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September 6, 2022

Via FedEx & CD-ROM

Dr. Susan Carlson
Director, Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5001 Campus Drive
College Park, MD 20740-3835

Re: GRAS Notice for Clara Foods Company dba THE EVERY Company for Non-animal egg white protein produced by *K. phaffii*

Dear Dr. Carlson:

We respectfully submit the attached GRAS Notice on behalf of our client, the Clara Foods Company dba THE EVERY Company (Clara Foods Co.), for a non-animal egg white protein produced by *K. phaffii*. Non-animal egg white protein produced by *K. phaffii* is intended for use as a source of protein and as a food ingredient in various food applications as a replacement for whole eggs (fresh and powdered) and egg white protein powder, since it has functional properties such as foaming, gelation, and binding. More detailed information regarding product identification, intended use levels, the manufacturing process, and safety of the ingredient is set forth in the attached GRAS Notice.

Clara Foods Co. has determined that its non-animal egg white protein produced by *K. phaffii* is GRAS for its intended uses based on scientific procedures in accordance with 21 CFR § 170.30(b) and in conformance with the guidance issued by the Food and Drug Administration (FDA) under 21 CFR § 170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). Therefore, the use of the non-animal egg white protein produced by *K. phaffii,* as described in this GRAS Notice, is exempt from the requirement of premarket approval as set forth in the Federal Food, Drug, and Cosmetic Act.

The analytical data, published studies, and information that are the basis for this GRAS Notice are available for FDA review and copying at reasonable times at Keller and Heckman LLP, 1001 G Street, NW, Suite 500W, Washington, DC 20001, or will be sent to the FDA upon request.

We look forward to the agency's review of this submission and would be happy to provide agency officials with any information they may need to complete their assessment. Thank you for your attention to this matter.

Cordially yours,

Evangelia C. Pelonis

Generally Recognized as Safe Notice for

Non-Animal Egg White Protein produced by *Komagataella phaffii*

Prepared for: Office of Food Additive Safety (FHS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Campus Drive
College Park, Maryland 20740

Prepared by: Clara Foods Co. DBA The EVERY Company
1 Tower Place, Suite 800
South San Francisco, CA 94080

Submitted by: Keller and Heckman LLP
1001 G St., NW
Suite 500W
Washington, DC 20001

Date: September 6, 2022

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Part 1: Generally Recognized as Safe (GRAS) Notice - Exemption Claim (21 CFR 170.225)

1.1 Exemption Claim for Non-animal egg white protein produced by *Komagataella phaffii*

Clara Foods Co. doing business as The EVERY Company (Clara Foods) located at 1 Tower Place, Suite 800, South San Francisco, 94080 CA, in accordance with FDA's final rule of August 17, 2016 (81 Fed. Reg. 54960) and 21 CFR §170.225(c)(1) relating to the filing of Generally Recognized as Safe (GRAS) notices, submits the following exemption claim as it relates to the use of Non-animal egg white protein produced by Komagataella phaffii (*K. phaffii)* as a protein ingredient in food at levels in accordance with current Good Manufacturing Practice (cGMP).

Specifically, Clara Foods has concluded, and an independent panel of experts has agreed, that the proposed use, as a source of protein and as a food ingredient, of Non-animal egg white protein produced by *K. phaffii* is GRAS by scientific procedures in accordance with both 21 CFR 170.30(a) and (b) and is thereby exempt from pre-market approval requirements of the Food, Drug and Cosmetic Act.

In conformity with the requirements outlined in the rule, the following information is included with this exemption claim.

1.2 Information about Notifier

Notifier:

Clara Foods Co. Doing Business As The EVERY Company
Kritika Mahadevan, Ph.D.
Director Quality Assurance and Regulatory Affairs
1 Tower Place, Suite 800
South San Francisco, CA 94080

All communications on this matter are to be sent to Counsel for Clara Foods who is authorized to act on behalf of the Notifier:

Evangelia C. Pelonis
Keller and Heckman LLP
1001 G St., NW
Suite 500W
Washington, DC 20001
pelonis@khlaw.com
202-434-4106

1.3 Basis for GRAS Determination and Exclusion from Premarket Approval

Keller and Heckman LLP, on behalf of Clara Foods, hereby notifies the Agency of its determination that Clara Foods' non-animal egg white protein produced by *K. phaffii* DFB-004 is GRAS under the conditions of the intended use, consistent with section 201(s) of the Federal Food, Drug and Cosmetic Act (FD&C Act). This GRAS conclusion is based on scientific procedures in accordance with 21 CFR 170.30(a) and (b) and conforms to the guidance issued by the Food and Drug Administration (FDA) under 21 CFR 170.36, 81 Fed. Reg. 54960 (Aug. 17, 2016). The GRAS status of the substance is based on data generally available in the public domain.

The notified substance is not subject to pre-market approval requirements under the FD&C Act based on our conclusion that the notified substance is GRAS under the conditions of its intended use.

1.4 Intended Use

Non-animal egg white protein produced microbially by *K. phaffii* DFB-004 is intended as a source of dietary protein when manufactured according to cGMP. It may be incorporated into food products to provide nutritional value, as a source of protein, or functionality. It can be used as an ingredient in various food applications as a replacement for whole eggs (fresh and powdered) and egg white protein powder, since it has functional properties such as foaming, gelation, and binding. Such uses will not increase the consumption of protein for the general population. Non-animal egg white protein produced microbially by *K. phaffii* is not intended for use in products regulated under USDA/FSIS jurisdiction or in infant formula.

1.5 Availability of Information

The information for this GRAS conclusion including analytical data, published studies, and information that are the basis for this GRAS determination are available to FDA upon request as required by 21 CFR § 170.225(c)(7)(ii)(A) or (B) by contacting Keller and Heckman LLP at the below address.

Evangelia C. Pelonis
Keller and Heckman LLP
1001 G Street, NW
Suite 500W
Washington, DC 20001
Tel: 202-434-4106
Email: pelonis@khlaw.com

1.6 Confidential Commercial Information

Clara Foods is not claiming any information in Parts 2 through 7 of this document as trade secret, confidential or financial information that is privileged or confidential. Thus, all information and data in this submission are not exempt from the Freedom of Information Act (FOIA), 5 U.S.C. Section 552.

1.7 Certification Statement

We certify in accordance with 21 CFR § 170.225, on behalf of our client, Clara Foods, that this GRAS conclusion is based on representative data from Clara Foods required to support the safety and GRAS status of non-animal egg white protein produced by *K. phaffii* DFB-004. To the best of our knowledge, it is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information, known to us and pertinent to the evaluation of the safety and GRAS status of the use of the substance.

1.8 Signature of Responsible Party or Agent

Date: September 6, 2022

Evangelia C. Pelonis
Partner
Keller and Heckman LLP

Part 2: Identity, Manufacturing, Specifications, Use (21 CFR 170.230)

2.1 Identity of the Substance

2.1.1 Common and Usual Name of the Substance:

Non-animal egg white protein
Non-animal egg white protein from yeast
Animal-free egg white protein
Fermentation-derived egg white protein
Microflora-derived egg white protein
Microflora-based egg white protein
Egg white protein (from yeast)
Egg white protein (from fermentation)

2.1.2 Chemical name: Phosphoglycoprotein

In 2019, approximately 9.9 x 10^{10} eggs were sold in the US which is an egg consumption of approximately 287.1 per capita/year, representing 6.2 x 10^{11} grams of egg protein introduced into the US market per year. In addition to consumption of whole eggs, eggs are used in prepared foods to provide flavor and texture in addition to processing advantages such as gelation, stabilizing, increasing volume and machineability (American Egg Board (2020).

Hen egg ovalbumin, also known as “Gal d 2”, is located in the egg white, comprises approximately 54% of the egg white protein, and has been consumed for as long as eggs have been used as food. In addition, the protein has a history of safe consumption as an endogenous component of whole eggs and egg whites.

The native Gal d 2 protein is a globular phosphoglycoprotein, with a molecular mass of approximately 42.8 kDa, and an isoelectric point of 4.5 (Alleoni, 2006). Its polypeptide chain consists of 385 amino acids.

2.1.3 Substantial Equivalence to native hen egg Gal d 2

Clara Foods has undertaken a robust evaluation of the non-animal egg white protein (NEWP) preparation and determined that it is substantially equivalent to the native hen egg Gal d 2 by

- Glycosylation analysis
- Amino acid analysis
- Molecular weight characteristics
- Immunoreactivity characteristics
- *In vitro* digestibility determinations

- Non-animal egg white protein (NEWP) Glycosylation Form

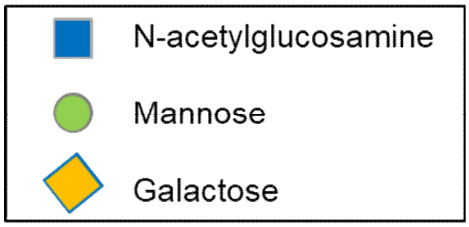
  The host strain used for production of NEWP is *Komagataella phaffii*. It is also known by the name *Pichia pastoris* as described in detail in Section 2.2.1. The context for providing both names of the production organism here is because some of the key pieces of information in references cited in this Notice use the *Pichia pastoris* nomenclature.

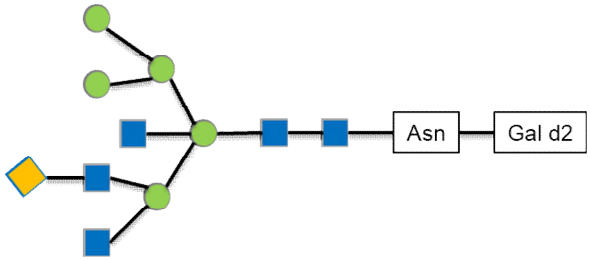
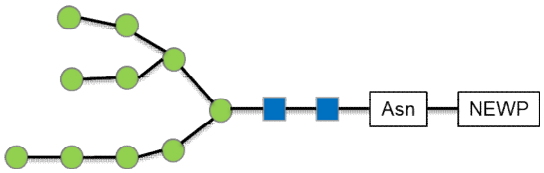
  LC MS and enzyme studies indicate that NEWP, produced by *Komagataella phaffii* DFB-004, is glycosylated via the standard asparagine N-linkage and both canonical sites (N292 and N311) are occupied. Quantitative LC-MS analysis of the glycans attached to NEWP indicates that the glycans attached to the protein have a range of 8 to 14 mannose residues, with the highest percentage being 10 mannoses, consistent with published work on Komagataella-based N-linked glycosylation (Trimble *et al*., 1991; reviewed in Gemmill and Trimble, 1999) and similar to published results for recombinant Gal d 2 from *Komagataella phaffii* (Ito & Matsudomi, 2005). Gemmill and Trimble provide an excellent comparison of typical glycan trees for yeasts and show that *Saccharomyces cerevisiae* typically adds much more extensive mannose trees compared to *Komagataella*. But in both cases, the trees are typically all mannose (Gemmill & Trimble, 1999). A likely structure for a 10-mannose tree for recombinant Gal d 2 (NEWP) is shown in Figure 1b. This basic structure has been demonstrated for other Komagataella-produced proteins (Jacobs, Geysens, Vervecken, Contreras, & Callewaert, 2009) (Trimble, et al., 2003).

  Harvey et al. (2000) showed that hen egg Gal d 2 molecule's N-linked glycosylation consisted of a mannose tree that was either with one branch capped with galactose or N-acetylglucosamine (GlcNac), and the whole structure connected to the asparagine residue by the standard 2x N-acetyl glucosamine (Figure 1a), although in some instances, the glycan was determined to be a Mannose 9 form linked to the di-GlcNac (Kato, Iwase, & Hotta, 1984). Native Gal d 2 has two potential N-linked glycosylation sites, both sites have glycans attached (Kato et al. 1986) during its production but one site can be deglycosylated by the animal leading to a protein with only one site occupied (Nisbet, Saundry, Moir, Fothergill, & Fothergill, 1981).

Figure 1. NEWP N-glycan structure compared to native Gal d 2 (adapted from Harvey et al., 2000), NEWP structure is shown for most abundant Man10 version and branching form is based on literature for *Komagataella* (Gemmill & Trimble, 1999)**.**

Legend:



1A. Gal d 2 from chicken egg (Harvey et al., 2000)	**1B. NEWP by *K. phaffii* DFB-004 (LC-MS/MS)**
	

- 'NEWP' LCMS amino acid composition:

NEWP produced by *K. phaffii* DFB-004 was compared with Gal d 2 from chicken egg white by liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS, a widely applied and preferred method in proteomics (Switzar, Giera, & Niessen, 2013), was used to identify Gal d 2 in NEWP.

The protein samples were first digested into peptides using trypsin. Tandem mass spectrometry data was searched against *in silico* tryptic digests of a custom-built database (Clara Foods, Inc.). The database (101,326 sequences) comprised the proteins of the Uniprot-KB *Gallus gallus* database (version 2019/11) appended with the *Komagataella phaffii* (version 2019/11) and additionally with a database of proteins commonly found in proteomics experiments that are present either by accident or through unavoidable contamination of protein samples (known as the common repository of adventitious proteins, cRAP). The search parameters were defined as: iodoacetamide modified for cysteine alkylation and trypsin as the digestion enzyme. Additional modifications and cleavages were defined previously (Colgrave, Goswami, Blundell,

Howitt, & Tanner, 2014). The database search results were manually curated to yield the protein identifications using a 1% global False Discovery Rate (FDR) determined by the in-built FDR tool within ProteinPilot software (Tang, Shilov, & Seymour, 2008).

The native form of *Gallus gallus* (chicken) Gal d 2 has 385 amino acids. NEWP contains the mature form of Gal d 2 expressed as a fusion protein with the *Saccharomyces cerevisiae* alpha factor pre-pro sequence in *Komagataella phaffii*. Post-translation modification results in the removal of the alpha factor pre-pro sequence. Edman N terminal sequencing (Smith, 2001) and LC-MS/MS analysis results showed imprecise cleavage of the N-terminal pro sequence by *Komagataella* host post- translational machinery. The majority of the NEWP starts with an N-terminal extension containing 4 extra amino acid residues (bolded font below) of the *Saccharomyces cerevisiae* alpha factor pre-pro sequence.

NEWP amino acid sequence has not been submitted to NCBI or UniProt databases. The accession number for native chicken egg Gal d 2 is P01012.2 [1] (Protein Database, 2021). We note that this UniProt entry is the pro+mature sequence of the protein that has a molecular mass of 42.88 kDa.

The molecular mass of the mature Gal d 2 protein, found in hen eggs, is ~ 42.75 kDa.

EAEAGSIGAA SMEFCFDVFK ELKVHHANEN IFYCPIAIMS
ALAMVYLGAK DSTRTQINKV VRFDKLPGFG DSIEAQCGTS
VNVHSSLRDI LNQITKPNDV YSFSLASRLY AEERYPILPE
YLQCVKELYR GGLEPINFQT AADQARELIN SWVESQTNGI
IRNVLQPSSV DSQTAMVLVN AIVFKGLWEK AFKDEDTQAM
PFRVTEQESK PVQMMYQIGL FRVASMASEK MKILELPFAS
GTMSMLVLLP DEVSGLEQLE SIINFEKLTE WTSSNVMEER
KIKVYLPRMK MEEKYNLTSV LMAMGITDVF SSSANLSGIS
SAESLKISQA VHAAHAEINE AGREVVGSAE AGVDAASVSE
EFRADHPFLF CIKHIATNAV LFFGR

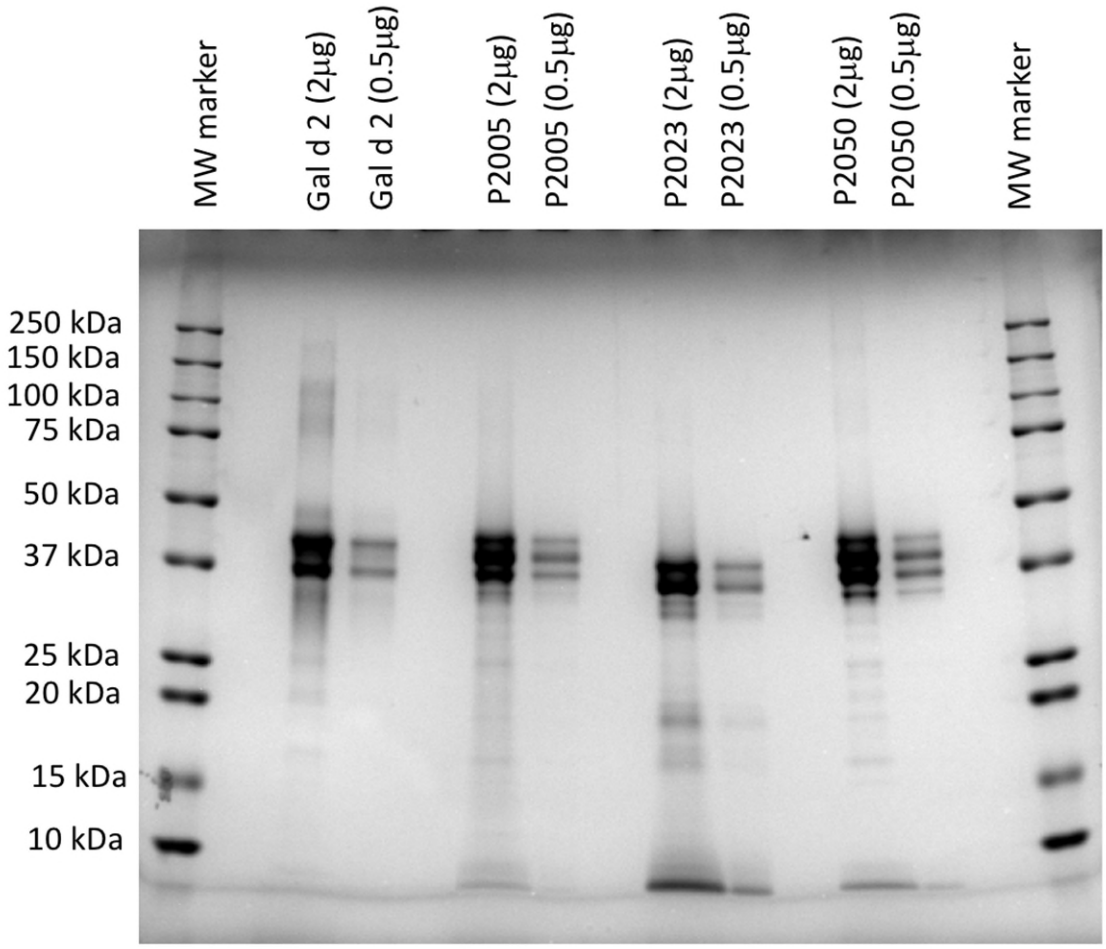
[1] Protein Database. 2021. *National Center for Biotechnology Information, U.S. National Library of Medicine.* Accessed Nov 2021. www.ncbi.nlm.nih.gov/protein/P01012.2.

The N-terminal extension (EAEA) was checked using BLAST analysis and compared to sequences of known protein allergens (Goodman, et al., 2016) and known toxins (Liu, Zheng, Jin, Chen, & Yang, 2019) (Virulence Factor Database) and there was no significant homology to related proteins of concern (Pearson & Lipman, 1988). We note specifically that the amino acid remnants of the *S. cerevisiae* pre-pro sequences are derived from an organism with a safe history of use in food. This, in conjunction with the sequence analysis, indicates that the added amino acid sequences do not present safety concerns.

- Molecular weight characteristics:

  Molecular weight of NEWP was compared against native chicken Gal d 2 using SDS-PAGE.

Figure 2. SDS-PAGE gel of native chicken Gal d 2 and NEWP produced by *K. phaffii* DFB-004 (P2005, P2023 and P2050) demonstrating equivalence in molecular weight.



- Immunoreactivity characteristics:

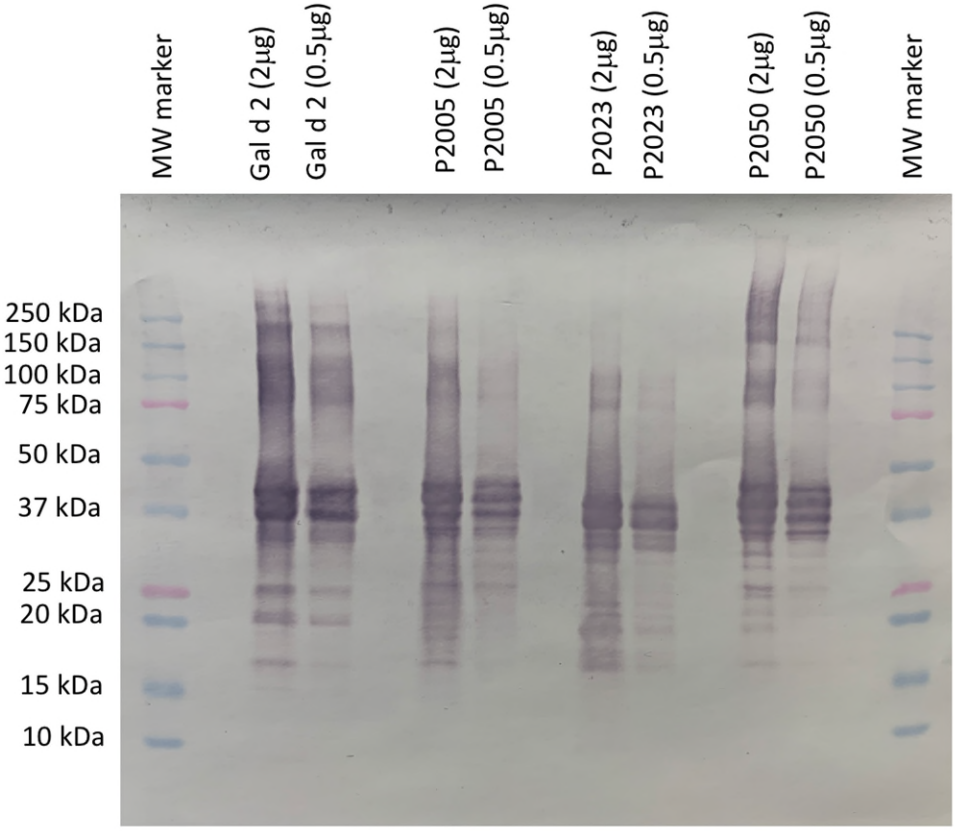
  Western blotting (immunoblotting, protein blotting) is a sensitive immunological method for detecting proteins separated by electrophoresis (Mahmood & Yang, 2012). This technique can detect target proteins as low as 1 ng due to high-resolution capacity of gel electrophoresis and the strong sensitivity and specificity of the immunoassay.

