022). Further, several clinical trials administering casein to human subjects were identified (Mariotti *et al.*, 2015; McDonald *et al.*, 2017; Wu *et al.*, 2017; Kaskous, 2020; Yuda *et al.*, 2021; Chen *et al*., 2022) with no reported adverse effects. These studies did not report results contradicting the conclusions reported in the SCOGS report. Therefore, critical studies from the SCOGS report are detailed below.

SCOGS conducted a comprehensive review of the use, exposure, and safety of casein and caseinate salts in 1979 and stated that casein and various caseinates occur in mammalian milk, and thus are part of the normal human diet (FASEB, 1979). SCOGS reported acute, short-term, and long-term feeding studies in a variety of species administered casein and caseinates. A selection of critical studies from the SCOGS report are detailed below.

Boyd *et al.* (1967) administered "high-protein" and "vitamin-free" casein[8] *via* intragastric cannula to young male albino rats (n=20/group) in doses of 50 mL/kg of 15% suspensions at 5 successive hourly intervals each day (37.5 g/kg/day) for 3 days; the dose was then increased to 9 administrations per day until death occurred or for 3 weeks, whichever came first. A mortality of approximately 40% resulted from stomach rupture after administration of 37.5 g/kg on Day 1. No further deaths occurred until the eighth day when daily administrations had increased to 7, at which point 50% of the rats had died of gastric rupture. The authors estimated the lethal oral dose of casein to be over 1,000 g/kg body weight when administered in multiple doses over a period of 2 weeks. Similar administration of sodium caseinate or calcium caseinate solutions in quantities up to 75 g caseinate/kg/day in multiple doses produced a number of deaths from stomach rupture. Necropsy of animals that died after 3 to 5 days of treatment without gastric rupture showed generalized organ degeneration to which the authors stated, "*high sodium or calcium intake was a contributing factor*." The median lethal dose (LD_{50}) of sodium or calcium caseinate was estimated to be 400 to 500 g/kg when administered over a 5-day period (Boyd *et al.*, 1967).

[8] The "high-protein" casein was prepared by lactic acid fermentation of skim milk and contained 85% protein, 11% moisture, 1.9% ash, 1.5% fat, and small quantities of vitamins. The "vitamin free" casein was prepared by multiple extractions of casein with hot alcohol and contained 89% protein, 8.0% moisture, 2.0% ash, and 0.5% fat.

Nayak and Higginson (1962) administered diets containing 20% casein to groups of 20 C3H or CFW mice for 100 days. The authors reported that at necropsy, 3 of 7 CFW mice, but none of 12 C3H mice, had generalized amyloidosis involving the liver, spleen, kidneys, adrenals, and small intestine. The authors stated that they had previously observed similar amyloidosis in 33 of 55 Hauschka mice fed a 20% casein diet for up to 400 days. It is unclear whether amyloidosis was attributed to casein administration or to contamination of the administered casein with *Bacillus cereus*, as subsequent studies have demonstrated that a bacteriologically sterile product does not produce amyloidosis (Stora *et al.*, 1968).

Newburgh and Curtis (1928) investigated the effect of high dietary levels of protein on urine composition and kidney histology in long-term rat feeding studies. Weanling rats (breed not stated) were fed diets containing 7 to 61% protein, with 8 to 75% of the diet consisting of casein. Other groups were fed 12 to 72% protein, with 14.8 to 92.5% of the diet consisting of beef muscle protein. Urine specimens were taken every second month and were analyzed for albumin content and presence of casts.[9] An abnormally large number of casts were reported in the urine of rats fed 33% casein or beef muscle protein for 240 days with the number being larger at 450 days. However, renal injury as indicated by number of casts was greater in the groups fed beef muscle for 240 and 450 days. Urine albumin content of the animals fed beef was also greater (1.1%) than that of the casein-fed group (0.1%) at 450 days. Histologic examination of the kidneys of rats fed a diet containing 75% casein for more than 1 year showed moderate degeneration of the epithelium of the convoluted tubules and some tubular dilatation. Beef muscle protein at a comparable dietary level caused more severe tubular injury, and injury also was evident in rats fed a 31% beef protein diet for 15 months. Microscopic examination of kidney tissues of animals euthanized at 350 days revealed marked cystic dilatation of some tubules and various forms of glomerular injury. The authors concluded that the amount of protein consumed is positively correlated with an increase in kidney damage, and that the "*nature of the protein fed is at least as important a factor as the amount of protein in both the production of injury and in the degree of injury*," such that the casein diet induced fewer lesions than the beef muscle diet (Newburgh and Curtis, 1928). Addis *et al.* (1926) reported histologically normal kidneys and no more casts in the urine of rats fed a diet containing 74% casein as compared to a control group (n=6 rats/group). Administration of the diet lasted for 330 days. Samples of fresh urine were examined for casts rather than the concentrates obtained by centrifuging all urine collected over a 24-hour period, as was reported by Newburgh and Curtis (1928).

Bras and Ross (1964) reported that renal damage in rats, described as progressive glomerulonephrosis (PGN), was influenced by both protein and caloric intake. In lifetime feeding studies with male Charles River Sprague-Dawley rats, a higher incidence (46/209) of PGN was found in Group A, fed a daily diet of 10 g containing 30% casein, than in Group B (16/233) that received 10 g of a similar diet containing 8% casein. The lowest incidence (1/119) of PGN was in Group C, fed 5.9 g of a diet containing 51% casein, which had the same daily consumption of protein (3 g) as Group A but at a lower caloric intake.

[9] Urinary casts can be made up of white blood cells, red blood cells, kidney cells, or substances such as protein or fat. The content of a cast can help determine whether kidney function is healthy or abnormal.

Adverse effects (primarily kidney injury) were reported in high-casein diets (75% dietary inclusion of casein, approximately equivalent to 75,000 mg/kg body weight/day);[10] however, SCOGS stated this toxicological effect is associated with high dietary protein intake and was not specific to casein (Lalich *et al.*, 1970). Of the studies reviewed by SCOGS, the administration of 20% dietary casein (equivalent to 20,000 mg/kg body weight/day)[11] elicited no major adverse effects.[12] While SCOGS did not establish an acceptable daily intake (ADI) from the studies presented, safety factors may be utilized to account for interspecies and interindividual toxicodynamic and toxicokinetic differences, to establish an ADI of 200 mg casein/kg body weight/day in humans (safety factors = 100).

The estimated intake of casein and caseinates was projected by SCOGS to be approximately 200 mg/person/day on a *per capita* basis (approximately equivalent to 3.33 mg/kg body weight/day for a 60-kg adult). It should be noted that SCOGS did not analyze casein or caseinates on a consumer-only basis. As casein and caseinates represent a minor contribution to the total average daily intake of protein, SCOGS stated:

> *There is no evidence in the available information on casein, sodium caseinate, or calcium caseinate that demonstrates or suggests reasonable grounds to suspect a hazard when they are used at levels that are now current or that may reasonably be expected in the future*. (FASEB, 1979)

It should be noted that the estimated exposure to protein ingredients based on their inclusion in sugar (see Section 3.3.2) demonstrated that the total population on a consumer-only basis would be exposed to a mean of approximately 122.66 mg protein/day (2.42 mg/kg body weight/day). At the 90th percentile level, exposure would be approximately 274.66 mg protein/day (4.06 mg/kg body weight/day). While this value is above the exposure level reported by SCOGS, is lower than the acceptable daily intake derived from animal toxicity studies (ADI = 200 mg/kg body weight/day). Additionally, the calculated exposure is 5,000 times lower than the NOAEL identified in the 100-day rodent toxicity study administering 20% casein in the diet (from which the ADI was derived), thus there is a sufficient threshold of safety to conclude that the use of casein and calcium caseinate as intended poses no safety concern.

6.3.2 Pea Protein

The published scientific literature has been reviewed in several previous GRNs, most recently in 2021 (GRN 948 – U.S. FDA, 2021c). An updated search of the published scientific literature was conducted through September 2022. Results from this search indicate that 1 toxicological study relevant to the safety of pea protein was published in the specified time frame (Hidayat *et al*., 2022). The details of this study are described below. Several studies administering casein in the diet to laboratory animals as part of efficacy studies were identified (Liu *et al.*, 2021; Salles *et al.*, 2021; Scuderi *et al*., 2022). A review of these studies indicates that the results do not contradict conclusions reported in GRN 851 nor GRN 948.

[10] Calculated using Priority Based Assessment of Food Additives (PAFA) Conversion Table (U.S. FDA, 1993).
[11] Calculated using PAFA Conversion Table (U.S. FDA, 1993).
[12] While Nayak and Higginson (1962) reported amyloidosis in a minority of CFW mice (3/7), it is unclear if the effect was attributed to casein administration or to contamination of the administered casein with *Bacillus cereus*, as subsequent studies demonstrated that a bacteriologically sterile product did not produce amyloidosis (Stora *et al*., 1968).

Once daily all rats were weighed and underwent visual observations for mortality, behavioral patterns, changes in physical appearance, injury, and signs of illness. At the end of the experiment, all animals were euthanized. Blood samples were collected for biochemical and hematological analyses. The organs were excised, weighed, and examined macroscopically and relative organ weight was calculated. The liver and kidney were preserved for histopathological study.

There were no deaths and no treatment-related adverse effects reported in any animals. After 28 days without treatment, the body weights of male and female control satellite rats were not statistically different from the average body weight of rats in the high-dose group ($p>0.05$). The food and water consumptions of the treated rats was not significantly different compared to control rats. The relative organ weights of each organ recorded at necropsy in the treatment groups did not show a significant difference ($p>0.05$) compared to the controls.

There were no treatment-related adverse effects in any hematological parameter evaluated including leucocyte (total white blood cell [WBC]), erythrocyte (red blood cell [RBC]), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte, monocyte, neutrophil, eosinophil, thrombocyte (platelet [PLT]), red cell distribution width (RDW), and platelet distribution width (PDW). The authors reported triglyceride (TG) levels of female control rats were significantly higher compared to all treated rats. No statistically significant differences in liver function parameters (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) were reported. In the highest dose group, it was reported that male rats had no impairment of renal function parameters (urea, creatinine [Cr], and uric acid [UA]). However, the low and moderate doses in male rats and low doses in female showed significant differences as compared to the control, even though the Cr levels were still within the normal range, and the urea levels of all rats, both control and treatment groups, were reported to be above the normal range. In female rats, the highest dose did not affect urea and Cr parameters, but there was an increase in UA levels. Other blood biochemical profiles (lipid profile: total cholesterol, low-density lipoprotein [LDL], high-density lipoprotein [HDL]) and glucose (GLUC) did not differ significantly from controls.

The authors reported no abnormalities in the macroscopic examination of the vital organs of treated animals when compared with the organs of the control group. The authors reported normal structure and absence of any gross pathological lesion in organs, except in the median score of liver histopathology parameters for lobular inflammation of the high-dose group was 3, which was higher as compared to the control group score, which was 1. Lobular inflammation indicates early-stage inflammation of the hepatocytes; however, the inflammation was mild to medium. After 28 days of recovery, the high-dose satellite group showed significant improvement (total score 0). The authors reported that the results of the median score of kidney histopathology using the Kruskal Wallis test was $p>0.05$, indicating no significant difference between groups.

The authors concluded that the no-observed-adverse-effect level (NOAEL) of the green pea protein hydrolysate "*in the sub-chronic toxicity study was the dose of 200 mg/kg* [body weight]" (Hidayat *et al*.,2022), the second highest dose tested, based on lobular inflammation in the liver observed in the highest dose groups**.** Given the crude production method of the test article in this study, it is considered of little relevance to Incredo's pea protein, which is produced according to cGMP and with various enzymes that are denatured during processing (unlike bromelain in this study).

Roquette (supplier of pea protein for the manufacture of Incredo Sugar®) conducted a series of safety studies detailed in GRN 851 (U.S. FDA, 2020). In the first study, 3 female Wistar rats and 3 CD1 female mice were administered pea protein isolate *via* oral intubation at 2,000 mg/kg body weight (Aouatif *et al.*, 2013a). The studies were conducted according to the Organisation for Economic Co-operation and Development (OECD) Guideline 423 (*Acute oral toxicity - acute toxic class method*). None of the animals exhibited any signs of dullness, abnormal body posture, tremors, seizures, restlessness, weight gain decrement, or any other signs of toxicity. The authors reported that an oral dose of 2,000 mg/kg body weight of pea protein isolate did not produce toxicity in any of the treated animals, and that the LD_{50} of pea protein isolate taken orally was higher than 2,000 mg/kg body weight. According to OECD Guideline 423, substances that have an oral LD_{50} higher than 2,000 mg/kg body weight can be considered nontoxic (OECD, 2001).

In the second study reported by Aouatif *et al.* (2013b), Wistar rats of both sexes were fed pea protein isolate in the diet at concentrations of 25,000, 50,000, and 100,000 parts per million (ppm), according to OECD Guideline 408 (*Repeated dose 90-day oral toxicity study in rodents*). After acclimation, animals were randomly distributed into 6 groups (n=10/sex/group) namely: control (0.0 ppm), low-dose (25,000 ppm), intermediate-dose (50,000 ppm), high-dose (100,000 ppm), satellite control (0.0 ppm), and satellite high-dose (100,000 ppm). The test substance was administered daily in the diet for 90 days. Food and water intake were measured once daily and reported weekly. Animals were weighed once weekly. Rats in the satellite groups were administered the control diet without the test article for an additional 28 days to evaluate any possible withdrawal effects. All animals were individually observed once daily for clinical signs. All animals were observed for functional observational battery (FOB) parameters prior to the administration of the test substance, during the 13th week for the main groups, and during the 17th week for the satellite groups.

There were no deaths or signs of toxicity on gross observation that were attributable to the ingestion of pea protein isolate. Feed consumption and weight gain during the study were comparable to the control group. The absolute and relative organ weights in the rats were comparable to controls, except an increase in the absolute weight of the spleen in females and decrease of the testes in male rats of the high- and low-dose groups, respectively. The authors reported these minimal alterations as attributed to intra-animal variation, since changes were not dose-dependent.

Gross pathological examination and histopathological findings did not reveal any treatment-related adverse changes. Minor but statistically significant changes were reported in a few biochemical assays (*e.g.*, AST, blood urea nitrogen [BUN], GLUC, and TG). The authors reported these changes were not test compound nor dose-dependent, but spurious. Pea protein isolate administration in rats did not alter normal liver or kidney function, nor produce any hematological alterations.

Ophthalmoscopic examination was unremarkable, and data from the FOB tests did not reveal any neurological toxicity induced by dietary administration of pea protein isolate. The authors reported that the NOAEL of pea protein isolate in Wistar rats can be defined as 100,000 ppm of diet (equivalent to 8,726 mg/kg body weight/day for males and 9,965 mg/kg body weight/day for females). Based on these findings, the authors concluded, "*Pea Protein can be considered as non-toxic when administered through diet*" (Aouatif *et al.*, 2013b).

Pea protein isolate was assessed for its mutagenic potential in the Ames assay with 5 tester strains of *Salmonella* typhimurium (TA100, TA102, TA1535, TA98, and TA1537), in presence and in absence of metabolic activation (S9) (Aouatif *et al.*, 2013c). The assay was performed according to OECD Guideline 471 (*Bacterial reverse mutation test*). Five test concentrations of 312.5, 625, 1,250, 2,500, and 5,000 g/plate with 10% S9 and without S9 along with solvent and positive controls were chosen for mutagenicity evaluation in the 5 tester strains. There was no concentration related or reproducible increase in the number of revertant colonies in any of the test concentrations in any of the tester strains. No 2- or 3-fold increase in the means of the revertant counts was reported in the test concentrations in all tester strains with and without S9, while positive controls exhibited a significant multi-fold increase in revertant counts ($p<0.05$, Dunnett's test). The authors reported that "*the negative result indicated that under these experimental conditions Pea Protein was nonmutagenic in the Ames Salmonella typhimurium reverse mutation assay*."

Pea protein isolate was also evaluated for its capacity to induce structural and numerical aberrations in an *in vitro* chromosomal aberration test using cultured human peripheral blood lymphocytes (Aouatif *et al.*, 2013c). Peripheral blood was obtained from 3 healthy adult (>30 years age) non-smoking male volunteers, without any recent history of illness, according to OECD Guideline 473 (*In Vitro mammalian chromosome aberration test*). The percentage aberrations of all pea protein isolate–treated cultures was not significantly different from the concurrent solvent control cultures, while positive controls exhibited a significant increase in the percentage aberrations ($p<0.05$, Dunnett's test). The authors concluded, "*Under the conditions of the test, pea protein isolate did not induce a genotoxic response in human lymphocytes when tested up to concentrations inducing acceptable levels of cytotoxicity*." Acceptable levels of cytotoxicity were reported at the highest concentration tested (1,000 µg/ml).

The genotoxic potential of pea protein isolate was evaluated *in vivo* using the mouse micronucleus assay (Aouatif *et al.*, 2013c). The assay was performed by assessing the induction of micronuclei in polychromatic erythrocytes (PCEs) and determining the ratio of immature and mature erythrocytes in bone marrow cells, in compliance with the OECD Guideline 474 (*Mammalian erythrocyte micronucleus test*). Healthy male and female CD1 mice of 6 to 8 weeks of age were used for the study as per the guideline's specification. A range-finding study was performed with doses of 320, 800, and 2,000 mg/kg body weight employing 2 mice/sex/dose with a concurrent vehicle control. The mice were treated orally and administered a single treatment. Mice were euthanized 24 hours after dosing. A limit test was performed administering single- and 2-day treatments (24 hours apart) with the highest dose (2,000 mg/kg body weight). No mortality was reported in any of the groups. In the preliminary test, there was a mild dose-dependent increase in the polychromatic erythrocyte:normochromatic erythrocyte ratio (PCE:NCE ratio) reported in females (PCE:NCE ratio of >1) without disturbance in cellularity, and a ratio of >1 was reported in males at 800 mg/kg body weight, exhibiting a similar trend to that of the concurrent vehicle control. In the limit, test no evident increase in the frequencies of micronucleated PCEs was reported in the dose group compared to that of the concurrent vehicle control groups at all time points of sacrifice. The authors concluded that pea protein isolate was "*nongenotoxic in single- and 2-day treatments under the test conditions employed*."

The estimated exposure to protein ingredients based on their inclusion in sugar (see Section 3.3.2) demonstrated that the total population on a consumer-only basis would be exposed to a mean of approximately 122.66 mg protein/day (2.42 mg/kg body weight/day). At the 90th percentile level, exposure would be approximately 274.66 mg protein/day (4.06 mg/kg body weight/day). This level is approximately 2,000 times lower than the NOAEL of 8,726 mg/kg body weight/day.

6.3.3 Rice Protein

A minimally processed rice protein ingredient has been previously concluded to be GRAS for uses in a variety of conventional food products (GRN 609 – U.S. FDA, 2016a) at use levels of up to 34.3%. The ingredient was produced from whole-grain brown rice (*Oryza sativa*) that is milled and hydrolyzed using amylase in water. The liquid hydrolysate is then removed leaving behind the crude rice protein, which is washed and dried and milled to produce a protein powder. Using information on the intended uses in conjunction with survey data from NHANES 2011-2012 (USDA, 2014; CDC, 2015), GRAS uses of rice protein were estimated to result in total population all user dietary intakes of rice protein of 10.3 g per person per day (181 mg/kg body weight) and 17.3 g per person per day at the mean and the 90th percentile, respectively. Data and information supporting the safety of rice protein included nutritional and compositional comparisons demonstrating that rice protein contains a similar amino acid composition to other protein ingredients, such as whey and soy proteins, that are generally considered safe. GRN 609 described data demonstrating that Chinese wild brown rice was not mutagenic as assayed by bone marrow micronucleus, sperm abnormality, and a reverse mutation assay using *S.* typhimurium. The safety of rice protein was also supported by published studies conducted in rats and humans that reported no adverse effects (Prakash *et al*., 1996; Zhai *et al.*, 1996; CIR, 2006; Gottlob *et al.*, 2006; Lasekan *et al.*, 2006; Koo and Lasekan, 2007; Khan *et al.*, 2011; Joy *et al.*, 2013; Sauer *et al.*, 2012; Axiom Foods, Inc. / SPRIM Strategy & Intelligent Innovation, 2015).

Another rice protein ingredient (hydrolyzed rice protein) was concluded to be GRAS in 2020 (GRN 944 – U.S. FDA, 2021b). This GRAS evaluation incorporated, by reference, data and information supporting safety previously described in GRN 609. The notifier conducted an updated literature search for the safety information on rice protein or hydrolyzed rice protein published since GRN 609 through April 2020 and reported that no new relevant safety studies were identified. Incredo notes that a review of literature was also conducted as part of GRN 1031 (U.S. FDA, 2022c). This search was conducted through 21 October 2021.

Incredo conducted an updated search of the published scientific literature to obtain data and information relevant to safety published since the latest safety evaluation of rice protein in 2020 and 2021 (GRN 994 and GRN 1031, respectively). Search results yielded 2 studies, Li *et al.* (2021) and Hajimohammadi *et al.* (2021), which contain information relevant to the safety evaluation of rice protein. These studies are described below. Additionally, 1 efficacy study in rodents performed using a genetically modified rice (Hajimohammadi *et al.*, 2022) and 1 human trial administering a rice protein isolate to healthy male individuals (Tiekou Lorinczova *et al.*, 2021) were identified, but have been omitted due to limited relevance.

Li *et al.* (2021) conducted a 90-day dietary toxicity study administering a genetically modified rice producing phytase-lactoferricin fusion protein, BPL9K-4, to Sprague-Dawley rats (n=10 rats/sex/group). Groups were administered either BPL9K-4 (10.9% protein), 9 K (a non-transgenic parental rice, 12.6% protein), or Weiyou64 common rice (7.8% protein). BPL9K-4 and 9 K rice were formulated into diets at concentrations of 15%, 30% and 60%, while Weiyou64 common rice was added to diets at a concentration of 60% (equivalent to 1.635%, 3.27%, and 6.54% rice protein in the diet for BPL9K-4; equivalent to 1.89%, 3.78%, and 7.56% rice protein in the diet for 9 K; equivalent to 4.68% rice protein in the diet for Weiyou64). AIN93G diet was set as a basal-diet control. The study was conducted in compliance with the guideline for safety assessment of genetically modified plant and derived products 90-day feeding test in rats (NY/T 1102–2006, Ministry of

Agriculture of China) which is generally consistent with the related OECD guideline,[13] and in accordance with OECD principles of Good Laboratory Practice.

