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METHODS OF ANALYSIS FOR PROVISIONS IN THE STANDARD FOR INFANT FORMULAS AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENTED FOR INFANTS (CXS 72-1981)

IN SESSION WORKING GROUP

(Prepared by the United States of America)

Introduction

The 41st Session of the Codex Committee on Methods of Analysis and Sampling (CCMAS41, May 2021) considered a referral from CCNFSDU41 (Rep20/NFSDU-20) Regarding Methods of analysis for provisions in the *Standard for Infant Formulas and Formulas for Special Medical Purposes Intended for Infants* (CXS 72-1981). CCMAS41 agreed to inform CCNFSDU that the methods for fructans, beta-carotene and lycopene were not endorsed as there were no accompanying provisions in CXS 72-1981 and to request CCNFSDU to provide a rationale to support their proposal for methods for these substances. CCNFSDU should be informed that all proposed methods of analysis must have direct pertinence to the Codex standard to which they are directed.

The main concern raised by CCMAS is a need for CCNFSDU to confirm that fructans, beta-carotene, and lycopene are optional ingredients based on the provisions established in the *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants*.

The purpose of this Conference Room Document (CRD) is to outline the basis for confirming that fructans (i.e., non-digestible oligosaccharides), beta-carotene, and lycopene suitable are optional ingredients based on the provisions of the standard and current use internationally. It should be emphasized that the factors addressed in this document for establishing that these optional ingredients are safe and suitable for inclusion in infant formulas are the initial foundational criteria for undertaking the process of validating methods of analysis for optional ingredients. Further, these factors apply to considering any optional ingredient for infant formulas beyond the three nutrients identified here.

***Infant Formula Standard* (CXS-72-1981) Optional Ingredients Provision, section 3.2**

3.2 Optional ingredients

3.2.1 In addition to the compositional requirements listed under 3.1.3, other ingredients may be added in order to provide substances ordinarily found in human milk and to ensure that the formulation is suitable as the sole source of nutrition for the infant or to provide other benefits that are similar to outcomes of populations of breastfed babies.

3.2.2 The suitability for the particular nutritional uses of infants and the safety of these substances shall be scientifically demonstrated. The formula shall contain sufficient amounts of these substances to achieve the intended effect, taking into account levels in human milk.

Consideration of Fructans, Beta-Carotene, and Lycopene as Optional Ingredients

Fructans

Fructans Definition and Research Supporting Use

Fructans, also called fructooligosaccharides (FOS) or oligofructose (OF), or oligofructan are non-digestible oligosaccharides commonly added as optional ingredients to Infant Formula internationally and have been shown in scientific studies to have a positive impact on the stool consistency and gut microbiota [1-5].

It is widely accepted that breast-fed infants have different stools and gut microbiota than formula-fed infants. This fact is presumed to be due to by the oligosaccharides naturally present (in concentrations of 10-12 g/L) in human breast milk [6,7].

There is evidence to support adding fructans to infant formula can provide benefits towards breastfed infants.

As measured by the different bacteria counts and by bacterial activity end products, such as short chain fatty acids, and prebiotic oligosaccharides (such as the GOS/short chain (sc) FOS blend), studies show that fructans modify the gut microflora of formula-fed infants into a microflora more closely resembling the one of breast-fed infants.[8]. These studies are briefly described below.

- A review from Meyer et al. [9] studied the bifidogenic effect of inulin and scFOS individually in infants, adults and the elderly. The study concluded that a bifidogenic effect of scFOS may be observed in infants and young children from 6 days to 19 months of age, although it was not observed in all reviewed studies.
- Brunser et al. [10] reported a significantly higher count of Bifidobacteria in a group supplemented 4.5 g/L of a prebiotic mix containing 70% scFOS and 30% inulin (p=0.029. The count of Lactobacilli was also higher in the experimental group, but not significantly (p=0.057).
- Waligora et al. [11] observed that after a 2 g/d scFOS supplementation for 21 days, 6–24-month-old subjects fed the experimental formula tended to have more Bifidobacteria than the control group, although the baseline was similar (p=0.095).
- In Zheng et al. [12], a 14-day supplementation of 2 g/L of 70% scFOS and 30% inulin mix in oncologic pediatric patients aged 1-12 years lead to significantly higher Lactobacilli counts (p=0.02) in the prebiotic group, and a similar trend was observed for Bifidobacteria.
- In Euler et al. [6], a 1-week supplementation of scFOS at both 1.5 g/L and 3 g/L did not lead to a significant difference in Bifidobacteria count between either scFOS supplemented group and breast-fed infants seven days after the treatment.
- Wernimont et al. [13] demonstrated that Bifidobacteria increased more in the Oligofructose (OF) group versus control and was not significantly different than the human milk (HM) group. Stool consistency was intermediate between OF group and control and HM.
- Closa-Monasterolo et al. [14] showed that an oligofructose enriched inulin group infants had a microbiota composition closer to that of breast-fed infants, with a trend towards higher

Bifidobacterium cell counts, softer stools and a higher deposition frequency compared to control.

- Neumer et al. [15] concluded that supplementation of infant formula with inulin-type prebiotic oligosaccharides containing shorter and longer chains was well tolerated and beneficially modulated the infant gut microbiota towards higher Bifidobacterium levels, accompanied by softer stool consistency.
- Veereman-Wauters, G et al. [16] demonstrated stool consistency and bacterial composition of infants taking oligofructose 0.8 g/dL or GOS:FOS-supplemented formula were closer to the breast-fed pattern.