  Western Blot was performed on three NEWP Lots (P2005, P2023, P2050) using primary anti-ovalbumin antibody from rabbit (ab181688 Abcam) at a 1:2500 dilution (Jensen, 2012). The secondary antibody used was goat anti-rabbit IgG conjugated to alkaline phosphatase (AP ab97048 Abcam). Molecular weight marker preparation used was from Bio Rad (161-0394).

Protein gels are stained with SimplyBlue™ SafeStain from Life Technologies (LC6065).

The Western blot comparison (Figure 3) shows the same immunoreactivity for the NEWP samples (P) and native Gal d 2 from chicken egg white.

Figure 3. Western Blot of native chicken Gal d 2 and NEWP produced by *K. phaffii* DFB-004 demonstrating equivalence in immunoreactivity



- *In-vitro* simulated gastric digestibility:

  The *in-vitro* digestibility of samples was measured using the AOAC 971.09 method in terms of the Pepsin (0.2%) Digestible Protein. The analysis demonstrated the equivalence between native chicken egg white Gal d 2 and NEWP in terms of Pepsin Digestible Protein calculated as a percentage of the crude protein i.e. (crude protein – indigestible protein) / crude protein (Table 1).

Table 1: Comparison of Gal d 2 and NEWP *in-vitro* digestibility

Product	In-vitro digestibility
P2005	99.6%
P2023	99.7%
P2050	99.3%
Native chicken Gal d 2	99.3%

In summary, the results of the amino acid sequence, molecular weight, immunoreactivity, and gastric enzyme *in vitro* digestibility determinations confirm that NEWP is substantially equivalent to the native Gal d 2 from chicken egg white.

2.2 Production Microorganism

2.2.1 Production host strain names and safe strain lineage

Komagataella phaffii is a nonpathogenic, non-toxigenic, and well-characterized yeast with a history of safe use in the food industry.

The type strain for *Komagataella phaffii* (originally called as *Pichia pastoris*), now part of the genus *Komagataella* (Yamada, 1995), was isolated in 1922 from a chestnut tree in France and described by A. Guillermond.[2] The type strain was given the accession number NRRL Y-1603 for the US-based stock center and CBS704 for a European stock center. Later versions of *Komagataella phaffii* were isolated by H. Phaff from trees in California (Phaff & Knapp, 1956). NRRL Y-1603 was used, along with other strains, by Phillips Petroleum to develop improved versions that were deposited back into the US stock center. One of these new strains, NRRLY-11430 (CBS7435), was the base strain for the development of *Komagataella phaffii* into a protein production platform (Cregg, Barringer, Hessler, & Madden, 1985).

Recent phylogenetic work, using molecular information such as 26S RNA sequence information (Kurtzman C. , 2005), established new species designations within the genus *Komagataella.* Additional analyses of the original type strain and the main strains being used for protein production determined that the modern strains actually represent two different species *K. pastoris* and *K. phaffii* (Kurtzman C. , 2009). *K. phaffii* was shown to be descended from the strain isolated by Phaff in the US (C. Kurtzman, 2009). The NRRL Y-11430 strain was used by the company BioGrammatics (Carlsbad, CA, USA) to develop strain BG08 that was further modified to create BG10 through the loss of endogenous plasmids. This work by

[2] http://gcm.wfcc.info/Strain_numberToInfoServlet?strain_number=CBS%20704

BioGrammatics is described, along with the genome sequence for BG10, in a recent publication (Sturmberger, et al., 2016). Clara Foods further modified BG10 to have a phenotype that reduces the strain's ability to consume methanol. This base strain is called DFB-001A.

The general taxonomy of *K. phaffii* is:

Name: *Komagataella phaffii*
Kingdom: *Fungi*
Phylum: *Ascoymycota*
Class: *Hemiascomycetes*
Order: *Saccharomycetales*
Family: *Endomycetaceae*
Genus: *Komagataella*
Species: *phaffii*

2.2.1 Production Strain

Production strain *K. phaffii* DFB-004 was constructed from recipient strain DFB-001A using transformations with different expression constructs in order to express NEWP. DFB-001A is a direct descendent of the BG10 strain whose genome was sequenced and published in Sturmberger et al. 2016. In addition to the protein coding sequence for Gal d 2, Strain DFB-004 also contains extra copies of the *K. phaffii* transcription factor HAC1, expressed under strong native *K. phaffii* methanol-induced promoters. Methanol-induced gene expression is a common strategy used to produce high levels of recombinant proteins after producing biomass on glycerol and glucose and inducing with methanol (Cereghino & Cregg, 2000). The identity and location of the inserted gene cassettes were confirmed via DNA sequencing. All introduced DNAs consisted of safe sequences that do not introduce any toxicity or pathogenicity issues. Introduced DNAs were native Komagataella sequences, including promoters, transcriptional terminators, and host genes under the control of new host promoters, DNA encoding a *Saccharomyces cerevisiae* secretion signal and the coding sequence for the mature form of the NEWP.

Strain *Komagataella phaffii* DFB-004 has been deposited in American Type Culture Collection (ATCC)[3] under the non-trade name *Komagataella phaffii* accession number GSD-1235.

[3] https://www.atcc.org/

The *K. phaffii* production strain background complies with the Organization for Economic Development (OECD) criteria for Good Industrial Large-Scale Practice (GILSP) microorganisms (OECD 1992, 1993). It also meets the criteria for a safe production microorganism as described by Pariza and Foster, Pariza and Johnson, and several expert groups (EU Scientific Committee for Food, 1992) (FAO/WHO, 1996) (International Food Biotechnology Council, 1990) (Jonas, et al., 1996) (OECD, 1993) (Pariza & Foster, 1983) (Pariza & Johnson, 2001).

2.2.2 Construction of Production Strain DFB-004: Strain Overexpressing Gal d 2 and HAC1 protein

Gal d 2 is a protein found in chicken (*Gallus gallus)* egg whites. Gal d 2 is expressed in animals as a pre+mature form that is secreted with removal of the "pre" sequence during the secretion process.
The *Saccharomyces cerevisiae (S. cerevisiae)* alpha mating factor pre-pro sequence (‘ScPrePro) is a common secretion signal for secretion of heterologous proteins by *K. phaffii.* The protein coding sequence of the mature form of *Gallus* Gal d 2 was synthesized as a fusion sequence with the ScPrePro for expression and secretion in *K. phaffii*.

HAC1 is a *K. phaffii* transcription factor that regulates genes involved in the Unfolded Protein Response (Guerfal, et al., 2010). Overexpression of HAC1 can improve production of heterologous proteins in several yeast systems, (Gasser, Maurer, Gach, Kunert, & Mattanovich, 2006) (Guerfal, et al., 2010).

Linear cassettes of methanol - inducible promoter :: ScPrePro :: NEWP :: AOX1term and a linear cassette of DNA containing a HAC1 gene under the control of methanol inducible promoters such as the pAOX1 promoter and AOX1 terminator were introduced into Strain DFB-001A using standard electroporation methods and host recombination machinery integrated the linear DNA into the chromosomal DNA (Lin-Cereghino, et al., 2005) (Li et al 2007). The linear pieces of introduced DNA are not replicated inside the host cell (Li et al, 2007).

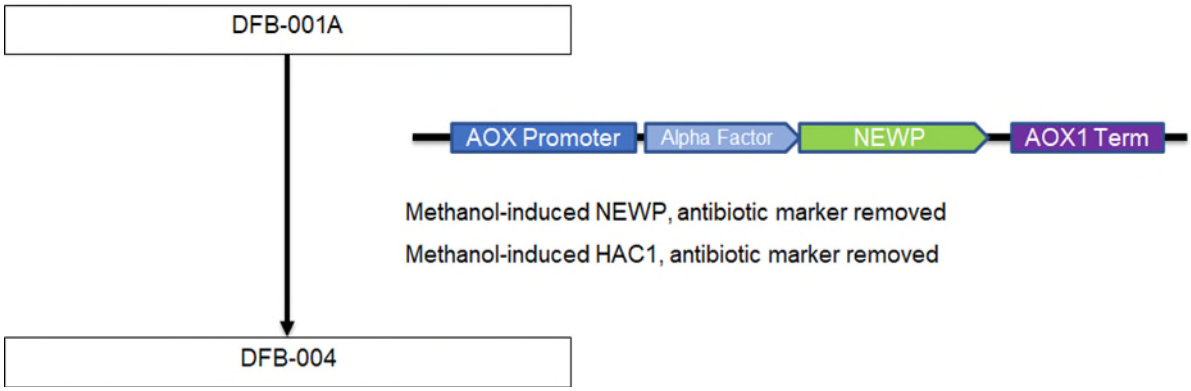
PCR analysis and protein expression assays identified the production strain, Strain DFB-004 which contains the ScPrePro :: NEWP :: AOX1term gene and the HAC1 gene.

As antibiotic resistance markers are present on the plasmid vector used to insert target genes into the DFB-001A chromosomes to produce DFB-004, a loop-out technique, Cre-Lox, commonly used in GRAS notices (e.g., GRN

825, GRN 922; Lambert et al, 2007) was used to remove the antibiotic resistance marker prior to use of DFB-004 as a production host.

A combination of PCR, antibiotic resistance testing and DNA sequencing analysis was used to demonstrate complete removal of all antibiotic resistance genes or bacterial origins of replications present in the original vector plasmid DNA in the production host DFB-004.

Figure 4. Construction of production strain using recipient strain DFB-004



2.2.3 Stability of the Production Strain

All changes introduced into production Strain DFB-004 are stably integrated in the genome and confirmed to be present after forty-five (45) generations of growth on non-selective growth media. The presence of the changes was confirmed by phenotype testing, in terms of NEWP titer, of 44 yeast colonies at the end of the fermentation compared to 44 yeast colonies at the beginning of the fermentation.

No vector plasmid sequences are present in the production strain. Hence, plasmid sequences will not be transferred from the production strain to a non-related organism.

2.2.4 Absence of Antibiotic Resistance Genes

The production strain DFB-004 does not contain antibiotic resistance genes. Antibiotic resistance markers used in strain construction were "looped out" of the production strain. The absence of the antibiotic resistance genes was confirmed by a combination of PCR, antibiotic resistance, and DNA sequencing analysis.

In addition to the absence of antibiotic resistance genes, none of the NEWP production lots contained Gal d 2 transformable DNA. The absence of

transformable Gal d 2 DNA has been established by PCR analysis based on the guidelines provided by the European Food Safety Authority (EFSA Panel on Genetically Modified Organisms (GMO), 2011). The PCR analysis (Appendix 1) concludes that no encoding pieces of recombinant DNA are present in Clara Foods' NEWP preparation. The level of detection of the PCR analysis was established to be 100 femtogram (fg) of recombinant DNA in the PCR reaction (Appendix 1, Figure B, Lane 6). Typical yeast transformations require microgram quantities of recombinant DNA for homologous integration. The PCR test protocol used is highly sensitive since it can detect ~10,000,000 times less transforming DNA than is required for homologous integration.

2.2.5 Absence of the DFB-004 Production Organism in the Final Product

The DFB-004 production organism is not detected in the NEWP samples in accordance with the recommendation of safety evaluation by the International Food Biotechnology Committee (IFBC, 1990). Procedure to ascertain absence of DFB-004 in the sample lots is presented in Appendix 1 (PCR method, demonstrating absence of organism through absence of transformable DNA) and Appendix 2 (Plating method, where no colonies of any organism were found to grow).

2.2.6 Absence of Potential Toxicants

The production strain and manufacturing process for NEWP do not produce any known toxicants. The extensive purification steps, including centrifugation, ultra-filtration and drying, add an additional level of confidence that the preparations are free from potential toxicants.

The safety decision tree (Pariza & Johnson, 2001) did not indicate the need for toxicological studies on NEWP. Previous toxicology studies, as reported in GRAS Notices GRN 204 and GRN 737, conducted subacute toxicity, repeated dose toxicity and mutagenicity/genotoxicity studies on test articles produced in the *K. phaffii* BG10 host background. No adverse test article effects were reported (See GRN 204 and GRN 737 included here by reference) (GRN 204, 2006) (GRN 737, 2018). Phospholipase C received FDA GRAS status in 2006 (GRN 204 2006) and has since been used for degumming vegetable oils for food use.

Komagataella phaffii is a yeast that is not known for making antibiotics, or toxic secondary metabolites. Yeasts, in general, are not known to make antibiotics (MacWilliams, 1959) and the Phaff Yeast Culture Collection (UC

Davis)[4] holds over 7500 strains of yeast, none of which are known to produce antibiotics.

Biopharmaceutical Jetrea® (Ocriplasmin), produced by *K. phaffii,* was approved by FDA in 2012 after Phase 3 clinical trials. Jetrea® is used for treatment of symptomatic vitreomacular adhesion (Research Corporation Technologies., 2012). Another biopharmaceutical approved by FDA in 2009 is Kalbitor® (DX-88 ecallantide), a recombinant kallikrein inhibitor protein produced using *K. phaffii*. It is used as an injection for the treatment of acute attacks of hereditary angioedema in patients aged 16 years or older (Research Corporation Technologies, 2020). Other products derived from *Komagataella* include recombinant human insulin, Insugen®, produced by Biocon; and the enzyme Phytase (Phytex, USA) used as an animal feed additive to provide phosphate by cleaving plant derived phytate.

Dried *K. phaffii* is an approved food additive for use in broiler poultry feed at up to a 10% inclusion rate as a source of protein as per 21 CFR 573.750. As noted in GRN 204, "In addition, *P. pastoris* (*K. phaffii)* itself has been approved by FDA as a source of animal feed protein for use in broiler feed up to 10% of the total feed (21 CFR 573.750). Toxicity studies done in support of the above-referenced *K. phaffii* - approved animal feed (including a pathogenicity study in mice, an acute oral toxicity study in rats, a subacute oral toxicity study in rats, and a two- generation teratology study in rats) also demonstrated-per FDA's review in 1993 - that *K. phaffii* is neither pathogenic nor toxigenic (21 CFR 573.750)"

Based on a comprehensive survey of the scientific literature,[5] Clara Foods concludes that there is no publicly available information that indicates or suggests safety concerns of the use of *K. phaffii* as a production organism for food substances.

2.2.7 Allergenicity concern

Gal d 2 protein is one of the predominant allergens in egg. Since NEWP is a fermentation-derived Gal d 2, products containing NEWP as an ingredient must be labeled as "Contains egg" or other appropriate ingredient descriptor that indicates that the product contains egg allergen according to allergen labelling requirements in the Food, Drug and Cosmetic Act (FD&C Act, Section 403(w)).

K. phaffii has a history of safe usage as a food ingredient production organism. The potential allergenicity of a product produced by the *K. phaffii*

[4] https://phaffcollection.ucdavis.edu/searchable-fields-strain-database#8
[5] Literature search July 2022

BG10 host background was evaluated and reported in GRN 737, page 58. The study concluded, and FDA had no objection, that there is no evidence of a risk of allergenicity from the carryover fermentation products of the production strain, *K. phaffii*.

2.2.8 Safe Strain lineage

Pariza and Johnson (Pariza & Johnson, 2001) recommend that microbial strains used to produce food-grade enzymes have a safe strain lineage. *K. phaffii* DFB-004 meets the following safe strain lineage characteristics:

- A well characterized nonpathogenic, nontoxigenic strain with a safe history of use in food enzyme manufacture
- Safety of all new DNA that has been introduced into the host organism
- Procedures used to modify the host organism are appropriate for food use

2.3 Intended Use

NEWP produced by *K. phaffii* DFB-004 is intended to be used as an ingredient in various food applications as a replacement for whole eggs (fresh and powdered) and egg white protein powder. It is GRAS at levels of use in accordance with cGMPs.

The intended uses of NEWP include but are not limited to product categories such as baked goods, prepared foods, breakfast category including ready-to-eat cereals, protein bars, prepared foods, salad dressings, eggnog, and egg white cocktails. Such uses will not increase the consumption of protein for the general population. It can also be used as a protein fining (clearing) agent in wines and juices just like egg white is currently used for this purpose.[6]

NEWP will be added to the product categories as a direct substitute for whole eggs (fresh and powdered) and hen egg white protein powder and therefore will not contribute to an additional exposure to egg white albumin for consumers.

NEWP is not intended for use in products regulated under USDA/FSIS jurisdiction or in infant formula.

[6] See 27 CFR 24.246.

2.4 Manufacturing, Production and Release Specifications

The NEWP is prepared in three (3) stages: construction of the production strain of *K. phaffii*, expression of protein in fermentation, and purification of the protein.

All materials used in the production of NEWP are standard food or pharmaceutical grade ingredients of a purity and quality suitable for the intended use, and processing conditions are appropriate for food production under cGMP as set forth in 21 CFR Part 117, Subpart B, hazard analysis and risk-based preventive controls (HARPC) requirements at 21 CFR Part 117, Subpart C, and otherwise adhere to all applicable U.S. food safety requirements.

2.4.1 Raw Materials

Raw materials used in the fermentation and recovery process for the NEWP are standard ingredients used in the food/enzyme industry. The specifications include limits on lead and other pertinent heavy metals. The raw materials are of a purity and quality suitable for the intended use in a food product; they are food grade and GRAS or certified USP or NF or ACS grade. The raw material fermentation ingredients are not major allergens and major allergens are not used in the final product formulation.

2.4.2 Fermentation

The NEWP is produced in a bioreactor using a *K. phaffii*-based fermentation process. The seed train for the fermentation process begins with the thawing of the cryo-stored *K. phaffii* in glycerol seed vials to room temperature. The contents of the thawed seed vials are used to inoculate liquid culture media in the primary fermenter.

The primary fermenter culture is grown at process temperature for a duration long enough to achieve target cell density after which the grown *K. phaffii* primary fermenter culture is transferred to a production scale reactor.

The culture is grown in the production bioreactor at target fermentation conditions and fed a series of substrates in accordance with the developed feed algorithm. At multiple times during the process, the fermentation is analyzed for culture purity.

2.4.3 Purification

The NEWP is purified by separating the cells from the liquid medium by centrifugation followed by micro-filtration. Further purification is accomplished using ultra-filtration, chromatography, and pH adjustments before a drying step to produce the final protein product.

Figure 5. Overview of the manufacturing steps for NEWP

2.4.4 In-process testing and monitoring

The fermentation process is carried out for a set number of days to ensure a standardized protein titer. To ensure the absence of significant microbial contamination, the inoculum is analyzed by microscopy or plate counts before inoculation of both the seed and the main fermentation and at critical process steps during fermentation and recovery.

In-process samples are taken at the completion of each unit operation in the production process. The in-process samples are analyzed for purity of culture and checked to ensure there is no microbial contamination. The identity of the protein produced is monitored by SDS PAGE. Identity and purity of the final spray dried NEWP preparation product is evaluated by a combination of analyses. Proximate analyses, heavy metal and microbial analyses are carried by an ISO 17025 accredited laboratory to ensure the sample meets the product specifications.

An LC MS/MS study was also performed on in-process samples and the final NEWP product. We investigated the samples pulled from the key downstream processing steps involved in the protein purification of the NEWP product (Figure 5). In-process samples were collected after cell separation and pre-purification (consisting of membrane filtration using microfiltration and ultrafiltration) as well as post purification (using column chromatography). These samples were then analyzed to identify the proteins present in them using LC MS/MS (Colgrave *et al*., 2014).

The purpose of this LC MS/MS study was to:

- Identify the host proteins present during the manufacture of NEWP
- Evaluate the inherent safety and allergenicity of these host proteins

The above investigation demonstrated that fractions of samples pulled after cell separation and pre-purification (Table 2) contained mostly the same safe host proteins that are present in Clara Foods recombinant protein preparation "Non animal Soluble Egg White Protein (NSEWP)" (GRN 967, reviewed by FDA, with no further questions) as well as other proteins considered to be safe. The data from the LC MS/MS analysis on the final spray dried powder (NEWP) that had gone through the subsequent purification steps (post-purification) is also presented in Table 2.

The protein sequences were checked by BlastP analysis for similarities to known allergens at the University of Nebraska Food Allergy Research and Resource Program (FARRP)[7] Allergens Online, to known bacterial virulence factors[8], and for similarity to other proteins at NCBI using BlastP[9] and all fell into safe categories such as strongly matching *Saccharomyces* proteins (e.g. Kar2p), or failing to match any known allergen or bacterial toxin. As noted in the GRN 737 filing, there is a strong history of safe consumption of *Saccharomyces* proteins, so these *Komagataella* proteins are expected to be as safe as other yeast proteins. Note a typical nutritional yeast product recommends a 15 g/day dose, and the nutritional label estimates the 15g serving to have 8 g protein[10], and people who maintain a vegan diet consume nutritional yeast as a source of certain B vitamins that they would normally get from consuming meat.

Table 2: Komagataella Host Proteins detected in samples pulled from downstream stages of production: post cell separation, pre-purification, and post-purification i.e., NEWP Product.

	Uniprot Protein ID	**Protein Description**	**Post cell separation**	**Pre-purification**	**Post-purification (NEWP powder)**	**Match to either Allergen or VFDB Database**	**Closest Protein *S. cerevisiae* (s288c)**
	P01012.2	Gal d 2 #	Yes	Yes	Yes	Yes - Allergen DB Match	N/A
1	F2R0E1	Cell wall biogenesis involved protein	Yes	No	No	No	Nca3
2	F2QUR1	Endochitinase	Yes	Yes	No	No	Cts1

[7] https://farrp.unl.edu/resources/allergenonline
[8] http://www.mgc.ac.cn/VFs/search_VFs.htm
[9]https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome
[10] https://www.bobsredmill.com/nutritional-yeast.html

	Uniprot Protein ID	Protein Description	Post cell separation	Pre-purification	Post-purification (NEWP powder)	Match to either Allergen or VFDB Database	Closest Protein *S. cerevisiae* (s288c)
3	F2QXH5	Extracellular protein X1	Yes	Yes	No	No	Pry2
4	F2QNG1	Putative glucanase	Yes	No	No	No	Scw4
5	F2QUV5	Cell wall beta-glucan assembly glycoprotein	Yes	Yes	No	No	Kre9
6	C4R3C4	Putative subtilisin-like GPI-anchored zonadhesion	Yes	Yes	No	No	No Hits
7	Q56D08	Kar2p, protein chaperone	Yes	No	No	Yes - Allergen DB Match	Kar2
8	Q9C1Z8	Protein disulfide-isomerase	Yes	Yes	No	No	Pdi1
9	Q0QCW1	1,3-beta-glucanosyltransferase	Yes	Yes	No	No	Gas1
10	F2QQJ0	Putative Tos1p-like cell wall protein *	Yes	Yes	No	No	Tos1p
11	F2QQ27	Putative lectin-like protein *	No	Yes	No	No	Flo9
12	F2QZP3	Thioredoxin *	No	Yes	Yes	No	Trx1/2

P01012.2 (Gal d 2) is an internal control demonstrating the detection of NEWP as homologous to the protein of interest

* Host proteins found in NEWP preparations but not in NSEWP samples in GRN-967 - these proteins were detected in the more concentrated pre-purification samples but not in the earlier stage where protein concentration was lower.