The authors reported that all animals survived well during the feeding trial. No obvious clinical signs were reported in any of the groups throughout the experimental period, and there were no remarkable clinical signs of toxicity reported from any experimental group in behavior, activity, posture, or other external appearance. Total body weight gain and total food consumption were not statistically different between groups, and while some transient differences were reported in the mean feed efficiency in male groups, the authors concluded that "*there were no biologically significant differences noted in body weight gain or food consumption attributed to administration of BPL9K-4 diets*."

Most hematological indexes were comparable between the transgenic rice groups and their corresponding non-transgenic parental rice groups. Similar results were reported in hematological parameters between groups. Only PLT in the male BPL9K-4 high-dose group were higher ($p<0.05$) than its corresponding parental rice diet group, and neutrophils were lower ($p<0.05$) in the female BPL9K-4 high-dose group than its conventional counterpart group on Day 90. On Day 45, Cr was lower in the male BPL9K-4 high-dose group compared with its corresponding 9 K group. On Day 90, alkaline phosphatase, urea nitrogen, and blood GLUC were lower in the female mid-dose BPL9K-4 group than in the mid-dose 9 K group; Cr and blood GLUC decreased in the female high-dose BPL9K-4 group compared to the high-dose 9 K group; and lactate dehydrogenase (LDH) decreased in the male high-dose BPL9K-4 group compared to its corresponding 9 K group. The authors reported that, in general, all alterations in hematological and biochemical parameters noted above were without dose–response, spontaneous in nature, and within historical control range, and were therefore considered toxicologically irrelevant. There were no remarkable changes reported in organ weights, macroscopic organ evaluation, or histopathology of any organs of animals related to administration of BPL9K-4 rice when compared with control groups in both sexes. The authors concluded that "*the BPL9K-4 transgenic rice exhibited no toxic effects on rats when compared with its conventional comparators as presented in this 90-day subchronic study*" (Li *et al.*, 2021). There are no findings in this study to call into question previous conclusions on the GRAS status of rice protein as a food ingredient since these findings support conclusions from previous studies of rice protein. Levels administered in this study (1.635% to 7.56% in the diet) are approximately equivalent to 1,635 mg/kg body weight/day and 7,560 mg/kg body weight/day.[14]

Hajimohammadi *et al.* (2021) conducted a 90-day dietary toxicity study administering a genetically modified rice expressing Cry1Ab protein, an insect-resistance protein, to Sprague-Dawley rats (n=20 rats/sex/group). Rats were divided into the following groups: Group A: standard feed with substitution of 50% carbohydrate with *Tarom Molaii* rice; Group B: standard feed with substitution of 50% carbohydrate with genetically modified *Bacillus thuringiensis* rice (GM Bt rice); and Group C: standard feed. Protein content of rice not reported. During the 90-day experimental period, clinical observations of the rats were conducted twice daily for mortality, abnormal signs, and unusual behaviors, while body weight and food consumption were measured each week. After 90 days, hematology,[15] biochemistry,[16] and urinalyses[17] analyses were

[13] This guideline does not contain certain clinical chemistry parameters such as sodium, potassium, and urea that are present in OECD guidelines.
[14] Calculated using PAFA Conversion Table (U.S. FDA, 1993).
[15] Parameters assessed included PLT, WBC, RBC, HGB, HCT, MCV, MCH, MCHC, mean platelet volume, RDW, and PDW.
[16] Parameters assessed included BUN, GLUC, cholesterol, TG, Cr, HDL, ALT, AST, total protein (TP), albumin, LDH, calcium, bilirubin (Bili), and UA.
[17] Parameters assessed included pH, ketones, urine specific gravity, TP, urobilinogen, blood GLUC, Bili, nitrite, and leukocytes.

conducted. At terminal sacrifice, the brain, spleen, liver, heart, uterus, ovary, testis, kidney, colon, thyroid, stomach, and esophagus were sampled for gross and histopathological examination.

There were no reported deaths and no observations indicating any adverse effects in physiology or clinical behavior. Body weight and mean weekly feed utilization were similar between groups. Hematological, and blood biochemistry values were similar between groups, except ALT, which was significantly decreased in the Group B male and female groups. The authors reported no effects on urinalysis, liver weight and histopathological findings. Organ weights, gross necropsy, and histopathology between groups were reported as unremarkable. The authors concluded that "*GM Bt rice showed no unintended obvious adverse effect on the health of rats*" (Hajimohammadi *et al.*, 2021). There are no findings in this study that call into question previous conclusions on the GRAS status of rice protein as a food ingredient.

Rice protein has been concluded as GRAS as an ingredient in a variety of food categories at levels ranging from 0.96 to 34.3% (GRN 609 – U.S. FDA, 2016a). There is a long history of safe uses of rice as a food staple, but there is lack of well-designed animal or human studies investigating the toxicity or adverse effects of rice or its constituents, including protein. Like all dietary protein, rice protein concentrate is digested in the human gastrointestinal tract. Based on amino acid profiles, rice protein is similar to whey and soy protein, both of which are GRAS. The notifiers reported the results of the available limited animal and human studies, which did not reveal any adverse effects of rice protein concentrate. The conclusion of GRAS status for rice protein as a food ingredient was therefore based on a comparison of dietary levels of rice intake to intake based on the proposed intake of rice protein. According to GRN 609, at levels ranging 0.96 to 34.3%, inclusion of rice protein in a variety of food categories will result in a daily maximum intake (90^{th} percentile) of 20.5 g/person/day. Compared to this value, the intake (90^{th} percentile) of protein (24.96 g/person/day) from the consumption of rice as a staple is higher. If rice protein were to be added to sugar, at the 90^{th} percentile intake level, exposure would be approximately 274.66 mg protein/day, or 4.06 mg/kg body weight/day. This level is inconsequential when compared to the dietary intakes discussed in GRN 609. Additionally, if the highest level of rice protein administered (7,560 mg/kg body weight/day) as reported in Li *et al.* (2021) is 1,800 times greater than the 90^{th} percentile intake level calculated for protein use in sugar. Therefore, there is reasonable certainty that rice protein is safe at inclusion levels in sugar up to 0.8%.

6.3.4 Sucrose

Incredo considered the safety of sucrose as unnecessary for discussion as part of this GRAS evaluation because sucrose is universally recognized as a safe food ingredient, is GRAS under 21 CFR §184.1854, and its use in food and beverage production is limited only by cGMP.

6.4 Human Data

Toxicological studies assessing the safety of sucrose, casein, calcium caseinate, pea protein, and rice protein in humans following oral exposure were not identified in the extensive search of the literature. Due to the ubiquity of protein in the human diet, and the low level of inclusion of the aforementioned ingredients in Incredo Sugar®, Incredo does not anticipate any associated human adverse effects following the proposed intake of Incredo Sugar®.

6.5 Allergenicity

Cases of pea allergy are relatively rare; however, a review of its allergenicity is incorporated by reference to its respective GRAS Notice (GRN 851 – Roquette Freres, 2019; Part 6(4)(d) pg. 39-40). The notifiers noted that pea protein is not a major allergen according to the *Food Allergen Labeling and Consumer Protection Act* (FALCPA) of 2004; Incredo notes that this remains the case in the *Food Allergy Safety, Treatment, Education & Research Act of 2021* (FASTER). Additionally, the notifiers stated that among individuals with food allergies, pea allergy prevalence is estimated at only 1%. Further, Incredo will ensure the ingredient will be identified on food product labels so that any pea allergic consumer will be aware of its presence in a food.

Similarly, cases of rice allergy are relatively rare; however, a review of its allergenicity is incorporated by reference to its respective GRAS Notice (GRN 609 – Axiom Foods, Inc. / SPRIM Strategy & Intelligent Innovation, 2015; Section 4.8 pg. 20-22). Similar to pea protein, the notifiers noted that rice protein is not a major allergen according to FALCPA and Incredo notes that this has not changed in the FASTER. The notifiers noted that there are some reported instances of rice allergy/sensitivity in the literature, but overall allergy to rice is rare and consumption of rice protein concentrate is unlikely to result in allergic reaction. Further, Incredo will ensure the ingredient will be identified on food product labels so that any rice allergic consumer will be aware of its presence in a food.

Milk-derived casein constitutes one of the most common allergies (U.S. FDA, 2022d) and is classified by FALCPA as 1 of the 8 major foods[18] or food groups responsible for a majority of food allergies. For this reason, Incredo will ensure all casein ingredients will be identified on food product labels so that the consumer will be aware of its presence in food.

6.6 GRAS Panel Evaluation

Incredo has concluded that protein-sucrose is GRAS for use in sugar, 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 sucrose, casein, calcium caseinate, pea protein, and rice protein, as used to manufacture protein-sucrose (Incredo Sugar®) 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 GRAS Panel consisted of the following qualified scientific experts: Joseph F. Borzelleca, Ph.D., (Virginia Commonwealth University School of Medicine), George C. Fahey Jr., Ph.D. (University of Illinois), and Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. (Soni & Associates Inc.).

The GRAS Panel, convened by Incredo (formerly DouxMatok Ltd.), independently and critically evaluated all data and information presented herein, and also concluded that protein-sucrose is GRAS for use in sugar as described in Section 1.3, based on scientific procedures. A summary of data and information reviewed by the GRAS Panel, and evaluation of such data as it pertains to the proposed GRAS uses of protein sucrose is presented in Appendix B.

[18] Nine as of the FASTER Act of 2021.

6.7 Conclusion

Based on the above data and information presented herein, Incredo has concluded that protein-sucrose is GRAS, on the basis of scientific procedures, for use in food and beverage products as described in Part 1. General recognition of Incredo's GRAS conclusion is supported by the unanimous consensus rendered by an independent Panel of Experts, qualified by experience and scientific training, to evaluate the use of protein-sucrose in food, who similarly concluded that the proposed uses of protein-sucrose are GRAS on the basis of scientific procedures.

Protein-sucrose (Incredo Sugar®) therefore can be marketed and sold for its intended purpose in the U.S. without the promulgation of a food additive regulation under 21 CFR §170.3.

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
135—Frozen desserts	135.110	Ice cream and frozen custard
	135.140	Sherbert
166—Margarine	166.110	Margarine
170—Food additives	170.3	Definitions
172—Food additives permitted for direct addition to food for human consumption	172.840	Polysorbate 80
182—Substances generally recognized as safe	182.1	Substances generally recognized as safe
182—Substances generally recognized as safe	182.90	Substances migrating to food from paper and paperboard products
184—Direct food substances affirmed as generally recognized as safe	184.1553	Peptones
	184.1854	Sucrose

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04.1/2023

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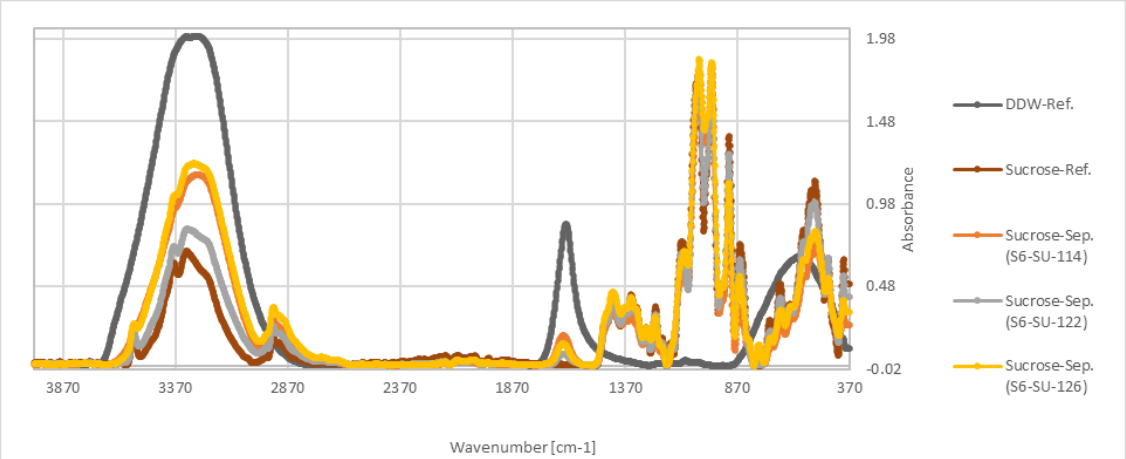
APPENDIX A. ADDITIONAL ANALYTICAL CHARACTERIZATION

1.1 Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) of Sucrose

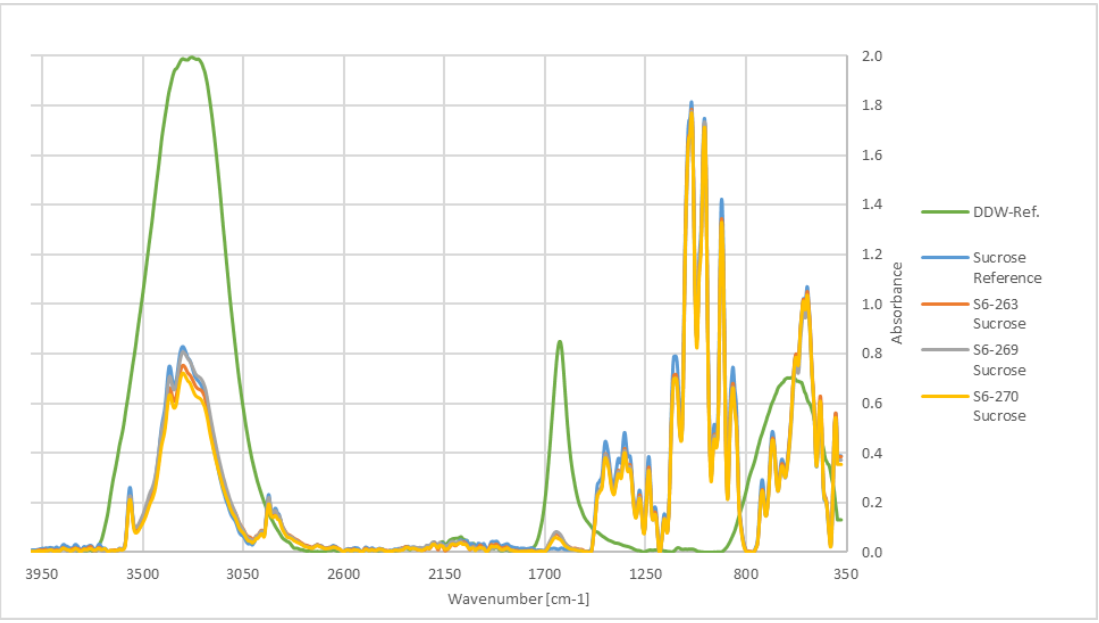
The attenuated total reflection with Fourier-transform infrared spectroscopy (ATR-FTIR) spectrum of pure sucrose and the separated sucrose are presented below. For the separated sucrose, a peak at 1,627 cm^{-1} is shown due to water molecules that are present in the samples (it is technically impossible to remove all trace of water molecules).

Figure 1.1-1 ATR-FTIR Spectrum of Pure Sucrose, Pure Di-Distilled Water and Separated Sucrose from Incredo Sugar® Concentrate 2nd Generation (50% Ca-caseinate/50% sucrose)



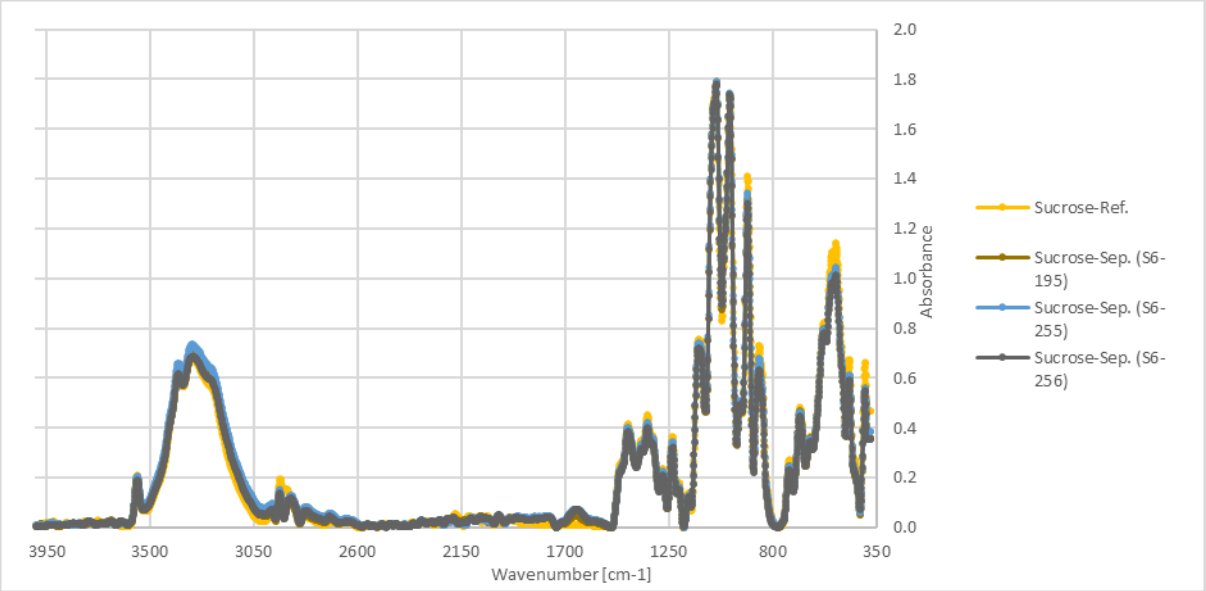
ATR-FTIR = attenuated total reflection with Fourier-transform infrared spectroscopy; DDW = di-distilled water; Ref. = reference; Sep. = separated.

Figure 1.1-2 ATR-FTIR Spectrum of Pure Sucrose, Pure Di-Distilled Water and Separated Sucrose from Incredo Sugar® Concentrate 2nd Generation (30% micellar casein/70% sucrose)



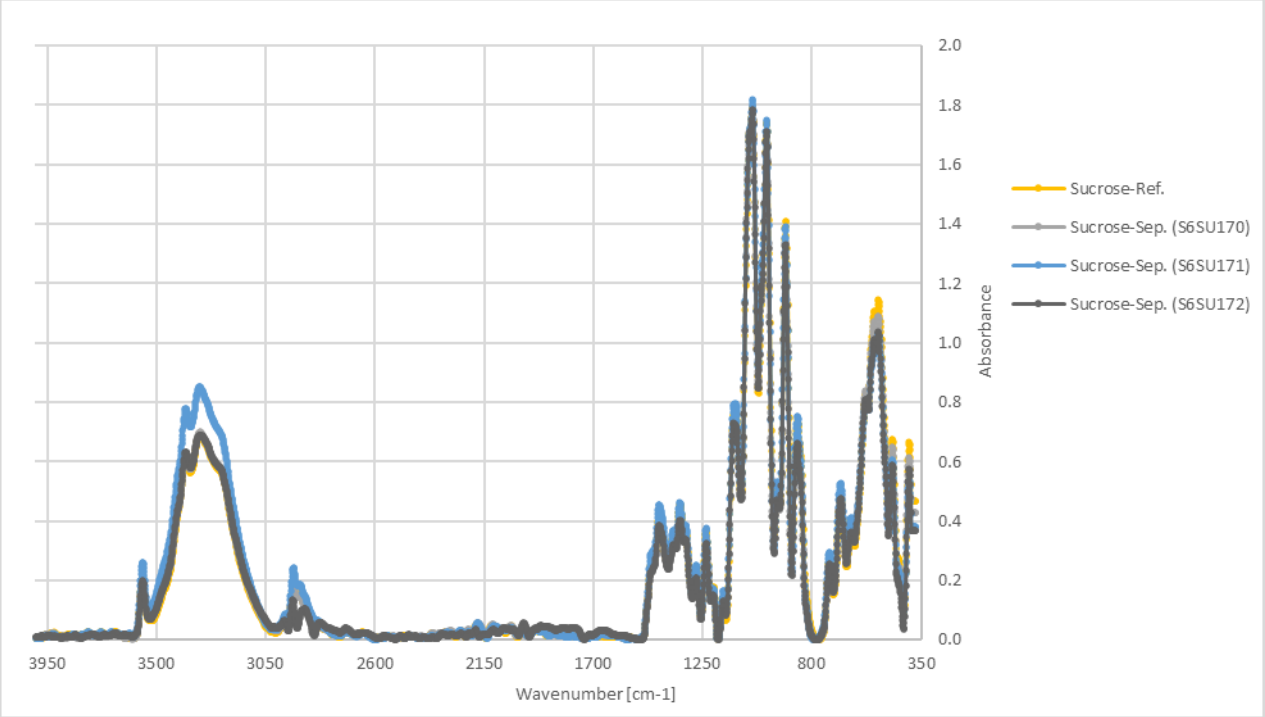
ATR-FTIR = attenuated total reflection with Fourier-transform infrared spectroscopy; DDW = di-distilled water; Ref. = Reference.

Figure 1.1-3 ATR-FTIR Spectrum of Pure Sucrose and Separated Sucrose from Incredo Sugar® Concentrate 2nd Generation (30% pea/70% sucrose)



ATR-FTIR = attenuated total reflection with Fourier-transform infrared spectroscopy; Ref. = reference; Sep. = separated.

