SUBSTANTIAL EQUIVALENCE OPINION

2'-*O*-Fucosyllactose

The Food Safety Authority of Ireland (FSAI) received an application in March of 2016 from Glycom A/S of Denmark for an opinion on the substantial equivalence of 2'-*O*-fucosyllactose (2'FL) to the same ingredient authorised for the EU market by Commission Implementing Decision (EU) 2016/376. The novel ingredient and the authorised comparator are manufactured by the same company.

The novel ingredient (2'FL) is a trisaccharide that occurs naturally in human milk and is comprised of L-fucose, D-galactose and D-glucose. In 2016, chemically synthesised 2'FL manufactured by Glycom A/S was authorised for the EU market by Commission Implementing Decision (EU) 2016/376. The same company has since developed a fermentation process which is more efficient at producing 2'FL and is seeking an opinion on its substantial equivalence to the authorised synthetic 2'FL. The production of 2'FL by fermentation occurs in two primary stages and is carried out to Good Manufacturing Practices in accordance with HACCP principles. Stage 1 involves fermentation of the substrate (D-lactose) by a genetically modified (GM) *Escherichia coli* K-12 using D-sucrose as a source of energy and carbon. The GM *E. coli* is considered a processing aid in the production process as the novel ingredient is purified and concentrated from the medium without disruption of the bacterial cells. Therefore the authorisation and labelling requirements specific to GM foods do not apply. Stage 2 involves a series of steps to isolate and purify 2'FL crystals which are washed and dried prior to packaging. Process controls and quality checks are in place to monitor the potential presence of undesirable substances and to ensure the purity of the final ingredient. The novel ingredient has a five year shelf life and is intended for inclusion in the same foods and at the same maximum levels as the authorised 2'FL. Batch analysis demonstrates that the novel ingredient produced by fermentation is very similar to the authorised comparator in terms of the specifications set out in Commission Implementing Decision (EU) 2016/376.

The 2'FL produced by fermentation is categorised as novel in line with *Article 1.2 (d)* of the Novel Food Regulation (EC) No. 258/97; "foods and food ingredients consisting of or isolated from microorganisms, fungi or algae".

Composition

The novel ingredient is described as a white to off-white powder or agglomerate (>94% purity) with a slight to intense acetic odour and sweet/sour taste. The applicant has demonstrated that the fermented 2'FL molecule is chemically and structurally identical to the synthetic comparator. Specifications for the novel ingredient are in line with those for the authorised comparator as described in Commission Implementing Decision (EU) 2016/376, though minor process-related differences are noted. These include slightly higher levels of sulphated ash, acetic acid and lactose. The applicant has introduced a specification for the sum of 2'FL, lactose, difucosyllactose and fucose or 'Human Identical Milk Saccharides (water free)' of not less than 96% to account for slight variations in the quantities of particular saccharides.

Parameter	**2'FL Produced by Chemical Synthesis**	**2'FL Produced by Fermentation**
	Specification	Specification
Identification [1]	= RT of standard ± 3 %	= RT of standard ± 3 %
Human-identical Milk Saccharides [2]	Not less than 96 %	Not less than 96 %
Assay 2'FL (water free)	Not less than 95 %	Not less than 94 %
D-Lactose	Not more than 1.0 %	Not more than 3.0 %
L-Fucose	Not more than 1.0 %	Not more than 1.0 %
Difucosyl-D-lactose [3]	Not more than 1.0 %	Not more than 1.0 %
2'-Fucosyl-D-lactulose	Not more than 0.6 %	Not more than 1.0 %
pH (20°C, 5% solution)	3.2 to 7.0	3.2 to 5.0
Water	Not more than. 9.0 %	Not more than 5.0 %
Ash, sulphated	Not more than 0.2 %	Not more than 1.5 %
Acetic acid	Not more than 0.3 %	Not more than 1.0 %
Residual solvents (methanol, 2-propanol, methyl acetate, acetone)	Not more than 50 mg/kg singly Not more than 200 mg/kg in combination	Not applicable
Residual proteins	Not more than 0.01 %	Not more than 0.01 %
Palladium (Pd)	Not more than 0.1 mg/kg	Not applicable
Nickel (Ni)	Not more than 3.0 mg/kg	Not applicable

2'FL = 2'-*O*-fucosyllactose; RT = retention time

[1] Specification not used in regulatory filing of 2'FL produced by chemical synthesis but applied.

[2] Human-identical Milk Saccharides = Sum of 2'FL, D-Lactose, L-Fucose and Difucosyllactose. Specification not used in regulatory filing of 2'FL produced by chemical synthesis but applied.

[3] Specified as "Sum of Difucosyllactose isomers" for 2'FL produced by chemical synthesis, but corresponding to the single naturally occurring isomer (2',3-*O*-difucosyllactose) in 2'FL produced by fermentation.

Nutritional Value and Metabolism

Regardless of the production method, 2'FL is considered a fibre, with a calorific value of 8 kJ/g (2kcal/g). Due to possible carryover from the fermentation process, trace elements and minerals including phosphorous, sodium, potassium, magnesium, calcium, zinc, copper, manganese and molybdenum can be present in the novel ingredient. However, the applicant concludes that the amounts concerned would have a negligible nutritional impact for consumers. Therefore, it can be concluded that nutritional value and metabolism of the novel ingredient is substantially equivalent to that of the authorised comparator.

Intended Uses

The novel ingredient is intended for use in the same food categories and at the same maximum use levels as the authorised comparator as set out in Commission Implementing Decision (EU) 2016/376. Food categories include infant and follow on formula, other foods for infants and young children, dietary foods for special medical purposes and food supplements. General foodstuffs to which the novel ingredient will be added include milk based products, dairy analogues, cereal bars, table-top sweeteners and beverages.

Level of Undesirable Substances

The production of 2'FL by fermentation does not involve the use of any organic solvents. The applicant has demonstrated through DNA analysis that any trace of the fermenting *E. coli* is absent from the final product, while protein is not detectable. Batch analyses demonstrate adherence to microbiological specifications in relation to endotoxins, yeasts and moulds, *Salmonella*, Enterobacteriaceae, *Escherichia coli*, *Cronobacter sakazakii*, *Listeria monocytogenes* and *Bacillus cereus*. Checks are carried out on the final product for the presence of heavy metals including palladium, nickel, chromium, aluminium, cobalt, lead, arsenic, mercury and cadmium. Traces of ethylenediaminetetraacetic acid (EDTA) and oxalate from the fermentation medium may be present in the final product, but not at levels that would pose a safety concern. To provide further reassurance about the safety of the novel ingredient, the applicant carried out preclinical toxicology studies, similar to those presented for the authorisation of the synthetic comparator. These studies included an adapted

subchronic 90-day oral toxicity study in rats, a bacterial reverse mutation assay and an in vitro micronucleus assay.

Conclusions

The FSAI is satisfied that the information provided by the applicant demonstrates that 2'FL produced by fermentation is substantially equivalent to the chemically synthesised comparator which was authorised for the EU market to Glycom A/S by Commission Implementing Decision (EU) 2016/376. The uses and maximum use levels of the novel 2'FL will be the same as for the EU-authorised synthetic 2'FL. The designation of the novel ingredient in foods to which it is added shall be "2'-*O*-fucosyllactose", while consumer information will be provided in accordance with the requirements set out in *Article 2* of Commission Implementing Decision 2016/376.