Note one protein (C4R3C4) in the post cell separation and pre-purification samples did not match any proteins from *Saccharomyces* cerevisiae. This protein was detected in the NSEWP preparation, which is the subject of GRN 967 that FDA reviewed and issued a “no questions” letter. Nevertheless, this protein was not detected in the final NEWP powder post purification. BlastP analysis of protein C4R3C4 showed similarities to a subtilisin-like protein. Subtilisin is used in the food industry and one version from *Bacillus subtilis* was tested in a 90 day feeding study and received GRAS status as a food processing aid (GRN 714).

2.4.5 Product release specification

The microbial and purity specifications are based on the Enzyme Preparations Monograph set forth in Food Chemicals Codex (FCC) 11th edition (Pharmacopeia, 2018) as a guide to appropriate food grade specifications for proteins. Final product release specifications are listed in Table 3.

The results from testing three (3) nonconsecutive lots of the NEWP product are presented in Table 4, verifying that the product meets or exceeds specifications for proteins used as ingredients as listed in the product release specifications in Table 3. This data confirms that the preparation method produces a consistent product.

Tables 3 and 4 are presented in the following two pages.

Table 3. Specification for Non-animal egg white protein (NEWP) produced by *K phaffii* DFB-004

Physical properties	**Specification**
Source	Yeast fermentation-derived
Appearance	White to off-white amorphous powder
Solubility	Soluble in water

Chemical Properties (in powder as is)	**Specification**	**Method**
Protein	> 75% (w/w)	AOAC 990.03[1a] AOAC 992.15[1b]
Moisture	Maximum 10.0%	AOAC 925.09[2]
Carbohydrate	Maximum 10%	Calculated
Ash	Maximum 5.0%	AOAC 942.05[3]
Fat by Acid Hydrolysis	< 0.4%	AOAC 954.02[4]
Mercury (Hg)	< 1 mg/kg	ICP-AES[5]
Lead (Pb)	< 1 mg/kg	ICP-AES[5]
Arsenic (As)	< 1 mg/kg	ICP-AES[5]
Cadmium (Cd)	< 1 mg/kg	ICP-AES[5]

Microbial Properties (in powder as is)	**Specification**	**Method**
Standard Plate Count	< 10000 CFU/g	AOAC 966.23[6]
Yeast & Mold	< 100 CFU/g	FDA BAM Chapter 18 mod.[7]
Salmonella	Not Detected / 25g	AOAC 2003.09[8]
E. coli	Not Detected / g	AOAC 991.14[9]
Total coliform	≤ 30 CFU/g	AOAC 991.14[9]

[1a] AOAC 2006. Protein (crude) in animal feed, combustion method, 990.03. *In:* Official methods of analysis of AOAC International. 18th ed. Gaithersburg: ASA-SSA Inc.

[1b] AOAC 2006. Proximate Analysis and Calculations Crude Protein Meat and Meat Products Including Pet Foods - item 80. *In:* Official methods of analysis Association of Analytical Communities, Gaithersburg, MD, 17th edition, Reference data: Method 992.15 (39.1.16); NFNAP; NITR; NT.

[2] AOAC 2005. Solids (total) and moisture in flour. In: *Official Methods of Analysis of AOAC International*, Methods 925.09: 18th Ed., AOAC International, Gaithersburg, MD

[3] AOAC 2005. Official Method 942.05, Ash of Animal Feed. In: Official Methods of Analysis of AOAC International, 18th edition, Chapter 4, p. 8, AOAC International, Gaithersburg, MD.

[4] AOAC International. 2012. Official Method Fat (crude) or ether extraction in pet food. Gravimetric method, 954.02. *In*: Official Methods of Analysis of AOAC International, 19th ed., AOAC International, Gaithersburg, MD, USA, 2012.

[5] Julshamn, K., Maage, A., Norli, H. S., Grobecker, K. H., Jorhem, L., Fecher, P. 2007. "Determination of Arsenic, Cadmium, Mercury, and Lead by Inductively Coupled Plasma/Mass Spectrometry in Foods after Pressure Digestion: NMKL Interlaboratory Study" Journal of AOAC International, Volume 90, Issue 3, 844–856

[6] AOAC International. 2000. Aerobic plate count, method 966.23. *In:* Official methods of analysis of AOAC International, 17th ed. Gaithersburg, MD.

[7] FDA Bacteriological Analytical Manual. 1998. 8th Edition, Revision A, Chapter 18: Yeasts, Molds and Mycotoxins

[8] AOAC International. 2005. *Salmonella* in selected foods, BAX automated system, method 2003.09. *In* Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD.

[9] AOAC International. 2005. *E. coli* count in foods, dry rehydratable film, method 991.14. *In:* Official methods of analysis of AOAC International, 17th ed. AOAC International, Gaithersburg, MD.

Table 4. Results for three lots of Non-animal egg white protein (NEWP) produced by *K phaffii* DFB-004

Analysis Parameter	Specification	P2005	P2023	P2050
Protein (%)	> 75% (w/w)	86.06	85.19	81.88
Protein (% dry weight powder)	> 80%	92.8	92.7	87.4
Moisture and Volatiles (%)	< 10%	7.3	8.1	6.3
Carbohydrates, Calculated (%)	< 10%	3.95	5.89	7.34
Ash (%)	< 5%	2.69	0.82	4.48
Fat by Acid Hydrolysis (%)	< 0.4%	<0.10	<0.10	<0.10
Arsenic (As) (mg/kg)	< 1 mg/kg	<0.010	<0.010	<0.010
Mercury (Hg) (mg/kg)	< 1 mg/kg	<0.010	<0.010	<0.010
Lead (Pb) (mg/kg)	< 1 mg/kg	<0.010	<0.010	0.018
Cadmium (Cd) (mg/kg)	< 1 mg/kg	<0.010	<0.010	<0.010
Aerobic Plate Count (CFU/g)	< 10000 CFU/g	<10	<10	<10
Molds (CFU/g)	< 100 CFU/g	<10	<10	<10
Yeast (CFU/g)	< 100 CFU/g	<10	<10	30 (est)
Salmonella (CFU/25g)	Not Detected / 25g	Not Detected	Not Detected	Not Detected
Escherichia Coli (CFU/g)	Not Detected / g	Not Detected	Not Detected	Not Detected
Coliforms (CFU/g)	< 30 CFU/g	<10	<10	<10
Absence of source organism from product	Not detected * / mg sample	Not detected	Not detected	Not detected
Absence of encoding DNA from product	Not detected ** / mg sample	Not detected	Not detected	Not detected

* Limit of detection for source organism = 11 CFU/mg sample

** Limit of detection for encoding DNA = 100 femtogram (Appendix 1)

Table 4 demonstrates that the production methods described yield a consistent product that meet the specifications listed in Table 3.

Part 3: Dietary Exposure (21 CFR 170.235)

Non-animal egg white protein (NEWP) is intended to be used as an ingredient in various food applications as a replacement for egg white protein powder and whole eggs (fresh and powdered), since it has functional properties such as foaming, gelation, binding similar to egg whites and whole eggs. The uses of NEWP include but are not limited to product categories and usage levels provided in Table 5. The GRAS position covers the use of NEWP in food at levels in accordance with current Good Manufacturing Practices (cGMPs). Table 5 below provides examples of typical product categories and use levels of NEWP.

Table 5: Typical product categories and use levels of NEWP

Category	**Representative Examples**	**Serving size [a]**	**% inclusion (as solids) [b]**	**NEWP usage level (g/serving size) [c]**
Bars	Protein bars	52 g	10 – 23%	6 – 15 g
	Snack bars	30 g (RACC = 40 g)	10%	3 – 4 g
Ready-to-eat cereal	Cereal / Granola	60 g	10%	7 - 8 g
Breakfast category	Scrambled egg	110 g	10%	14 g
	Egg white patty	70 g	10%	9 g
	Pancakes	116 g	3 – 5%	4 – 7 g
	Waffles	70	3 – 5%	2 – 4 g
Baked goods	Cakes	80 g	2 – 6%	2 – 6 g
	Protein Cookies	40 g	10 - 25%	4 – 12 g
	Quiche	140 g	10%	17 g
	Souffle	136 g	8%	13 g
Prepared foods (plant-based meat analogues)	Meat Alternative Burgers (Ready To Eat & Ready To Cook)	120 g	4 – 8%	6 – 12 g
	Veggie Dogs / Corn Dogs	86 g	4 – 8%	4 – 9 g

Category	Representative Examples	Serving size [a]	% inclusion (as solids) [b]	NEWP usage level (g/serving size) [c]
Prepared foods (plant-based meat analogues)	Meat Alternative Sausage Links	46 g	4 – 6%	2 – 3 g
	Meat Alternative Sausage Patties	38 g	6 – 8%	2 – 4 g
	Appetizers	84 g	2 – 6%	2 – 6 g
	Nuggets	86 g	4 – 8%	4 – 9 g
	Meatless Crumbles	55 g	3 – 5%	2 – 3 g
	Plant-based Bacon	16 g	4 – 8%	1 – 2 g
	Meat Alternative Whole muscle cut	85 g	3 – 6%	3 – 6 g
	Sandwich Breakfast	105 g	15 – 20%	19 – 26 g
Prepared foods	Egg noodles / pasta	55 g	8 – 10%	4 – 5 g
Miscellaneous	Egg white cocktail	110 mL	2%	2 – 3 g
	Eggnog	120 mL	2 – 3%	3 – 4 g
	Dressing	30 mL	5 – 10%	1.5 – 3 g
	Powdered Shake Mix	200 – 240 mL	11 – 13%	28 – 33 g

[a] Based on potential applications and Reference Amounts Customarily Consumed (RACCs) per Eating Occasion established in 21 CFR 101.12(b)

[b] Based upon current usage rates of egg white protein

[c] Adjusted for 80g protein in 100g NEWP

NEWP will be added to various foods as a direct substitute for whole eggs (fresh and powdered) and hen egg white protein powder and therefore, will not contribute to an additional exposure to egg white for consumers. If used as a fining agent to clarify and stabilize wine, the level of usage will be comparable to

the current application levels of egg albumin that ranges from 30 to 150 mg/L (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2000).

As part of the protein consumption in the US, consumers currently consume hen eggs as a routine component of the daily diet. In addition to consumption of whole eggs, eggs are used in prepared foods to provide flavor and texture in addition to functional uses as emulsifiers, stabilizers, and increasing volume and machineability (American Egg Board, 2020).

In 2019, approximately 9.9 x 10^{10} hen eggs were sold in the US or 6.2 x 10^{11} grams of egg protein introduced into the US market representing an egg consumption of approximately 287 eggs per capita (American Egg Board, 2020). An average large whole egg contains 6.25g of protein. This provides 11.2% and 13.6% of the daily recommended protein intake for adult males and females respectively (Institute of Medicine 2005).

Based on the USDA, Agricultural Research Service (USDA/ARS) Usual Nutrient Intakes from Food and Beverage by Gender and Age, based on What We Eat in America, (WWEIA, NHANES) 2013-2016 data (*See* Table A2), [11] we conservatively estimate the mean and 90th percentile exposure to NEWP for all persons 1 year and older to not exceed 79.2 g/person/day and 158.4 g/person/day, respectively. The 90th percentile exposure was estimated by doubling the mean intake of protein reported by WWEIA.

These estimates are based on the assumption that NEWP will replace existing protein in the food supply and will not increase a consumer's overall exposure to protein. We note that these estimates are very conservative as NEWP will not be used in every category of food that contains protein, we would not expect NEWP to penetrate the market completely, it is highly unlikely if not impossible that a person would consume all the listed food categories containing NEWP in a day, and many sources of protein in the diet are unlikely to be completely replaced by NEWP.

[11] USDA USUAL NUTRIENT INTAKE from Food and Beverages, by Gender and Age What We Eat in America, NHANES 2013-2016: https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/usual/Usual_Intake_gender_WWEIA_2013_2016.pdf

Part 4: Self-limiting Levels of Use (21 CFR 170.240)

When used as an ingredient, the level of use of NEWP is self-limiting due to sensory and technical limitations.

Part 5: Experience Based on Common Use in Food before 1958 (21 CFR 170.245)

While it is obvious that Gal d 2 has been in human food for millennia as a constituent protein of hen egg white, the substantially equivalent NEWP produced in *K. phaffii* was not present in the food supply prior to 1958, and a GRAS determination based upon a common use in food before 1958 alone does not apply.

Part 6: Safety Narrative (21 CFR 170.250)

6.1 Background

Plant and animal-derived proteins are important ingredients in food manufacture and are frequently used by consumers to augment their protein intake. Traditionally, these proteins were extracted from their respective plant and animal sources. Over the last few decades, it has been possible to produce these proteins in higher yield and enhanced purity by virtue of fermentation processes utilizing either bacteria or yeast as production organisms. Many such "microbially-produced" food ingredients have been reviewed by the US FDA and are listed on the FDA GRAS Notice Inventory.[12]

NEWP produced by *K. phaffii* DFB-004 is an excellent example wherein a protein (Gal d 2) that is utilized as a safe and suitable nutrient (normally extracted from hen egg whites) can now be produced using a microbial production system. The two preparations (native hen egg Gal d 2 and NEWP) contain essentially the same protein but are produced with different primary production processes.

The safe history of use of hen egg Gal d 2 is well established since it is the major protein in hen egg whites. The use of avian eggs as food predates recorded history and proteins derived from eggs in food processing have a long history of safe use and are considered GRAS (21 CFR 170.30(d)). In addition to consumption of whole eggs, eggs used in prepared foods provide flavor and texture in addition to processing advantages such as emulsification, stabilization, increasing volume and machinability (American Egg Board, 2020).

6.2 Regulatory History

There are no regulations in the U.S. that limit the consumption of eggs or the consumption of food products containing egg components. Reinforcing the justification for the lack of regulatory limitation, FDA states "Proteins derived from egg whites do not raise toxicity concerns because egg whites have been safely consumed by humans as a source of food throughout recorded history without any reports of toxicity (FDA, 1998)."

As NEWP is a novel protein preparation, there is no direct history of regulatory oversight. However, US FDA has reviewed the use of the same *K. phaffii* host background as part of the GRN 205 and GRN 737 GRAS Notice and had "no questions" concerning the safety of the proposed use. Dry *K. phaffii*

[12] Examples of GRAS Notice for microbially-produced ingredients: GRN 967, GRN 863, GRN 737, GRN 205, GRN 1001.

is also allowed for use in poultry food at a usage rate of up to 10% (w/w) as described in 21 CFR 573.750. In addition, US FDA has recently reviewed a similar filing (GRN 967) from Clara Foods, that uses the same *K. phaffii* host background to make recombinant egg protein and had "no questions" regarding the safety.

6.3 Safety Data

6.3.1 NEWP substantial equivalence to the native hen egg Gal d 2

NEWP has been shown to be equivalent to the native hen egg Gal d 2 in amino acid sequence, immunoreactivity, and digestibility in simulated gastric tests. NEWP contains minor modifications to its sequence that are not found in the native hen egg-derived preparation leading to a difference in molecular weight and n-terminal amino acid sequence.

Specifically, NEWP contains 4 extra amino acids in the N-terminal derived from *S. cerevisiae*, as a result of post-translation processing. A protein sequence safety evaluation of the resulting proteins has demonstrated that the NEWP amino acid sequence has no significant similarity to known proteinaceous toxins or known allergens other than the expected amino acid sequence equivalence to the native Gal d 2 (Pearson & Lipman, 1988) (Liu, Zheng, Jin, Chen, & Yang, 2019) (Virulence Factor Database). Further, the very minor amino acid changes to the Gal d 2 sequence are derived from the *S. cerevisiae* that has a history of safe use in food. Thus, the NEWP structure is highly unlikely to present safety risks that are not present with consumption of hen egg.

Further, the physical characteristics of the mature protein forms of native Gal d 2 and recombinant Gal d 2 (NEWP) described in Table 6 (below) are substantially equivalent in molecular weight, isoelectric point, glycosylation sites. The two proteins differ in glycosylation as described in detail in Section 2.1.3.

Table 6: Physical characteristics of native Gal d 2 and NEWP

Protein	Native Gal d 2 (mature form)	NEWP
Amino acid sequence of mature protein without glycosylation	GSIGAASMEFCFDVFKELKVHHANE NIFYCPIAIMSALAMVYLGAKDSTRT QINKVVRFDKLPGFGDSIEAQCGTS VNVHSSLRDILNQITKPNDVYSFSLA SRLYAEERYPILPEYLQCVKELYRG GLEPINFQTAADQARELINSWVESQ TNGIIRNVLQPSSVDSQTAMVLVNAI VFKGLWEKAFKDEDTQAMPFRVTE QESKPVQMMYQIGLFRVASMASEK MKILELPFASGTMSMLVLLPDEVSG LEQLESIINFEKLTEWTSSNVMEERK IKVYLPRMKMEEKYNLTSVLMAMGI TDVFSSSANLSGISSAESLKISQAVH AAHAEINEAGREVVGSAEAGVDAAS VSEEFRADHPFLFCIKHIATNAVLFF GRCVSP	***EAEA***GSIGAASMEFCFDVFKELKVH HANENIFYCPIAIMSALAMVYLGAKD STRTQINKVVRFDKLPGFGDSIEAQ CGTSVNVHSSLRDILNQITKPNDVYS FSLASRLYAEERYPILPEYLQCVKEL YRGGLEPINFQTAADQARELINSWV ESQTNGIIRNVLQPSSVDSQTAMVL VNAIVFKGLWEKAFKDEDTQAMPFR VTEQESKPVQMMYQIGLFRVASMA SEKMKILELPFASGTMSMLVLLPDE VSGLEQLESIINFEKLTEWTSSNVME ERKIKVYLPRMKMEEKYNLTSVLMA MGITDVFSSSANLSGISSAESLKISQ AVHAAHAEINEAGREVVGSAEAGVD AASVSEEFRADHPFLFCIKHIATNAV LFFGRCVSP
Molecular Mass (Calculated)	42.88 kDa	43.15 kDa
Isoelectric point without glycosylation[a]	4.6	4.84
Number of residues glycosylated	1[b]	2
Carbohydrate identities [c]	N-acetyl glucosamine, mannose, galactose	N-acetyl glucosamine, mannose

[a] Calculated isoelectric point
[b] Nisbet *et al*., 1981. "The Complete Amino-Acid Sequence of Hen Ovalbumin." *Eur J Biochem 115,* pages 335-345.
[c] Refer to Figure 1 for description of Carbohydrate residues and the relevant glycoforms.

The glycosylation pattern of native protein is slightly different (Figure 1) compared to the NEWP produced by *Komagataella*. LC-MS analysis of the glycans attached to NEWP indicates that the glycans attached to the protein have a range of 8 to 14 mannose residues, with the highest percentage being 10 mannoses, consistent with published work on Komagataella-based N-linked glycosylation (Trimble et al., 1991; reviewed in Gemmill and Trimble, 1999) and similar to published results for recombinant Gal d 2 from *Komagataella* (Ito and Matsudomi, 2005). Gemmill and Trimble, 1999 provide an excellent comparison of typical glycan trees for yeasts and show that *Saccharomyces cerevisiae* typically adds much more extensive mannose trees compared to *Komagataella*. Consumption of yeast-glycosylated protein has no safety or

health concern. Glycosylated yeast proteins are consumed on a daily basis as part of nutritional yeast and yeast extract products. Ciofalo et al. (2006), performed a 90-day toxicity study with rats fed up to 2000 mg/kg body weight/day of a glycoprotein produced using *Komagataella phaffii*. The authors concluded that the glycoprotein was not toxic at any dose tested. This publication was part of the GRAS notice from Diversa Corp. (GRN 204) which received a No Questions letter from US FDA. People are consuming *Komagataella* proteins on a regular basis by way of Impossible Foods Burger (GRN 737) with no reported ill effects. Some of these Komagataella proteins may be glycosylated with essentially the same glycans as found in the NEWP preparation.

As shown in Table 1, the *in-vitro* digestibility analysis demonstrated the equivalence between native chicken egg white Gal d 2 and NEWP. Since NEWP has essentially the same amino acid sequence, gastric digestibility profile and immunoreactivity as the native hen egg Gal d 2, NEWP is substantially equivalent to the native Gal d 2 and thus, can be said to have the same safe history of consumption as hen egg protein.

Finally, NEWP will be added to the product categories as a direct substitute for whole eggs (fresh and powdered) and hen egg white protein powder and therefore will not contribute to an additional exposure to egg white albumin for consumers.

There are no known toxicological limits established for the daily intake of amino acids from protein ingested as food, including egg proteins (Institute of Medicine, 2005). Thus, the consumption of NEWP at the levels envisioned herein do not present a safety concern.

6.3.2 Allergenicity concern

Gal d 2 protein is one of the predominant allergens in egg. Since NEWP is a fermentation-derived Gal d 2, products containing NEWP as an ingredient must be labeled as “Contains egg” or other appropriate ingredient descriptor that indicates that the product contains egg allergen according to allergen labelling requirements in the Food, Drug and Cosmetic Act (FD&C Act, Section 403(w)).

K. phaffii has a history of safe usage as a food ingredient production organism. The potential allergenicity of a product produced by the *K. phaffii* BG10 host background was evaluated and reported in GRN 737, page 58. The study concluded, and FDA had no objection, that there is no evidence of a risk of allergenicity from the carryover fermentation products of the production strain, *K. phaffii*.

6.3.3 Safety of the production organism

Once the safety of the microbially-produced NEWP is assured, it is appropriate that changes in the means of production should be evaluated to ensure that no increase in the likelihood of hazard to consumers is created.

Clara Foods has inserted the hen egg Gal d 2 gene into a yeast production host organism, *K. phaffii*, that has a history of safe use in the production of proteins for food use, including enzymes. The following describes the safety evaluation, following the guidelines of Pariza and Johnson (Pariza & Johnson, 2001), undertaken to determine with reasonable assurance that the *K. phaffii*-produced NEWP is safe for use in food.

Clara Foods has determined by amino acid sequencing, immunoreactivity, glycosylation analysis, and digestibility that NEWP produced by *K. phaffii* DFB-004 is substantially chemically and structurally equivalent to hen egg Gal d 2. Hen egg Gal d 2, as a constituent of egg white, has a history of safe use in food, and therefore, the substantially equivalent protein produced by *K. phaffii* DFB-004 is presumed to be safe. Pariza and Johnson advise that if an enzyme product has a history of safe use in food that the "safety of the production strain should be the primary consideration in evaluating enzyme safety" (Pariza and Johnson 2001). While NEWP is not an enzyme product, it is a protein, and we have followed the Pariza and Johnson decision tree and Delaney *et al* (2008) as a guide to appropriate scientific procedures for the safety determination of the protein.