Figure 1.1-4 ATR-FTIR Spectrum of Pure Sucrose and Separated Sucrose from Incredo Sugar® Concentrate 2nd Generation (30% rice protein/70% sucrose)

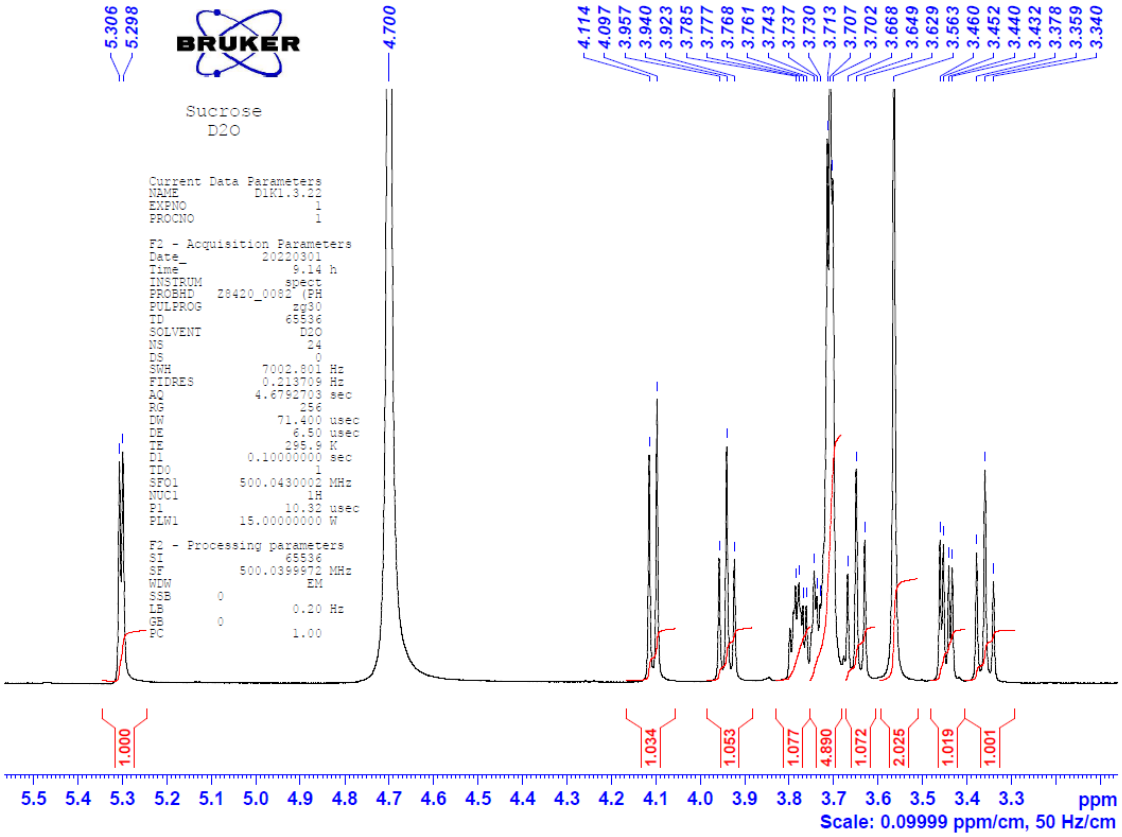


ATR-FTIR = attenuated total reflection with Fourier-transform infrared spectroscopy; Ref. = reference; Sep. = separated.

1.2 Nuclear Magnetic Resonance

Incredo conducted proton nuclear magnetic resonance (H^1-NMR), carbon-13 nuclear magnetic resonance (C^{13}-NMR), and carbon-13 distortionless enhancement by polarization transfer (C^{13}-DEPT)[1] measurements of separated sucrose from Incredo Sugar® manufactured with calcium caseinate, micellar casein, pea protein, and rice protein and their spectra compared to pure sucrose (Figures 9.2-1 to 9.2-15). Results from this experiment indicate that the samples only contains sucrose. However, it is possible that some samples will contain minor inversion products (hydrolysis of sucrose to fructose and glucose). This minor inversion is within the limit of normal occurrence in commercial sucrose and typically under the limit of reporting based on the sugar analysis by the ion chromatography (Table 2.4.1-1).

Figure 1.2-1 H^1 NMR of Pure Sucrose



[1] DEPT is used to determine the multiplicity of carbon atoms, that is, whether they are C, CH, CH2, or CH3.

Figure 1.2-2 H^1 NMR of Sucrose Isolated from Ca-Caseinate Incredo Sugar® (S6SU126)

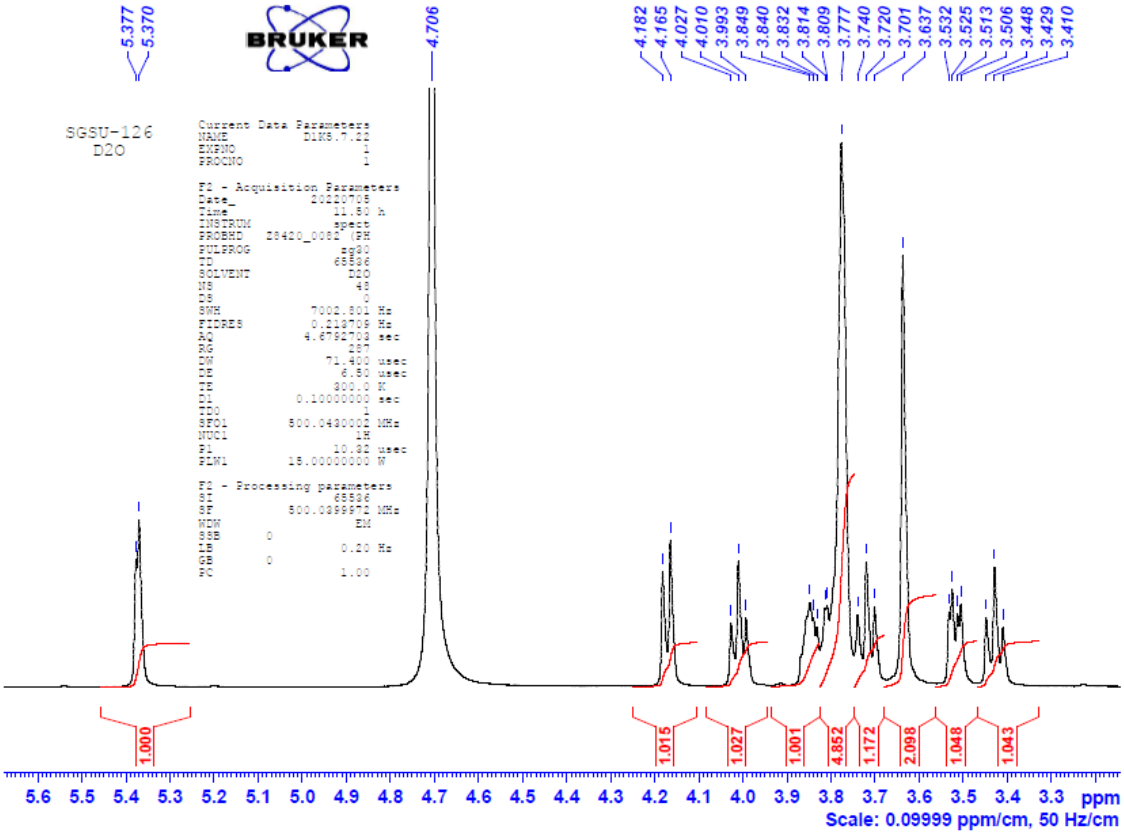


Figure 1.2-3 **H^1 NMR of Sucrose Isolated from Micellar Casein Incredo Sugar® (S6-269)**

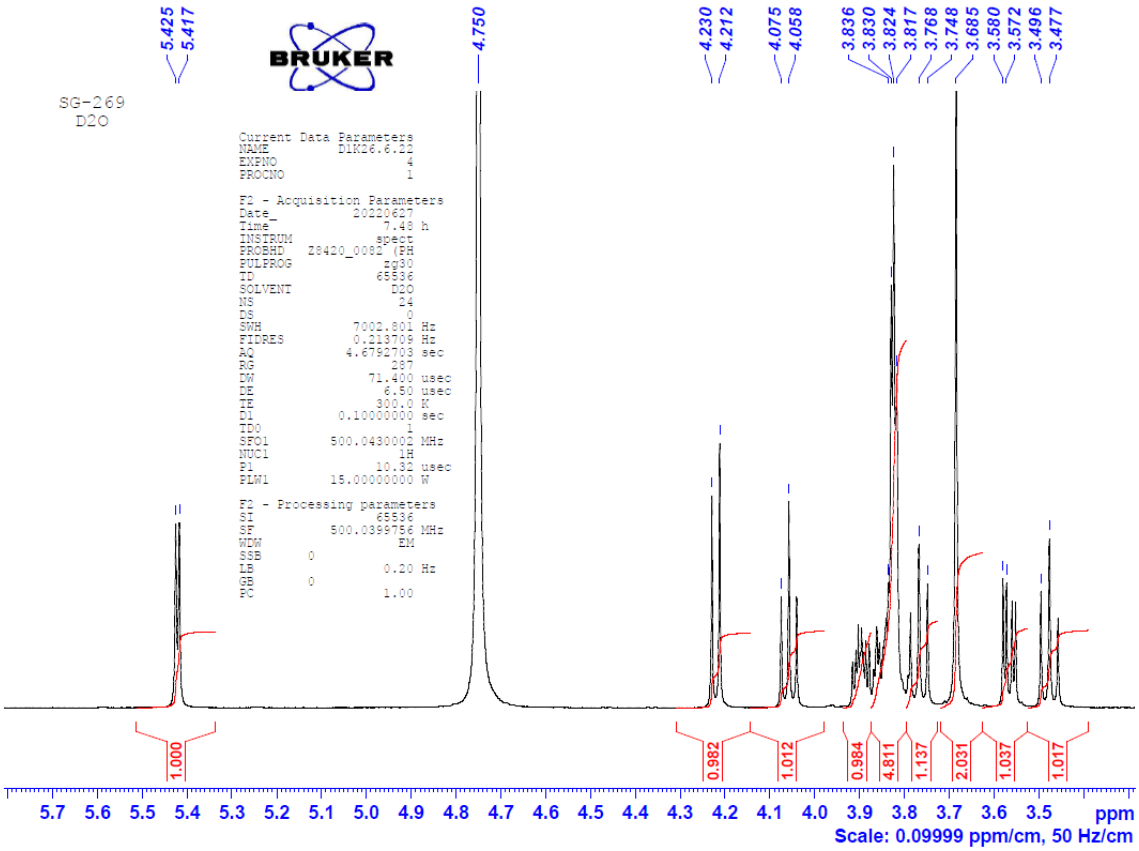


Figure 1.2-4 **H^1 NMR of Sucrose Isolated from Pea Protein Incredo Sugar® (S6-195)**

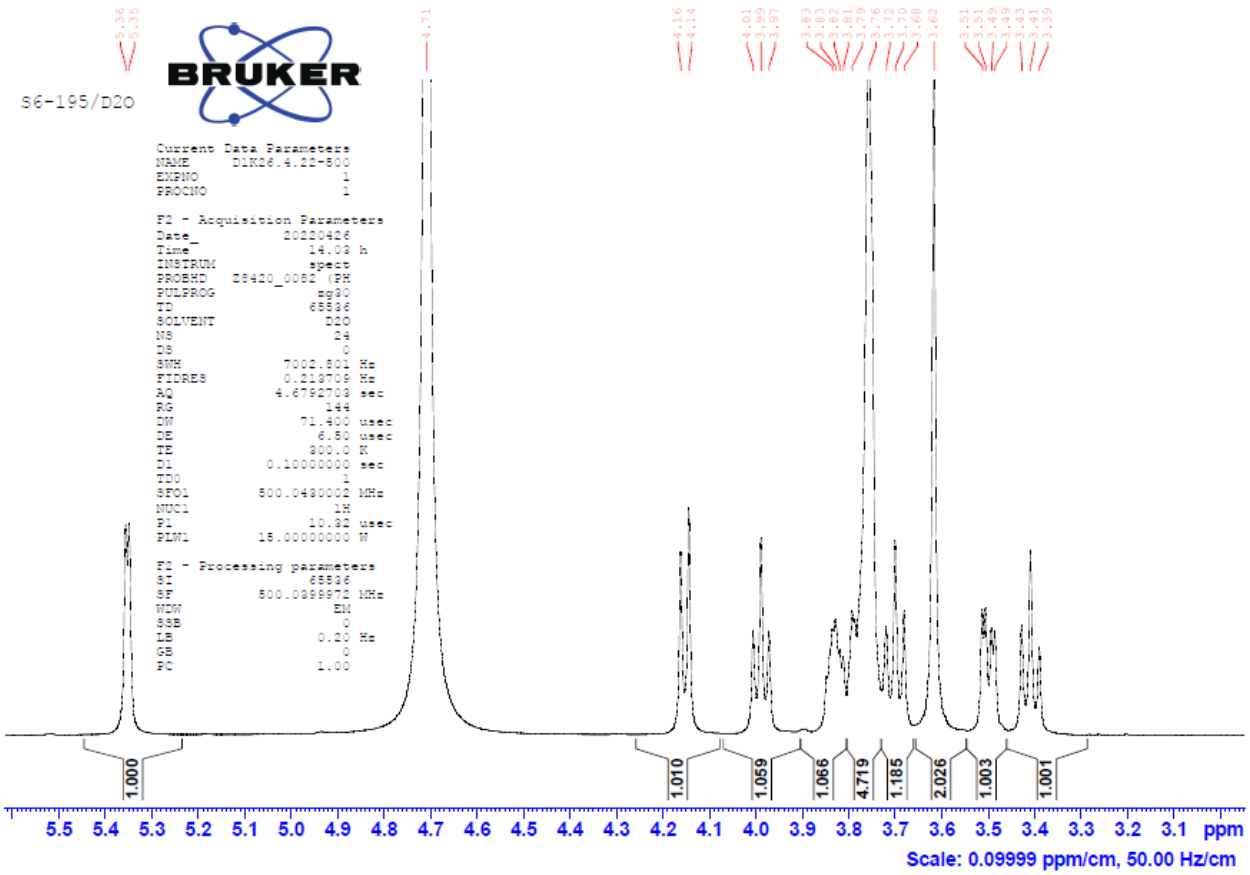


Figure 1.2-5 **H^1 NMR of Sucrose Isolated from Rice Protein Incredo Sugar® (S6SU170)**

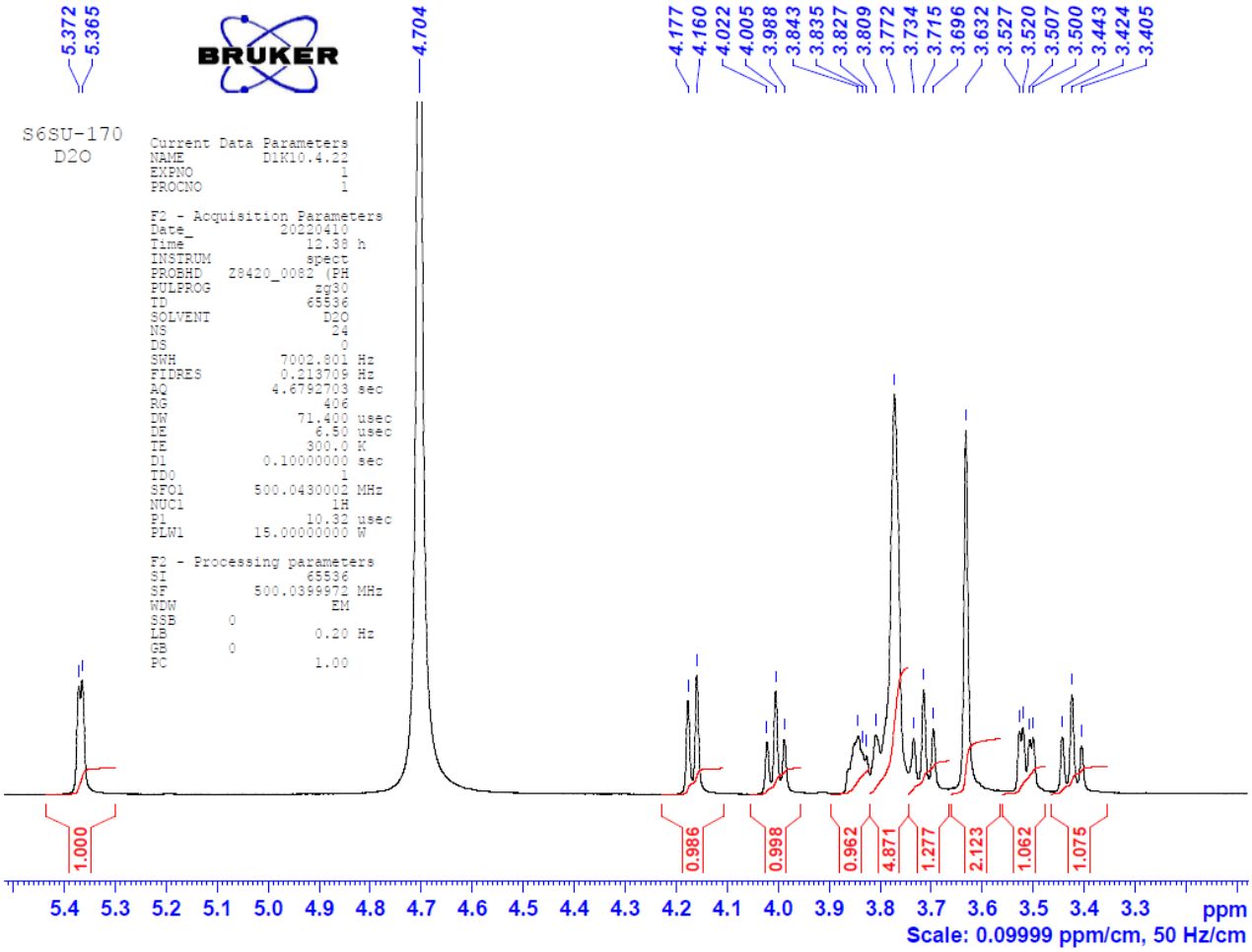
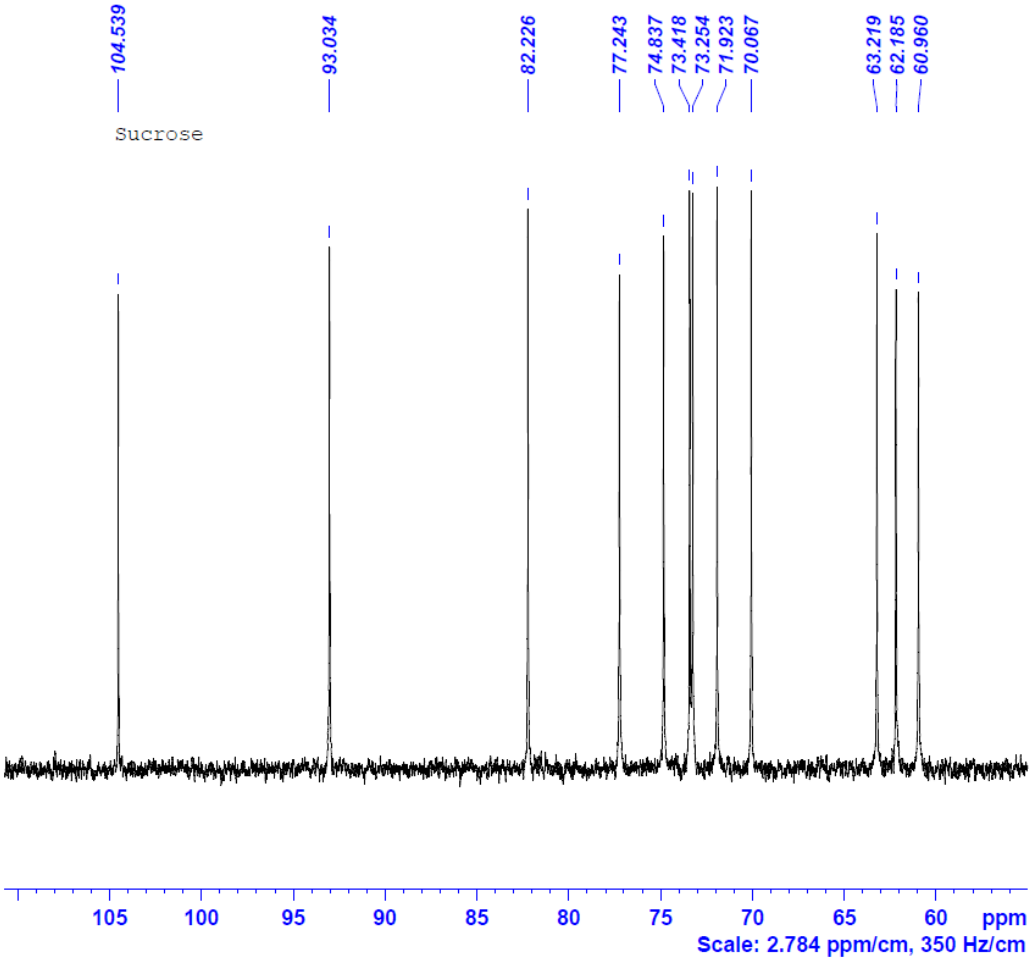


Figure 1.2-6 **C^{13} NMR of Pure Sucrose**

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DE                       6.50 usec
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Figure 1.2-7 **C^{13} NMR of Sucrose Isolated from Ca-Caseinate Incredo Sugar® (S6SU126)**

