Biotechnology Notification File No. 000154
Note to the File

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To: Administrative Record, BNF No. 000154

Subject: Event NF872, reduced polyphenol oxidases, apple

Keywords:
Apple, *Malus x domestica*, Arctic apple, Fuji, NF872, RNA interference (RNAi), polyphenol oxidase (PPO), reduced polyphenol oxidases, enzymatic browning, *nptII* from *Escherichia coli* transposon Tn5, OECD unique identifier OKA-NBØØ3-1

Purpose

This document summarizes the Food and Drug Administration's (FDA, our) evaluation of biotechnology notification file (BNF) No. 000154. Okanagan Specialty Fruits (OSF) submitted a safety and nutritional assessment of genetically engineered apple with reduced levels of polyphenol oxidases, transformation event NF872, which we received on April 11, 2016. We received additional information from OSF on December 21, 2016, June 4, 2017, September 7, 2017, January 5, 2018, and March 9, 2018. We evaluated the information in OSF's submissions to ensure that regulatory and safety issues regarding human and animal food derived from NF872 apple have been resolved prior to commercial distribution.

In our evaluation, we considered all information provided by OSF as well as publicly available information and information in the agency's files. Here, we discuss the outcome of the consultation, but do not intend to restate the information provided in the final consultation in its entirety.

Intended Effect

The intended effect of the modification in NF872 is reduced enzymatic browning. This was achieved by suppressing expression of polyphenol oxidase (PPO) genes through the RNA interference (RNAi) pathway. More details about the intended effects can be found in FDA's memorandum from BNF No. 000132.

Regulatory Considerations

The purposes of this evaluation are (1) to assess whether OSF has introduced into human or animal food a substance requiring premarket approval as a food additive and (2) to determine whether use of the new plant variety in human or animal food raises other regulatory issues under the Federal Food, Drug and Cosmetic Act (FD&C Act).

Genetic Modification and Characterization

Introduced DNA and Method

OSF introduced DNA into Fuji apple from the GEN-03 plasmid using *Agrobacterium*-mediated transformation. The functional sequences in the vector included an RNAi suppression cassette containing partial sequences from four apple PPO genes and an expression cassette for the selectable marker neomycin phosphotransferase (NPTII). For more details about the GEN-03 plasmid vector, see FDA's memorandum from BNF No. 000132.

Characteristics, Inheritance, and Stability of the Introduced DNA

OSF characterized the DNA insertions in NF7872 using whole genome sequencing followed by bioinformatics analysis to identify junction sequences matching both the GEN-03 plasmid vector and the GDDH13_V1-1 apple reference genome. OSF determined its average haploid genome coverage to be 29.2×.

OSF identified six classes of junction sequences, consistent with three T-DNA insertions. The apple sequences flanking the insertions derive from chromosomes 3, 13, and 17. The arrangement of vector sequences in the junctions indicates that all three insertions were complex, comprising multiple vector fragments. OSF identified four internal junction sequence classes, each representing a junction between vector sequence fragments within an insertion. OSF used the six vector/apple junctions and four internal junctions to propose a model for each insertion. The insertion in chromosome three contains vector backbone DNA, which lacks functional sequences. The insertion in chromosome 17 contains a sequence fragment with a partial CaMV 35S promoter oriented towards apple genomic sequence. OSF does not consider this promotor fragment capable of initiating transcription of unintended expression products, because it lacks essential functional elements.

Commercial apples are vegetatively propagated and do not undergo processes associated with genetic variation such as meiosis, recombination, or segregation. Therefore, genotypes and phenotypes of apple varieties are expected to remain relatively stable. OSF developed NF872 in 2003, propagated the variety through multiple generations of tissue culture, and grafted it onto rootstocks in 2005. Molecular characterization, phenotypic analysis, and compositional analysis were performed on samples collected in 2015 and 2016. PCR analysis confirmed the presence of the *nptII* selectable marker gene in leaf samples collected in 2016. These results are consistent with the long-term stability of the insertions and of the non-browning trait.

Using bioinformatics analysis, OSF assessed putative translation products of open reading frames (ORFs) generated by the insertion events for homology to known toxic or allergenic proteins. For comparison to known allergens, OSF used the Food Allergy Research and Resource Program (FARRP) allergen protein database (Version 17). For comparison to known toxins, OSF used the NCBI non-redundant protein sequence database. OSF concludes that in the unlikely event that the identified ORFs were translated, none would be sufficiently similar to known allergens or toxins to raise safety concerns.

Protein Characterization

Identity and Function of Introduced Protein

OSF affirms that the only additional protein intentionally expressed in NF872 is NPTII. The *nptII* gene confers resistance to the antibiotic kanamycin and was used as a selectable marker during the transformation of the parental apple variety.