Pariza and Johnson (2001) clearly state that the safety of the production strain should be the primary consideration in evaluating the safety of expressed proteins, enzymes, and other microbial products. Clara Foods has fully characterized *K. phaffii* DFB-004 to ensure that it is a safe and suitable production host.

The *K. phaffii* DFB-004 production host background, *K. phaffii* BG10, is a commercially available, non-toxigenic and non-pathogenic, safe, and suitable food production organism that has been reviewed by FDA in GRAS Notices GRN 205, GRN 737 and GRN 967 for the production of enzyme and protein food ingredients. *K. phaffii* BG10 background complies with the OECD criteria for Good Industrial Large-Scale Practice (GILSP) microorganisms (OECD, 1992; OECD, 1993) and meets the criteria for a safe production microorganism as described by Pariza and Foster (1983), Pariza and Johnson (2001), and several expert groups, including the EU Scientific committee for Food, FAO/WHO and the International Food Biotechnology Council. *K. phaffii* BG10 has a safe strain lineage "through which improved strains may be derived via genetic modification either by using traditional/classical or rDNA strain improvement strategies" (IFBC, 1990).

Pariza and Johnson (2001) also state that the elements needed to establish a safe strain lineage for the production host include characterization of the host organism, determining the safety of all new DNA introduced into the host, and ensuring that the procedures used to modify the host are appropriate for food use. Clara Foods has rigorously followed the guidance provided in Pariza and Johnson (2001) to establish the safety of the production host *K. phaffii* DFB-004 for the production of NEWP.

The hen egg Gal d 2 gene sequence is a well characterized, contiguous gene sequence and results in the production of Gal d 2 in hen eggs. The Gal d 2 gene is inserted into the DFB-004 production host in two locations in Chromosome 1.

The microbial production host has been characterized by DNA sequencing. Sequence analysis demonstrates that *K. phaffii* DFB-004 is identical to *K. phaffii* BG10 background with the addition of well-defined genetic elements added to generate the NEWP production organism.

K. phaffii DBF-004 was constructed employing well-defined genetic modification techniques using only DNA that is safe for use in the production of food. DNA introduced to the production organism includes the hen egg Gal d 2 gene, copies of *K. phaffii* methanol-inducible promoters, and *Saccharomyces cerevisiae* alpha mating factor pre-pro sequence for the expression of heterologous proteins in *K. phaffii*.

Insertion of the complete hen egg Gal d 2 gene and loci of insertion in the host genome were confirmed. DNA sequence analysis confirms that the native Gal d 2 gene and the production enhancing DNA cassettes were present in *K. phaffii* DFB-004 at the specific insertion locations in the production host *K. phaffii* DFB-004 and no known potentially hazardous genetic modifications were made in the construction of the production host organism.

Standard recombinant techniques were used to remove antibiotic resistance markers from the production host and standard methods, i.e., a combination of PCR, antibiotic resistance, and DNA sequencing analysis, were used to demonstrate the absence of antibiotic resistance genes or bacterial origins of replication present in the production host *K. phaffii* DFB-004.

The *K. phaffii* BG10 host background has been used to produce three (3) proteins that are the subject of GRN 205, GRN 737 and GRN 967 (incorporated herein by reference). Toxicity testing of the preparations (GRN 205 and GRN 737) indicated no treatment related adverse effects, indicating the safety of the production organism and the target proteins.

Dry *Komagataella phaffii* is permitted in poultry feed at an inclusion rate of up to 10% (w/w) (21 CFR 573.750). If we assume a feed ration of 1/4 pound or 113 g of ration per broiler hen per day and a harvest weight of 3.2 kg per bird, the daily intake of *Komagataella phaffii* would be 3.4 g Komagataella/kg body weight/day. While we understand that chickens are not the recommended toxicity test model, the intake of *Komagataella phaffii* fed to animals that are destined to be human food at these levels is further evidence of the safety of the organism. A comprehensive literature search for toxicity or pathogenicity of *K. phaffii* did not identify any references that raised safety concerns, thereby supporting the status of the organism as non-toxigenic and non-pathogenic organism that is safe for use in the production of food.

6.4 Manufacturing and Labeling

Critical to the production of safe and suitable food, manufacturing facilities must meet US regulations for food production. The NEWP produced by *K. phaffii* meets production specifications (Table 3) and is produced in accordance with cGMP as defined in 21 CFR 117, Subpart B and hazard analysis and risk-based preventive controls (HARPC) requirements at 21 CFR Part 117, Subpart C employing standard food fermentation industry practices. All ingredients used in the production of the NEWP are GRAS ingredients, approved food additives, and other food-grade materials appropriate for food production. NEWP is an egg protein and will be labelled as such on foods that contain it; no other major food allergens are used as fermentation raw materials or ingredients in the production of NEWP.

6.5 Conclusion of the Pariza and Johnson Decision Tree

Pariza and Johnson (2001) provide a peer-reviewed description of the scientific procedures to be followed in determining the safety of microbially-produced proteins. Clara Foods has rigorously followed this guidance in assessing the safety of NEWP produced by *K. phaffii*. The conclusion of the decision tree is that NEWP produced by *K. phaffii* is accepted as a safe and suitable food ingredient. The GRAS conclusion is based on the substantial equivalence of the microbially produced NEWP to the hen egg native Gal d 2 that has a history of safe use in food, the demonstrated safety of the production *K. phaffii* strain, and manufacturing processes that meet or exceed food cGMP requirements.

The decision tree, in question-and-answer format is included below:

1. Is the production strain genetically modified?
   Yes. If yes, go to 2.

2. Is the production strain modified using rDNA techniques?
Yes. If yes, go to 3.

3. Issues relating to the introduced DNA are addressed in 3a-3e.

3a. Do the expressed enzyme [protein] product(s) which are encoded by the introduced DNA have a history of safe use in food?
Yes. If yes, go to 3c.

3c. Is the test article free of transferable antibiotic resistance gene DNA?
Yes. If yes, go to 3e.

3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce food-grade products?
Yes. If yes, go to 4.

4. Is the introduced DNA randomly integrated into the chromosome?
Yes. If yes, go to 5.

5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed?
Yes. If yes, go to 6.

6. Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via this evaluation procedure?
Yes. If yes, the test article is **ACCEPTED**.

6.6 Summary

The Non-animal egg white protein (NEWP) and the production organisms have been shown by scientific procedures to be safe and suitable for use in food production.

The NEWP produced by *K. phaffii* is substantially chemically and structurally equivalent to hen egg Gal d 2. The native Gal d 2 has a long and safe history of use in food, since it is present in egg white. The production host *K. phaffii* DFB-004 has been fully characterized and satisfies the criteria for a safe strain lineage as recommended by Pariza and Johnson (2001). The host background has been used to produce GRAS substances. The wild type organism is also an approved food additive in poultry feed.

Toxicological testing of the host background as notified to FDA in previous GRAS Notices indicate no safety concerns for the use of the production organism and no allergen concerns.

The intended use of NEWP is substitutional for current uses of egg white protein and whole eggs. Therefore, there would be no increase in dietary exposure to egg white protein from the intended use. Eggs and egg proteins have been consumed for millennia without any evidence of toxicity, providing further evidence for the safety of NEWP.

6.7 GRAS Conclusion

Clara Foods has concluded by scientific procedures that NEWP, produced by *K. phaffii,* is Generally Recognized as Safe (GRAS) for use in food when manufactured according to cGMP pursuant to both 21 CFR 170.30 (a) and (b) and is thereby exempt from pre-market approval requirements of the Food, Drug and Cosmetic Act.

Part 7: List of supporting data and information (21 CFR 170.255)

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Appendix 1: Standard Operating Procedure and Results for Absence of encoding DNA by PCR

Purpose:
This method is used to internally confirm the absence of transformable DNA in NEWP final powder samples.

Materials:
- 2X Taq MasterMix from NEB
- Primers appropriate for cPCR and chicken Gal d 2
- For Gal d2 orf 3 FOR:
GGTTCCATTGGCGCAGCATCTATGGAGTTCTGCTTCG
- For Gal d 2 orf 3 REV: CTAAGGGGACACGCATCTACCGAAAAACAGCAC
- Tm = 63
- Gal d 2 transforming DNA (1X Gal d 2 orf 3 (NHOVA109Z)
- PCR water
- 25mM sodium hydroxide diluted in PCR water
- PCR tubes
- Purified recombinant Gal d 2 Product (NEWP)
- Agarose
- SYBR green
- DNA loading dye
- Thermocycler
- Gel electrophoresis system with power supply
- Gel documentation system

Methods:

Dilute the Gal d 2 powder to 100mg powder/mL in 25mM sodium hydroxide (this may require extensive vortexing in order to get into solution. After the powder is fully dissolved, transfer 250μL to two new tubes with 250μL of 25mM sodium hydroxide (this will create two new 500μL tubes of 50mg/mL NEWP powder). From this dilution, make an additional 10x dilution to have two tubes at a final concentration of 5 mg/mL by adding 35μl of 50mg/mL Gal d 2 to 315μl of 25mM NaOH. To one of these tubes, add positive control Gal d 2 plasmid DNA to get a final concentration of 1ng/μL (this will serve as the positive control for the assay).

In an 8-tube PCR strip tube aliquot 45uL of un-spiked 5mg/mL NEWP powder in 25mM NaOH into Tubes 2-8. In Tube 1, add 50μL of the 5mg/mL NEWP solution spiked with 1ng/μL Gal d 2 plasmid then transfer 5μL from Tube 1 to Tube 2 and mix well. Continue dilutions until the last tube. Be sure to mix very well after transferring the 5μL to each tube to ensure proper mixing. Based on the starting spiked control of 1ng/μL, you will have 1fg of control plasmid in Tube 7 which is near the detection limit of this assay. Final volumes for Tubes 1-7 will be 45μL

while Tube 8 will have 50μL. Repeat this for any number of samples you may have. The samples generated in this step is simply for the limit of detection of Gal d 2 plasmid DNA in 5mg/mL NEWP product in 25mM sodium hydroxide.

Set up the master mix as seen below for the appropriate number of reactions you will need +10% for volume loss. Load the test sample of 5mg/mL NEWP without spiked DNA in duplicate. For positive control reaction load 1μL of 1ng/μL of Gal d 2 plasmid DNA. For negative control, load 1μL of 25mM sodium hydroxide. For 5mg/mL NEWP product in duplicate, the 8 dilutions to show limit of detection of each sample Lot of NEWP, there will be a total of 12 tubes in the cPCR test: plasmid DNA, one positive and one negative control. For all samples, load 1μL into the PCR reaction.

Component	x1	x 15
2X TaqMM	10	15
wz_pr900 Gal d 2_FOR 10μM	1	15
wz_pr901 Gal d 2_REV 10μM	1	15
DNA	1	-
water	7	105
PCR conditions Gal d 2 ORF 3		
Standard cPCR Taq protocol		
95C 3' denature		
95C 30" 30 cycle		
63C 30" 30 cycle		
68C 1'10" 30 cycle		
68C 5'		
4C forever		

After PCR reactions are finished, add loading buffer, and then load 20μL of the sample on a 1% agarose gel and run for 35 minutes at 110V. Product for Gal d 2 ORF should be 570bp.

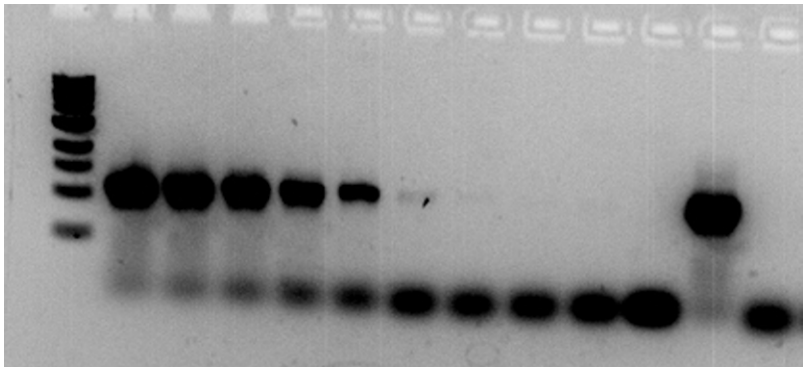
Presence of coding DNA is determined by presence of the respective products showing up on the DNA gel. Limit of detection is around 100 femtogram (fg) of positive control plasmid.

Gel Results for **P2005**:

Lane description is provided below the gel picture (Figure A). Limit of detection is ~10fg to 100fg of plasmid DNA as observed from the thin band in Lane 7 and

a more visible band in Lane 6 respectively. There is no detectable Gal d 2 DNA in the duplicate test samples (Lanes 10 and 11).

Figure A: Gel picture illustrating absence of Gal d 2 coding DNA in P2005 NEWP sample (Lanes 10 and 11)



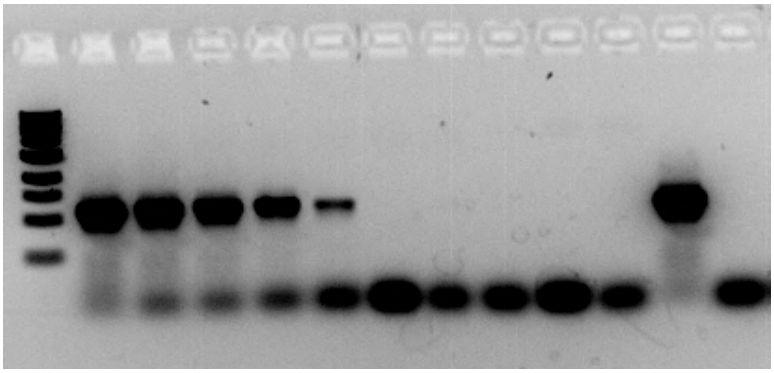
Lane # 1 2 3 4 5 6 7 8 9 10 11 12 13

Lane #	
1	1kb DNA ladder
2	1ng
3	0.1ng Gal d 2 plasmid DNA
4	0.01ng Gal d 2 plasmid DNA
5	1pg Gal d 2 plasmid DNA
6	0.1pg (i.e., 100fg) Gal d 2 plasmid DNA
7	0.01pg (i.e., 10fg) Gal d 2 plasmid DNA
8	1fg Gal d 2 plasmid DNA
9	0.1fg Gal d 2 plasmid DNA
10	5mg/mL NEWP lot#2005
11	5mg/mL NEWP lot#2005
12	positive control (1ng Gal d 2 plasmid DNA)
13	negative control

Gel Results for P2023:

Lane description is provided below the gel picture (Figure B). Limit of detection is 100 femtogram (fg) of plasmid DNA as observed from the thin band in Lane 6. There is no detectable Gal d 2 DNA in the duplicate test samples (Lane 10 and 11).

Figure B: Gel picture illustrating absence of Gal d 2 coding DNA in P2023 NEWP sample (Lanes 10 and 11)



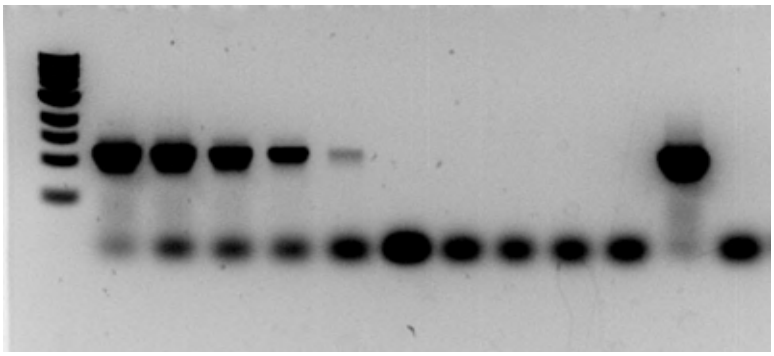
Lane # 1 2 3 4 5 6 7 8 9 10 11 12 13

Lane #	
1	1kb DNA ladder
2	1ng
3	0.1ng Gal d 2 plasmid DNA
4	0.01ng Gal d 2 plasmid DNA
5	1pg Gal d 2 plasmid DNA
6	0.1pg (i.e., 100fg) Gal d 2 plasmid DNA
7	0.01pg (i.e., 10fg) Gal d 2 plasmid DNA
8	1fg Gal d 2 plasmid DNA
9	0.1fg Gal d 2 plasmid DNA
10	5mg/mL NEWP lot#2023
11	5mg/mL NEWP lot#2023
12	positive control (1ng Gal d 2 plasmid DNA)
13	negative control

Gel Results for P2050:

Lane description is provided below the gel picture (Figure C). Limit of detection is 100fg of plasmid DNA as observed from the thin band in Lane 6. There is no detectable Gal d 2 DNA in the duplicate test samples (Lanes 10 and 11).

Figure C: Gel picture illustrating absence of Gal d 2 coding DNA in P2050 NEWP sample (Lanes 10 and 11)



Lane # 1 2 3 4 5 6 7 8 9 10 11 12 13

Lane #	
1	1kb DNA ladder
2	1ng
3	0.1ng Gal d 2 plasmid DNA
4	0.01ng Gal d 2 plasmid DNA
5	1pg Gal d 2 plasmid DNA
6	0.1pg (i.e., 100fg) Gal d 2 plasmid DNA
7	0.01pg (i.e., 10fg) Gal d 2 plasmid DNA
8	1fg Gal d 2 plasmid DNA
9	0.1fg Gal d 2 plasmid DNA
10	5mg/mL NEWP lot#2050
11	5mg/mL NEWP lot#2050
12	positive control (1ng Gal d 2 plasmid DNA)
13	negative control

Appendix 2: Standard Operating Procedure to determine Absence of Production Organism by Plating

Purpose

- This method internally confirms the presence of the recombinant *K. phaffii* species used to manufacture the Clara Foods product of interest.
- This protocol is only necessary if the internal bioburden check assay resulted in colonies on the PGA plates.
- This protocol should be done for each colony type present on the PGA plate. If there are numerous colonies of each type, select at least 5 of each type to complete the protocol.

Materials

- *Minimal methanol (MM) agar plates*
- *Potato Glucose Agar (PGA) plates*
- *Sterile deionized water*
- *Incubating cabinet*
- *Biosafety cabinet*
- *Inoculum spreading loops*

Procedure

1) Collect partial sample of the colony in question with inoculation loop and streak onto minimal media plate in 2 quadrants.

2) Collect remaining colony in question with inoculation loop and streak onto PGA plate in 2 quadrants.

3) Incubate PGA plates for 48 hours at 30 °C.

4) Incubate *Minimal methanol* plates for 120 hours at 30 °C.

5) If colonies grow on *Minimal methanol* plates within 120 hours at 30C, select single colonies and run colony PCR with cassette specific primers (see PCR method, Appendix 1). If colony PCR confirms presence of production cassette, it can be concluded that the manufacturing organism is present.

Appendix 3: Expert Panel Report

REPORT OF THE GRAS PANEL ON THE *GENERALLY RECOGNIZED AS SAFE* (GRAS) STATUS OF THE INTENDED USES OF NON-ANIMAL EGG WHITE PROTEIN PRODUCED BY *PICHIA PASTORIS*

December 2021

GRAS Panel Members

Joseph F. Borzelleca, Ph.D. and Michael W. Pariza, Ph.D.

Introduction

Clara Foods doing business as The EVERY Company ("the company") proposes to use "non-animal egg white protein", produced by a genetically-modified strain of *Pichia pastoris*, as a direct replacement for all current food uses of hen egg white and whole egg. The "non-animal egg white protein" is intended as a source of dietary protein and as a food ingredient when manufactured according to cGMP. It can be used as an ingredient in various food applications as a replacement for egg white (fresh and powdered) and whole eggs (fresh and powdered), since it has functional properties such as foaming, gelation and binding. Such uses will not increase the consumption of protein for the general population. The company convened a panel of independent scientists (the "GRAS Panel"), qualified by their scientific training and national and international experience to evaluate the safety of food ingredients, to conduct an independent and critical evaluation of the available information on the safety of its genetically-modified *P. pastoris* production strain for non-animal egg white protein, and to determine whether the proposed uses of the non-animal egg white protein manufactured using this strain are safe and suitable and *Generally Recognized As Safe* (GRAS) based on scientific procedures. The members of the GRAS Panel were Professors Michael W. Pariza and Joseph F. Borzelleca.

Summary and Basis for GRAS

The GRAS Panel, individually and collectively, critically evaluated a dossier prepared by Clara Foods doing business as The EVERY Company entitled, "Non-animal egg white protein produced by *Pichia pastoris* Generally Recognized as Safe Notice," dated November 2021. This dossier described:

(1) the history of safe use of non-animal egg white protein as a food ingredient;
(2) the biology of *P. pastoris* and its history of safe use in food manufacture;
(3) the non-animal egg white protein production strain DFB-004 and its fully sequenced genome;
(4) the cloning methodology that was utilized to create *P. pastoris* DFB-004;

(5) protein sequence data showing that the non-animal egg white protein produced by *P. pastoris* DFB-004 is chemically identical to naturally occurring chicken egg white protein (i.e., hen egg ovalbumin);
(6) the manufacturing process;
(7) product specifications; and
(8) projected consumer intake.

The GRAS Panel participated in a teleconference on December 2, 2021, with Dr. Kritika Mahadevan, Director of Quality Assurance and Regulatory Affairs, Clara Foods doing business as The EVERY Company.

Following its independent and collective critical evaluation of the publicly available information, the GRAS Panel unanimously concluded that the proposed uses of Clara Food's non-animal egg white protein preparation are safe and suitable and *Generally Recognized As Safe* (GRAS) based on scientific procedures.

Conclusions

We, the GRAS Panel, independently and collectively critically evaluated the information and data summarized above and unanimously conclude that Clara Food's *Pichia pastoris* production strain DFB-004 is safe and suitable for the manufacture of food-grade non-animal egg white protein.

We further conclude that Clara Food's non-animal egg white protein preparation, produced using *Pichia pastoris* DFB-004, manufactured in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is safe and suitable and *Generally Recognized as Safe* (GRAS) based on scientific procedures for use as a direct replacement for all current food uses of hen egg white. It can be used as an ingredient in various food applications as a replacement for egg white (fresh and powdered) and whole eggs (fresh and powdered) since it has functional properties such as foaming, gelation and binding. The non-animal egg white protein can also be used for protein fortification of conventional foods such as protein supplement powders, bread, protein cookies and nutritional bars.

It is our opinion that other experts qualified to assess the safety of food ingredients would concur with these conclusions.