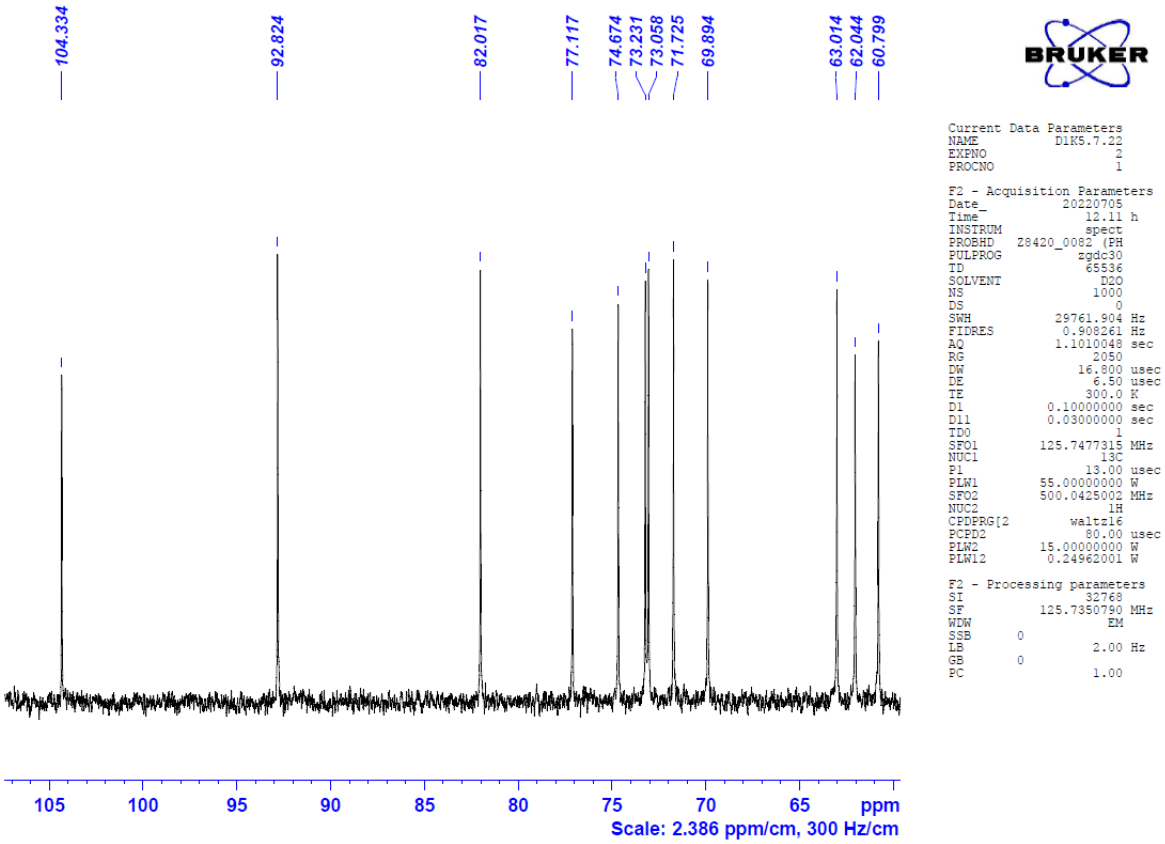

Figure 1.2-8 **C^{13} NMR of Sucrose Isolated from Micellar Casein Incredo Sugar® (S6-269)**

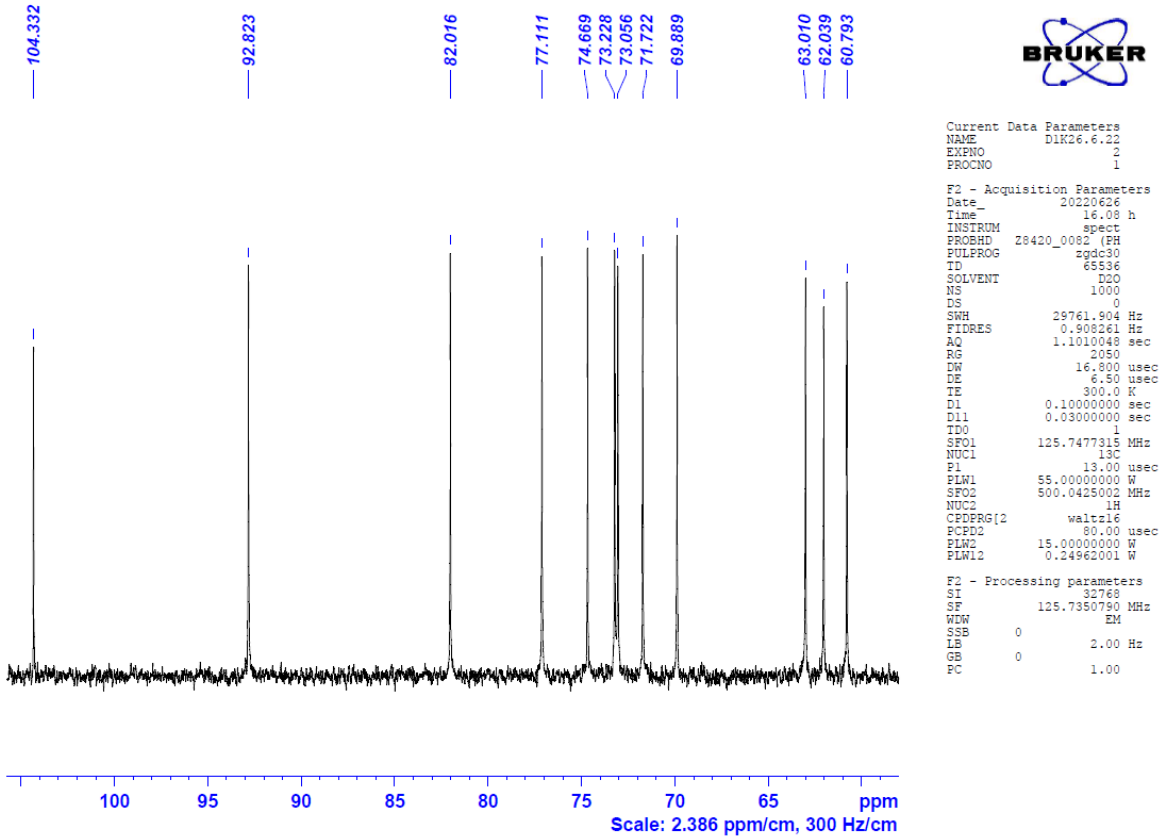

Figure 1.2-9 **C^{13} NMR of Sucrose Isolated from Pea Protein Incredo Sugar® (S6-195)**

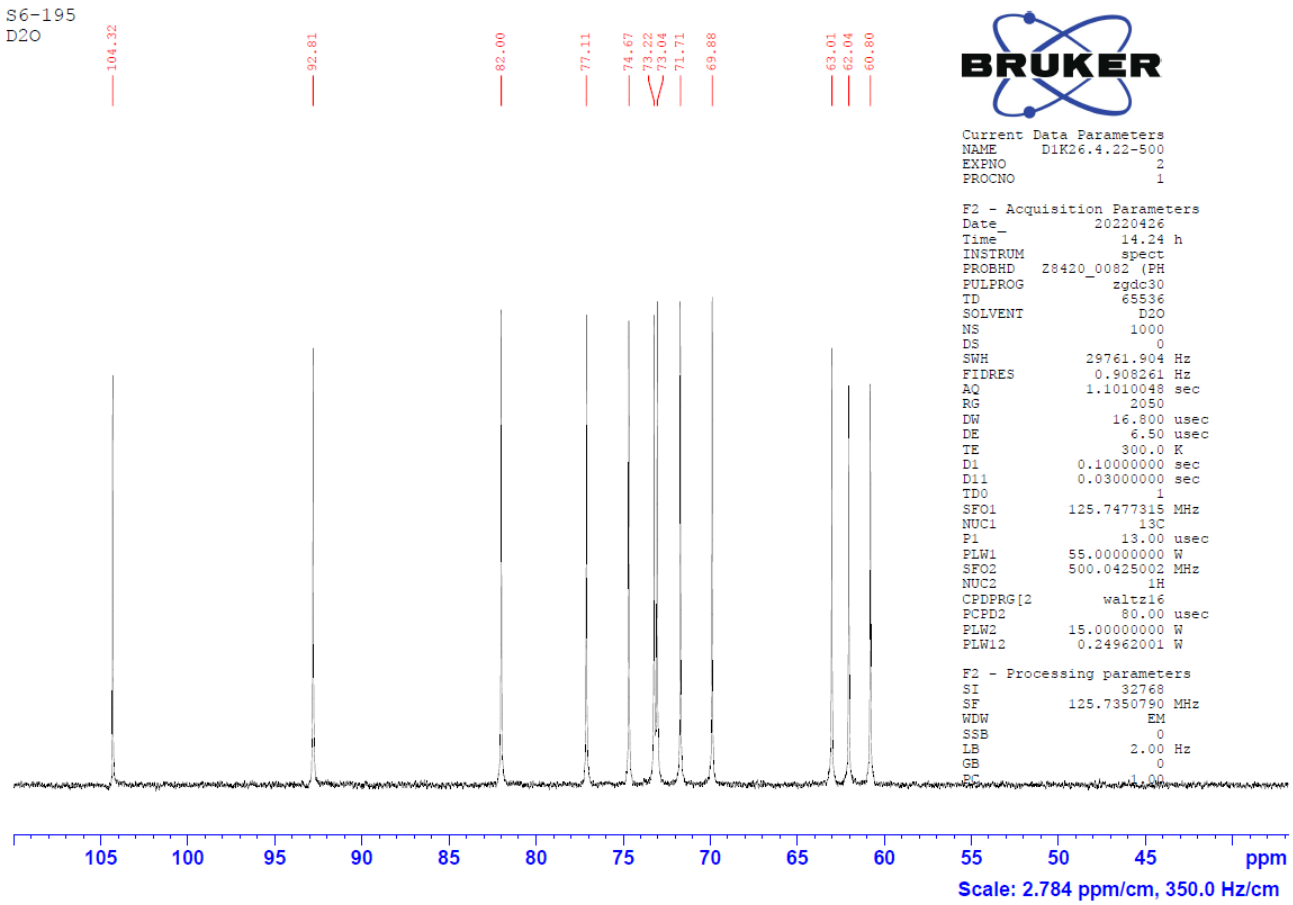

Figure 1.2-10 **C^{13} NMR of Sucrose Isolated from Rice Protein Incredo Sugar® (S6SU170)**

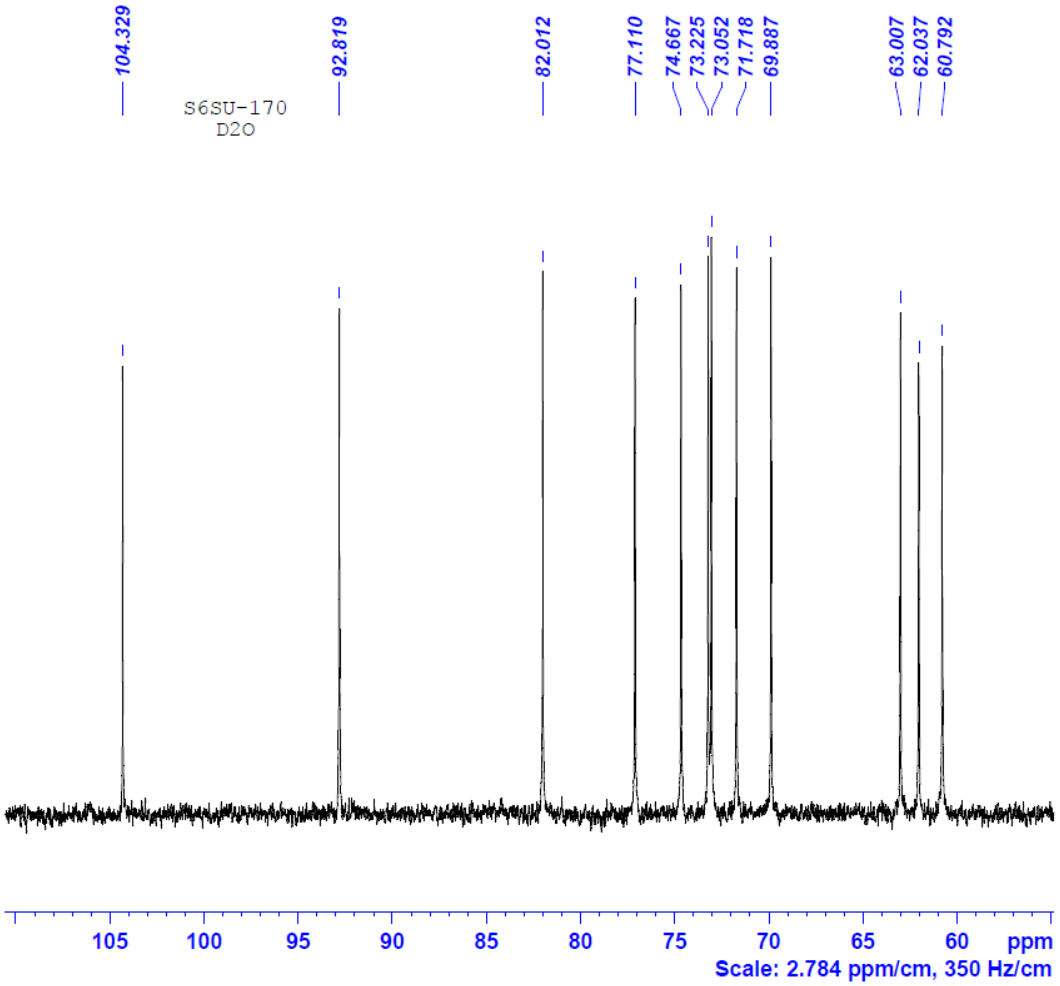


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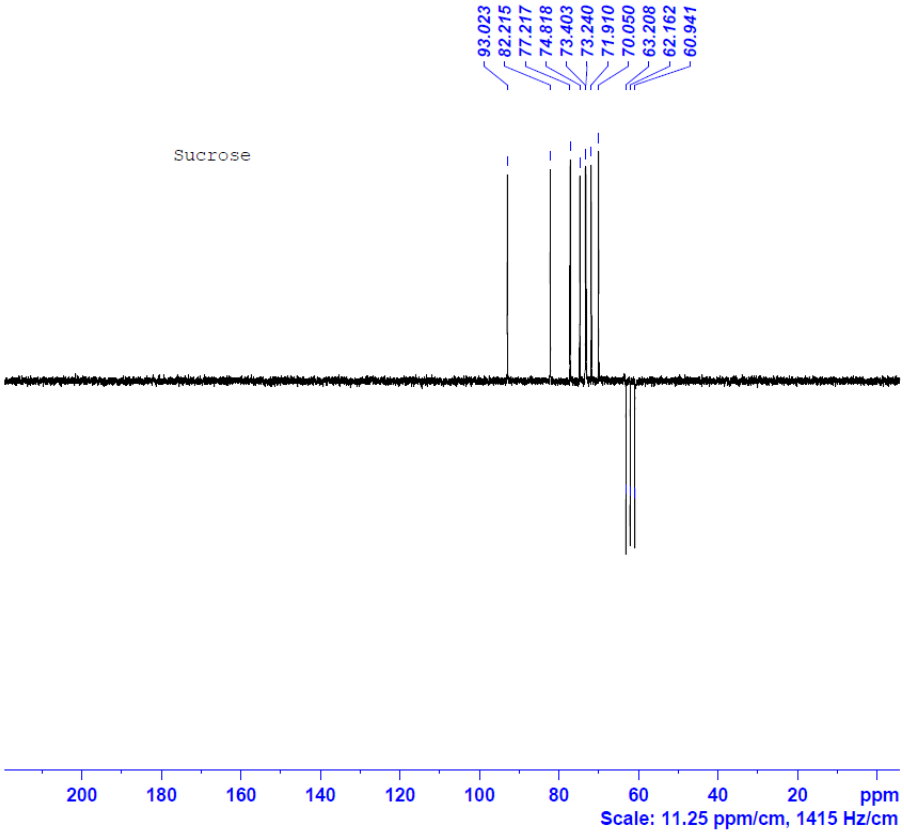
Figure 1.2-11 C^{13} DEPT of Pure Sucrose



BRUKER

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PLW1                55.00000000 W
SFO2                500.0425002 MHz
NUC2                        1H
CPDPRG[2                waltz16
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P4                       20.64 usec
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PLW12                0.24962001 W

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Figure 1.2-12 C^{13} DEPT of Sucrose Isolated from Ca-Caseinate Incredo Sugar® (S6SU126)

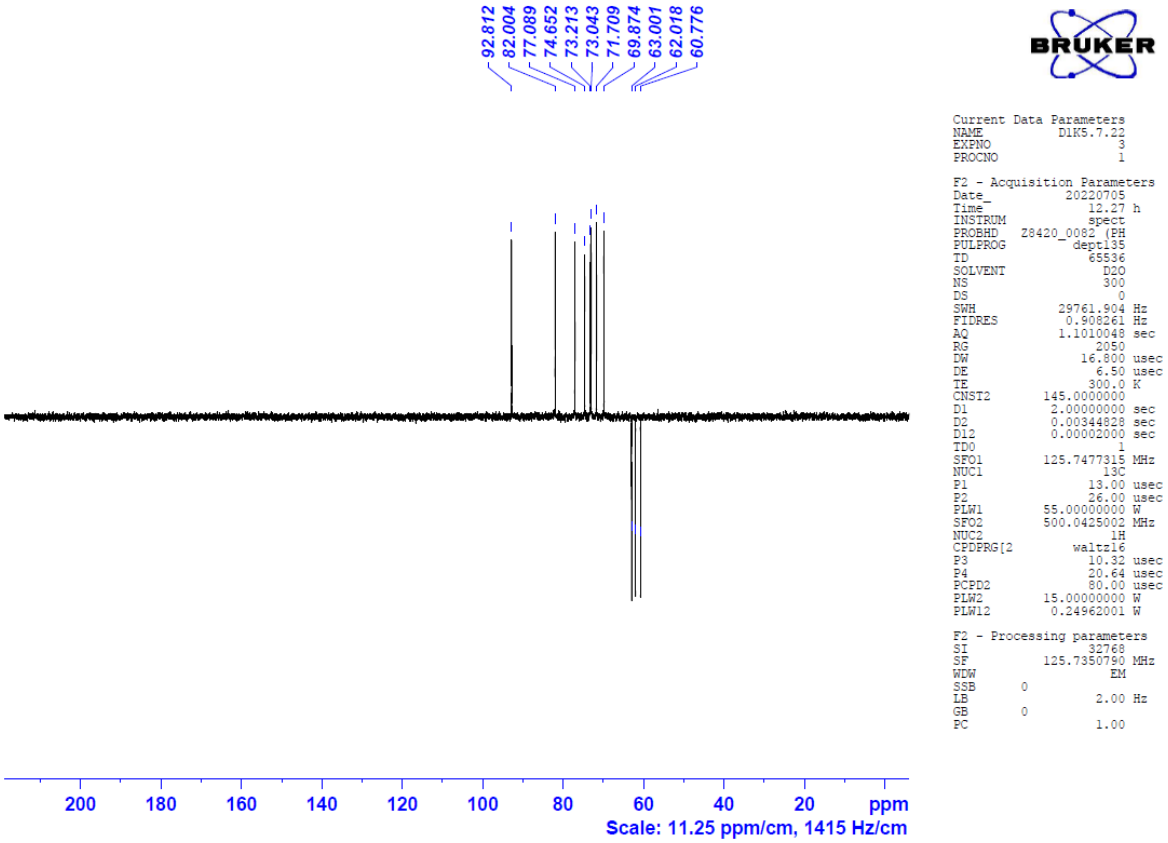


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Figure 1.2-13 C^{13} DEPT of Sucrose Isolated from Micellar Casein Incredo Sugar® (S6-296)

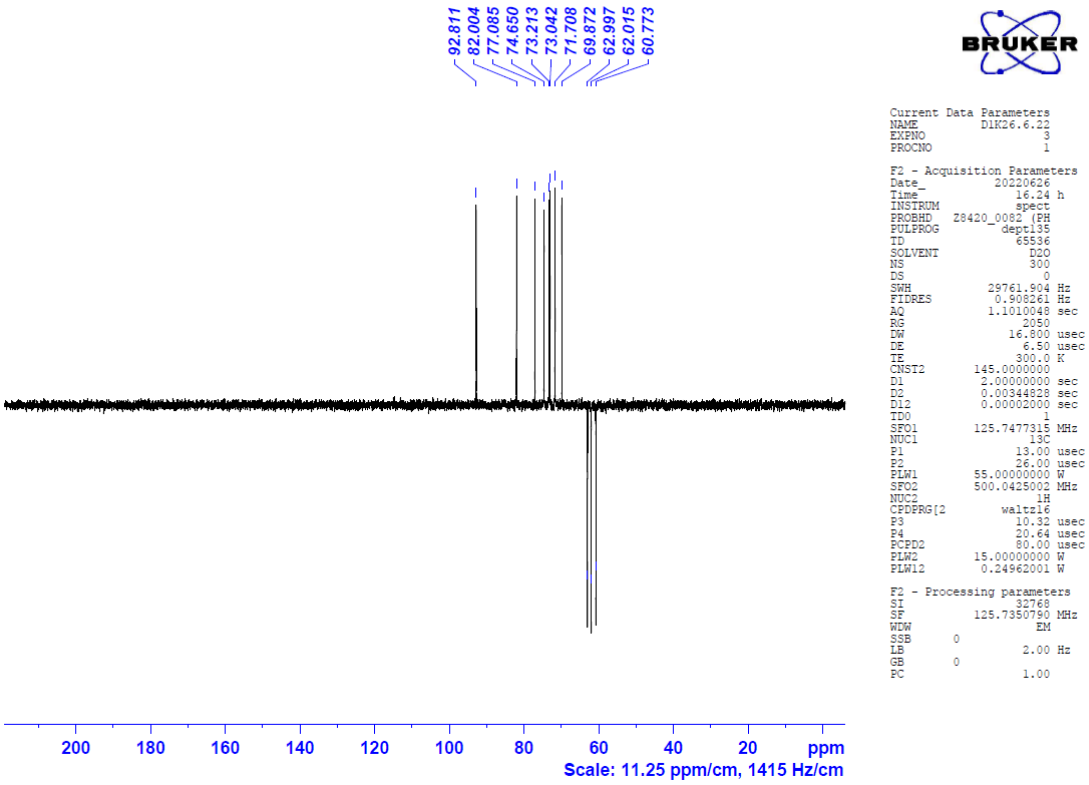


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Figure 1.2-14 **C^{13} DEPT of Sucrose Isolated from Pea Protein Incredo Sugar® (S6-195)**