Protein Expression Level

OSF estimated NPTII protein levels in mature NF872 fruit using a semi-quantitative enzyme-linked immunosorbent assay (ELISA) approach. OSF analyzed 6 fruits of NF872 and 6 fruits of untransformed

Fuji control (NF) harvested in 2015. Levels of NPTII in all samples were below the limit of quantitation, or 10 ng/g fresh weight.

Potential for Toxicity and Allergenicity of the Introduced Protein

OSF notes that NPTII is the only introduced protein in NF872 and that the NPTII protein does not accumulate to detectable levels in mature apple fruit. Further, OSF notes that FDA has previously evaluated the safety of the use of the *nptII* gene in the development of other genetically engineered plants (59 FR 26700, May 23, 1994[1], 21 CFR 173.170, 573.130). Therefore, no new protein that may be toxic or allergenic is expressed in NF872.

Evidence for Lower PPO Activity in Tissues

OSF measured PPO activity in multiple plant tissues using a functional assay. In skin samples from mature fruit, PPO activity was suppressed by 98% in NF872 relative to NF control. Bruising experiments found significantly reduced color change from bruising in NF872. OSF also observed the non-browning phenotype following other forms of mechanical damage, including slicing and juicing. OSF concludes that these data support the conclusion that the targeted PPO genes are functionally suppressed in NF872.

Human and Animal Food Use

Fuji is one of the top five apple varieties grown in the United States and is popular as a dessert apple. OSF refers to its previous consultation with FDA (BNF 000132), in which OSF reviewed the historical uses of apples in human food. Apples are consumed fresh and are processed into juice, pie filling, sauce, alcoholic cider, juice concentrate, fruit leather, dehydrated fruit bars and other products, and as food ingredients in the flavor industry. Fresh apples are the primary apple product consumed as food in the U.S., with processed apple products accounting for less than 50 percent of the U.S. crop on average. Byproducts of manufacturing, such as pomace left over from juice production, may be fed to animals.

Composition

Scope of Analysis

OSF analyzed the composition of mature fruit from NF872 apple and from NF control.

Study Design - Compositional Analyses

OSF harvested fruit in 2015 from plantings of NF872 and NF control in New York State and Washington State. For each variety and from each site, OSF analyzed 6 samples, each sample derived from one randomly selected apple from the planting. OSF measured fat, protein, moisture, ash, carbohydrates, calories, total dietary fiber, potassium, vitamin C, and phenolics. OSF repeated its sampling procedure in the 2016 growing season and measured values for sugars and potassium. OSF pooled data across locations for each variety and presented the mean, standard deviation, and range for each component. OSF compared its results to values from publicly available USDA databases for apple as well as for Fuji apple specifically.

Results of analyses

OSF found no differences that would significantly affect nutrition between levels of components in NF872 and NF control, although levels of phenolics and vitamin C appeared higher in NF872. OSF considers the values obtained for phenolics and vitamin C in NF872 to result from reduced activity of PPO, which would normally be expected to degrade these components following tissue damage and

[1] FDA also assessed the safety of NPTII protein (referred to as Aminoglycoside 3' phosphotransferase II in FDA's regulations) in 59 FR 26700, May 23, 1994 at 26702.

analytical sample preparation.[2] OSF notes that values for vitamin C and phenolics in NF872 were similar to values for apples in the USDA database. OSF concludes that NF872 is nutritionally equivalent to its parental variety.

Human Food Labeling Considerations

It is a producer's or distributor's responsibility to ensure that labeling of the foods it markets meets applicable legal requirements, including disclosure of any material differences in the food. It is our understanding that NF872 apple may be used in various food applications. Depending on the particular food application, the non-browning aspect of the apples may be considered material information requiring disclosure under Section 201(n) and Section 403(a)(1) of the FD&C Act. Companies marketing NF872 apples or products containing NF872 apple are advised to consult with FDA's Center for Food Safety and Applied Nutrition's Office of Nutrition and Food Labeling, Food Labeling and Standards Staff, to discuss any required or voluntary labeling including statements relating to attributes of this apple variety.

Conclusion

FDA evaluated OSF's submission to determine whether NF872 apple raises any safety or regulatory issues with respect to the intended modifications or with respect to its uses in human or animal food. Based on the information provided by the company and other information available to the agency, FDA did not identify any safety or regulatory issues under the FD&C Act that would require further evaluation at this time.

OSF has concluded that its non-browning apple variety, NF872 apple, and the human and animal foods derived from it are as safe as and are not materially different in composition or any other relevant parameter from other apple varieties now grown, marketed, and consumed. At this time, based on OSF's data and information, the agency considers OSF's consultation on NF872 apple to be complete.

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[2] FDA does not consider the observed changes in levels of vitamin C and phenolics in NF872 following tissue damage to be nutritionally consequential.