[redacted]

Date: 7 December 2021

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Virginia Commonwealth University School of Medicine
Richmond, VA

GRAS Notice No. GRN 001104

Responses to questions

1. **On page 11, Clara presents the amino acid sequence of egg white protein (EWP) containing 4 extra amino acid residues (EAEA). However, we note that the number of amino acid residues in the sequence for EWP on page 11 (385 amino acids) is different from that in the sequence (389 amino acids) presented in Table 6 (page 37). For the administrative record, please clarify this discrepancy.**

Response:

The correct amino acid sequence for Clara EWP is 389 amino acids long as presented in Table 6 (page 37), and presented here:

EAEAGSIGAASMEFCFDVFKELKVHHANENIFYCPIAIMSALAMVYLGAKDSTRTQI
NKVVRFDKLPGFGDSIEAQCGTSVNVHSSLRDILNQITKPNDVYSFSLASRLYAEER
YPILPEYLQCVKELYRGGLEPINFQTAADQARELINSWVESQTNGIIRNVLQPSSVD
SQTAMVLVNAIVFKGLWEKAFKDEDTQAMPFRVTEQESKPVQMMYQIGLFRVASM
ASEKMKILELPFASGTMSMLVLLPDEVSGLEQLESIINFEKLTEWTSSNVMEERKIKV
YLPRMKMEEKYNLTSVLMAMGITDVFSSSANLSGISSAESLKISQAVHAAHAEINEA
GREVVGSAEAGVDAASVSEEFRADHPFLFCIKHIATNAVLFFGRCVSP

2. On pages 10-12, Clara discusses the "EWP LCMS amino acid composition" in conjunction with the sequence analysis, but there is no discussion on *the percentages of each amino acid in the sequence of that protein*. Please discuss the amino acid composition of EWP compared with that of the native hen egg Gal d 2 to support that the chemical identity of EWP is substantially equivalent to the native Gal d 2.

Response:

The amino acid composition of native hen egg Gal d 2 from Protein Database (2021) was used to compare against the measured amino acid composition of two Lots of EWP.

The comparison (Table 1) shows the amino acid composition of EWP is substantially equivalent to the native hen egg Gal d 2.

Table 1: Comparison of amino acid composition of native Gal d 2 and EWP

Amino Acid	Theoretical values for native Gal d 2[1] (Mole%)	Measured values for EWP Lot P2023 (Mole%)	Measured values for EWP Lot P2005 (Mole%)
Histidine	1.8	1.9	1.9
Serine	9.9	9.2	9.2
Arginine	3.9	4.0	4.0
Glycine	4.9	5.0	5.2
Aspartic acid*	8.1	8.2	8.3
Glutamic acid*	12.5	13.6	13.8
Threonine	3.9	4.0	3.9
Alanine	9.1	9.1	10.0
Proline	3.6	3.9	3.8
Lysine	5.2	5.5	5.3
Tyrosine	2.6	2.5	2.3
Methionine	4.2	4.1	3.9
Valine	8.1	7.9	8.0
Isoleucine	6.5	6.5	6.3
Leucine	8.3	8.9	8.6
Phenylalanine	5.2	5.6	5.4

*Asparagine and glutamine bear amides in their side chains and are, therefore, completely converted to their corresponding carboxylic acids during acid hydrolysis,

[1] Protein Database. (2021). *National Center for Biotechnology Information, U.S. National Library of Medicine.* Accessed Nov 2021. www.ncbi.nlm.nih.gov/protein/P01012.2.

forming aspartic acid and glutamic acid, respectively. The values are reported here as the sums of Asp + Asn and Glu + Gln (Rutherford and Gilani, 2009)[2].

Cysteine and tryptophan were not included in this table due to stability issues during processing for amino acid analysis and so are not part of the % composition calculation.

3. On page 10, Clara describes the differences in the glycosylation form between hen egg Gal d 2 protein and EWP (Fig. 1). Additionally, Clara notes that "The glycosylation pattern of native protein is slightly different compared to the EWP produced by *Komagataella*" (page 37). Considering that EWP has a different number of glycosylated residues compared to the native hen egg Gal d 2, please describe the thermostability of EWP by comparing with that of hen egg Gal d 2.

Response:

The thermostability of EWP was compared against that of native hen egg Gal d2. Melting temperatures of three Lots of EWP and a sample of Gal d 2 (OVA) from Sigma (A5503) were determined using Thermal Shift Assay, based on the published method by Huynh and Partch (2015)[3]. Results are presented in Table 2 below.

Table 2: Thermal Stability of native hen egg Gal d 2 and EWP

Sample	Melting temperature (ºC)
Gal d 2	74.4 ± 0.07
P2005	72.7 ± 0.40
P2023	72.6 ± 0.05
P2050	73.2 ± 0.34

n = 4

The thermostability of EWP is very close to that of native Gal d 2 from hen egg (Table 2), measured under the same conditions. The small difference in glycosylation did not have an impact on the thermostability.

[2] Rurtherford, S. M. and Gilani, G. S. (2009). Amino Acid Analysis *Current Protocols in Protein Science.* 11.9.1-11.9.37. DOI: 10.1002/0471140864.ps1109s58

[3] Huynh, K. and Partch, C.L. (2015) Curr Protoc Protein Sci., 79: 28.9.1–28.9.14. doi:10.1002/0471140864.ps2809s79

The above results resonate with the observations published by Ito and Matsudomi (2005)[4] where they showed the thermostability of the recombinant proteins, made using *Pichia pastoris*, was almost identical to hen egg OVA (Gal d 2).

4. On page 23, Clara states that the EWP is purified by centrifugation followed by micro-filtration, and then further purified by ultrafiltration, chromatography, and adjustment of the pH. Please describe how the final EWP preparation is standardized to a final concentration of protein (>75% w/w). In addition, please describe what analytical methods are used to monitor the identity and purity of the in-process samples and final product.

Response:

The fermentation process is carried out for a set number of days to ensure a standardized protein titer. The NEWP preparation is then centrifuged, washed and filtered. The protein concentration, protein composition and microbial purity are monitored at each unit operation. The purified preparation is then spray dried. The unit operations during production ensure the protein concentration in the spray dried powder meets the specification.

In-process samples are taken at the completion of each unit operation in the production process. The in-process samples are analyzed for purity of culture and checked to ensure there is no microbial contamination. Identity of the protein produced is monitored by SDS PAGE. Identity and purity of the final dried NEWP product is evaluated by a combination of analyses. The concentration of recombinant Gal d 2 in the preparation is quantified using a validated HPLC method. Proximate analysis, heavy metal and microbial analyses are carried out by an ISO 17025 accredited laboratory using validated methods of analyses to ensure the sample meets the product specifications.

5. In Table 4, Clara provides the results from the batch analyses demonstrating levels of arsenic, mercury, and cadmium to be below 0.01 mg/kg, and levels of lead to be below 0.02 mg/kg. However, the specifications for these heavy metals were stated to be <1 mg/kg (page 29). We recommend that Clara lower the specification limits for heavy metals to better reflect the results of the batch analyses and to be as low as possible.

Response:

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[4] Ito, K., & Matsudomi, N. (2005). Structural Characteristics of Hen Egg Ovalbumin Expressed in Yeast Pichia pastoris. *Biosci. Biotechnol. Biochem., 69(4)*, 755– 761.

Clara Foods understands FDA's interest in ensuring that heavy metal concentrations in foods are as low as possible and will consider lowering specification limits as appropriate.

We note that there is a limited data set of quality testing results during the product development stage of EWP and we believe it is premature to lower the specifications below the currently accepted QC limits for proteins in the industry.

6. Please provide a statement that all analytical methods are validated for their particular purpose.

Response:

All analytical methods used to evaluate the product are validated for their particular purpose.

7. On page 21, Clara states that EWP "... can also be used as a protein fining (clearing) agent in wines and juices just like egg white is currently used for this purpose". However, we note that egg white is currently approved for use in wine with specific *Tax and Trade Bureau* (*TTB*) limitations, but not for use in juice (27 CFR 24.246). Further, we note that wine and juice are not listed as food categories in which EWP is intended to be used (Table 5; pages 30 and 31). Please clarify whether the use of EWP as a fining agent in wine and juice is included in the intended uses of EWP in GRN 001104.

Response:

Yes, wine and juice fining are included in the intended uses of EWP in GRN 001104. We have ensured to list them in the "intended uses of EWP" in Table 1 in Addendum A as part of the dietary exposure estimate (provided by Exponent, Inc.), attached at the end of this document. Typically, the protein will be removed from the final beverage product due to precipitation during processing.

8. On pages 21 and 30, Clara indicates that the intended use is not limited to selected food categories. Please clarify if the intended use is in all conventional foods or only in the listed food categories. If the intended use is in all conventional foods or in food categories that are in addition to those listed, the dietary exposure estimate should be revised to be reflective of all the intended uses of EWP.

Response:

We have calculated the dietary exposure estimate of EWP in a comprehensive list of food categories and the Report is presented in Addendum A and the findings are discussed as a response to Question 9.

9. On page 32, Clara estimates dietary exposure to EWP for the U.S. population aged 1 year and older to not exceed 79.2 g/person (p)/d at the mean and 158.4 g/p/d at the 90th percentile, respectively. However, we note that these estimates represent the total dietary protein intake including both animal (including dairy) and plant proteins based on food consumption data from the 2013-2016 National Health and Nutrition Examination Survey (NHANES), but not dietary exposure from the intended uses of the recombinant EWP, as presented in Table 5 (pages 30 and 31). We also note that more recent food consumption data are available. Please provide a dietary exposure estimate for EWP at the mean and 90th percentile for the U.S. population aged 2 years and older based on the intended uses using available recent

Response:

Please see the 2-day average dietary exposure estimate (provided by Exponent, Inc.) in Addendum A for EWP preparation at the mean and 90th percentile for the U.S. population aged 2 years and older based on the intended use in food using available recent U.S. food consumption surveys (WWEIA / NHANES 2017 - March 2020 consumption data), as requested.

Intake of EWP was developed from reported intakes of foods representative of the intended uses. The full intake assessment report and the list of food codes identified as representative of the intended uses and used in the analysis of intakes is provided in Addendum A.

The “per capita” estimates of EWP intake for the U.S. population 2 years and older were a mean of 7.8 g/day and 15.7 g/day at the 90th percentile. The “per user” estimated daily intake of EWP for the U.S. population 2 years and older is a mean of 8.6 g/day and 16.3 g/day at the 90th percentile.

The cumulative dietary exposure to protein would not increase based on the intended uses of EWP. The inclusion of EWP would serve as a substitute source of protein in the diet.

10. There are reports in the literature that oral tolerance to allergic proteins may sometimes break down resulting in new episodes of sensitization. Although the mechanisms of the breakdown of tolerance are not clearly understood, one of the possibilities is the high exposure to the allergenic protein, with the threshold of exposure varying significantly across the sensitized population. FDA previously reviewed the safety of individual allergenic proteins from milk and had no

questions on the notifiers' GRAS conclusions when the source was labeled, and the proposed use levels were close to the lower end of the Acceptable Macronutrient Distribution Range (AMDR) for proteins (the range is 10-35%; please see the Institute of Medicine (IOM) recommendation on daily protein intake). If Clara is planning to use the dietary exposure based on total protein intakes in the What We Eat in America (WWEIA) survey for EWP, please address the following:

a) **Please discuss using publicly available information, why the proposed high use level of EWP is not expected to have any unintended safety consequences.**

b) **Additionally, please discuss if replacement of total dietary protein with EWP would meet the IOM recommendations for recommended daily allowance (RDA) of essential amino acids.**

Response:

Based on the dietary exposure levels of EWP provided in the response to the Question 9 above and in Addendum A, the estimated use level of EWP (16.3 g/day at the 90th percentile) is well below the lower end of the AMDR for proteins. The estimated daily intake of EWP at a mean value of 0.14 g/kg bw/day (0.28 g/kg bw/day at the 90th percentile) is significantly lower than the RDA for protein at 0.8 g/kg bw/day recommended by Institute of Medicine[5]. In addition, EWP will always be accompanied with an allergen warning label as stated in Sections 2.2.7 and 6.3.2 in the original Notice GRN 001104. Given the low estimated intake levels of EWP and the clear allergen labeling, EWP is not expected to have any unintended safety consequences.

EWP is substantially equivalent to Gal d 2 from hen eggs, in terms of its amino acid composition and good digestibility, making it a very good source of essential amino acids. However, consumers will likely include EWP as one among many sources of protein in their diet. EWP intake will not totally replace all dietary proteins. In reality, the maximum intended use level of EWP may not be in all products, and not all consumers may select products with EWP at all eating occasions.

11. Please clarify the parameters of the allergenicity/toxigenicity assessment. What sequence was queried: EWP (including N-terminal extension) vs. EWP fusion protein vs. N-terminal extension?

Response:

The allergenicity and bacterial toxin bioinformatic assessment was done with EWP including the N-terminal extension of EAEA.

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12. In Appendix 3: Expert Panel Report, the authors stated that "This dossier described the non-animal egg white protein production strain DFB-004 and its fully sequenced genome". Please state whether the full genomic sequence of the production strain is publicly available and provide the corresponding National Center for Biotechnology Information (NCBI) accession number.

Response:

The genome sequence information for DFB-004 is confidential business information and has not been deposited in a publicly accessible manner such as NCBI.

13. Please describe the origin and source of the donor genes (e.g., are they de novo synthesized or of bacterial origin).

Response:

The genes added by Clara Foods doing business as the EVERY company to make DFB-004 were synthesized *de novo* by Integrated DNA Technologies company.

14. In the legends of Figures A, B and C in Appendix 1, please complete the label of Lane 2.

Response:

The Lane 2 label should read as "1ng Gal d 2 plasmid DNA" in the legends of Figures A, B and C in Appendix 1. An example is provided for Figure A (Appendix 1 in GRN 001104):

Lane #	
1	1kb DNA ladder
2	1ng Gal d 2 plasmid DNA
3	0.1ng Gal d 2 plasmid DNA
4	0.01ng Gal d 2 plasmid DNA
5	1pg Gal d 2 plasmid DNA
6	0.1pg (i.e. 100fg) Gal d 2 plasmid DNA
7	0.01pg (i.e. 10fg) Gal d 2 plasmid DNA
8	1fg Gal d 2 plasmid DNA

9	0.1fg Gal d 2 plasmid DNA
10	5mg/mL NEWP lot#2005
11	5mg/mL NEWP lot#2005
12	positive control (1ng Gal d 2 plasmid DNA)
13	negative control (1 µL of 25mM sodium hydroxide)

15. In support of the safety conclusion, Clara lists three GRAS notices where different strains of *K. phaffii* were used to produce various substances for use in food (i.e., GRN 000205, GRN 000737 and GRN 000967). The subject of GRN 000205 is pullulanse enzyme preparation from *Bacillus subtilis* expressing the pullulanase gene from *B. acidopullulyticus* for use in the beer brewing industry as an enzyme for the saccharification of liquefied starch. Please clarify whether this is the GRAS notice the notifier intended to reference. Further, as each GRAS notice stands on its own, for the administrative record, please briefly discuss these GRAS notices in the context of the notifier's safety conclusion.

Response:

GRN 000205 is a typo. All references to GRN 000205 should have been to GRN 000204 throughout the current GRN 001104. The following table summarizes GRN 000204, GRN 000737 and GRN 000967 in support of the safety conclusion of GRN 001104, followed by a more detailed summary of each GRAS Notice. All these GRAS Notices use the same *P. pastoris* (or *K. phaffii*) as the production microorganism for the production of the GRAS substances for use in food. The *K. phaffii* DFB-004 production host background for GRN 001104, *K. phaffii* BG10, is a commercially available, non-toxigenic and non-pathogenic, safe, and suitable food production organism. It has been reviewed by FDA in GRN 000204, GRN 000737 and GRN 000967 for the production of enzyme and protein food ingredients.

Table 3. GRAS notices using *P. pastoris* (or *K. phaffii*) for the production of substances used in food

GRN No.	Substance	Intended Use	Production microorganism	Status

204	Phospholipase C enzyme preparation from *Pichia pastoris* expressing a heterologous phospholipase C gene.	For use as an enzyme in degumming vegetable oils for food use.	*P. pastoris* (or *K. phaffii*) strain SMD 1168	FDA issued a “no questions” letter on Dec. 5, 2006.
737	Soy leghemoglobin preparation from a strain of *Pichia pastoris.*	For use at levels up to 0.8% soybean leghemoglobin protein to optimize flavor in ground beef analogue products intended to be cooked.	*P. pastoris* (or *K. phaffii*) strain MXY0291	FDA issued a “no questions” letter on Jul 23, 2018.
967	Soluble egg-white protein produced by *Komagataella phaffii* strain GSD-1209.	For use as a substitute for egg-white protein in foods containing eggs; and as a source of protein in nutritional powders and drinks; bars; and certain snack foods at levels in accordance with current good manufacturing practices (excluding infant formula, or in any products under the jurisdiction of the United States Department of Agriculture).	*P. pastoris* DFB-003 (or *K. phaffii* strain GSD-1209)	FDA issued a “no questions” letter on Sep 9, 2021.

GRN 000204

GRN 000204 was for Phospholipase C enzyme preparation from *Pichia pastoris* expressing a heterologous phospholipase C gene (BD16449 Phospholipase C enzyme preparation) to be used as an enzymatic processing aid for degumming vegetable oils (intended for use in foods) at a recommended level of 100-1000 grams of formulated enzyme preparation per metric ton of vegetable oil (i.e., at 100-1000 ppm).

The BD16449 phospholipase C enzyme preparation was derived from a non-toxigenic, non-pathogenic, genetically modified strain of *P. pastoris.* The *P. pastoris* strain SMD 1168 was a commercially available protease-derivative of the wild-type strain NRRL Y-11430, which has been genetically modified (strain DVSA-PLC-004) for production of BD16449 phospholipase C. This genetically modified production organism was said to comply with the OECD (Organization for Economic Development) criteria for GILSP (Good Industrial Large-Scale Practice) microorganisms. It also met the criteria for a safe production microorganism as described by Pariza and Foster, Pariza and Johnson, and several expert groups. The safety of *P. pastoris* strain SDM 1168 was discussed in the GRAS notice including the history of safe use of *P. pastoris*, absence of pathogenicity and toxicity, and safety of inserted genetic material.

GRN 000204 pointed out that, "In addition, *P. pastoris* itself has been approved by FDA as a source of animal feed protein for use in broiler feed up to 10% of the total feed (FDA, 1993). Toxicity studies done in support of the above-referenced *P. pastoris*-approved animal feed (including a pathogenicity study in mice, an acute oral toxicity study in rats, a subacute oral toxicity study in rats, and a two-generation teratology study in rats) also demonstrated, per FDA's review in 1993, that *P. pastoris* is neither pathogenic nor toxigenic (FDA, 1993)".

In addition, the safety of the BD 16449 phospholipase C enzyme preparation was supported by a series of published safety studies such as genotoxicity tests and 90-day oral toxicity study in rats on the enzyme preparation. The results from the genotoxicity tests such as Ames assay, chromosomal aberrations test in human lymphocytes, and mouse micronucleus assay demonstrated that the enzyme was not genotoxic. There were no test article-related clinical signs of toxicity noted from the 90-day study and the No-Observed-Adverse-Effect-Level (NOAEL) was determined to be greater than 2000 mg/kg/day, the highest dose tested in the study. The NOAEL was approximately 133,000 times higher than the maximum estimated daily intake (EDI) of 0.015 mg/kg/day for the intended uses of BD 16449 phospholipase C. The EDI assumed a "worst case" scenario of possible, daily, human exposure by assuming that all of the enzyme, if added to the crude oil at the highest recommended use rate, was retained in the final refined vegetable oil. FDA had no questions regarding the notifier's determination that BD16449 Phospholipase C enzyme preparation produced by *P. pastoris* is GRAS under the conditions of use specified in the notification.

GRN 000737

GRN 000737 was for Soy leghemoglobin preparation (LegH Prep) from a strain of *Pichia pastoris* for use at levels up to 0.8% soybean leghemoglobin protein to optimize flavor in ground beef analogue products intended to be cooked. Soy leghemoglobin protein is expressed during submerged fed-batch fermentation using the *P. pastoris* MXY0291 production strain, which was constructed from recipient strain Bg11(MXY0051) using a series of transformations with different expression constructs.

P. pastoris Bg11 is a derivative of Bg10, both of which are commercially available and were derived from the well-characterized strain Y-11430, which is deposited in the collection at the Northern Regional Research Laboratories (NRRL). The lineage of *P. pastoris* strain NRRL Y-11430 was previously included in GRN 204. *P. pastoris* was discussed in the GRAS notice as a nonpathogenic, non-toxigenic, and well-characterized yeast with a history of safe use in the food industry.

Genotoxicity of LegH Prep was assessed using the bacterial reverse mutation assay and the chromosomal aberration assay in human lymphocytes. LegH Prep was found to be non-mutagenic and non-clastogenic in each assay. The NOAEL from a 28-day feeding study in rats showed no evidence of toxicity at the highest dose tested, 750 mg/kg/day soy leghemoglobin. An ADI of 7.5 mg/kg/day for soy leghemoglobin was derived by applying a standard safety factor of 100 to the NOAEL. The 90th percentile EDI for soy leghemoglobin was estimated to be 6.67 mg/kg/day. Since the EDI is lower than the ADI, the notifier concluded that there were no safety concerns. FDA had no questions regarding the notifier's determination that LegH Prep produced by *P. pastoris* is GRAS under the conditions of use specified in the notification.

GRN 000967

GRN 000967 was submitted by Clara Foods Co. for Soluble egg-white protein produced by *Komagataella phaffii* strain GSD-1209, for use as a substitute for egg-white protein in foods containing eggs; and as a source of protein in nutritional powders and drinks; bars; and certain snack foods at levels in accordance with current good manufacturing practices. The production organism *P. pastoris* DFB-003 (or *K. phaffii* GSD-1209) is genetically engineered from the commercially available base strain, *K. phaffii* strain "BG10" (or Bg10 per GRN 000737) to express hen egg ovomucoid. *K. phaffii* (or *P. pastoris*) strain BG08 is a single colony isolate from the Phillips Petroleum strain NRRL Y-11430 obtained from the Agriculture Research Service culture collection. *P. pastoris* BG10 was derived from BG08 and Clara Foods further modified BG10 to develop a base strain called DFB-001. The production strain *P. pastoris* DFB-003 was constructed from recipient strain DFB-001 using transformations with different expression constructs in order to express NSEWP.

The production organism was constructed through transformation and chromosomal-integration of an expression cassette carrying a gene sequence that encodes hen egg ovomucoid that is identical to the protein sequence from chicken egg (Gallus gallus). The genome of *K. phaffii* GSD-1209 was fully sequenced. The production organism did not contain any antibiotic resistance genes, nor vector plasmid sequences and therefore, was not capable of DNA transfer to other organisms.