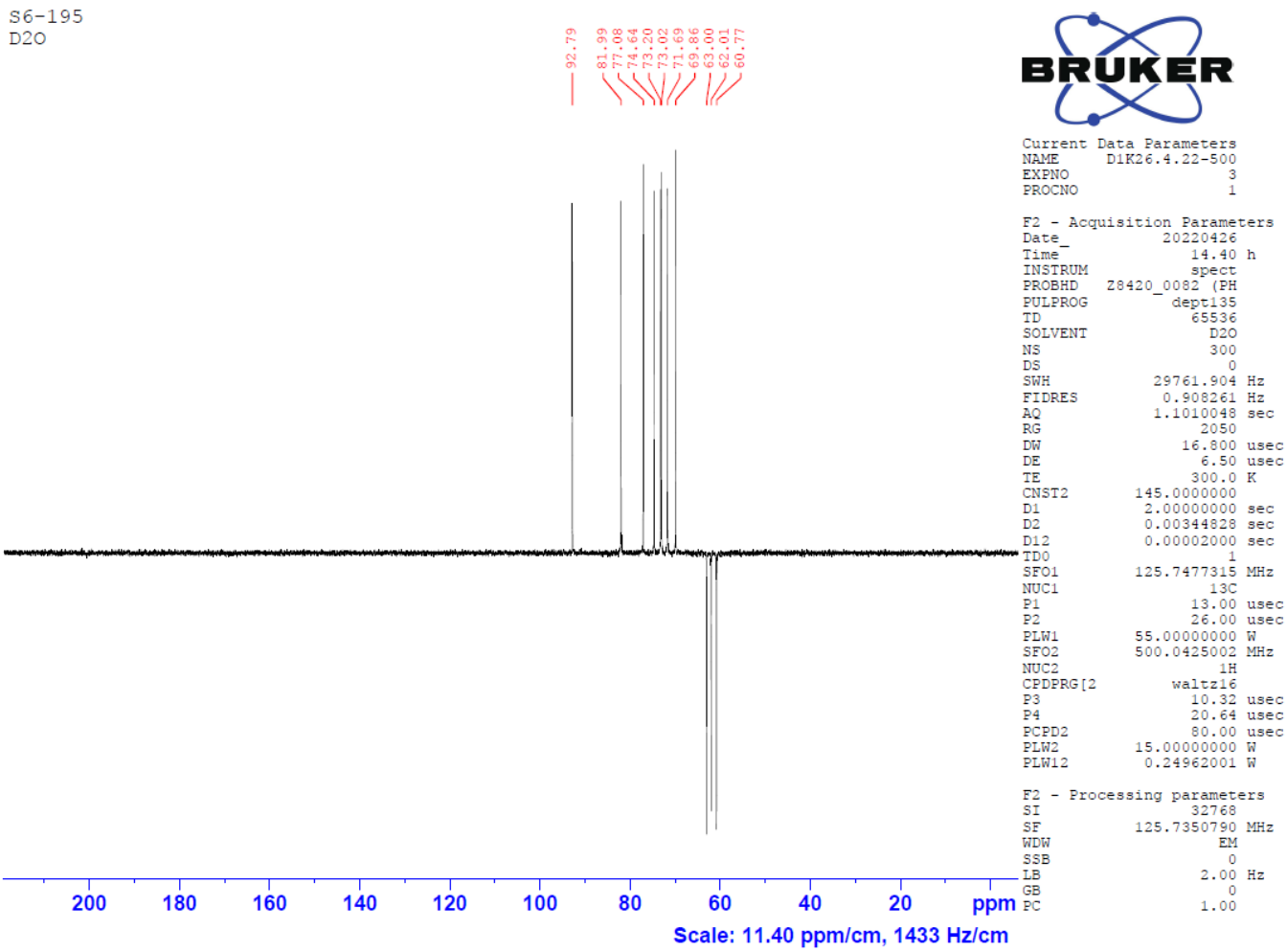
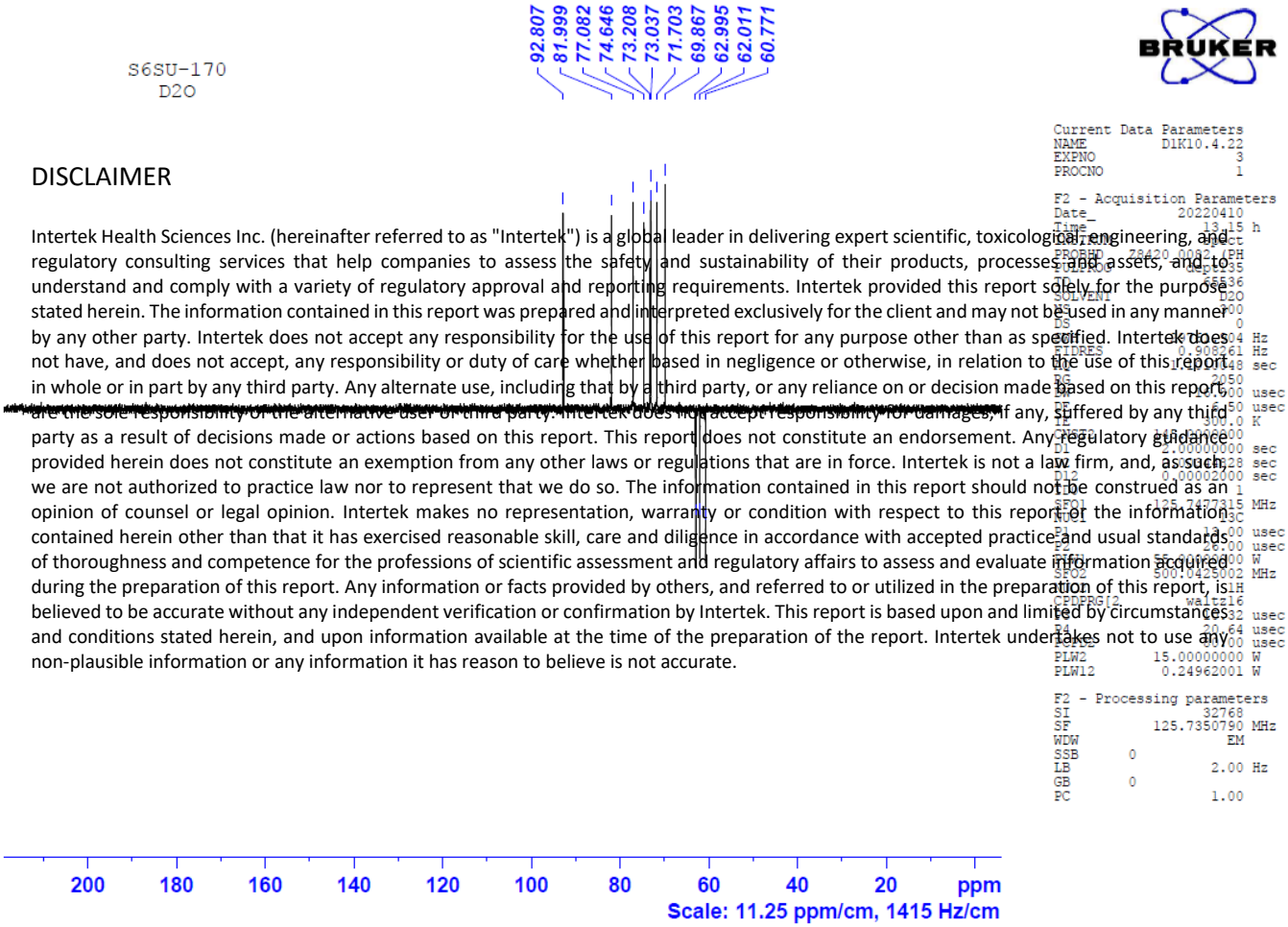


Figure 1.2-15 C^{13} DEPT of Sucrose Isolated from Rice Protein Incredo Sugar® (S6SU170)



GRAS Panel Evaluation of the Proposed Uses of Incredo Sugar® as an Ingredient in Foods and Beverages

23 September 2022

Introduction

At the request of DouxMatok Inc. (DouxMatok), a GRAS Expert Panel (the "Panel") of independent scientists, qualified by their relevant national and international 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, on Incredo Sugar® and determine whether, under the conditions of intended use as an ingredient in foods and beverages, Incredo Sugar® would be "Generally Recognized as Safe" (GRAS), based on scientific procedures. The Panel consisted of the below-signed qualified scientific experts:
Joseph F. Borzelleca, Ph.D., (Virginia Commonwealth University School of Medicine), George C. Fahey Jr., Ph.D. (University of Illinois), and Madhusudan G. Soni, Ph.D., F.A.C.N., F.A.T.S. (Soni & Associates Inc.).

The 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 searches of the published scientific literature conducted through September 2022 by Intertek. In addition, the Panel evaluated other information deemed appropriate or necessary, including data and information provided by DouxMatok. The data evaluated by the 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 summaries of the comprehensive literature on the safety of Incredo Sugar® and its individual components.

Following independent, critical evaluation of such data and information, the Panel met *via* teleconference on 21 September 2022, and unanimously concluded that under the conditions of intended use in traditional foods described herein, Incredo Sugar®, meeting appropriate food-grade specifications, and manufactured consistent with current good manufacturing practice, is GRAS based on scientific procedures. A summary of the basis for the Panel's conclusion is provided below.

Identity, Manufacturing, and Specifications

DouxMatok has developed a proprietary technology for increasing the sweetness perception of nutritive and non-nutritive sweeteners. This technology involves the creation of non-covalent complexes between the sweetener and various common food-grade protein ingredients. Food-grade proteins, in the form of either casein, calcium caseinate, pea protein, or rice protein, are mixed mechanically with sucrose using a high-shear mixer and then dried together to produce Incredo Sugar®. No chemical bonds are formed between the sweetener molecules and the protein; instead, the sweetener and proteins are held together *via* hydrogen and van der Waals bonding. Incredo Sugar® is not a new substance because no covalent bonds are formed in its production. Incredo Sugar® is an association between sucrose and protein ingredients; therefore, the subject of the GRAS Panel evaluation was the new intended use of the Incredo Sugar® and its components, sucrose and food-grade proteins. The safety of sucrose was not discussed as part of this GRAS evaluation because sucrose is universally recognized as a safe food ingredient, is GRAS under 21 CFR 182.1 and its use in food and beverage production is limited only by cGMP.

Protein Ingredients

The casein and calcium caseinate purchased and used in the production of Incredo Sugar® meet the specifications of the Food Chemicals Codex (FCC) monograph. Casein is manufactured from fresh, pasteurised skim milk using a low-heat membrane filtration process to ensure protein is undenatured. Casein undergoes additional microfiltration to slightly increase the casein-to-whey ratio from that which naturally occurs in milk. After membrane separation, casein is immediately spray dried and packaged in multi-wall paper bags with a polyethylene liner (net weight of 20 kg), palletized, and wrapped to units of 800 kg. It is also available in polyethylene-lined totes (net unit weight of 500 kg). Calcium caseinate is manufactured *via* acid preparation of casein from fresh skimmed milk. The casein is converted into its calcium salt by the addition of calcium hydroxide and the resulting product is milled and dried.

Pea protein is purified from the dry common yellow pea *Pisum sativum.* Pea proteins purchased for production of Incredo Sugar® are GRAS as nutritional protein ingredients and adhere to food-grade specifications provided by the supplier. The pea protein purchased from Roquette for production of Incredo Sugar® meets the specifications described in its corresponding GRAS notice: GRN 851. Manufacture of pea protein occurs *via* the following processes, as reported in GRN 851. Peas are physically cleaned and ground to remove hulls to produce a pea flour, which is a mixture of protein, starch, fibre, sugar, and fat. Water is added to the pea flour, and the pea starch and fibre are then removed. The protein goes through separation flocculation steps to adjust the pea protein at the isoelectric point (pI), which is where the proteins have the minimum solubility levels and are able to separate (isoelectric precipitation). The soluble pea protein is then removed from the pea protein isolate. The pea protein is then coagulated, purified, and re-buffered to neutral pH. Following the extraction process, a heat treatment is used to effectuate microbial reduction and reduce moisture. Food-grade enzymes (*e.g*., exopeptidase, endopeptidase, and aminopeptidase) are then used to enhance pea protein isolate functionalities, such as by decreasing viscosity. These added enzymes are destroyed with a thermal heat treatment before spray drying. The function of the enzymes is to split pea proteins *via* hydrolysis. This releases lower molecular weight peptides of shorter chain length, and amino acids. The final processing step includes drying the pea protein product in a spray dryer before it is packaged and stored.

Rice protein is derived from non-GMO *Oryza sativa* whole-grain brown rice. Rice protein purchased from Axiom Foods for production of Incredo Sugar® meets the specifications described in GRN 609. Rice protein is derived from the bran, germ, and endosperm extracted from whole-grain brown rice through a low-heat process. The whole-grain brown rice is received, tested, and approved for further processing. A hydrolysis process is performed to obtain whole brown rice protein (40 to 60% concentration). Amylase is used to separate protein from syrup solids, and only the whole brown rice protein concentrate is kept. A separation process is conducted to obtain whole brown rice protein concentrate. The concentrate is then washed, milled into the appropriate mesh, dried, and sterilised. It is packaged in 25-kg bags with inner polyethylene liners.

Incredo Sugar

For the production of Incredo Sugar®, food-grade protein is mixed mechanically with sucrose in solution and then dried. Analysis of 7 non-consecutive lots of Incredo Sugar® demonstrated that the manufacturing process produces a product that reproducibly meets appropriate food-grade specifications (see Table 1 in Appendix A).

Incredo Sugar® was further characterized by DouxMatok using differential scanning calorimetry, ion chromatography with electrochemical detection, and nuclear magnetic resonance spectroscopy on three batches of each protein type of Incredo Sugar® (casein, calcium caseinate, pea protein, or rice protein). The

results demonstrate that no other molecules are created during the manufacture of Incredo Sugar®, and the inclusion of protein ingredients achieve the intended effect of increasing the dissolution of sugar.

DouxMatok also conducted the following tests on three batches of each protein type of Incredo Sugar®: attenuated total reflection with Fourier-transform infrared spectroscopy and sodium dodecyl sulfate–polyacrylamide gel electrophoresis. This data was evaluated by the Panel and the Panel concluded that no sugar-protein interactions occurred following the dissolution of Incredo Sugar® in water. Incredo Sugar® is completely dissociated into protein and sugar.

DouxMatok conducted stability studies to analyze accelerated microbiology stability studies. The temperature and relative humidity utilized in this study was 40° ± 2 C and 75% ± 5% relative humidity, respectively. Batches were tested in 4-time intervals to be equivalent to 24 months (*e.g.*, T0, T1 = 8 months, T2 = 16 months, T3= 24 months). Data from T3 has yet to be analyzed. However, it may be concluded that Incredo Sugar® is stable for at least 16 months.

Intended Use and Estimated Exposure

DouxMatok intends to market Incredo Sugar® as an ingredient in sugar at levels ranging from 0.01 to 0.8% protein, as shown in Table 2 in Appendix B.

Consumption data and information pertaining to the intended food uses of protein ingredients were used to estimate the *per capita* and consumer-only intakes of protein ingredients for specific demographic groups and for the total U.S. population. There were a number of assumptions included in the assessment that render exposure estimates suitably conservative. For example, it was assumed that all food products within a food category contain protein ingredients 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 protein ingredients will have 100% market penetration in the food category of sugar.

On a consumer-only basis, the resulting mean and 90th percentile intakes of protein ingredients by the total U.S. population from proposed food uses of Incredo Sugar® in the U.S. were estimated to be 122.66 mg/person/day (1.74 mg/kg body weight/day) and 274.66 mg/person/day (4.06 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes of protein ingredients were determined to be 141.34 mg/person/day (1.70 mg/kg body weight/day) and 368 mg/person/day (4.16 mg/kg body weight/day), respectively, as identified among male adults. While infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 32 and 48 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest mean daily intake of 2.70 mg/kg body weight/day while young children had the highest 90th percentile intake estimate of 5.50 mg/kg body weight/day. Data relevant to estimated exposure are presented in Table 3 and 4 in Appendix B.

The intake of additional sucrose from the proposed uses of Incredo Sugar® is not necessary to evaluate due to the long history of safe consumption of sucrose and to the universal recognition of this by regulatory agencies world-wide.

Data Pertaining to Safety

The safety of Incredo Sugar® is based on a substantial body of evidence supporting the safety of sucrose, casein, calcium caseinate, pea protein, and rice protein. It has been definitively established that there are no chemical interactions between the proteins used in the manufacture of Incredo Sugar® and sucrose. These proteins are chemically representative of the protein ingredients that were either (i) concluded as GRAS by the Select Committee on GRAS Substances (SCOGS) or (ii) previously concluded to be GRAS by an Expert Panel with notification to FDA (*i.e.*, GRNs 609 and 851). A discussion of publicly available data and information relevant to the safety of these protein ingredients is incorporated by reference to pivotal studies discussed in the SCOGS report or GRNs 609 and 851. The safety of sucrose is not discussed as part of this narrative because it is universally recognized as a safe food ingredient and is GRAS under 21 CFR 182.1.

To identify new data pertinent to the safety of each selected protein published since its GRAS status was last evaluated, a comprehensive search of the published scientific literature was conducted by Intertek through September 2022. The search was conducted using the electronic search tool, ProQuest Dialog™, with several databases, including Adis Clinical Trials Insight, AGRICOLA, AGRIS, Allied & Complementary Medicine™, BIOSIS® Toxicology, BIOSIS Previews®, CAB ABSTRACTS, Embase®, Foodline®: SCIENCE, FSTA®, MEDLINE®, NTIS: National Technical Information Service, and ToxFile®. Based on this updated search of the literature, it was by concluded by the Panel and by DouxMatok that there are no newly published studies that suggest that the selected proteins would be unsafe when used as a food ingredient.

A summary of the pertinent toxicological studies from prior GRAS notifications and newly identified studies or publicly available scientific evaluation relevant to the safety of each selected protein was critically evaluated by the Panel. Based on conclusions from previous expert panels on the GRAS status of each protein, corresponding “no questions” letters issued by the FDA, the widespread history of use of such proteins and sucrose as food ingredients globally, and conclusions from other authoritative and scientific bodies on the safety of selected proteins (*e.g.*, SCOGS) and sucrose, the Panel concluded that the current GRAS status of each protein and sucrose can be extended to their use in the manufacture of Incredo Sugar®.

The Panel, therefore, concluded that sucrose, casein, calcium caseinate, pea protein, and rice protein, as described herein, are GRAS, for use in the production of Incredo Sugar®, based on scientific procedures. The available data related to the safety of each protein and sucrose are summarised below.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The metabolic pathway of all protein ingredients is expected to imitate any other protein ingredient in the human diet.

Toxicological Studies on the Ingredients Used in the Manufacture of Incredo Sugar

Casein and Calcium Caseinate

The published scientific literature regarding the safety of casein and calcium caseinate was previously reviewed by SCOGS in 1979. Since the SCOGS review, several GRAS conclusions were notified to the FDA and received "no questions" responses (GRN 011, GRN 633). An updated search of the published scientific literature conducted from late-2015 did not reveal new toxicological studies relevant to the safety of casein or calcium caseinate. However, several studies administering casein in the diet to laboratory animals as part of efficacy studies, or as controls in safety studies of unrelated test articles were identified (Jones *et al.*, 2015; Singh *et al.*, 2016; Fuc *et al.*, 2019; Shahkhalili *et al.*, 2020; de Gaudry *et al*., 2021; Roman *et al.*, 2021; AL Tamimi *et al*., 2022; Menikdiwela *et al*., 2022; Zhang *et al*., 2022; Zhao *et al*., 2022). Further, several clinical trials administering casein to human subjects were identified (Mariotti *et al.*, 2015; McDonald *et al.*, 2017; Wu *et al.*, 2017; Kaskous, 2020; Yuda *et al.*, 2021; Chen *et al*., 2022) with no reported adverse effects. These studies did not report results contradicting the conclusions reported in the SCOGS report. Therefore, critical studies from the SCOGS report are detailed below.

The SCOGS conducted a comprehensive review of the use, exposure, and safety of casein and caseinate salts in 1979 and stated that casein and various caseinates occur in mammalian milk, and thus are part of the normal human diet (FASEB, 1979). The SCOGS reported acute, short-term, and long-term feeding studies in a variety of species administered casein and caseinates. Adverse effects (primarily kidney injury) were reported in high-casein diets (75% dietary inclusion of casein, approximately equivalent to 75,000 mg/kg body weight/day); however, SCOGS stated this toxicological effect is associated with high dietary protein intake, and not specific to casein (Lalich *et al.*, 1970). Of the studies reviewed by SCOGS, the administration of 20% dietary casein (equivalent to 20,000 mg/kg body weight/day) elicited no major adverse effects. While SCOGS did not establish an acceptable daily intake (ADI) from the studies presented, safety factors may be utilized to account for interspecies and interindividual toxicodynamic and toxicokinetic differences, to establish an ADI of 200 mg casein/kg body weight/day in humans (safety factors = 100).

The estimated intake of casein and caseinates was projected by SCOGS to be approximately 200 mg/person/day on a *per capita* basis (approximately equivalent to 3.33 mg/kg body weight/day for a 60-kg adult). It should be noted that SCOGS did not analyze casein or caseinates on a consumer-only basis. As casein and caseinates represent a minor contribution to the total average daily intake of protein, SCOGS stated, "*there is no evidence in the available information on casein, sodium caseinate, or calcium caseinate that demonstrates or suggests reasonable grounds to suspect a hazard when they are used at levels that are now current or that may reasonably be expected in the future*" (FASEB, 1979). It should be noted that the estimated exposure to protein ingredients based on their inclusion in sugar (Section 3.3.2) demonstrated that the total population on a consumer-only basis would be exposed to a mean of approximately 122.66 mg protein/day (2.42 mg/kg body weight/day). At the 90th percentile level, exposure would be approximately 274.66 mg protein/day (4.06 mg/kg body weight/day). While this value is above the exposure level reported by SCOGS, it is lower than the acceptable daily intake derived from animal toxicity studies (ADI = 200 mg/kg body weight/day).

Pea Protein

The published scientific literature has been reviewed in several previous GRNs, most recently in 2021 (GRN 948). An updated search of the published scientific literature was conducted through September 2022. Results from this search indicate that one toxicological study relevant to the safety of pea protein was published in the

specified time frame (Hidayat *et al*., 2022). The details of this study are described below. Several studies administering casein in the diet of laboratory animals as part of efficacy studies were identified (Liu *et al.*, 2021; Salles *et al.*, 2021; Scuderi *et al*., 2022). A review of these studies indicates that the results do not contradict conclusions reported in GRN 851 or GRN 948.