In summary, a wide range of scientific data exist to show that fructans provide benefits related to softer stools and abundance of Bifidobacteria that are similar to outcomes of populations of breastfed babies.

Fructans Regulatory Status

Fructans have been added to formula for many years, they are approved for use and regulated around the world, examples of regulated limits can be seen in the table below.

Lower Limit (g/L)	Upper Limit (g/L)	details	Countries
-	0.8	As consumed	Brazil (IF/FUF/FSMP)
-	4	As consumed	Algeria (FSMP) Caribbean (FSMP) Israel (FSMP) Pakistan (FSMP)
-	6	As consumed	Malaysia (IF)
-	8	As consumed	Hungary (FSMP) India (IF/FSMP) Singapore (IF/FUF) Sri Lanka (FUF) US (IF/FSMP/FUF)
-	3	As consumed	Australia (IF/FUF) New Zealand (IF/FUF)

The fructans (in combination with other oligosaccharides, such as galactooligosaccharides (GOS)) can be added up to levels of 8g/L (regulatory maximum is up to 8 g/L) [4].

The European Commission Scientific Committee on Food (2003) [17] referred to the safety of a mixture of GOS (90%) and FOS (10%) up to 8g/L in infant formula and follow-on formula.

Methods of Analysis for Fructans

Existing methods for fructans referenced in the *Recommended Methods of Analysis and Sampling (*CXS 234-1999) (i.e., AOAC 997.08 and 999.03) have not been validated for application to infant formula or adult nutritionals. AOAC 997.08 is not optimal because the error in determination of free sugars in the matrix (a necessary part of the method) may be higher than the amount of fructan present and as such reliable results cannot be obtained.
AOAC 999.03 is not suitable for the determination of short chain fructans (FOS) because of the conversion of terminal fructose residues of the FOS to sugar alcohols before measurement. That

conversion results in a significant underestimation (~25%) of fructans when FOS is used in the product. To address potential regulatory disputes and because fiber is already referenced in CXS 234-1999, it is appropriate to propose a Type II method for the determination of fructans in infant formula.

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Beta-carotene and Lycopene

Beta-Carotene and Lycopene Definition and Research Supporting Use

Carotenoids are a family of antioxidant nutrients that are found naturally in food and can provide nutritional benefits. Beta-carotene and lycopene are among the most predominant carotenoids present in human milk [1]. Levels of these carotenoids in human milk are highly variable and thought to be influenced by maternal diet. A multinational study of carotenoid concentrations in human milk from well-nourished mothers reported a range of beta-carotene from 0-72 mcg/L and lycopene from 0-33 mcg/L[2].

Although beta-carotene and lycopene are present in human tissue and human breast milk, these carotenoids are not synthesized in humans and are instead obtained through the diet. Infants obtain their nutrition exclusively from human milk or infant formula preparations in the first few months of life. Human milk-fed infants are exposed to beta-carotene and lycopene through breastfeeding. In contrast, infants who consume dairy-based formulas have low or no intake of these carotenoids because they are not highly concentrated in cow's milk. In studies, plasma levels of carotenoids in formula fed infants were shown to be lower than in human milk fed infants[3].

Beta-carotene and lycopene are added to infant formulas to simulate the nutritional quality of human breast milk. Feeding infants a formula supplemented with the carotenoids beta-carotene, lutein, and lycopene was shown to increase plasma carotenoid levels within the range of human milk-fed infants[4]. The formulas with added carotenoids in the study had up to 92.7 mcg/L of beta-carotene and 80.5 mcg/L of lycopene. The study suggests carotenoids in infant formula may be less bioavailable compared to human milk, thus higher levels may need to be added to infant formula to reach a similar plasma concentration range as human milk fed infants.

Additionally, beta-carotene has provitamin A activity and may be added to infant formula as a source of vitamin A. To that effect, beta-carotene is listed as a source of provitamin A in infant formula according to CAC/GL 10-1979.

Beta-carotene and Lycopene Regulatory Status

Beta-carotene is allowable in infant and other formulas under the regulatory provisions for safe and suitable ingredients in the United States, Canada, and China. The safety of synthetic lycopene has

been reviewed by the European Food Safety Authority (EFSA) for use in food supplements, general food categories, and foods for special medical purposes. Based on the evidence provided, the panel concluded that synthetic lycopene is safe [5].

Beta-carotene

Lower Limit (g/L)	Upper Limit (g/L)	details	Countries
-	-	As a source of vitamin A	United States (IF)
-	-	As a source of vitamin A, subject to regulatory limits for vitamin A	Canada (IF/FUF/FSDU)
-	-	As a source of vitamin A	China (IF/FUF)

Lycopene

Lower Limit (g/L)	Upper Limit (µg/L)	details	Countries
-	81	As consumed	United States (IF self-GRAS)

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Conclusion

Discussion/Recommendations

The general factors to consider an ingredient as a safe and suitable "optional" ingredient in the frame of the *Infant Formula Standard* (CXS-72-1981) include:

- The substance is ordinarily found in human milk or used to provide other benefits that are similar to outcomes of populations of breast-fed babies.
- There is adequate scientific evidence of suitability for use of the substance.
- The substance is present in sufficient amounts for the intended effect, taking into account the levels in human milk.
- The substance is applied to infant formula under regulatory allowances in multiple countries.

The subject optional ingredients – fructans, beta-carotene and lycopene -- meet these factors and, thus, should considered as recognized optional ingredients for use in the *Standard for Infant Formulas and Formulas for Special Medical Purposes Intended for Infants* (CXS 72-1981)-. Recommend CCMAS type and endorse the methods of analysis previously submitted.