Clara Foods Co. estimated a cumulative dietary exposure from the substitutional uses in foods and the use as a source of protein in the select food categories to be 21.5 g/p/d at the mean and 47.9 g/p/d at the 90th percentile for the U.S. population aged 2 years and older. It was not anticipated that soluble egg-white protein will substitute for all egg white proteins, and that it's use as a source of protein in the select food categories would be a subset of the current uses of egg white protein. Therefore, it

was concluded that there would be no increase in the cumulative dietary exposure to ovomucoid-related protein. FDA had no questions regarding the notifier's determination that Soluble egg-white protein produced by *K. phaffii* (or *P. pastoris*) is GRAS under the conditions of use specified in the notification.

16. Please provide an updated literature search including the date (month and year) the literature search was performed and discuss the safety of *K. phaffii.*

Response:

We conducted an updated literature search on June 23, 2023. The following public databases were searched: Pubmed, Google Scholar.

The following search terms were used: "Komagataella phaffii", "K. phaffii", " Pichia pastoris", " P. pastoris", "Komagataella phaffii AND safety", "K. phaffii AND safety", " Pichia pastoris AND safety", and " P. pastoris AND safety".

No studies were identified that would give rise to safety concerns.

17. We note that some of the references appear twice in the provided lists of references in the notice. This is a just comment for the administrative record. We are not asking for a revised list of references.

Response:

Noted, thank you.

Exponent®

EXTERNAL MEMORANDUM

To:	Dr. Kritika Mahadevan, Clara Foods Co. (DBA The EVERY Company)
From:	Exponent, Inc.
Date:	June 19, 2023
PROJECT:	2305062.000
SUBJECT:	Estimated Daily Intake of Egg White Protein (EWP) produced by *Komagataella phaffii* strain ATCC GSD-1235 from the Intended Use in Select Foods

Introduction

Exponent Inc. (Exponent) was engaged by Clara Foods Co. (DBA The EVERY Company) to develop estimates of intake of egg white protein (EWP) produced by *Komagataella phaffii* strain ATCC GSD-1235 from the intended use in select foods. The estimates of intake are presented below.

Methods

Estimates of intake from the intended use of EWP produced by *Komagataella phaffii* strain ATCC GSD-1235 were developed by Exponent from food consumption records collected in the What We Eat in America, National Health and Nutrition Examination Survey, 2017-March 2020 Pre-pandemic (WWEIA/NHANES 2017-March 2020).[1] The NHANES datasets provide nationally representative nutrition and health data and prevalence estimates for nutrition and health status measures in the United States. As part of the examination, trained dietary interviewers collected detailed information on all foods and beverages consumed by respondents in the previous 24-hour time period (midnight to midnight). A second dietary recall was administered by telephone three-to-ten days after the first dietary interview, but not on the same day of the week as the first interview. A total of 10,830 individuals in the survey period 2017-March 2020 provided two complete days of dietary recalls.

The intended use of EWP produced by *Komagataella phaffii* strain ATCC GSD-1235 per food category and the intended use levels (g per 100 g in food) are shown in Table 1. The list of all food codes reported consumed in WWEIA/NHANES 2017-March 2020 was reviewed and food codes corresponding to the intended use (as a food or an ingredient in a mixture) were identified. The weight proportions of ingredients in mixtures were identified with WWEIA supporting data. The list of food codes identified as representative of the intended use and used in the analysis of intakes is provided in Appendix A.

[1] https://wwwn.cdc.gov/nchs/nhanes/Default.aspx

Table 1. Intended use and use level of egg white protein (EWP) produced by *Komagataella phaffii* strain ATCC GSD-1235

Category	Representative Examples	Serving size [a]	% inclusion (as solids) [b]
Bars	Protein bars	40 g	10 – 23%
	Snack bars	40 g	10%
Breakfast cereals, ready-to-eat	Cereal / granola	15-60 g	10%
Egg products	Scrambled egg	110 g	12%
	Egg white patty	50 g	12%
	Quiche	140 g	10%
	Souffle	140 g	8%
Baked goods and baking mixes	Cakes, brownies, cookies (including macarons), muffins, doughnuts	30-125 g	2 – 6%
	Protein cookies	30 g	10 – 25%
	Gluten free bread	50 g	4%
	Pancakes	110 g	3 – 5%
	Waffles	85 g	3 – 5%
Plant protein products	Meat alternative burgers (ready-to-eat & ready-to-cook)	85-110 g	4 – 8%
	Veggie dogs / corn dogs	85 g	4 – 8%
	Meat alternatives, e.g., sausage patties, sausage links	55 g	6 – 8%
	Nuggets	85 g	4 – 8%
	Meatless crumbles	55 g	3 – 5%
	Plant-based bacon	15 g	4 – 8%
	Meat alternative whole muscle cut	85-110 g	3 – 6%
Grain products and pastas	Egg noodles (dry weight, includes dry noodles ready-to-eat)	25-55 g	8 – 10%
Beverages, alcoholic	Egg white cocktail	110 mL	2%
Milk products	Eggnog	120 mL	2 – 3%
Fats and oils	Dressing	15-30 g	5 – 10%
Beverages and beverage bases, nonalcoholic	Powdered shake mix (as consumed)	240 mL	11 – 13%
Wine and juice fining	Wine and juice	150-240 mL	0.6 – 3% [c]

[a] Based on potential applications and Reference Amounts Customarily Consumed (RACCs) per Eating Occasion established in 21 CFR 101.12(b).
[b] Based upon current usage rates of egg white protein.
[c] Protein will be removed from the end product due to precipitation during processing.

Using the WWEIA/NHANES 2017-March 2020 consumption data, Exponent estimated the 2-day average daily intake on a *per capita* and *per user* basis. *Per capita* estimates refer to consumption based on the entire population of interest, whereas *per user* estimates refer to consumption among those who reported consuming any foods of interest on either survey day. For each subject with a complete 2-day dietary recall, a 2-day average estimate of EWP produced by *Komagataella phaffii* strain ATCC GSD-1235 intake was derived by multiplying the reported intake of foods from the 24-hour recall with the maximum intended use level (see Table 1); the cumulative sum was divided by two.

The mean and 90th percentile of the 2-day average intakes were calculated for the total U.S. population two years and older (2+ years) and subpopulations of children ages 2-5 years, children ages 6-12 years, teenagers ages 13-18 years, and adults ages 19 years and older (19+ years). The analysis was limited to individuals who provided two complete and reliable dietary recalls as determined by the National Center for Health Statistics.

The 2-day average intakes by each individual were estimated using Exponent's Foods Analysis and Residues Evaluation Program (FARE® version 15.11) software. FARE® incorporates statistical weights that compensate for variable probabilities of selection, adjusted for non-response, and provides intake estimates that are representative of the U.S. population.

Results

Estimated daily intakes (EDI) of EWP produced by *Komagataella phaffii* strain ATCC GSD-1235 from the intended use are presented in Table 2 in units of g per day (g/day); estimates of intake in units of g per kilogram body weight per day (g/kg bw/day) are presented in Table 3.

Table 2. 2-day average estimated daily intake **(g/day)** of EWP produced by *Komagataella phaffii* strain ATCC GSD-1235 from the intended use in food; WWEIA/NHANES 2017-March 2020

Population	Total Sample, n	Users, n	% User	Per Capita (g/day)		Per User (g/day)	
				Mean	90th Percentile	Mean	90th Percentile
US 2+ years	10116	9160	91	7.8	15.7	8.6	16.3
Children 2-5 years	801	773	96	5.7	11.9	5.9	12.0
Children 6-12 years	1381	1328	97	7.3	14.3	7.5	14.4
Adolescents 13-18 years	1135	1025	92	7.1	14.6	7.7	15.2
Adults 19+ years	6799	6034	90	8.1	16.3	9.0	16.9

Intended use as shown in Table 1.

Table 3. 2-day average estimated daily intake **(g/kg bw/day)** of EWP produced by *Komagataella phaffii* strain ATCC GSD-123 from the intended use in food; WWEIA/NHANES 2017-March 2020

Population	Total Sample, n	Users, n	% User	Per Capita (g/kg bw/day)		Per User (g/kg bw/day)	
				Mean	90th Percentile	Mean	90th Percentile
US 2+ years	10116	9160	91	0.12	0.27	0.14	0.28
Children 2-5 years	801	773	96	0.34	0.68	0.35	0.71
Children 6-12 years	1381	1328	97	0.22	0.45	0.22	0.45
Adolescents 13-18 years	1135	1025	92	0.11	0.24	0.12	0.25
Adults 19+ years	6799	6034	90	0.10	0.21	0.11	0.22

Intended use as shown in Table 1.

Appendix A. Food Codes Used in Analysis

Food code	Food description
Bars	
Protein bars	
53720100	Nutrition bar (Balance Original Bar)
53720200	Nutrition bar (Clif Bar)
53720210	Nutrition bar (Clif Kids Organic Zbar)
53720300	Nutrition bar (PowerBar)
53720400	Nutrition bar (Slim Fast Original Meal Bar)
53720500	Nutrition bar (Snickers Marathon Protein Bar)
53720600	Nutrition bar (South Beach Living Meal Bar)
53720610	Nutrition bar (South Beach Living High Protein Bar)
53720700	Nutrition bar (Tiger's Milk)
53720800	Nutrition bar (Zone Perfect Classic Crunch)
53729000	Nutrition bar or meal replacement bar, NFS
Snack bars	
53710400	Cereal or granola bar (General Mills Fiber One Chewy Bar)
53710500	Cereal or granola bar (Kellogg's Nutri-Grain Cereal Bar)
53710502	Cereal or granola bar (Kellogg's Nutri-Grain Yogurt Bar)
53710504	Cereal or granola bar (Kellogg's Nutri-Grain Fruit and Nut Bar)
53710600	Milk 'n Cereal bar
53710700	Cereal or granola bar (Kellogg's Special K bar)
53710800	Cereal or granola bar (Kashi Chewy)
53710802	Cereal or granola bar (Kashi Crunchy)
53710810	Cereal or granola bar (KIND Fruit and Nut Bar)
53710900	Cereal or granola bar (General Mills Nature Valley Chewy Trail Mix)
53710902	Cereal or granola bar, with yogurt coating (General Mills Nature Valley Chewy Granola Bar)
53710904	Cereal or granola bar (General Mills Nature Valley Sweet and Salty Granola Bar)
53710906	Cereal or granola bar (General Mills Nature Valley Crunchy Granola Bar)
53711000	Cereal or granola bar (Quaker Chewy Granola Bar)
53711002	Cereal or granola bar (Quaker Chewy 90 Calorie Granola Bar)
53711004	Cereal or granola bar (Quaker Chewy 25% Less Sugar Granola Bar)
53711006	Cereal or granola bar (Quaker Chewy Dipps Granola Bar)
53712000	Snack bar, oatmeal
53712100	Cereal or Granola bar, NFS
53712200	Cereal or granola bar, lowfat, NFS
53712210	Cereal or granola bar, nonfat
53713010	Cereal or granola bar, fruit and nut
53714200	Cereal or granola bar, chocolate coated, NFS
53714210	Cereal or granola bar, with coconut, chocolate coated
53714220	Cereal or granola bar with nuts, chocolate coated

Food code	Food description
53714230	Cereal or granola bar, oats, nuts, coated with non-chocolate coating
53714250	Cereal or granola bar, coated with non-chocolate coating
53714300	Cereal or granola bar, high fiber, coated with non-chocolate yogurt coating
53714400	Cereal or granola bar, with rice cereal
53714500	Breakfast bar, NFS
53714520	Breakfast bar, cereal crust with fruit filling, lowfat
Breakfast cereals, ready-to-eat	
Cereal/granola	
11435100	Yogurt, Greek, with oats*
11446000	Yogurt parfait, low fat, with fruit*
57000100	Cereal, oat, NFS
57100100	Cereal, ready-to-eat, NFS
57101000	Cereal (Kellogg's All-Bran)
57103000	Cereal (Post Alpha-Bits)
57103100	Cereal, O's, flavored
57104000	Cereal (Kellogg's Apple Jacks)
57106050	Cereal (Post Great Grains Banana Nut Crunch)
57106060	Cereal (General Mills Cheerios Banana Nut)
57106260	Cereal (General Mills Cheerios Berry Burst)
57117000	Cereal (Quaker Cap'n Crunch)
57117500	Cereal (Quaker Christmas Crunch)
57119000	Cereal, crunch
57120000	Cereal (Quaker Cap'n Crunch's Peanut Butter Crunch)
57123000	Cereal, O's, plain
57124030	Cereal (General Mills Chex Chocolate)
57124050	Cereal (General Mills Chex Cinnamon)
57124100	Cereal (General Mills Cheerios Chocolate)
57124200	Cereal, chocolate puffs
57124300	Cereal (General Mills Lucky Charms Chocolate)
57125000	Cereal, cinnamon toast
57125010	Cereal (General Mills 25% Less Sugar Cinnamon Toast Crunch)
57125900	Cereal (General Mills Honey Nut Clusters)
57126000	Cereal, chocolate crispy
57127000	Cereal (Post Cocoa Pebbles)
57128000	Cereal (General Mills Cocoa Puffs)
57130000	Cereal (General Mills Cookie Crisp)
57132000	Cereal, corn squares
57134000	Cereal, corn flakes, plain
57135000	Cereal (Kellogg's Corn Flakes)
57137000	Cereal, corn puffs
57139000	Cereal (General Mills Count Chocula)

Food code	Food description
57143000	Cereal (Kellogg's Cracklin' Oat Bran)
57143500	Cereal (Post Great Grains, Cranberry Almond Crunch)
57148000	Cereal (Kellogg's Crispix)
57151000	Cereal, rice crispy, plain
57206700	Cereal (General Mills Fiber One)
57206710	Cereal (General Mills Fiber One Honey Clusters)
57206715	Cereal (General Mills Fiber One Raisin Bran Clusters)
57207000	Cereal, bran flakes, plain
57208000	Cereal (Kellogg's All-Bran Complete Wheat Flakes)
57209000	Cereal (Post Bran Flakes)
57211000	Cereal (General Mills Frankenberry)
57213000	Cereal (Kellogg's Froot Loops)
57213010	Cereal (Kellogg's Froot Loops Marshmallow)
57213850	Cereal (General Mills Cheerios Frosted)
57214000	Cereal, shredded wheat, flavored
57216000	Cereal, rice crispy, flavored
57221700	Cereal, fruit rings
57221810	Cereal (General Mills Cheerios Fruity)
57223000	Cereal, fruit crispy
57224000	Cereal (General Mills Golden Grahams)
57227000	Cereal, granola
57229000	Cereal (Kellogg's Low Fat Granola)
57230000	Cereal (Post Grape-Nuts)
57231200	Cereal (Post Great Grains Raisins, Dates, and Pecans)
57237100	Cereal, oat bunches
57237200	Cereal (Post Honey Bunches of Oats with Vanilla Bunches)
57237300	Cereal (Post Honey Bunches of Oats with Almonds)
57238000	Cereal (Post Honeycomb)
57240100	Cereal, corn squares, flavored
57241000	Cereal, O's, honey nut
57241200	Cereal (Post Shredded Wheat Honey Nut)
57243000	Cereal (Kellogg's Honey Smacks)
57301500	Cereal (Kashi 7 Whole Grain Puffs)
57301505	Cereal (Kashi Autumn Wheat)
57301510	Cereal (Kashi GOLEAN)
57301511	Cereal (Kashi GOLEAN Crunch)
57301512	Cereal (Kashi GOLEAN Crunch Honey Almond Flax)
57301530	Cereal (Kashi Heart to Heart Honey Toasted Oat)
57301600	Cereal, multigrain
57303100	Cereal (General Mills Kix)
57303105	Cereal (General Mills Honey Kix)

Food code	Food description
57303200	Cereal (Kellogg's Krave)
57304100	Cereal, oat squares
57305100	Cereal (General Mills Lucky Charms)
57305150	Cereal, frosted oats with marshmallows
57305160	Cereal (Malt-O-Meal Blueberry Muffin Tops)
57305165	Cereal (Malt-O-Meal Cinnamon Toasters)
57305170	Cereal (Malt-O-Meal Coco-Roos)
57305174	Cereal (Malt-O-Meal Colossal Crunch)
57305175	Cereal (Malt-O-Meal Cocoa Dyno-Bites)
57305180	Cereal (Malt-O-Meal Corn Bursts)
57305210	Cereal (Malt-O-Meal Frosted Flakes)
57305300	Cereal (Malt-O-Meal Fruity Dyno-Bites)
57305400	Cereal (Malt-O-Meal Honey Graham Squares)
57305500	Cereal (Malt-O-Meal Honey Nut Toasty O's)
57305600	Cereal (Malt-O-Meal Marshmallow Mateys)
57306500	Cereal (Malt-O-Meal Golden Puffs)
57306700	Cereal (Malt-O-Meal Toasted Oat Cereal)
57306800	Cereal (Malt-O-Meal Tootie Fruities)
57308190	Cereal, muesli
57308400	Cereal, O's, multigrain
57309100	Cereal (Nature Valley Granola)
57316380	Cereal (General Mills Cheerios Oat Cluster Crunch)
57316385	Cereal (General Mills Cheerios Protein)
57316450	Cereal (General Mills Oatmeal Crisp with Almonds)
57316710	Cereal (Quaker Honey Graham Oh's)
57320500	Cereal (Quaker Granola with Oats, Honey, and Raisins)
57321900	Cereal (Nature's Path Organic Flax Plus)
57326000	Cereal (Barbara's Puffins)
57327450	Cereal (Quaker Toasted Oat Bran)
57327500	Cereal (Quaker Oatmeal Squares)
57329000	Cereal, bran flakes, flavored
57330000	Cereal (Kellogg's Raisin Bran)
57330010	Cereal (Kellogg's Raisin Bran Crunch)
57331000	Cereal (Post Raisin Bran)
57332100	Cereal (General Mills Raisin Nut Bran)
57335550	Cereal (General Mills Reese's Puffs)
57336000	Cereal, rice squares
57337000	Cereal, rice flakes
57339000	Cereal (Kellogg's Rice Krispies)
57339500	Cereal (Kellogg's Rice Krispies Treats Cereal)
57340000	Cereal, puffed rice

Food code	Food description
57341200	Cereal (Kellogg's Smart Start Strong)
57341300	Cereal (Kellogg's Smorz)
57344000	Cereal, K's, plain
57344001	Cereal (Kellogg's Special K Blueberry)
57344005	Cereal (Kellogg's Special K Chocolatey Delight)
57344010	Cereal, K's, flavored
57344015	Cereal (Kellogg's Special K Fruit & Yogurt)
57344020	Cereal (Kellogg's Special K Vanilla Almond)
57344025	Cereal (Kellogg's Special K Cinnamon Pecan)
57347000	Cereal, flavored puffs
57348000	Cereal, corn flakes, flavored
57349000	Cereal (Kellogg's Frosted Flakes)
57355000	Cereal (Post Golden Crisp)
57401100	Cereal, O's, NFS
57407100	Cereal (General Mills Trix)
57408100	Cereal (Uncle Sam)
57411000	Cereal, wheat squares
57416000	Cereal, plain puffs
57416010	Cereal, puffed wheat, sweetened
57417000	Cereal, shredded wheat, plain
57418000	Cereal, wheat flakes
57420100	Cereal, other, NFS
57420110	Cereal, other, plain
57420120	Cereal, other, fruit flavored
57420130	Cereal, other, chocolate
57420140	Cereal, other, peanut butter
57420150	Cereal, other, honey
Egg products	
Scrambled egg	
27520140	Bacon and egg sandwich*
32105180	Huevos rancheros*
32105190	Egg casserole with bread, cheese, milk and meat*
32105200	Egg foo yung, NFS*
32105210	Chicken egg foo yung*
32105220	Pork egg foo yung*
32105230	Shrimp egg foo yung*
32105240	Beef egg foo yung*
32129990	Egg omelet or scrambled egg, NS as to fat
32130000	Egg omelet or scrambled egg, made with margarine
32130010	Egg omelet or scrambled egg, made with oil
32130020	Egg omelet or scrambled egg, made with butter

Food code	Food description
32130040	Egg omelet or scrambled egg, made with animal fat or meat drippings
32130060	Egg omelet or scrambled egg, made with cooking spray
32130065	Egg omelet or scrambled egg, NS as to fat type
32130070	Egg omelet or scrambled egg, no added fat
32130080	Egg omelet or scrambled egg, from fast food / restaurant
32130100	Egg omelet or scrambled egg, with cheese, made with margarine*
32130110	Egg omelet or scrambled egg, with cheese, made with oil*
32130120	Egg omelet or scrambled egg, with cheese, made with butter*
32130140	Egg omelet or scrambled egg, with cheese, made with animal fat or meat drippings*
32130160	Egg omelet or scrambled egg, with cheese, made with cooking spray*
32130170	Egg omelet or scrambled egg, with cheese, no added fat*
32130190	Egg omelet or scrambled egg, with meat, NS as to fat*
32130200	Egg omelet or scrambled egg, with meat, made with margarine*
32130210	Egg omelet or scrambled egg, with meat, made with oil*
32130220	Egg omelet or scrambled egg, with meat, made with butter*
32130260	Egg omelet or scrambled egg, with meat, made with cooking spray*
32130270	Egg omelet or scrambled egg, with meat, no added fat*
32130290	Egg omelet or scrambled egg, with cheese and meat, NS as to fat*
32130300	Egg omelet or scrambled egg, with cheese and meat, made with margarine*
32130310	Egg omelet or scrambled egg, with cheese and meat, made with oil*
32130320	Egg omelet or scrambled egg, with cheese and meat, made with butter*
32130360	Egg omelet or scrambled egg, with cheese and meat, made with cooking spray*
32130365	Egg omelet or scrambled egg, with cheese and meat, NS as to fat type*
32130370	Egg omelet or scrambled egg, with cheese and meat, no added fat*
32130400	Egg omelet or scrambled egg, with tomatoes, fat added*
32130410	Egg omelet or scrambled egg, with tomatoes, no added fat*
32130420	Egg omelet or scrambled egg, with tomatoes, NS as to fat*
32130430	Egg omelet or scrambled egg, with dark-green vegetables, fat added*
32130450	Egg omelet or scrambled egg, with dark-green vegetables, NS as to fat*
32130460	Egg omelet or scrambled egg, with tomatoes and dark-green vegetables, fat added*
32130470	Egg omelet or scrambled egg, with tomatoes and dark-green vegetables, no fat added*
32130480	Egg omelet or scrambled egg, with tomatoes and dark-green vegetables, NS as to fat*
32130490	Egg omelet or scrambled egg, with vegetables other than dark green and/or tomatoes, fat added*
32130500	Egg omelet or scrambled egg, with vegetables other than dark green and/or tomatoes, no added fat*
32130510	Egg omelet or scrambled egg, with vegetables other than dark green and/or tomatoes, NS as to fat*
32130600	Egg omelet or scrambled egg, with cheese and tomatoes, fat added*
32130610	Egg omelet or scrambled egg, with cheese and tomatoes, no added fat*
32130620	Egg omelet or scrambled egg, with cheese and tomatoes, NS as to fat*
32130630	Egg omelet or scrambled egg, with cheese and dark-green vegetables, fat added*