Once daily, all rats were weighed and underwent visual observations for mortality, behavioral patterns, changes in physical appearance, injury, and signs of illness. At the end of the experiment, all animals were euthanized. Blood samples were collected for biochemical and hematological analyzes. The organs were excised, weighed, and examined macroscopically and relative organ weight was calculated. The liver and kidney were preserved for histopathological study.

There were no deaths and no treatment-related adverse effects reported in any animals. After 28 days without treatment, the bodyweights of male and female control satellite rats were not statistically different from the average body weight of rats in the high dose group ($p>0.05$). The food and water consumptions of the treated rats was not significantly different compared to control rats. The relative organ weights of each organ recorded at necropsy in the treatment groups did not show a significant difference ($p>0.05$) compared to the controls.

There were no treatment-related adverse effects in any hematological parameter evaluated including leucocyte (total white blood cell), erythrocyte (red blood cell), hemoglobin, hematocrit, MCV, MCH, MCHC, lymphocyte, monocyte, neutrophil, eosinophil, thrombocyte (platelet), RDW (red cell distribution width), and PDW (platelet distribution width). The authors reported triglyceride levels of female control rats were significantly higher compared to all treated rats. No statistically significant differences in liver function parameters (ALT and AST) were reported. In the highest dose group, it was reported that male rats had no impairment of renal function parameters (urea, creatinine, and uric acid). However, the low and moderate doses in male rats and low doses in female showed significant differences as compared to the control, even though the creatinine levels were still within the normal range, and the urea levels of all rats, both control and treatment groups, were reported to be above the normal range. In female rats, the highest dose did not affect urea and creatinine parameters, but there was an increase in uric acid levels. Other blood biochemical profiles (lipid profile: total cholesterol, LDL, HDL) and glucose, did not differ significantly from controls.

The authors reported no abnormalities in the macroscopic examination of the vital organs of treated animals when compared with the organs of the control group. The authors reported normal structure and absence of any gross pathological lesion in organs, except in the median score of liver histopathology parameters for lobular inflammation of the high dose group, which was 3, higher as compared to the control group which was 1. Lobular inflammation indicates early-stage inflammation of the hepatocytes; however, the inflammation was mild to medium. After 28 days of recovery, the high-dose satellite group showed significant improvement (total score 0). The authors reported that the results of the median score of kidney histopathology using the Kruskal Wallis test was $p>0.05$, indicating no significant difference between groups.

The authors concluded that the NOAEL of the green pea protein hydrolysate “in the sub-chronic toxicity study was the dose of 200 mg/kg bw” (Hidayat *et al*., 2022), the second highest dose tested, based on lobular inflammation in the liver observed in the highest dose groups**.** Given the crude production method of the test article in this study, it is considered of little relevance to DouxMatok’s pea protein, which is produced according to cGMP and with various enzymes that are denatured during processing (unlike bromelain in this study).

Roquette (supplier of pea protein for the manufacture of Incredo Sugar®) conducted a series of safety studies detailed in GRN 851. In the first study, 3 female Wistar rats and 3 CD1 female mice were administered pea protein isolate *via* oral intubation at 2,000 mg/kg body weight (Aouatif *et al.*, 2013a). The studies were

conducted according to the Organization for Economic Cooperation and Development (OECD) Guideline 423 (*Acute oral toxicity - acute toxic class method*). None of the animals exhibited any signs of dullness, abnormal body posture, tremors, seizures, restlessness, weight gain decrement, or any other signs of toxicity. The authors reported that an oral dose of 2,000 mg/kg body weight of pea protein isolate did not produce toxicity in any of the treated animals, and that the LD_{50} of pea protein isolate taken orally was higher than 2,000 mg/kg body weight. According to OECD Guideline 423, substances that have an oral LD_{50} higher than 2,000 mg/kg body weight can be considered non-toxic (OECD, 2001).

In the second study reported by Aouatif *et al.* (2013b), Wistar rats of both sexes were fed pea protein isolate in the diet at concentrations of 25,000 ppm, 50,000 ppm, and 100,000 ppm, according to OECD Guideline 408 (*Repeated dose 90-day oral toxicity study in rodents*). After acclimation, animals were randomly distributed into 6 groups (10/sex/group) namely: control (0.0 ppm), low-dose (25,000 ppm), intermediate-dose (50,000 ppm), high-dose (100,000 ppm), satellite control (0.0 ppm), and satellite high-dose (100,000 ppm). The test substance was administered daily in the diet for 90 days. Food and water intake were measured once daily and reported weekly. Animals were weighed once weekly. Rats in the satellite groups were administered the control diet without the test article for an additional 28 days to evaluate any possible withdrawal effects. All animals were individually observed once daily for clinical signs. All animals were observed for functional observational battery (FOB) parameters prior to the administration of the test substance, during the 13th week for the main groups, and during the 17th week for the satellite groups. The authors reported no test-article-induced toxigenic effects, and concluded that the no-observed-adverse-effect level (NOAEL) of pea protein isolate in Wistar rats can be defined as 100,000 ppm of diet (equivalent to 8,726 mg/kg body weight/day for males and 9,965 mg/kg body weight/day for females). Based on these findings, the authors concluded that "*Pea Protein can be considered as non-toxic when administered through diet*" (Aouatif *et al.*, 2013b).

The genotoxic and mutagenic potential of pea protein isolate was assessed in the Ames assay with 5 tester strains of *Salmonella* typhimurium (TA100, TA102, TA1535, TA98, and TA1537), in presence and in absence of metabolic activation (S9). It was also evaluated *in vivo* utilizing the mouse micronucleus assay and evaluated for its capacity to induce structural and numerical aberrations in an *in vitro* chromosomal aberration test using cultured human peripheral blood lymphocytes (Aouatif *et al.*, 2013c). Under the conditions of these assays, pea protein was reported to neither induce genotoxicity nor mutagenicity.

The estimated exposure to protein ingredients based on their inclusion in sugar demonstrated that the total population on a consumer-only basis would be exposed to a mean of approximately 122.66 mg protein/day (2.42 mg/kg body weight/day). At the 90th percentile level, exposure would be approximately 274.66 mg protein/day (4.06 mg/kg body weight/day). This level is approximately 2,000 times lower than the NOAEL of 8,726 mg/kg body weight/day.

Rice Protein

A minimally processed rice protein ingredient has been previously concluded to be GRAS for uses in a variety of conventional food products (GRN 609) at use levels of up to 34.3% (identical to the rice protein sourced by DouxMatok). The ingredient was produced from whole grain brown rice (*Oryza sativa*) that is milled and hydrolyzed using amylase in water. The liquid hydrolysate is then removed leaving behind the crude rice protein, which is washed and dried and milled to produce a protein powder. Using information on the intended uses in conjunction with survey data from NHANES 2011-2012 (USDA, 2014; CDC, 2015), GRAS uses of rice protein were estimated to result in total population all-user dietary intakes of rice protein of 10.3 g per person per day (181 mg/kg body weight) and 17.3 g per person per day at the mean and the 90th percentile, respectively. Data and information supporting the safety of rice protein included nutritional and compositional

comparisons demonstrating that rice protein contains a similar amino acid composition to other protein ingredients such as whey and soy proteins that are generally considered safe. The notice described data demonstrating that Chinese wild brown rice was not mutagenic as assayed by bone marrow micronucleus, sperm abnormality, and a reverse mutation assay using *S. typhimurium*. The safety of rice protein was also supported by published studies conducted in rats and humans that reported no adverse effects (Prakash *et al.,* 1996; Zhai *et al.*, 1996; CIR, 2006; Gottlob *et al.*, 2006; Lasekan *et al.*, 2006; Koo and Lasekan, 2007; Khan *et al.*, 2011; Joy *et al.*, 2013; Sauer *et al.*, 2012; Axiom Foods, Inc. / SPRIM Strategy & Intelligent Innovation, 2015).

Another rice protein ingredient (hydrolyzed rice protein) was concluded to be GRAS in 2020 (GRN 944). This GRAS evaluation incorporated, by reference, data and information supporting safety previously described in GRN 609. The notifier conducted an updated literature search for the safety information on rice protein or hydrolyzed rice protein published since GRN 609 through April 2020 and reported that no new relevant safety studies were identified.

DouxMatok conducted an updated search of the published scientific literature to obtain data and information relevant to safety published since the latest safety evaluation of rice protein in 2020 (GRN 994). Search results yielded 2 studies, Li *et al.* (2021) and Hajimohammadi *et al.* (2021), which contain information that is relevant to the safety evaluation of rice protein, and that are described below. An additional efficacy study in rodents performed utilizing a genetically modified rice (Hajimohammadi *et al.*, 2022) and 1 human trial administering a rice protein isolate to healthy male individuals (Tiekou Lorinczova *et al.*, 2021) were identified, but have been omitted due to limited relevance.

Li *et al.* (2021) conducted a 90-day dietary toxicity study administering a genetically modified rice producing phytase-lactoferricin fusion protein, BPL9K-4, to Sprague-Dawley rats (10 rats/sex/group). Groups were administered either BPL9K-4 (10.9% protein), 9 K (a non-transgenic parental rice, 12.6% protein), or Weiyou64 common rice (7.8% protein). BPL9K-4 and 9 K rice were formulated into diets at concentrations of 15%, 30%, and 60%, while Weiyou64 common rice was added to diets at a concentration of 60% (equivalent to 1.635%, 3.27%, and 6.54% rice protein in the diet for BPL9K-4; equivalent to 1.89%, 3.78%, and 7.56% rice protein in the diet for 9 K; equivalent to 4.68% rice protein in the diet for Weiyou64). AIN93G diet was set as a basal diet control. The study was conducted in compliance with the guideline for safety assessment of genetically modified plant and derived products 90-day feeding test in rats (NY/T 1102–2006, Ministry of Agriculture of China) which is generally consistent with the related OECD guideline,[1] and in accordance with OECD Good Laboratory Practice. The authors reported no test-article-induced toxigenic changes occurring from the administration of different rice ingredients. The authors concluded that "*the BPL9K-4 transgenic rice exhibited no toxic effects on rats when compared with its conventional comparators as presented in this 90-day subchronic study*" (Li *et al.*, 2021). There are no findings in this study to call into question previous conclusions on the GRAS status of rice protein as a food ingredient, since these findings support conclusions from previous studies of rice protein. Levels administered in this study (1.635% to 7.56% in the diet) are approximately equivalent to 1635 mg/kg body weight/day and 7560 mg/kg body weight/day.[2]

Hajimohammadi *et al.* (2021) conducted a 90-day dietary toxicity study administering a genetically modified rice expressing Cry1Ab protein, an insect-resistant protein, to Sprague-Dawley rats (20 rats/sex/group). Rats were divided into the following groups: Group A: standard feed with substitution of 50% carbohydrate with *Tarom Molaii* rice; Group B: standard feed with substitution of 50% carbohydrate with genetically modified

[1] This guideline does not contain certain clinical chemistry parameters such as sodium, potassium, and urea that are present in OECD guidelines.
[2] Calculated using PAFA Conversion Table (U.S. FDA, 1993).

Bacillus thuringiensis rice (GM Bt rice); and Group C: standard feed. Protein content of rice was not reported. During the 90-day experimental period, clinical observations of the rats were conducted twice daily for mortality, abnormal signs, and unusual behaviours, while bodyweight and food consumption were measured each week. After 90 days, haematology, biochemistry, and urinalysis analyses were conducted. At terminal sacrifice, organs were sampled for gross and histopathological examination. The authors reported no test-article-related toxigenic effects and concluded that "*GM Bt rice showed no unintended obvious adverse effect on the health of rats*" (Hajimohammadi *et al.*, 2021). There are no findings in this study that call into question previous conclusions on the GRAS status of rice protein as a food ingredient.

Rice protein has been concluded as GRAS as an ingredient in a variety of food categories at levels ranging from 0.96 to 34.3% (GRN 609). There is a long history of safe uses of rice as a food staple, but there is lack of well-designed animal or human studies investigating the toxicity or adverse effects of rice or its constituents, including protein. Like all dietary protein, rice protein concentrate is digested in the human gastrointestinal tract. Based on amino acid profiles, rice protein is similar to whey and soy protein, both of which are GRAS. The notifiers reported the results of the available limited animal and human studies, which did not reveal any adverse effects of rice protein concentrate. The conclusion of GRAS status for rice protein as a food ingredient was, therefore, based on a comparison of dietary levels of rice intake to intake based on the proposed intake of rice protein. According to GRN 609, at levels ranging from 0.96% to 34.3%, inclusion of rice protein in a variety of food categories will result in a daily maximum intake (90th percentile) of 20.5 g/person/day. Compared to this value, the intake (90th percentile) of protein (24.96 g/person/day) from the consumption of rice as a staple is higher. If rice protein were to be added to sugar, at the 90th percentile intake level, exposure would be approximately 274.66 mg protein/day, or 4.06 mg/kg body weight/day. This level is inconsequential when compared to the dietary intakes discussed in GRN 609. Additionally, the highest level of rice protein administered (7560 mg/kg body weight/day) as reported in Li *et al.* (2021) is 1,800 times greater than the 90th percentile intake level calculated for protein use in sugar. Therefore, there is reasonable certainty that rice protein is safe at inclusion levels in sugar up to 0.8%.

Sucrose

The Panel considered the safety of sucrose as unnecessary for discussion as part of this GRAS evaluation because sucrose is universally recognized as a safe food ingredient, is GRAS under 21 CFR 182.1, and its use in food and beverage production is limited only by cGMP.

Clinical Studies and Allergenicity

Toxicological studies assessing the safety of sucrose, casein, calcium caseinate, pea protein, and rice protein in humans following oral exposure were not identified in the extensive search of the literature. Due to the ubiquity of protein in the human diet, and the low level of inclusion of the aforementioned ingredients in Incredo Sugar®, the Panel does not anticipate any associated human adverse effects following the proposed intake of Incredo Sugar®.

Cases of pea and rice allergy are relatively rare (GRNs 851, 944). However, milk-derived casein constitutes one of the most common allergies (U.S. FDA, 2022). For this reason, all protein ingredients will be identified on food product labels so that the consumer will be aware of its presence in food.

Conclusions

We, the GRAS Expert Panel, have independently and collectively, critically evaluated the data and information summarized above and conclude that the intended uses as an ingredient in foods and beverages of Incredo Sugar®, meeting appropriate food grade specifications and produced consistent with current good manufacturing practice, are Generally Recognized as Safe (GRAS) based on scientific procedures.

[redacted]

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

Part	Section §	Section Title
170—Food additives	170.3	Definitions
182—Substances generally recognized as safe	182.1	Substances that are generally recognized as safe

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APPENDIX A: Specifications

Table 1 Specifications and Methods of Analysis for Incredo Sugar

Parameter	Specification	Method*
Appearance	Characteristic	Appearance
Sucrose (%)	>30	AM/C/1014 by Ion Exchange Chromatography
Protein (%)	<70	AM/C/224 by Dumas method
Ash	<2.5	AM/C/803 based on BS 4401: Part 1:1998
Loss on Drying (%)	≤10	AM/C/801 based on Feeding Stuff Regulations 2000
Total Fat	<1.5	AM/C/1015 by oven drying and Pulsed NMR
Microbial Contaminants (SI 885/3)		
Total Count (CFU/g)	<10,000	ESGMM300 using PCA pour plate technique
Yeast Count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Mould Count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
E. coli (CFU/g)	<10	ESGMM561 based on ISO 16649-3:2015
Salmonella (negative in 25 g)	negative	ESGMM515 Solus ELISA Kit method and DYNEX equipment

BS = British Standard; CFU = colony-forming units; ELISA = enzyme-linked immunosorbent assay; ISO = International Organization for Standardization; NMR = Nuclear Magnetic Resonance; PCA = Plate Count Agar.

* Testing conducted at an ISO 17025:2005 accredited laboratory.

APPENDIX B: Intended Use and Dietary Exposure

Table 2 Proposed Food Use and Use Level of Protein in Sugar in the U.S.

Food Category (21 CFR §170.3) (U.S. FDA, 2021a)	Proposed Food Uses	Protein Use Levels (g/100 g)
Sugar, white, granulated	White Sugar	0.01 to 0.8

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

Table 3 Summary of the Estimated Daily Intake of Protein Ingredients from Proposed Food Uses in the U.S. by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	*Per Capita* Intake (mg/day)		Consumer-Only Intake (mg/day)			
		Mean	90th Percentile	%	n	Mean	90th Percentile
Infants and Toddlers	0 to <2	5.34	8.00	20.6	87	32.00	48.00
Young Children	2 to 5	16.00	53.34	37.8	212	45.34	93.34
Children	6 to 11	26.66	72.00	48.5	398	56.00	133.34
Female Teenagers	12 to 19	53.34	152.00	49.7	210	109.34	224.00
Male Teenagers	12 to 19	40.00	120.00	38.2	189	106.66	240.00
Female Adults	20 and older	66.66	184.00	53.5	1,302	125.34	282.66
Male Adults	20 and older	72.00	192.00	51.3	1,083	141.34	368.00
Total Population	2 and older	61.34	157.34	50.4	3,394	122.66	274.66

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

Table 4 Summary of the Estimated Daily Per Kilogram Body Weight Intake of Protein Ingredients 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 Toddlers	0 to <2	0.56	0.78	20.6	87	2.70	4.90
Young Children	2 to 5	0.98	3.54	37.9	210	2.64	5.50
Children	6 to 11	0.86	2.30	48.5	397	1.76	4.18
Female Teenagers	12 to 19	0.88	2.86	49.8	206	1.76	4.38
Male Teenagers	12 to 19	0.62	1.90	38.4	189	1.58	3.76
Female Adults	20 and older	0.90	2.42	53.5	1,293	1.70	3.98
Male Adults	20 and older	0.88	2.16	51.4	1,072	1.70	4.16
Total Population	2 and older	0.88	2.42	50.5	3,367	1.74	4.06

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

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04 December 2023

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Re: GRAS Notice No. GRN 001137

Dear Dr. Deng,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 27 November 2023 pertaining to the amendment for GRN 001137 provided by Incredo Ltd. dated November 5, 2023.

FDA.1. *We note that in Tables 2.3-2 and 2.3-3 (page 3 of the amendment) some of the results for yeast count, mold count, and heavy metals are reported as "<" [a value]. Please clarify if "<" [a value] represents the limit of quantitation (LOQ) or the limit of detection (LOD) of the analytical methods, and provide either the LOQ or the LOD, as appropriate, of the analytical method(s) used to analyze the batches for these specification parameters.*

The "<" [a value] for heavy metals represents the LOQ of the analytical method. The LOQ for each heavy metal parameter for all batches detailed in Table 2.3-3 (page 3 of the amendment) excluding S6-273 is detailed in Table 1 below. For S6-273, the LOQ for each heavy metal parameter is detailed in Table 2 below. This variation in LOQs stems from logistical limitations at the time of analysis that prompted us to conduct analyses on batches at different laboratories. In the future, the LOQ for lead will be 0.005 mg/kg.

Table 1 Limit of Quantitation for Heavy Metal Contaminant Parameters (Excluding Batch S6-273)

Parameter	Limit of Quantitation	Method
Heavy Metal Contaminants		
Lead (mg/kg)	0.005	ICP-MS
Arsenic (mg/kg)	0.01	ICP-MS
Mercury (mg/kg)	0.003	ICP-MS
Cadmium (mg/kg)	0.003	ICP-MS

Table 2 Limit of Quantitation for Heavy Metal Contaminant Parameters (Batch S6-273)

Parameter	Limit of Quantitation	Method
Heavy Metal Contaminants		
Lead (mg/kg)	0.01	ICP-MS
Arsenic (mg/kg)	0.01	ICP-MS
Mercury (mg/kg)	0.003	ICP-MS
Cadmium (mg/kg)	0.005	ICP-MS

The "<" [a value] for yeast count and mold count, represents the LOD of the analytical method. The LOD for these parameters is detailed in Table 3 below.

Table 3 Limit of Detection for Yeast and Mold Count Parameters

Parameter	Limit of Detection	Method
Microbial Contaminants		
Yeast count (CFU/g)	<20	ESGMM308 based on BS ISO 21527-1:2008
Mold count (CFU/g)	<20	ESGMM308 based on BS ISO 21527-1:2008
CFU = colony forming units.		

FDA.2. *In Table 2.3-3 (page 3 of the amendment), we note that the specification limit for lead is <0.3 mg/kg whereas all provided results are <0.1 mg/kg or close to this value. In line with FDA's "Closer to Zero" initiative that focuses on reducing dietary exposure to heavy metals, we recommend that you establish a lower specification limit for lead that reflects the actual measured levels of lead in the analyzed batches (Table 2.3-3) and is as low as possible.*

The lead results for different types of protein-sucrose vary greater than the other assessed heavy metals. Additionally, the result for batch S6DD11 is 0.124 mg/kg, a level above the FDA's proposed specification of <0.1 mg/kg. Incredo considered the lead specifications of the constituent protein raw materials in establishing of the lead specification for protein-sucrose. The constituent protein raw material specifications for lead are <0.25 mg/kg (rice protein), <0.2 mg/kg (pea protein), and not more than 1 mg/kg (according to FCC monograph standards for casein and calcium caseinate). After

reassessing the lead results for each type of protein-sucrose, Incredo has established the lead specification limit of protein-sucrose to be <0.15 mg/kg, which aligns closely with the batch analysis and the calculated contribution of proteins to the overall lead content in protein-sucrose. This change can be identified in Table 2.3-1 below in Incredo's response to FDA.3.

FDA.3. *In Table 2.3-2 (page 3 of the amendment), the notifier provides the specification limit for ash and the results from the batch analyses. We note that the results from two batches are higher than the specification limit for ash (<2.5%). If these values are typo errors, please provide the correct values. Otherwise, please establish a higher specification limit for ash that reflects the actual measured level of ash in the analyzed batches.*

Incredo confirms that the two ash values higher than the previous specification limit for ash (<2.5%) are correct. As shown in Table 2.3-1 below, Incredo has updated the specification limit for ash to <5%.