Food code	Food description
32130640	Egg omelet or scrambled egg, with cheese and dark-green vegetables, no added fat*
32130650	Egg omelet or scrambled egg, with cheese and dark-green vegetables, NS as to fat*
32130660	Egg omelet or scrambled egg, with cheese, tomatoes, and dark-green vegetables, fat added*
32130680	Egg omelet or scrambled egg, with cheese, tomatoes, and dark-green vegetables, NS as to fat*
32130690	Egg omelet or scrambled egg, with cheese and vegetables other than dark green and/or tomatoes, fat added*
32130700	Egg omelet or scrambled egg, with cheese and vegetables other than dark green and/or tomatoes, no added fat*
32130710	Egg omelet or scrambled egg, with cheese and vegetables other than dark green and/or tomatoes, NS as to fat*
32130800	Egg omelet or scrambled egg, with meat and tomatoes, fat added*
32130810	Egg omelet or scrambled egg, with meat and tomatoes, no added fat*
32130830	Egg omelet or scrambled egg, with meat and dark-green vegetables, fat added*
32130840	Egg omelet or scrambled egg, with meat and dark-green vegetables, no added fat*
32130850	Egg omelet or scrambled egg, with meat and dark-green vegetables, NS as to fat*
32130890	Egg omelet or scrambled egg, with meat and vegetables other than dark-green and/or tomatoes, fat added*
32130900	Egg omelet or scrambled egg, with meat and vegetables other than dark-green and/or tomatoes, no added fat*
32130910	Egg omelet or scrambled egg, with meat and vegetables other than dark-green and/or tomatoes, NS as to fat*
32131000	Egg omelet or scrambled egg, with cheese, meat, and tomatoes, fat added*
32131020	Egg omelet or scrambled egg, with cheese, meat, and tomatoes, NS as to fat*
32131030	Egg omelet or scrambled egg, with cheese, meat, and dark-green vegetables, fat added*
32131040	Egg omelet or scrambled egg, with cheese, meat, and dark-green vegetables, no added fat*
32131050	Egg omelet or scrambled egg, with cheese, meat, and dark-green vegetables, NS as to fat*
32131060	Egg omelet or scrambled egg, with cheese, meat, tomatoes, and dark-green vegetables, fat added*
32131070	Egg omelet or scrambled egg, with cheese, meat, tomatoes, and dark-green vegetables, no added fat*
32131080	Egg omelet or scrambled egg, with cheese, meat, tomatoes, and dark-green vegetables, NS as to fat*
32131090	Egg omelet or scrambled egg, with cheese, meat, and vegetables other than dark-green and/or tomatoes, fat added*
32131100	Egg omelet or scrambled egg, with cheese, meat, and vegetables other than dark-green and/or tomatoes, no added fat*
32131110	Egg omelet or scrambled egg, with cheese, meat, and vegetables other than dark-green and/or tomatoes, NS as to fat*
32131200	Egg omelet or scrambled egg, with potatoes and/or onions, fat added*
32131210	Egg omelet or scrambled egg, with potatoes and/or onions, no added fat*
32131220	Egg omelet or scrambled egg, with potatoes and/or onions, NS as to fat*
32202000	Egg, cheese, ham, and bacon on bun*

Food code	Food description
32202010	Egg, cheese, and ham on English muffin*
32202025	Egg, cheese and ham on bagel*
32202030	Egg, cheese, and sausage on English muffin*
32202034	Egg, cheese, and sausage on bun*
32202045	Egg, cheese, and steak on bagel*
32202050	Egg, cheese, and sausage on biscuit*
32202055	Egg, cheese, and sausage griddle cake sandwich*
32202060	Egg and sausage on biscuit*
32202070	Egg, cheese, and bacon on biscuit*
32202075	Egg, cheese, and bacon griddle cake sandwich*
32202080	Egg, cheese, and bacon on English muffin*
32202085	Egg, cheese and bacon on bagel*
32202090	Egg and bacon on biscuit*
32202110	Egg and ham on biscuit*
32202120	Egg, cheese and sausage on bagel*
32202130	Egg and steak on biscuit*
32202200	Egg and cheese on biscuit*
32204010	Scrambled egg sandwich*
32400055	Egg white omelet, scrambled, or fried, NS as to fat
32400060	Egg white omelet, scrambled, or fried, made with margarine
32400065	Egg white omelet, scrambled, or fried, made with oil
32400070	Egg white omelet, scrambled, or fried, made with butter
32400075	Egg white omelet, scrambled, or fried, made with cooking spray
32400078	Egg white omelet, scrambled, or fried, NS as to fat type
32400080	Egg white omelet, scrambled, or fried, no added fat
32400100	Egg white, omelet, scrambled, or fried, with cheese*
32400200	Egg white, omelet, scrambled, or fried, with meat*
32400300	Egg white, omelet, scrambled, or fried, with vegetables*
32400400	Egg white, omelet, scrambled, or fried, with cheese and meat*
32400500	Egg white, omelet, scrambled, or fried, with cheese and vegetables*
32400600	Egg white, omelet, scrambled, or fried, with meat and vegetables*
32400700	Egg white, omelet, scrambled, or fried, with cheese, meat, and vegetables*
33001010	Egg substitute, omelet, scrambled, or fried, fat added
33001050	Egg substitute, omelet, scrambled, or fried, no added fat
33401000	Egg substitute, omelet, scrambled, or fried, with cheese*
33401100	Egg substitute, omelet, scrambled, or fried, with meat*
33401200	Egg substitute, omelet, scrambled, or fried, with vegetables*
33401300	Egg substitute, omelet, scrambled, or fried, with cheese and meat*
33401400	Egg substitute, omelet, scrambled, or fried, with cheese and vegetables*
33401500	Egg substitute, omelet, scrambled, or fried, with meat and vegetables*
33401600	Egg substitute, omelet, scrambled, or fried, with cheese, meat, and vegetables*

Food code	Food description
34001100	Egg sandwich on white bread*
34001110	Egg sandwich on white bread, with cheese*
34001120	Egg sandwich on white bread, with meat*
34001130	Egg sandwich on white bread, with meat and cheese*
34001200	Egg sandwich on wheat bread*
34001210	Egg sandwich on wheat bread, with cheese*
34001220	Egg sandwich on wheat bread, with meat*
34001230	Egg sandwich on wheat bread, with meat and cheese*
34001300	Egg sandwich on English muffin*
34001310	Egg sandwich on English muffin, with sausage*
34001320	Egg sandwich on English muffin, with bacon*
34001330	Egg sandwich on English muffin, with ham*
34001400	Egg sandwich on croissant*
34001410	Egg sandwich on croissant, with sausage*
34001420	Egg sandwich on croissant, with bacon*
34001430	Egg sandwich on croissant, with ham*
34001500	Egg sandwich on biscuit*
34001510	Egg sandwich on biscuit, with sausage*
34001520	Egg sandwich on biscuit, with sausage and cheese*
34001530	Egg sandwich on biscuit, with bacon*
34001540	Egg sandwich on biscuit, with bacon and cheese*
34001600	Egg sandwich on bagel*
34001610	Egg sandwich on bagel, with sausage*
34001620	Egg sandwich on bagel, with bacon*
34001630	Egg sandwich on bagel, with ham*
34001710	Egg sandwich on griddle/pancake, with meat*
34002160	Sausage griddle/pancake sandwich*
34003100	Egg burrito*
34003120	Egg burrito, with sausage*
34003130	Egg burrito, with bacon*
34003140	Egg burrito, with ham*
58100000	Burrito, taco, or quesadilla with egg*
58100005	Burrito, taco, or quesadilla with egg and potato*
58100010	Burrito, taco, or quesadilla with egg and breakfast meat*
58100013	Burrito, taco, or quesadilla with egg and breakfast meat, from fast food*
58100015	Burrito, taco, or quesadilla with egg, potato, and breakfast meat*
58100017	Burrito, taco, or quesadilla with egg, potato, and breakfast meat, from fast food*
58100020	Burrito, taco, or quesadilla with egg, beans, and breakfast meat*
58104790	Quesadilla, egg, with meat*
58104900	Taquito, egg*
58104905	Taquito or flauta with egg and breakfast meat*

Food code	Food description
58109210	Breakfast pizza with egg*
58124220	Pastry, egg and cheese filled*
58126400	Turnover or breakfast pocket, egg*
58127270	Croissant sandwich with sausage and egg*
58127290	Croissant sandwich with bacon and egg*
58127310	Croissant sandwich with ham, egg, and cheese*
58127330	Croissant sandwich with sausage, egg, and cheese*
58127350	Croissant sandwich with bacon, egg, and cheese*
58145170	Macaroni or noodles with cheese and egg*
Egg white patty	
31108100	Egg, white, cooked, NS as to fat
31108110	Egg, white, cooked, no added fat
31108120	Egg, white, cooked, fat added
34002000	Egg white sandwich*
34002010	Egg white sandwich, with cheese*
34002020	Egg white sandwich, with meat*
Quiche	
58125110	Quiche with meat, poultry or fish
58125120	Spinach quiche, meatless
58125180	Cheese quiche, meatless
Souffle	
14630200	Cheese souffle
73305020	Squash, winter, souffle
75411010	Corn, scalloped or pudding
Baked goods and baking mixes	
Cakes, brownies, cookies	
13120550	Ice cream cookie sandwich*
13252600	Tiramisu
51187020	Anisette toast
52301000	Muffin, NFS
52302010	Muffin, fruit
52302020	Muffin, fruit, low fat
52302500	Muffin, chocolate chip
52302600	Muffin, chocolate
52303010	Muffin, whole wheat
52303500	Muffin, wheat
52304010	Muffin, wheat bran
52304040	Muffin, bran with fruit, lowfat
52304100	Muffin, oatmeal
52304150	Muffin, oat bran
52306010	Muffin, plain

Food code	Food description
52306500	Muffin, pumpkin
52306550	Muffin, zucchini
53100100	Cake or cupcake, NFS
53101100	Cake, angel food
53101200	Cake, angel food, with icing or filling
53102100	Cake or cupcake, applesauce, without icing or filling
53102200	Cake or cupcake, apple
53102700	Cake or cupcake, banana
53102800	Cake or cupcake, Black Forest
53103000	Cake, Boston cream pie
53104100	Cake or cupcake, carrot, without icing or filling
53104260	Cake or cupcake, carrot
53104400	Cake or cupcake, coconut
53105262	Cake or cupcake, chocolate with white icing, bakery
53105264	Cake or cupcake, chocolate with white icing, from mix
53105270	Cake or cupcake, chocolate with chocolate icing, bakery
53105272	Cake or cupcake, chocolate with chocolate icing, from mix
53105275	Cake or cupcake, chocolate, no icing
53105300	Cake or cupcake, German chocolate
53105396	Cake, chocolate, flourless
53106500	Cake, cream
53108200	Snack cake, chocolate
53109200	Snack cake, white
53109220	Snack cake, not chocolate, with icing or filling, reduced fat and calories
53110000	Cake, fruit cake
53111000	Cake or cupcake, gingerbread
53112100	Ice cream cake*
53113000	Cake, jelly roll
53114000	Cake or cupcake, lemon, without icing or filling
53114100	Cake or cupcake, lemon
53115100	Cake or cupcake, marble, without icing or filling
53115200	Cake or cupcake, marble
53115310	Cake or cupcake, nut, without icing or filling
53115320	Cake or cupcake, nut, with icing or filling
53115410	Cake or cupcake, oatmeal
53115450	Cake or cupcake, peanut butter
53116000	Cake, pound
53116020	Cake, pound, with icing or filling
53116270	Cake, pound, chocolate
53116500	Cake or cupcake, pumpkin, without icing or filling
53116510	Cake or cupcake, pumpkin

Food code	Food description
53116520	Cake or cupcake, red velvet
53116600	Cake, rice flour, without icing or filling
53116650	Cake, Quezadilla, El Salvadorian style
53117100	Cake or cupcake, spice, without icing or filling
53117200	Cake or cupcake, spice
53118100	Cake, sponge
53118110	Cake or cupcake, strawberry
53118200	Cake, sponge, with icing or filling
53118410	Rum cake, without icing
53118500	Cake, torte
53118550	Cake, tres leche
53119000	Cake, pineapple, upside down
53120270	Cake or cupcake, white with white icing, bakery
53120272	Cake or cupcake, white with white icing, from mix
53120275	Cake or cupcake, white, without icing or filling
53121270	Cake or cupcake, white with chocolate icing, bakery
53121272	Cake or cupcake, white with chocolate icing, from mix
53121275	Cake or cupcake, white, no icing
53122070	Cake, shortcake, biscuit type, with whipped cream and fruit
53122080	Cake, shortcake, biscuit type, with fruit
53123070	Cake, strawberry shortcake
53123080	Cake, shortcake, sponge type, with fruit
53124110	Cake or cupcake, zucchini
53200100	Cookie, batter or dough, raw
53201000	Cookie, NFS
53202000	Cookie, almond
53203000	Cookie, applesauce
53203500	Cookie, biscotti
53204000	Cookie, brownie, NS as to icing
53204010	Cookie, brownie, without icing
53204100	Cookie, brownie, with icing or filling
53205250	Cookie, butterscotch, brownie
53205260	Cookie, bar, with chocolate
53206000	Cookie, chocolate chip
53206020	Cookie, chocolate chip, made from home recipe or purchased at a bakery
53206100	Cookie, chocolate chip sandwich
53206500	Cookie, chocolate, made with rice cereal
53207000	Cookie, chocolate or fudge
53208000	Cookie, marshmallow, chocolate-covered
53208200	Cookie, marshmallow pie, chocolate covered
53209005	Cookie, chocolate, with icing or coating

Food code	Food description
53209010	Cookie, sugar wafer, chocolate-covered
53209015	Cookie, chocolate sandwich
53209100	Cookie, chocolate, sandwich, with extra filling
53209500	Cookie, chocolate and vanilla sandwich
53210000	Cookie, chocolate wafer
53210900	Cookie, graham cracker with chocolate and marshmallow
53211000	Cookie bar, with chocolate, nuts, and graham crackers
53215500	Cookie, coconut
53220000	Cookie, fruit-filled bar
53220030	Cookie, fig bar
53222010	Cookie, fortune
53222020	Cookie, cone shell, ice cream type, wafer or cake
53223000	Cookie, gingersnaps
53224000	Cookie, ladyfinger
53224250	Cookie, lemon bar
53225000	Cookie, macaroon
53226500	Cookie, marshmallow, with rice cereal, no bake
53226550	Cookie, marshmallow, with rice cereal and chocolate chips
53226600	Cookie, marshmallow and peanut butter, with oat cereal, no bake
53228000	Cookie, meringue
53230000	Cookie, molasses
53233000	Cookie, oatmeal
53233010	Cookie, oatmeal, with raisins
53233050	Cookie, oatmeal sandwich, with creme filling
53233060	Cookie, oatmeal, with chocolate chips
53233100	Cookie, oatmeal, with chocolate and peanut butter, no bake
53234000	Cookie, peanut butter
53234100	Cookie, peanut butter, with chocolate
53234250	Cookie, peanut butter with rice cereal, no bake
53235000	Cookie, peanut butter sandwich
53235500	Cookie, with peanut butter filling, chocolate-coated
53236000	Cookie, Pizzelle
53236100	Cookie, pumpkin
53237000	Cookie, raisin
53237010	Cookie, raisin sandwich, cream-filled
53238000	Cookie, sandwich-type, not chocolate or vanilla
53239000	Cookie, shortbread
53239050	Cookie, shortbread, with icing or filling
53239100	Pocky
53240000	Cookie, animal
53240010	Cookie, animal, with frosting or icing

Food code	Food description
53241500	Cookie, butter or sugar
53241510	Marie biscuit
53241600	Cookie, butter or sugar, with fruit and/or nuts
53242000	Cookie, sugar wafer
53243000	Cookie, vanilla sandwich
53243010	Cookie, vanilla sandwich, extra filling
53244010	Cookie, butter or sugar, with chocolate icing or filling
53244020	Cookie, butter or sugar, with icing or filling other than chocolate
53246000	Cookie, tea, Japanese
53247000	Cookie, vanilla wafer
53247500	Cookie, vanilla with caramel, coconut, and chocolate coating
53251100	Cookie, rugelach
53270100	Cookies, Puerto Rican style
53420000	Cream puff, eclair, custard or cream filled, NS as to icing
53420100	Cream puff, eclair, custard or cream filled, not iced
53420200	Cream puff, eclair, custard or cream filled, iced
53520000	Doughnut, NFS
53520100	Doughnut, cake type, plain
53520120	Doughnut, chocolate
53520130	Doughnut, cake type, powdered sugar
53520135	Doughnut, cake type, with icing
53520140	Doughnut, cake type, chocolate icing
53520160	Doughnut, chocolate, with chocolate icing
53520170	Doughnut holes
53520200	Churros
53520510	Beignet
53521110	Doughnut, yeast type
53521130	Doughnut, yeast type, with chocolate icing
53521140	Doughnut, jelly
53521210	Doughnut, custard-filled
53521230	Doughnut, custard-filled, with icing
53610100	Coffee cake, crumb or quick-bread type
53610170	Coffee cake, crumb or quick-bread type, with fruit
55801000	Funnel cake with sugar
55801010	Funnel cake with sugar and fruit
Protein cookies	
53204840	Cookie, brownie, reduced fat, NS as to icing
53204860	Cookie, brownie, fat free, NS as to icing
53206030	Cookie, chocolate chip, reduced fat
53207020	Cookie, chocolate or fudge, reduced fat
53207050	Cookie, chocolate, with chocolate filling or coating, fat free

Food code	Food description
53209020	Cookie, chocolate sandwich, reduced fat
53220040	Cookie, fig bar, fat free
53233040	Cookie, oatmeal, reduced fat, NS as to raisins
53239010	Cookie, shortbread, reduced fat
53243050	Cookie, vanilla sandwich, reduced fat
53247050	Cookie, vanilla wafer, reduced fat
53260030	Cookie, chocolate chip, sugar free
53260200	Cookie, oatmeal, sugar free
53260300	Cookie, sandwich, sugar free
53260400	Cookie, sugar or plain, sugar free
53260500	Cookie, sugar wafer, sugar free
53260600	Cookie, peanut butter, sugar free
53261000	Cookie, gluten free
Gluten free bread	
51806010	Bread, rice
51807000	Injera, Ethiopian bread
51808000	Bread, gluten free
51808010	Bread, gluten free, toasted
51808100	Roll, gluten free
54408485	Pretzels, soft, gluten free
55301025	French toast, gluten free*
58109100	Pizza, cheese, gluten-free thin crust*
58109120	Pizza, with meat, gluten-free thin crust*
58109140	Pizza, cheese and vegetables, gluten-free thin crust*
58109150	Pizza, cheese and vegetables, gluten-free thick crust*
Pancakes	
27560660	Sausage griddle cake sandwich*
32202055	Egg, cheese, and sausage griddle cake sandwich*
32202075	Egg, cheese, and bacon griddle cake sandwich*
34001710	Egg sandwich on griddle/pancake, with meat*
34002160	Sausage griddle/pancake sandwich*
53400200	Blintz, cheese-filled
53430100	Crepe, chocolate filled
53430200	Crepe, fruit filled
55100005	Pancakes, NFS
55100010	Pancakes, plain, frozen
55100020	Pancakes, fruit, frozen
55100025	Pancakes, chocolate, frozen
55100030	Pancakes, whole grain, frozen
55100040	Pancakes, gluten free, from frozen
55100050	Pancakes, plain, fast food / restaurant

Food code	Food description
55100055	Pancakes, fruit, fast food / restaurant
55100060	Pancakes, chocolate, fast food / restaurant
55100065	Pancakes, whole grain, fast food / restaurant
55100080	Pancakes, school
55101000	Pancakes, plain
55101015	Pancakes, plain, reduced fat
55103000	Pancakes, fruit
55103020	Pancakes, pumpkin
55103100	Pancakes, chocolate
55105000	Pancakes, buckwheat
55105100	Pancakes, cornmeal
55105200	Pancakes, whole grain
55105205	Pancakes, whole grain, reduced fat
55106000	Pancakes, gluten free
55400010	Crepe, NFS
55401000	Crepe, plain
58120110	Crepe, with meat
58120120	Crepe, filled with meat, poultry, or seafood, no sauce
58310310	Pancakes and sausage, frozen meal*
Waffles	
55200010	Waffle, NFS
55200020	Waffle, plain, frozen
55200030	Waffle, plain, reduced fat
55200040	Waffle, fruit, frozen
55200050	Waffle, chocolate, frozen
55200060	Waffle, whole grain, frozen
55200070	Waffle, whole grain, reduced fat
55200080	Waffle, whole grain, fruit, frozen
55200090	Waffle, gluten free, from frozen
55200100	Waffle, plain, fast food / restaurant
55200110	Waffle, chocolate, fast food / restaurant
55200120	Waffle, fruit, fast food / restaurant
55200130	Waffle, whole grain, fast food / restaurant
55200200	Waffle, school
55201000	Waffle, plain
55203000	Waffle, fruit
55203600	Waffle, chocolate
55203700	Waffle, cinnamon
55204000	Waffle, cornmeal
55205000	Waffle, whole grain
55208000	Waffle, gluten free

Food code	Food description
55211050	Waffle, plain, reduced fat
Plant protein products	
Meat alternative burgers	
41811800	Meatball, meatless
41811890	Veggie burger patty, no bun
41901010	Veggie burger, on bun*
41901020	Veggie burger, on bun, with cheese*
Veggie dogs/corn dogs	
27564420	Hot dog sandwich, vegetarian, on bun*
41811400	Hot dog, vegetarian
Meat alternatives, e.g., sausage patties, sausage links	
41810400	Breakfast link, pattie, or slice, meatless
41811600	Luncheon slice, meatless-beef, chicken, salami or turkey
Nuggets	
41810610	Chicken, meatless, breaded, fried
59003000	Meat substitute, cereal- and vegetable protein-based, fried
Meatless crumbles	
41440000	Textured vegetable protein, dry**
41812400	Pot pie, no meat*
41812450	Vegetarian chili, made with meat substitute*
Plant-based bacon	
27212050	Beef and macaroni with cheese sauce*
41810200	Bacon strip, meatless
41810250	Bacon bits
75140500	Broccoli salad with cauliflower, cheese, bacon bits, and dressing*
Meat alternative whole muscle cut	
41810600	Chicken, meatless, NFS
41811950	Swiss steak, with gravy, meatless*
Grain products and pastas	
Egg noodles/pasta	
27212000	Beef and noodles, no sauce*
27212100	Beef and noodles with tomato-based sauce*
27212150	Beef goulash with noodles*
27212200	Beef and noodles with gravy*
27212300	Beef and noodles with cream or white sauce*
27212350	Beef stroganoff with noodles*
27220210	Ham and noodles, no sauce*
27242000	Chicken or turkey and noodles, no sauce*
27242250	Chicken or turkey and noodles with mushroom sauce*
27242300	Chicken or turkey and noodles with cream or white sauce*
27242310	Chicken or turkey and noodles with cheese sauce*