Table 2.3-1 Specifications and Methods of Analysis for Incredo Sugar®

Parameter	Specification	Method
Appearance	Characteristic	Appearance
Sucrose (%)	>30	AM/C/1014 by ion exchange chromatography
Protein (%)	<70	AM/C/224 by Dumas method
Ash (%)	<5	AM/C/803 based on BS 4401: Part 1:1998
Loss on drying (%)	≤10	AM/C/801 based on Feeding Stuff Regulations 2000
Total fat (%)	<1.5	AM/C/1015 by oven drying and pulsed NMR
Microbial Contaminants (SI 885/3)		
Total count (CFU/g)	<10,000	ESGMM300 using PCA pour plate technique
Yeast count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Mold count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Escherichia coli (not detected/1g)	Not detected	ESGMM561 based on ISO 16649-3:2015
Salmonella (not detected/25g)	Not detected	ESGMM515 Solus ELISA Kit method and DYNEX equipment
Heavy Metal Contaminants		
Lead (mg/kg)	<0.15	ICP-MS
Arsenic (mg/kg)	<0.05	ICP-MS
Mercury (mg/kg)	<0.02	ICP-MS
Cadmium (mg/kg)	<0.05	ICP-MS

BS = British Standard; CFU = colony-forming units; ELISA = enzyme-linked immunosorbent assay; ISO = International Organization for Standardization; NMR = nuclear magnetic resonance; PCA = plate count agar; SI = Israeli Standard.

We hope this information adequately addresses the Agency's questions regarding GRN 001137. If the Agency requires any additional information or further clarification, Incredo will be happy to provide it upon request.
Sincerely,

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Re: GRAS Notice No. GRN 001137

Dear Dr. Deng,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 06 September 2023 pertaining to information provided within Incredo, Ltd. (Incredo)'s Generally Recognized as Safe (GRAS) Notice for the intended use of protein-sucrose filed by the Agency under GRN 001137.

FDA.1. *In Table 2.3-1 (page 13), Incredo Ltd. (Incredo) provides the specifications for protein-sucrose that do not include limits for heavy metals. We request that you include the limits for lead, arsenic, cadmium, and mercury in the specifications for protein-sucrose and provide the results for heavy metals from the analyses of a minimum of three non-consecutive batches for each protein-sucrose. Keeping in line with FDA's Closer to Zero initiative that focuses on reducing dietary exposure to heavy metals, we recommend that the specification limits for the heavy metals reflect the results of the batch analyses and be as low as possible. For the record, please provide an updated Table 2.3-1 including the limits for the heavy metals along with the corresponding analytical methods used to analyze the batches.*

Incredo has included limits for lead, arsenic, cadmium, and mercury in the specifications for protein-sucrose. An updated Table 2.3-1 is presented below. In addition, 3 batches of each type of protein-sucrose were analyzed to demonstrate adherence to the specifications. The results of these analyses are presented in Table 2.3-2 and 2.3-3 as part of our response to question FDA.2.

Table 2.3-1 Specifications and Methods of Analysis for Incredo Sugar®

Parameter	Specification	Method
Appearance	Characteristic	Appearance
Sucrose (%)	>30	AM/C/1014 by ion exchange chromatography
Protein (%)	<70	AM/C/224 by Dumas method
Ash	<2.5	AM/C/803 based on BS 4401: Part 1:1998
Loss on drying (%)	≤10	AM/C/801 based on Feeding Stuff Regulations 2000
Total fat	<1.5	AM/C/1015 by oven drying and pulsed NMR
Microbial Contaminants (SI 885/3)		
Total count (CFU/g)	<10,000	ESGMM300 using PCA pour plate technique
Yeast count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Mold count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Escherichia coli (not detected/1g)	Not detected	ESGMM561 based on ISO 16649-3:2015
Salmonella (not detected/25g)	Not detected	ESGMM515 Solus ELISA Kit method and DYNEX equipment
Heavy Metal Contaminants		
Lead (mg/kg)	<0.3	ICP-MS
Arsenic (mg/kg)	<0.05	ICP-MS
Mercury (mg/kg)	<0.02	ICP-MS
Cadmium (mg/kg)	<0.05	ICP-MS

BS = British Standard; CFU = colony-forming units; ELISA = enzyme-linked immunosorbent assay; ISO = International Organization for Standardization;; NMR = nuclear magnetic resonance; PCA = plate count agar; SI = Israeli Standard.

FDA.2. *In Table 2.3-2 (page 13), Incredo provides the results from the analyses of seven batches of protein-sucrose. Please specify a protein (casein, calcium caseinate, pea protein, or rice protein) for each of the seven batches in Table 2.3-2. In addition, we request that you provide the results from the analyses of additional batches to ensure that there are results from a minimum of three non-consecutive batches for each protein-sucrose. For the record, please also provide an updated Table 2.3-2 including the results from the additional batches.*

Batch Nos. S6SU122, S6SU126 and S6SU183 are from calcium caseinate-based protein-sucrose. Batch No. S6SU180 is from casein-based protein-sucrose. Batch Nos. S6SU200, S6SU201 (replaced with S6DD132 in the updated table), and S6SU202 are from rice protein-based protein-sucrose. An updated Table 2.3-2 with results from the analysis of additional batches is provided below. Incredo some analytical results are pending from Batch Nos. S6DD179 and S6-277. As soon as results from these batches are received a final Table 2.3-2 will be transmitted to the FDA.

Table 2.3-2 Summary of the Chemical Product Analysis for Each Protein-Type of Incredo Sugar®

Parameter	Specification	Calcium Caseinate			Casein			Pea Protein			Rice Protein		
		S6SU122	S6SU126	S6SU183	S6SU180	S6DD179	S6DD141	S6-273	S6-275	S6-277	S6SU200	S6DD132	S6SU202
Appearance	Characteristic	C	C	C	C	C	C	C	C	C	C	C	C
Sucrose (%)	>30	51.3	49.7	71.6	73.6	53.14	52.24	53.66	56.43	52.84	58.9	52.06	59.2
Protein (%)	<70	40.9	45.3	24.8	24.6	41.4	41.4	39.1	36.2	39	35.1	41.7	35.4
Ash (%)	<2.5	1.8	1.9	1	1.9	3.5	3.8	1.9	1.6	2	0.4	1.2	0.5
Loss on drying (%)	≤10	5.1	6.2	5.2	2.6	2.7	2.9	3.3	2.8	3.4	4.7	2.6	4.4
Total fat (%)	<1.5	0.4	0.5	0.3	0.2	0.3	0.5	1.8	1.8	1.9	0.8	1.2	0.8
Microbial Contaminants													
Total count (CFU/g)	<10,000	260	210	370	20	50	220	<10	<10	10	60	<10	70
Yeast count (CFU/g)	<100	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Mold count (CFU/g)	<100	<20	<20	<20	<20	<20	<20	20	<20	<20	<20	<20	<20
*Escherichia coli (*not detected/1g)	Not detected	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salmonella (not detected/25 g)	Not detected	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

C = conforms; CFU = colony-forming units; ND = not detected.

Table 2.3-3 Summary of the Heavy Metal Analysis for Each Protein-Type of Incredo Sugar®

Parameter	Specification	Calcium Caseinate			Casein			Pea Protein			Rice Protein		
		S6DD4	S6DD7	S6DD11	DMC1	DMC6	S6DD139	S6-273	S6-277	S6-283	DRP13	DRP23	DRP56
Lead (mg/kg)	<0.3	0.044	0.044	0.124	0.011	0.0056	0.0054	<0.01	0.012	0.011	0.056	0.062	0.055
Arsenic (mg/kg)	<0.05	0.018	0.017	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.028	0.031	0.03
Mercury (mg/kg)	<0.02	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	0.0065	0.0068	0.0069
Cadmium (mg/kg)	<0.05	0.0056	0.0054	0.0031	<0.003	<0.003	<0.003	0.024	0.025	0.024	0.018	0.018	0.018

FDA.3. *In Table 2.3-1 (page 13), we note that ISO 16649-3:2015 method is for enumeration of β-glucuronidase positive E.coli by most probable number (MPN). The detection limit is 1 MPN/g instead of 1 CFU/g. Please clarify whether ESGMM561 is based on the ISO 16649-3:2015 method.*

BS EN ISO 16649-3:2015 refers to the Detection and Most Probable Number Technique. ESGMM561 follows the detection portion of the ISO and as such the results are reported as Detected/Not Detected in x g of product. The LOD for this method is 1 CFU per quantity of product tested. Incredo has updated the specification of *E. coli* to "Not detected/1g."

FDA.4. *Please confirm that all analytical methods used to analyze the batches for the specification parameters are validated for their respective uses.*

Incredo confirms that all analytical methods used to analyze the batches for the specification parameters are validated for their respective uses.

FDA.5. *On page 9, Incredo states that pea protein is manufactured as described in GRN 000851 and includes Table 2.1.2-1 titled "Specifications for Pea Protein per GRN 851" (page 10). We note that there are some discrepancies between the specifications in Table 2.1.2-1 and in GRN 000851. For example, the protein content is 85% (please clarify if this is a minimum content) and the limit for mercury is ≤ 0.03 mg/kg in Table 2.1.2-1 whereas these values are > 84% and < 0.2 mg/kg, respectively, in GRN 000851. Considering these discrepancies, please clarify whether Incredo intends to use only pea protein that meets the specifications provided in Table 2.1.2-1 or pea protein that meets the specifications provided in GRN 000851. Please also address this question in the context of the specifications for rice protein in Table 2.1.3-1 (page 11) and in GRN 000609.*

Incredo intends to use only pea protein that meets the specifications provided in GRN 000851. In addition, Incredo intends to use only rice protein that meets the specifications provided in GRN 000609. The specifications for pea protein and rice protein are presented in an updated Table 2.1.2-1 and Table 2.1.3-1, respectively.

Table 2.1.2-1 Specifications for Pea Protein

Parameter	Specification
Physical and Chemical	
Appearance	Beige powder
Loss on drying	10% max.
Protein content (dry basis)	84% min.
Particle size on 200 µm	10% max.
Ash*	5%
Poured bulk density	0.35 to 0.50 kg/L

Table 2.1.2-1 Specifications for Pea Protein

Parameter	Specification
pH at 10% (w/w)	6.5 to 8.0
Aqueous solubility (pH 7)*	55%
Microbiological	
Total plate count	5,000 CFU/g max.
Yeasts	50 CFU/g max.
Molds	50 CFU/g max.
Enterobacteriaceae	10 CFU/g max.
Escherichia coli	Absent in 1 g
Salmonella	Absent in 25 g
Staphylococcus aureus	Absent in 1 g
Bacillus cereus	100 CFU/g max.
Mycotoxin	
Ochratoxin A	<20 mg/kg
Heavy Metals	
Lead (mg/kg)	<0.2 mg/kg
Arsenic (mg/kg)	<0.2 mg/kg
Mercury (mg/kg)	<0.2 mg/kg
Cadmium (mg/kg)	<0.2 mg/kg

CFU = colony-forming units; GRN = Generally Recognized as Safe (GRAS) Notice; max. = maximum; min. = minimum.
* Not specified in GRN 000851 as a minimum or maximum limit.
Source: GRN 000851 – (Roquette Freres, 2019)

Table 2.1.3-1 Specifications for Rice Protein

Parameter	Specification
Proximates	
Protein (dry basis)	≥80%
pH	4.5 to 7.0
Fat	≤5%
Fiber	≤18%
Moisture	≤6%
Ash	≤3.5%
Total carbohydrate	≤18%
Gluten	≤20 ppm
Heavy Metals	
Arsenic	<0.20 ppm
Cadmium	<0.30 ppm
Lead	<0.25 ppm
Mercury	<0.045 ppm
Microbiological	
Total plate count	≤15,000 CFU/g
Coliform	≤30 CFU/g

Table 2.1.3-1 Specifications for Rice Protein

Parameter	Specification
Yeasts and molds	≤100 CFU/g
Salmonella	Absent in 10 g
Escherichia coli	Absent in 10 g
Staphylococcus aureus	Absent in 10 g
Mycotoxin	
Aflatoxin B1	<5 µg/kg
Aflatoxin B2	<5 µg/kg
Aflatoxin G1	<5 µg/kg
Aflatoxin G2	<5 µg/kg
Ochratoxin A	<5 µg/kg

CFU = colony-forming units; GRN = Generally Recognized as Safe (GRAS) Notice; ppm = parts per million.
Source: GRN 000609 – (Axiom Foods, Inc. / SPRIM Strategy & Intelligent Innovation, 2015)

FDA.6. *In Table 2.1.1-1 (page 9), Incredo provides the specifications for casein and caseinate salts from the Food Chemicals Codex (FCC) Monograph Standards for Casein and Caseinate Salts. Please specify the FCC edition and year of publication.*

The monograph standards for casein and caseinate salts adhere to the 13th edition of the *Food Chemicals Codex* (FCC, 2023).

FDA.7. *On page 12, Incredo states that protein-sucrose can be milled to a specific particle size distribution. Please provide the range of particle sizes and confirm that the ingredient is not intended to be manufactured to have dimensions/properties of a nanomaterial.*

Incredo has analyzed the particle size distribution (PSD) of 3 batches of casein-, calcium caseinate-, rice protein-, and pea protein-based protein-sucrose. As shown in Figures 7-1 and 7-2, the lowest value (Dv (5)) reported in the PSD for casein-based and rice protein sucrose is 13.5 (Dv 10 – 23) µm and 8.96 (Dv 10 – 16.7) µm, respectively. The average PSD of the 3 batches was reported as 152 µm and 98 µm for casein-based and rice protein-based protein-sucrose, respectively. Similarly, for calcium caseinate- and pea protein-based protein-sucrose (Figures 7-3 and 7-4), the lowest value (Dv (10)) reported in the PSD is 43.5 and 53.6 µm, respectively. The average PSD of the 3 batches was reported as 156 µm and 273 µm for calcium caseinate-based and pea protein-based protein-sucrose, respectively. Therefore, the data from these batches indicate that protein-sucrose has a PSD that exists above 1000 nm. Further, Incredo confirms that protein-sucrose is not intended to be manufactured to have dimensions/properties of a nanomaterial.

Figure 7-1 Particle Size Distribution for 3 Batches of Casein-Based Protein-Sucrose

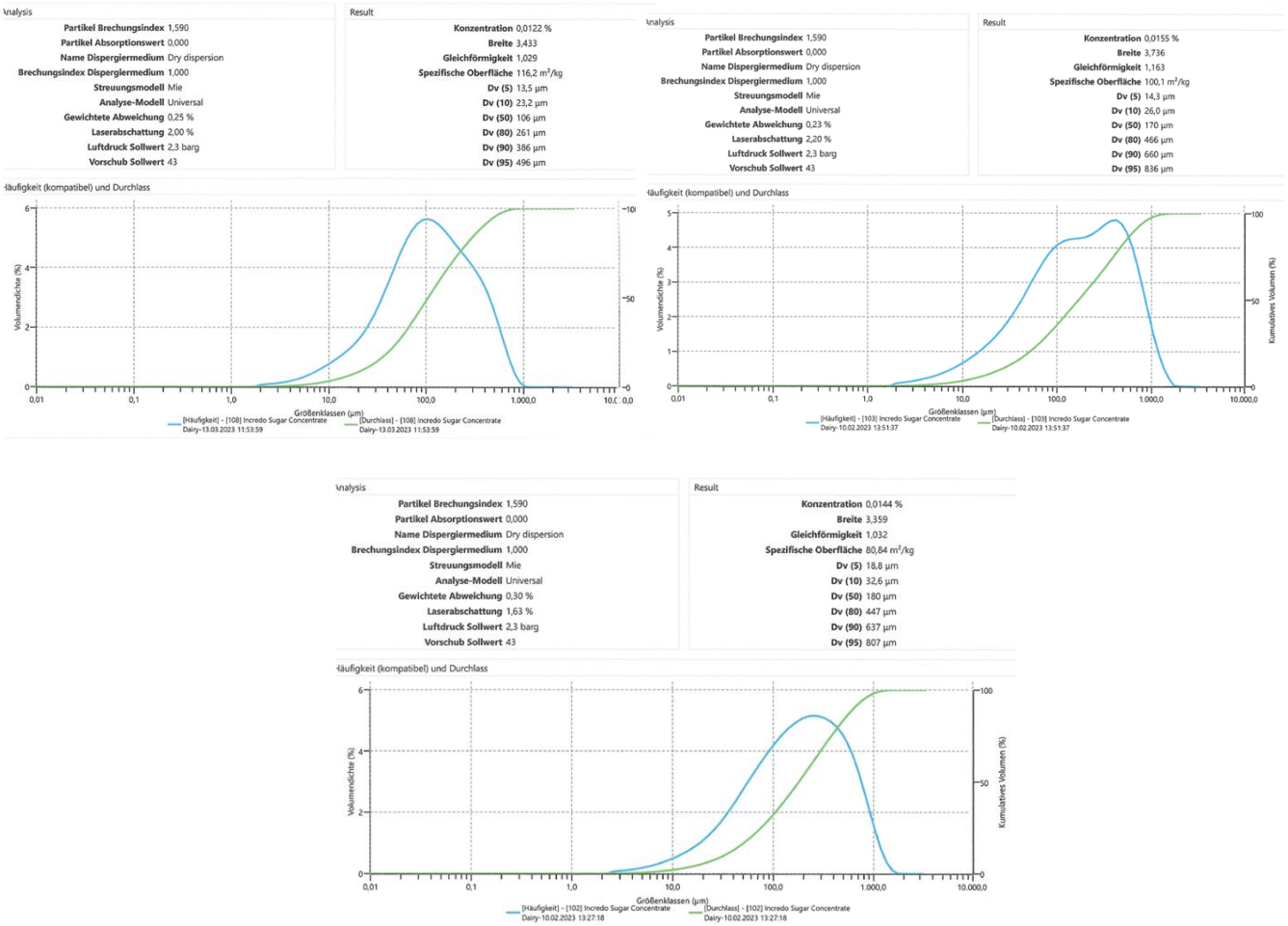


Figure 7-2 Particle Size Distribution for 3 Batches of Rice Protein-Based Protein-Sucrose

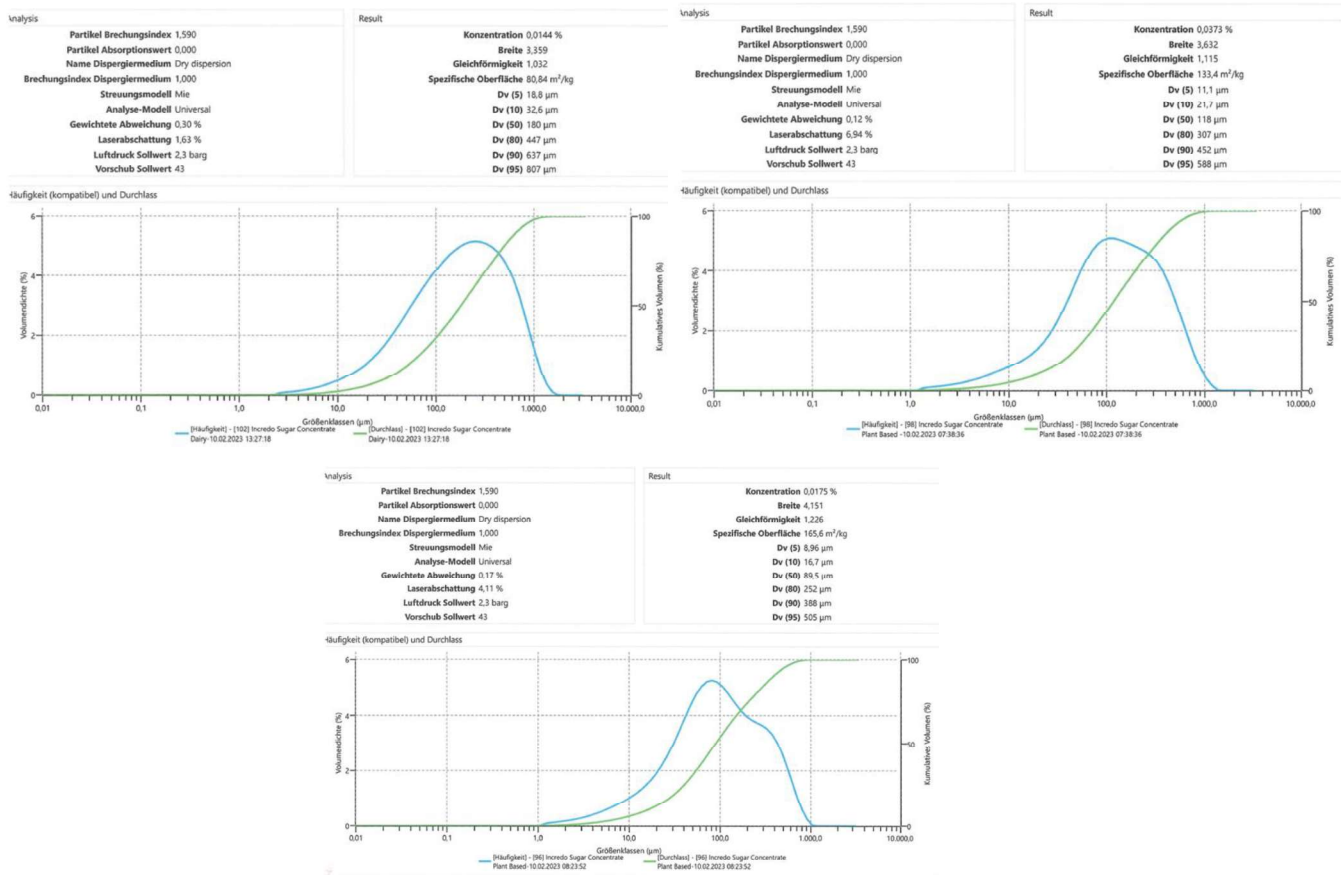


Figure 7-3 Particle Size Distribution for 3 Batches of Calcium Caseinate-Based Protein-Sucrose

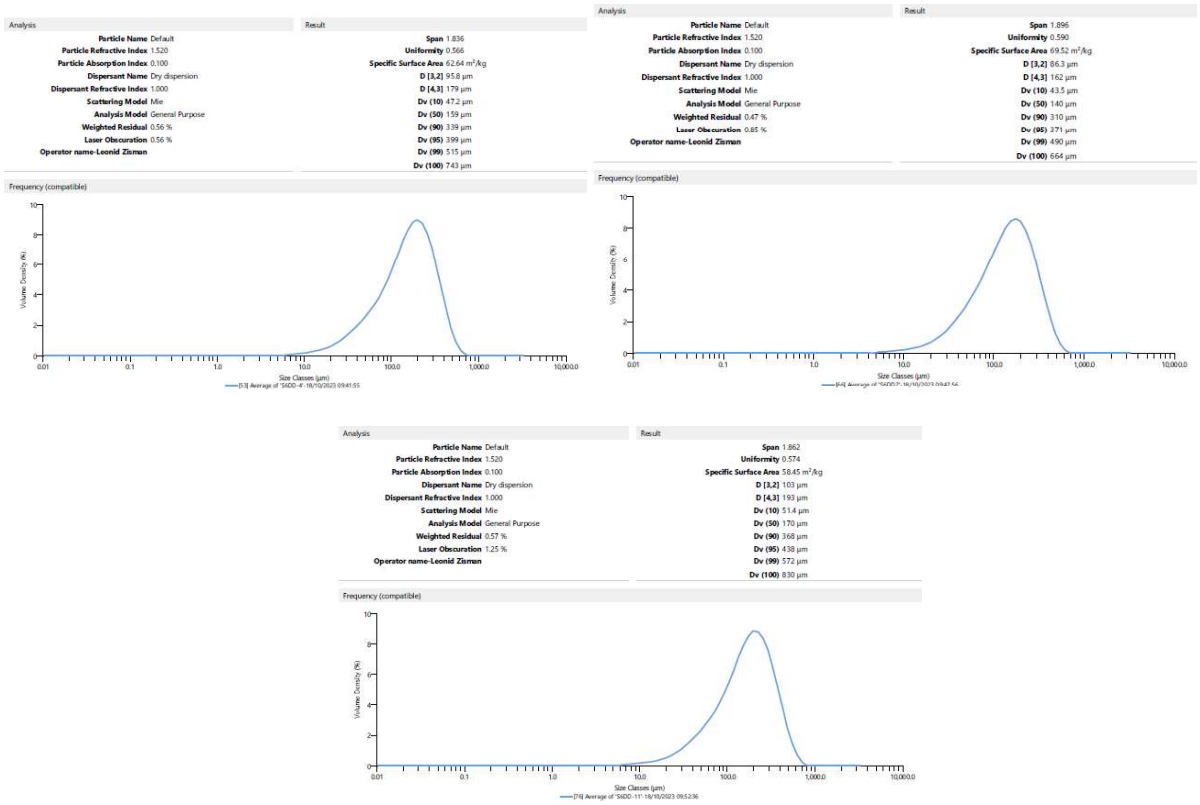
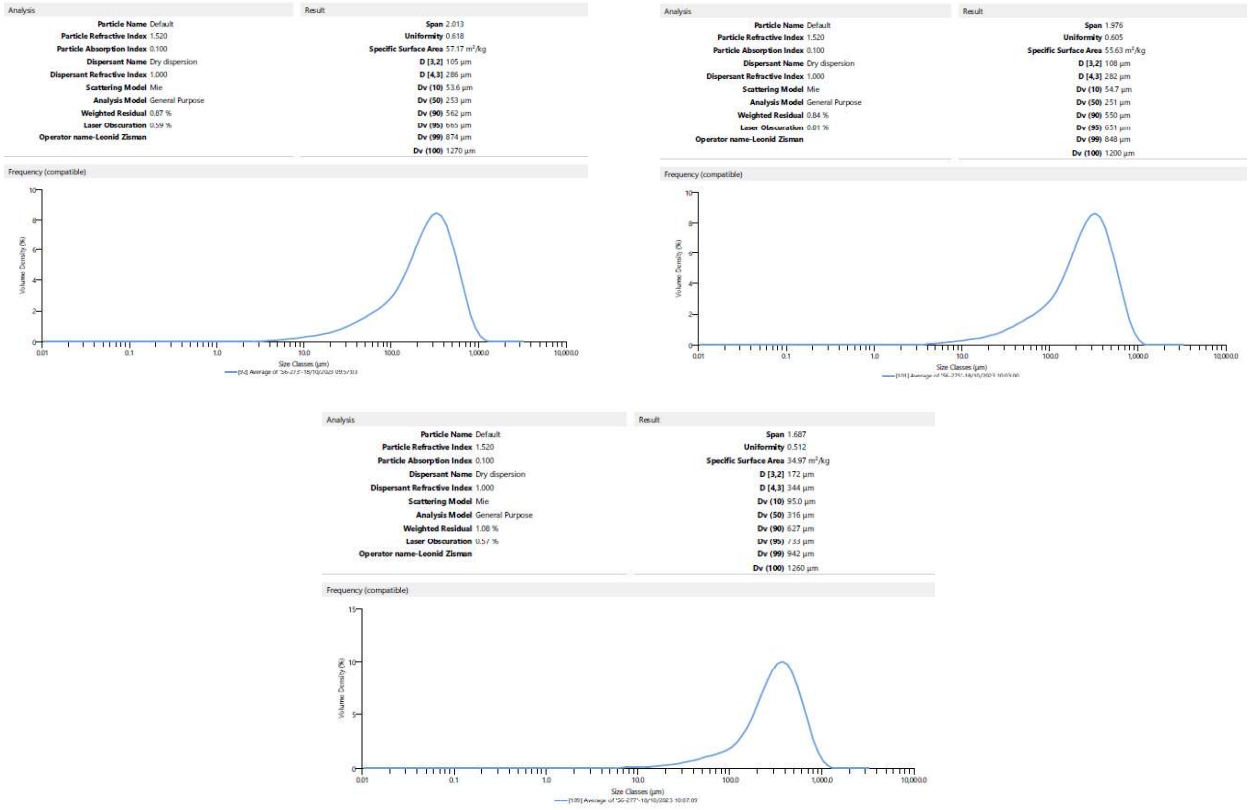


Figure 7-4 Particle Size Distribution for 3 Batches of Pea Protein-Based Protein-Sucrose



FDA.8. *On page 15, Incredo states that protein-sucrose may contain "minor inversion products" (i.e., glucose and fructose resulting from sucrose hydrolysis) and that their content in protein-sucrose is within limit of normal occurrence in commercial sucrose. Please provide the levels of "minor inversion products" in commercial sucrose and a reference(s) reporting these levels.*

According to the *Codex Alimentarius* "Standard for Sugars" (CXS 212-1999), minor inversion products (*i.e.,* invert sugar) may be present in white sugar and powdered sugar at levels of ≤0.04% *m/m*, and in plantation or mil white sugar at levels of ≤0.1% *m/m* (Codex Alimentarius, 2022).

FDA.9. *On page 7, Incredo states that protein-sucrose contains not more than 70% protein while the protein content when the ingredient is added to sugar is 0.01 to 0.8%. However, in Table 3.2-1 (page 26), the use level of protein-sucrose is 0.01 to 0.8 g/100 g sugar. For the record, please confirm that the use level of protein-sucrose in Table 3.2-1 is on a protein basis.*

Incredo confirms that the use level of protein-sucrose in Table 3.2-1 is on a protein basis.

FDA.10.
In Part 3, Incredo provides the estimates of dietary exposure to protein from the intended use of protein-sucrose. Please discuss whether the estimated dietary exposure to protein from the intended use of protein-sucrose is expected to result in an increase in the current cumulative dietary exposure to total protein and if yes, please explain why this increase would not raise safety concerns.

The estimated dietary exposure to protein from the intended use of protein-sucrose is expected to result in an increase in the current cumulative dietary exposure to total protein. The increase in cumulative dietary exposure to total protein in the diet from the proposed use of protein-sucrose was estimated to range from 48 mg/day to 368 mg/day, with the total population exposure estimated to be 275 mg/day (90th percentile consumer-only intakes; see Table 3.3.2-1, page 28). The daily reference value (DVR) for protein is 50 g/day for adults and children 4 or more years of age (U.S. FDA, 2023). The Institute of Medicine (IOM) established dietary reference intakes (DRIs) for total protein as 56 g/day for adult males, and 46 g/day for adult females (IOM, 2005). Additionally, the IOM determined there were insufficient data to provide dose-response relationships to establish a tolerable upper intake level (UL) for total protein. Based on Incredo's conservative estimate of protein intake from the proposed use of protein-sucrose, it is unlikely that the consumption of foods with protein-sucrose will result in a consumption greater than the DRI, such that the proposed use of protein-sucrose contributes only a trivial amount of protein (at most, 0.74% daily value of protein) to the diet.

FDA.11. *Incredo Ltd. has proposed a new intended use for calcium caseinate in sugar, which would be expected to increase the cumulative exposure to calcium in the diet. We note that the tolerable upper intake level (UL) for calcium has been set by the Institute for Medicine (IOM), and that this UL varies across life stages.[1] IOM considered a variety of safety endpoints when setting the UL, including the risk of hypercalcemia and nephrolithiasis. Since these levels were set in 2011, meta-analyses and epidemiological studies on total, dietary, and supplemental calcium intake and cardiovascular disease risk have also been published.[2,3,4] Please provide a narrative using publicly available information to support the conclusion that increased exposure to calcium in the diet from this new intended use does not pose a safety concern.*

[1]Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC):National Academies Press (US); 2011. 6, Tolerable Upper Intake Levels: Calcium and Vitamin D.
[2]Yang B., et al. (2016). Calcium intake and mortality from all causes, cancer, and cardiovascular disease: the cancer prevention study II nutrition cohort. Am J Clin Nutr. 103(3): 886-94.
[3]Asemi Z., et al. (2015). Total, dietary, and supplemental calcium intake and mortality from all-causes, cardiovascular disease, and cancer: a meta-analysis of observational studies. Nutr Metab Cardiovasc Dis.25(7): 623-34.
[4]Yang, C.et al. (2019).The evidence and controversy between dietary calcium intake and calcium supplementation and the risk of cardiovascular disease: a systematic review and meta-analysis of cohort studies and randomized controlled trials. J Am Coll Nutr.39(4): 352-370.

The calcium caseinate used in the production of protein-sucrose is guaranteed to contain no more than 1.2% calcium. As the maximum protein content in protein-sucrose is specified to be no more than 70%, the maximum calcium content in protein sucrose is estimated to be 0.84%. Based on the dietary intake assessment (Section 3), the mean and 90th percentile consumer-only exposure to calcium from the proposed use of calcium caseinate-based protein-sucrose is presented in Table 11-1 below.

Table 11-1 Summary of the Consumer-only Estimated Daily Intake of Calcium from the Proposed Food Uses of Calcium Caseinate-based Protein-sucrose by Population Group (2015-2016 NHANES Data)

Population Group	Age Group (Years)	Absolute Basis (mg/day)		Body Weight Basis (mg/kg body weight/day)	
		Mean	90th Percentile	Mean	90th Percentile
Infants and toddlers	0 to <2	0.269	0.403	0.023	0.041
Young children	2 to 5	0.381	0.784	0.022	0.046
Children	6 to 11	0.470	1.120	0.015	0.035
Female teenagers	12 to 19	0.919	1.882	0.015	0.037
Male teenagers	12 to 19	0.896	2.016	0.013	0.032
Female adults	20 and older	1.053	2.374	0.014	0.033
Male adults	20 and older	1.187	3.091	0.014	0.035
Total population	2 and older	1.030	2.307	0.015	0.034

NHANES = National Health and Nutrition Examination Survey.

On a consumer-only basis, the resulting mean and 90th percentile intakes of calcium by the total U.S. population from proposed food uses of calcium caseinate–based protein-sucrose in the U.S. were estimated to be 1.030 mg/person/day (0.015 mg/kg body weight/day) and 2.307 mg/person/day (0.034 mg/kg body weight/day), respectively. Among the individual population groups, the highest mean and 90th percentile intakes calcium were determined to be 1.187 mg/person/day (0.014 mg/kg body weight/day) and 3.091 mg/person/day (0.035 mg/kg body weight/day), respectively, as identified among male adults. While infants and toddlers had the lowest mean and 90th percentile consumer-only intakes of 0.269 and 0.403 mg/person/day, respectively, on an absolute basis, when expressed on a body weight basis, this age group had the highest mean daily intake of 0.023 mg/kg body weight/day, while young children had the highest 90th percentile intake estimate of 0.046 mg/kg body weight/day.

Incredo notes that the conclusions of meta-analyses and epidemiological studies on total, dietary, and supplemental calcium intake, and cardiovascular disease (CVD) risk indicate that supplemental calcium, rather than dietary calcium, is associated with increased CVD risk. For example, Yang *et al.* (2016) concluded for men, supplemental calcium intake of ≥1,000 mg/day may be associated with higher all-cause and CVD-specific mortality; notably, dietary calcium was not associated with all-cause mortality in either males or females. Additionally, Asemi *et al.* (2015) and Yang *et al.* (2020) similarly concluded that that calcium intake from calcium supplements might raise CVD risk, while dietary sources do not increase the risk of CVD. As the highest 90th percentile consumer-only intake of calcium from the proposed use of calcium caseinate protein-sucrose is 3.091 mg/day on an absolute basis, Incredo considers the increased exposure to calcium trivial in the context of the supplemental levels of intake associated with increased CVD risk (*e.g.*, ≥1,000 mg/day).

Further, Incredo considered the potential cumulative intake of calcium using the 90th percentile total usual intake of calcium from food, beverages, and dietary supplements by gender and age in the U.S. from the summary tables published for NHANES 2017 to March 2020 Pre-pandemic (USDA ARS, 2023). As shown in Table 11-2, at the 90th percentile intake level, calcium intake does not exceed the tolerable upper limits (UL) for calcium by age group established by the IOM (IOM, 2011). Assuming an addition intake of 3.091 mg calcium/day (the maximum estimated intake of calcium from the proposed use of calcium caseinate—based protein-sucrose), the cumulative intake of calcium would not exceed the UL for calcium. Incredo does note that at the 95th percentile (data not shown), the background intake of calcium does exceed the UL for calcium in certain groups (females, 51+). However, Incredo considers the

increased exposure to calcium as a result of protein-sucrose trivial compared to levels of calcium from dietary exposure.

Table 11-2 90^{th} Percentile of Total Usual Intake of Calcium from Food, Beverages, and Dietary Supplements, by Gender and Age, in the United States, 2017-March 2020 Prepandemic as Compared to the Tolerable Upper Limit for Calcium

Age Group (Years)	Tolerable Upper Limit (mg/day)	90^{th} Percentile Background Intake (mg/day)	Percent Tolerable Upper Limit (%)
Males:			
1 to 3	2500	1356	54.24
4 to 8	2500	1446	57.84
9 to 13	3000	1463	48.77
14 to 18	3000	1526	50.87
19 to 30	2500	1583	63.32
31 to 50	2500	1714	68.56
19 to 50	2500	1668	66.72
51 to 70	2000	1742	87.10
71+	2000	1701	85.05
51+	2000	1730	86.50
19+	N/A	1695	N/A
Females:			
1 to 3	2500	1287	51.48
4 to 8	2500	1281	51.24
9 to 13	3000	1400	46.67
14 to 18	3000	1202	40.07
19 to 30	2500	1329	53.16
31 to 50	2500	1461	58.44
19 to 50	2500	1413	56.52
51 to 70	2000	1656	82.80
71+	2000	1902	95.10
51+	2000	1741	87.05
19+	N/A	1562	N/A
All:			
1+	N/A	1578	N/A

N/A = not applicable.

Therefore, Incredo has concluded that increased exposure to calcium in the diet from the new intended use of calcium caseinate-based protein-sucrose does not pose a safety concern.

We hope this information adequately addresses the Agency's questions regarding GRN 001137. If the Agency requires any additional information or further clarification, Incredo will be happy to provide it upon request.

Sincerely,

Tali Rydlo
RA/QA Manager
Incredo, Ltd.

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04 December 2023

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Re: GRAS Notice No. GRN 001137

Dear Dr. Deng,

Please see the below responses to the United States (U.S.) Food and Drug Administration (FDA)'s letter dated 27 November 2023 pertaining to the amendment for GRN 001137 provided by Incredo Ltd. dated November 5, 2023.

FDA.1. *We note that in Tables 2.3-2 and 2.3-3 (page 3 of the amendment) some of the results for yeast count, mold count, and heavy metals are reported as "<" [a value]. Please clarify if "<" [a value] represents the limit of quantitation (LOQ) or the limit of detection (LOD) of the analytical methods, and provide either the LOQ or the LOD, as appropriate, of the analytical method(s) used to analyze the batches for these specification parameters.*

The "<" [a value] for heavy metals represents the LOQ of the analytical method. The LOQ for each heavy metal parameter for all batches detailed in Table 2.3-3 (page 3 of the amendment) excluding S6-273 is detailed in Table 1 below. For S6-273, the LOQ for each heavy metal parameter is detailed in Table 2 below. This variation in LOQs stems from logistical limitations at the time of analysis that prompted us to conduct analyses on batches at different laboratories. In the future, the LOQ for lead will be 0.005 mg/kg.

Table 1 Limit of Quantitation for Heavy Metal Contaminant Parameters (Excluding Batch S6-273)

Parameter	Limit of Quantitation	Method
Heavy Metal Contaminants		
Lead (mg/kg)	0.005	ICP-MS
Arsenic (mg/kg)	0.01	ICP-MS
Mercury (mg/kg)	0.003	ICP-MS
Cadmium (mg/kg)	0.003	ICP-MS

Table 2 Limit of Quantitation for Heavy Metal Contaminant Parameters (Batch S6-273)

Parameter	Limit of Quantitation	Method
Heavy Metal Contaminants		
Lead (mg/kg)	0.01	ICP-MS
Arsenic (mg/kg)	0.01	ICP-MS
Mercury (mg/kg)	0.003	ICP-MS
Cadmium (mg/kg)	0.005	ICP-MS

The "<" [a value] for yeast count and mold count, represents the LOD of the analytical method. The LOD for these parameters is detailed in Table 3 below.

Table 3 Limit of Detection for Yeast and Mold Count Parameters

Parameter	Limit of Detection	Method
Microbial Contaminants		
Yeast count (CFU/g)	<20	ESGMM308 based on BS ISO 21527-1:2008
Mold count (CFU/g)	<20	ESGMM308 based on BS ISO 21527-1:2008
CFU = colony forming units.		

FDA.2. *In Table 2.3-3 (page 3 of the amendment), we note that the specification limit for lead is <0.3 mg/kg whereas all provided results are <0.1 mg/kg or close to this value. In line with FDA's "Closer to Zero" initiative that focuses on reducing dietary exposure to heavy metals, we recommend that you establish a lower specification limit for lead that reflects the actual measured levels of lead in the analyzed batches (Table 2.3-3) and is as low as possible.*

The lead results for different types of protein-sucrose vary greater than the other assessed heavy metals. Additionally, the result for batch S6DD11 is 0.124 mg/kg, a level above the FDA's proposed specification of <0.1 mg/kg. Incredo considered the lead specifications of the constituent protein raw materials in establishing of the lead specification for protein-sucrose. The constituent protein raw material specifications for lead are <0.25 mg/kg (rice protein), <0.2 mg/kg (pea protein), and not more than 1 mg/kg (according to FCC monograph standards for casein and calcium caseinate). After

reassessing the lead results for each type of protein-sucrose, Incredo has established the lead specification limit of protein-sucrose to be <0.15 mg/kg, which aligns closely with the batch analysis and the calculated contribution of proteins to the overall lead content in protein-sucrose. This change can be identified in Table 2.3-1 below in Incredo's response to FDA.3.

FDA.3. *In Table 2.3-2 (page 3 of the amendment), the notifier provides the specification limit for ash and the results from the batch analyses. We note that the results from two batches are higher than the specification limit for ash (<2.5%). If these values are typo errors, please provide the correct values. Otherwise, please establish a higher specification limit for ash that reflects the actual measured level of ash in the analyzed batches.*

Incredo confirms that the two ash values higher than the previous specification limit for ash (<2.5%) are correct. As shown in Table 2.3-1 below, Incredo has updated the specification limit for ash to <5%.

Table 2.3-1 Specifications and Methods of Analysis for Incredo Sugar®

Parameter	Specification	Method
Appearance	Characteristic	Appearance
Sucrose (%)	>30	AM/C/1014 by ion exchange chromatography
Protein (%)	<70	AM/C/224 by Dumas method
Ash (%)	<5	AM/C/803 based on BS 4401: Part 1:1998
Loss on drying (%)	≤10	AM/C/801 based on Feeding Stuff Regulations 2000
Total fat (%)	<1.5	AM/C/1015 by oven drying and pulsed NMR
Microbial Contaminants (SI 885/3)		
Total count (CFU/g)	<10,000	ESGMM300 using PCA pour plate technique
Yeast count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Mold count (CFU/g)	<100	ESGMM308 based on BS ISO 21527-1:2008
Escherichia coli (not detected/1g)	Not detected	ESGMM561 based on ISO 16649-3:2015
Salmonella (not detected/25g)	Not detected	ESGMM515 Solus ELISA Kit method and DYNEX equipment
Heavy Metal Contaminants		
Lead (mg/kg)	<0.15	ICP-MS
Arsenic (mg/kg)	<0.05	ICP-MS
Mercury (mg/kg)	<0.02	ICP-MS
Cadmium (mg/kg)	<0.05	ICP-MS

BS = British Standard; CFU = colony-forming units; ELISA = enzyme-linked immunosorbent assay; ISO = International Organization for Standardization; NMR = nuclear magnetic resonance; PCA = plate count agar; SI = Israeli Standard.

We hope this information adequately addresses the Agency's questions regarding GRN 001137. If the Agency requires any additional information or further clarification, Incredo will be happy to provide it upon request.
Sincerely,

Tali Rydlo
RA/QA Manager
Incredo, Ltd.