Food code	Food description
27242400	Chicken or turkey and noodles with tomato-based sauce*
27250120	Shrimp and noodles, no sauce*
27250126	Shrimp and noodles with cream or white sauce*
27250130	Shrimp and noodles with cheese sauce*
27250132	Shrimp and noodles with tomato sauce*
27250610	Tuna noodle casserole with cream or white sauce*
27250630	Tuna noodle casserole with mushroom sauce*
27313010	Beef, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; no sauce*
27313020	Beef, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; no sauce*
27313110	Beef chow mein or chop suey with noodles*
27313220	Beef, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; tomato-based sauce*
27313410	Beef, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; gravy*
27313420	Beef, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; gravy*
27320025	Ham or pork, noodles and vegetables excluding carrots, broccoli, and dark-green leafy; no sauce*
27320027	Ham or pork, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; no sauce*
27320070	Ham or pork, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; tomato-based sauce*
27320080	Sausage, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; tomato-based sauce*
27320310	Pork chow mein or chop suey with noodles*
27343010	Chicken or turkey, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; no sauce*
27343020	Chicken or turkey, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; no sauce*
27343410	Chicken or turkey, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; gravy*
27343420	Chicken or turkey, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; gravy*
27343470	Chicken or turkey, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; cream sauce, white sauce, or mushroom sauce*
27343480	Chicken or turkey, noodles, and vegetables excluding carrots, broccoli, and/or dark-green leafy; cream sauce, white sauce, or mushroom sauce*
27343510	Chicken or turkey, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; tomato-based sauce*
27343520	Chicken or turkey, noodles, and vegetables excluding carrots, broccoli, and dark-green leafy; tomato-based sauce*
27343910	Chicken or turkey chow mein or chop suey with noodles*
27343950	Chicken or turkey, noodles, and vegetables including carrots, broccoli, and/or dark-green leafy; cheese sauce*
27350050	Shrimp chow mein or chop suey with noodles*
27350080	Tuna noodle casserole with vegetables, cream or white sauce*

Food code	Food description
27360010	Goulash, NFS*
27446355	Asian chicken or turkey garden salad with crispy noodles, chicken and/or turkey, lettuce, fruit, nuts, crispy noodles, no dressing*
28140100	Chicken dinner, NFS, frozen meal*
28143170	Chicken in cream sauce with noodles and vegetable, frozen meal*
28144100	Chicken and vegetable entree with noodles and cream sauce, frozen meal*
28310320	Beef noodle soup, Puerto Rican style*
28340510	Chicken or turkey noodle soup, chunky style, canned or ready-to-serve*
28340590	Chicken or turkey corn soup with noodles, home recipe*
28340640	Chicken or turkey vegetable soup with noodles, stew type, chunky style, canned or ready-to-serve*
56112000	Noodles, cooked*
56116000	Noodles, chow mein
58134610	Tortellini, meat-filled, with tomato sauce*
58134613	Tortellini, meat-filled, with tomato sauce, canned*
58134623	Tortellini, cheese-filled, meatless, with tomato sauce, canned*
58134710	Tortellini, spinach-filled, with tomato sauce*
58136120	Lo mein, meatless*
58136130	Lo mein, with shrimp*
58136140	Lo mein, with pork*
58136150	Lo mein, with beef*
58136160	Lo mein, with chicken*
58147520	Yat Ga Mein with meat, fish, or poultry*
58305250	Pasta with vegetable and cheese sauce, diet frozen meal*
58400100	Noodle soup, NFS*
58402010	Beef noodle soup, canned or ready-to-serve*
58402100	Beef noodle soup, home recipe*
58403010	Chicken or turkey noodle soup, canned or ready-to-serve*
58403040	Chicken or turkey noodle soup, home recipe*
58403050	Chicken or turkey noodle soup, cream of, home recipe, canned, or ready-to-serve*
58403060	Chicken or turkey noodle soup, reduced sodium, canned or ready-to-serve*
58407010	Instant soup, noodle*
58407030	Soup, mostly noodles*
58407035	Soup, mostly noodles, reduced sodium*
58408500	Noodle soup with vegetables, Asian style*
58409000	Noodle soup, with fish ball, shrimp, and dark green leafy vegetable*
72202010	Broccoli casserole with noodles*
74604010	Tomato beef noodle soup, prepared with water*
74604500	Tomato noodle soup, canned, prepared with water or ready-to-serve*
74606020	Tomato vegetable soup with noodles, prepared with water*
75460900	Chow mein or chop suey, meatless, with noodles*
75649150	Vegetable noodle soup, home recipe*

Food code	Food description
75651000	Minestrone soup, home recipe*
75651030	Vegetable beef noodle soup, prepared with water*
75651040	Vegetable noodle soup, canned, prepared with water, or ready-to-serve*
75652040	Vegetable beef soup with noodles or pasta, home recipe*
Beverages, alcoholic	
Egg white cocktail	
93301000	Cocktail, NFS
93301020	Bacardi cocktail
93301040	Daiquiri
93301111	Martini, flavored
93301132	Orange Blossom
93301160	Whiskey sour
93301250	White Russian
93301310	Mai Tai
Milk products	
Eggnog	
11531000	Eggnog
93301550	Eggnog, alcoholic*
Fats and oils	
Dressing	
11440010	Chipotle dip, yogurt based*
11440040	Ranch dip, yogurt based*
11440050	Spinach dip, yogurt based*
11440070	Vegetable dip, yogurt based*
12350010	Dip, NFS*
12350200	Chipotle dip, regular*
12350205	Chipotle dip, light*
12350210	Dill dip, regular*
12350220	Onion dip, regular*
12350225	Onion dip, light*
12350230	Ranch dip, regular*
12350235	Ranch dip, light*
12350240	Spinach dip, regular*
12350245	Spinach dip, light*
12350250	Vegetable dip, regular*
14620110	Artichoke dip*
14620115	Spinach and artichoke dip*
14620130	Seafood dip*
14640026	Cheese sandwich, American cheese, on white bread, with mayonnaise*
14640028	Cheese sandwich, American cheese, on wheat bread, with mayonnaise*
14640030	Cheese sandwich, American cheese, on whole wheat bread, with mayonnaise*

Food code	Food description
14640032	Cheese sandwich, Cheddar cheese, on white bread, with mayonnaise*
14640034	Cheese sandwich, Cheddar cheese, on wheat bread, with mayonnaise*
14640036	Cheese sandwich, Cheddar cheese, on whole wheat bread, with mayonnaise*
14640042	Cheese sandwich, reduced fat American cheese, on whole wheat bread, with mayonnaise*
25240000	Meat spread or potted meat, NFS*
25240110	Chicken salad spread*
25240220	Ham salad spread*
27220080	Ham croquette*
27246300	Chicken or turkey cake, patty, or croquette*
27250030	Codfish ball or cake*
27250040	Crab, cake*
27250050	Fish, cake or patty*
27250070	Fish, salmon cake or patty*
27250300	Mackerel cake or patty*
27420020	Ham or pork salad*
27446200	Chicken or turkey salad, made with mayonnaise*
27446205	Chicken or turkey salad with nuts and/or fruits*
27446220	Chicken or turkey salad with egg*
27446225	Chicken or turkey salad, made with light mayonnaise*
27446230	Chicken or turkey salad, made with mayonnaise-type salad dressing*
27446235	Chicken or turkey salad, made with light mayonnaise-type salad dressing*
27446240	Chicken or turkey salad, made with creamy dressing*
27446245	Chicken or turkey salad, made with light creamy dressing*
27446260	Chicken or turkey salad, made with any type of fat free dressing*
27450010	Crab salad*
27450030	Salmon salad*
27450060	Tuna salad, made with mayonnaise*
27450061	Tuna salad, made with light mayonnaise*
27450062	Tuna salad, made with mayonnaise-type salad dressing*
27450063	Tuna salad, made with light mayonnaise-type salad dressing*
27450068	Tuna salad, made with any type of fat free dressing*
27450070	Shrimp salad*
27450080	Seafood salad*
27450090	Tuna salad with cheese*
27450100	Tuna salad with egg*
27450130	Crab salad made with imitation crab*
27500050	Sandwich, NFS*
27500100	Meat sandwich, NFS*
27500300	Sandwich wrap, NFS*
27510000	Beef sandwich, NFS*
27510171	Whopper Jr with cheese (Burger King)*

Food code	Food description
27510235	Cheeseburger submarine sandwich with lettuce, tomato and spread*
27510552	Whopper Jr (Burger King)*
27510950	Reuben sandwich*
27513040	Roast beef submarine sandwich, with lettuce, tomato and spread*
27513041	Roast beef submarine sandwich, with cheese, lettuce, tomato and spread*
27520150	Bacon, lettuce, and tomato sandwich with spread*
27520155	Bacon, lettuce, and tomato submarine sandwich, with spread*
27520156	Bacon, lettuce, tomato, and cheese submarine sandwich, with spread*
27520310	Ham sandwich with lettuce and spread*
27520320	Ham and cheese sandwich, with lettuce and spread*
27520340	Ham salad sandwich on white*
27520370	Hot ham and cheese sandwich, on bun*
27520390	Ham and cheese submarine sandwich, with lettuce, tomato and spread*
27540110	Sliced chicken sandwich, with spread*
27540111	Sliced chicken sandwich, with cheese and spread*
27540120	Chicken salad sandwich on white*
27540121	Chicken salad sandwich on wheat*
27540122	Chicken salad sandwich wrap*
27540285	Chicken, bacon, and tomato club sandwich, with lettuce and spread*
27540290	Chicken submarine sandwich, with lettuce, tomato and spread*
27540291	Chicken submarine sandwich, with cheese, lettuce, tomato and spread*
27540296	Buffalo chicken submarine sandwich with cheese*
27540310	Turkey sandwich, with spread*
27540350	Turkey submarine sandwich, with cheese, lettuce, tomato and spread*
27540360	Turkey and bacon submarine sandwich, with lettuce, tomato and spread*
27540361	Turkey and bacon submarine sandwich, with cheese, lettuce, tomato and spread*
27541000	Turkey, ham, and roast beef club sandwich, with lettuce, tomato and spread*
27541001	Turkey, ham, and roast beef club sandwich with cheese, lettuce, tomato, and spread*
27550110	Crab cake sandwich*
27550120	Salmon cake sandwich*
27550720	Tuna salad sandwich on white*
27550730	Tuna salad sandwich on white, with cheese*
27550735	Tuna salad sandwich on wheat*
27550737	Tuna salad sandwich on wheat, with cheese*
27550740	Tuna salad sandwich, on bun*
27550745	Tuna salad sandwich, on bun, with cheese*
27550755	Tuna salad sandwich wrap*
27550800	Seafood salad sandwich*
27560120	Bologna and cheese sandwich, with spread*
27560500	Pepperoni and salami submarine sandwich, with lettuce, tomato and spread*
27560910	Cold cut sumarine sandwich, with cheese, lettuce, tomato and spread*

Food code	Food description
32102000	Egg, deviled*
32103000	Egg salad, made with mayonnaise*
32103015	Egg salad, made with light mayonnaise*
32103020	Egg salad, made with mayonnaise-type salad dressing*
32202025	Egg, cheese and ham on bagel*
32203010	Egg salad sandwich on white*
32203020	Egg salad sandwich on wheat*
58127500	Vegetable submarine sandwich, with fat free spread*
58148110	Macaroni or pasta salad, made with mayonnaise*
58148111	Macaroni or pasta salad, made with light mayonnaise*
58148112	Macaroni or pasta salad, made with mayonnaise-type salad dressing*
58148118	Macaroni or pasta salad, made with any type of fat free dressing*
58148120	Macaroni or pasta salad with egg*
58148130	Macaroni or pasta salad with tuna*
58148150	Macaroni or pasta salad with shrimp*
58148160	Macaroni or pasta salad with tuna and egg*
58148170	Macaroni or pasta salad with chicken*
58148180	Macaroni or pasta salad with cheese*
58148550	Macaroni or pasta salad with meat*
63401010	Apple salad with dressing*
63402950	Fruit salad, excluding citrus fruits, with salad dressing or mayonnaise*
63402980	Fruit salad, excluding citrus fruits, with marshmallows*
63403040	Fruit salad, including citrus fruits, with marshmallows*
71600950	Potato salad with egg, from restaurant*
71601010	Potato salad with egg, made with mayonnaise*
71601015	Potato salad with egg, made with light mayonnaise*
71601020	Potato salad with egg, made with mayonnaise-type salad dressing*
71601025	Potato salad with egg, made with light mayonnaise-type salad dressing*
71601030	Potato salad with egg, made with creamy dressing*
71601050	Potato salad with egg, made with any type of fat free dressing*
71602010	Potato salad, German style*
71602950	Potato salad, from restaurant*
71603010	Potato salad, made with mayonnaise*
71603015	Potato salad, made with light mayonnaise*
71603020	Potato salad, made with mayonnaise-type salad dressing*
71603050	Potato salad, made with any type of fat free dressing*
73101110	Carrots, raw, salad*
75140500	Broccoli salad with cauliflower, cheese, bacon bits, and dressing*
75140510	Broccoli slaw salad*
75140990	Coleslaw, fast food / restaurant*
75141000	Coleslaw*

Food code	Food description
75141005	Cabbage salad or coleslaw, made with light coleslaw dressing*
75141030	Cabbage salad or coleslaw, made with creamy dressing*
75141035	Cabbage salad or coleslaw, made with light creamy dressing*
75141040	Cabbage salad, NFS*
75141100	Coleslaw, with fruit*
75141200	Cabbage salad or coleslaw with pineapple, with dressing*
75142500	Cucumber salad, made with sour cream dressing*
75302080	Bean salad, yellow and/or green string beans*
75416500	Pea salad*
75416600	Pea salad with cheese*
81302040	Sandwich spread
81302050	Tartar sauce
81308100	Fry sauce*
83100100	Salad dressing, NFS, for salads
83100200	Salad dressing, NFS, for sandwiches
83101000	Blue or roquefort cheese dressing
83102000	Caesar dressing
83103000	Coleslaw dressing
83104000	French or Catalina dressing
83105500	Honey mustard dressing
83107000	Mayonnaise, regular
83108000	Vegan mayonnaise
83109000	Russian dressing
83110000	Mayonnaise-type salad dressing
83112000	Avocado dressing
83112400	Creamy Italian dressing
83112500	Creamy dressing
83112950	Poppy seed dressing
83112990	Sesame dressing
83113500	Ranch dressing
83114000	Thousand Island dressing
83115000	Yogurt dressing
83200100	Salad dressing, light, NFS
83201000	Blue or roquefort cheese dressing, light
83201500	Creamy Italian dressing, light
83202020	French or Catalina dressing, light
83203000	Caesar dressing, light
83204000	Mayonnaise, light
83204030	Mayonnaise, reduced fat, with olive oil
83204050	Mayonnaise-type salad dressing, light
83204500	Honey mustard dressing, light

Food code	Food description
83205450	Italian dressing, light
83205560	Ranch dressing, light
83206500	Sesame dressing, light
83207000	Thousand Island dressing, light
83210100	Creamy dressing, light
83300100	Blue or roquefort cheese dressing, fat free
83300200	Caesar dressing, fat free
83300300	Creamy dressing, fat free
83300400	French or Catalina dressing, fat free
83300500	Honey mustard dressing, fat free
83300700	Mayonnaise, fat free
83300750	Ranch dressing, fat free
83300900	Salad dressing, fat free, NFS
83301000	Thousand Island dressing, fat free
Beverages and beverage bases, nonalcoholic	
Powdered shake mix	
95102000	Nutritional drink or shake, ready-to-drink (Carnation Instant Breakfast)
95106010	Nutritional drink or shake, ready-to-drink, light (Muscle Milk)
95201000	Nutritional powder mix (Carnation Instant Breakfast)**
95201010	Nutritional powder mix, sugar free (Carnation Instant Breakfast)**
95201500	Nutritional powder mix, high protein (Herbalife)**
95201600	Nutritional powder mix (Isopure)**
95201700	Nutritional powder mix (Kellogg's Special K20 Protein Water)**
95202000	Nutritional powder mix (Muscle Milk)**
95210000	Nutritional powder mix (Slim Fast)**
95210020	Nutritional powder mix, high protein (Slim Fast)**
95220000	Nutritional powder mix, NFS**
95220010	Nutritional powder mix, high protein, NFS**
95230000	Nutritional powder mix, whey based, NFS**
95230010	Nutritional powder mix, protein, soy based, NFS**
95230020	Nutritional powder mix, protein, light, NFS**
95230030	Nutritional powder mix, protein, NFS**

* Only the proportion of food corresponding to the intended use was included in the analysis.

** Dry/powdered amount was adjusted to the prepared/reconstituted amount.

GRAS Notice No. GRN 001104

Response to Additional Questions

1. **In the amendment dated June 30, 2023, Clara provided the food categories and the corresponding use levels (Table 1 in Addendum A). In addition, Clara also provided the representative National Health and Nutrition Examination Survey (NHANES) food codes for each food category (Appendix A in Addendum A).**

   Clara stated that the use levels for certain food codes representing foods in dried or powder form were adjusted based on reconstitution factors, and the use levels for other food codes were adjusted based on the portion of food corresponding to the intended use (Appendix A). However, Clara did not provide those factors used to adjust use levels. In order to validate Clara's dietary exposure estimate, please provide an applied adjustment factor and resulting use level for each NHANES food code included in Appendix A. We suggest that the notifier provide this information in a spreadsheet.

RESPONSE:

Estimates of intake of egg white protein (EWP) produced by *Komagataella phaffii* strain ATCC GSD-1235 were developed from reported intakes of foods in WWEIA that represent an intended use of the ingredient. The intended uses include use of EWP in 100% (by weight) of representative foods (e.g., protein bars or snack bars), and use of EWP in the fraction (percent by weight) of foods corresponding to an ingredient that may contain EWP. When only a component of a food corresponds to the proposed use (e.g., mayonnaise on a sandwich, egg in an omelet), USDA's Food and Nutrient Database for Dietary Studies (FNDDS) was used to identify relevant ingredients in each food as grams per 100 gram (g/100 g) food. The most recent version of FNDDS available for each food code was used to identify relevant ingredients (USDA 2020, USDA 2022).

The food code list provided in Appendix A presents each code included in the assessment by category of intended use. Food code descriptions with a designation of "*"are foods for which an intended use of EWP represents use as an ingredient. Only the fraction of food corresponding to the intended use was included in the analysis. For all other foods, the proposed use of EWP was applied to 100% of the food in the analysis. A list of the NHANES food codes with the applied adjustment factor and resulting use level is provided in the Attachment.

As noted in Appendix A, some of the food codes (n=15) representing an intended use of EWP are a dry or powder form of food. All intended uses of EWP represent use in foods as consumed, with the exception of egg noodles. An adjustment factor of 0.35 was applied to cooked noodles to convert to dry weight; this factor was calculated using USDA's moisture content for cooked and dry noodles (USDA, 2018). In order to estimate intake of EWP from the intended use in these foods, it was necessary to apply an adjustment factor to account for the amount of dry/powder food consumed in the reconstituted food or beverage. All adjustment factors were derived from the gram weight corresponding to a serving as specified by USDA's WWEIA 2019-2020 FNDDS Food Portions and Weights Component file, which is provided by USDA as supporting documentation in FNDDS for the processing of dietary intake data (USDA 2022).

2. **In the amendment dated June 30, 2023, Clara stated that they will consider lowering the specification limits as appropriate. Clara also noted that it is premature to lower the limits based on the limited data currently available for egg- white protein produced by *Komagataella phaffii* strain ATCC GSD-1235.**

   We note that the FDA's recent Closer to Zero initiative specifically focuses on reducing dietary exposure to heavy metals in foods. We also note that we would not expect heavy metals to be present at levels as high as 1 mg/kg in ingredients produced by controlled fermentation and following current good manufacturing practices. We typically see levels of heavy metals (and therefore corresponding specification limits) of an order of magnitude lower or even below that (see GRNs 001075, 001070, 001062, 0o1056 and some other GRAS notices for fermentation- derived ingredients that were recently posted on our GRAS Notices inventory webpage). Keeping in line with FDA's Closer to Zero initiative and based on the provided batch analyses indicating that levels of heavy metals lower than 1 mg/kg can be achieved, we suggest that Clara reconsider our recommendation regarding lowering the specification limits for heavy metals.

Response:

In keeping with FDA's Closer to Zero initiative, Clara Foods Co. DBA The EVERY Company would like to lower the specification limits for heavy metals (Lead, Mercury, Arsenic and Cadmium) to < 0.4 mg/kg.

REFERENCES

U.S. Department of Agriculture, Agricultural Research Service. 2022. USDA Food and Nutrient Database for Dietary Studies 2019-2020. Food Surveys Research Group Home Page, http://www.ars.usda.gov/nea/bhnrc/fsrg

U.S. Department of Agriculture, Agricultural Research Service. 2020. USDA Food and Nutrient Database for Dietary Studies 2017-2018. Food Surveys Research Group Home Page, http://www.ars.usda.gov/nea/bhnrc/fsrg

U.S. Department of Agriculture, Agricultural Research Service. SR Legacy Foods. 2018. Noodles, egg, enriched, cooked, NDB No, 20110; Noodles, egg, dry, enriched, NDB No, 20109. https://fdc.nal.usda.gov/

From: Pelonis, Evangelia C. <pelonis@khlaw.com>
Sent: Tuesday, September 12, 2023 11:42 AM
To: Deng, Kaiping <Kaiping.Deng@fda.hhs.gov>
Cc: Kritika Mahadevan <kritika@clarafoods.com>; Ke, Qingdong Karin <ke@khlaw.com>
Subject: [EXTERNAL] RE: quick meeting Clara-FDA

Dear Kaiping,

We are responding to the discussion we had with FDA on August 23. The notifier of GRN 1104, Clara Foods Co. DBA The EVERY Company has agreed to lower the heavy metal specifications for arsenic, cadmium, lead, and mercury to ≤0.1 mg/kg.

Please let us know if FDA needs anything else to complete your review of this GRN.

Best,
Eve

Evangelia C. Pelonis
Partner

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