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**Supporting Document 1- Risk and technical assessment report**

A1178 – AOAC 2017.16 rapid integrated total dietary fibre method of analysis

# Executive summary

Food Standards Australia New Zealand (FSANZ) has assessed an application made by the Grains and Legumes Nutrition Council to permit the use of AOAC Official Method 2017.16 (AOAC 2017.16) as a new method of analysis for total dietary fibre. AOAC 2017.16 is an analytical method for the determination of total dietary fibre[[1]](#footnote-2) in foods and food ingredients. Currently, section S11—4 of the Australia New Zealand Food Standards Code (the Code) prescribes three methods for analysing total dietary fibre and four methods for analysing certain specifically named fibres. As part of the risk assessment, FSANZ considered AOAC 2009.01 (the predecessor method to AOAC 2017.16). AOAC 2009.01 is not permitted in the Code but is accepted as a method of analysis for total dietary fibre by Codex and some countries comparable to Australia and New Zealand such as Canada and the United States, and in the European Union. The Codex Committee on Methods of Analysis and Sampling (CCMAS) has supported the adoption of AOAC 2017.16 in place of AOAC 2009.01 (agenda item at CAC44 in early November 2021[[2]](#footnote-3)). Based on best available scientific evidence, FSANZ considers AOAC 2017.16 is a more suitable method than those currently permitted in the Code for analysis of foods containing a wide range of high and low molecular weight dietary fibres because it:

* is more comprehensive than older methods in the Code for measuring total dietary fibre
* has a similar level of precision to older methods in the Code for total dietary fibre (AOAC 985.29, 991.43 and 2001.03)
* has good recovery (mean recovery of 97.4% from seven samples)
* avoids the need to account for the double counting of specific dietary fibre fractions if total dietary fibre is measured by two or more methods
* has an incubation temperature that matches physiological conditions (37°C) and incubation time (4 h) that, compared with existing methods, aligns more closely to conditions for the digestion of dietary fibre in the small intestine
* has substantially increased enzyme levels (compared to AOAC 985.29, 991.43 and 2009.01) so that it more closely measures resistant starch values in line with those seen in AOAC 2002.02, and resolves the underestimation of fructo-oligosaccharide and overestimation of resistant maltodextrin as seen in AOAC 2009.01.

AOAC 2017.16 measures the components of dietary fibre that are measured by methods of analysis currently permitted by the Code for total dietary fibre (i.e. 985.29, 991.43 and 2001.03) and for specifically named dietary fibres. An exception is GOS. AOAC 2017.16 includes GOS in its measurement of total dietary fibre. FSANZ therefore considered whether GOS meets the Code’s definition of dietary fibre.

GOS has been found to meet certain criteria for the definition: fraction of the edible part of plants or their extracts, or synthetic analogues (naturally-occurring GOS in dairy foods does not meet this); resistance to digestion and absorption in the small intestine; usually partial or complete fermentation in the large intestine; the minimum degree of polymerisation (by virtue of analytical methods); and is not lignin.

The definition also requires that GOS promotes at least one of three beneficial physiological effects: laxation; reduction in blood cholesterol; and/or modulation of blood glucose.

The body of evidence about the physiological effects of GOS only includes results from clinical trials which used synthetic analogues, not the natural forms. FSANZ’s assessment found that the consumption of GOS does not promote any of the three beneficial physiological effects listed in the Code. Therefore, insofar as naturally occurring GOS is concerned, similar physiological effects are inferred on the basis of structural similarities to synthetic analogues and extend from an indirect body of evidence (i.e. synthetic analogues). Based on available evidence FSANZ concludes GOS in any form does not meet all criteria for the Code’s definition of dietary fibre.

The above means that the use of AOAC 2017.16 would result in an overestimate of total dietary fibre in GOS-containing foods based on the Code’s definition of dietary fibre.

Based on the best available data, plant-based foods contain naturally occurring GOS at levels on average of 0.85 g/100 g and up to 4 g/100 g for a small number of foods, and dairy products up to 0.6 g/100 g. GOS was present in about 25% of the surveyed plant foods. Likely due to its cost, GOS is not added to many foods in Australia and New Zealand beyond infant formula products, infant food and formulated supplementary foods for young children. Halmos et al. (2015) estimated a daily GOS consumption of 1.1 g/day. Based on the Code’s definition of dietary fibre, a small number of GOS-containing foods measured with AOAC 2017.16 will have a slight overestimation of dietary fibre values.

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## Abbreviations

|  |  |
| --- | --- |
| Dietary fibre insoluble in water | IDF |
| Dietary fibre soluble in water and precipitated by 78% ethanol | SDFP |
| Dietary fibre soluble in water and soluble in 78% ethanol (also referred to as Low Molecular Weight Dietary Fibre) | SDFS (LMWDF) |
| Fructo-oligosaccharides | FOS |
| Galacto-oligosaccharides | GOS |
| High molecular weight dietary fibre | HMWDF |
| Polydextrose | PD |
| Relative repeatability Standard Deviation (RSDr) (within a laboratory)  | RSDr  |
| Relative reproducibility Standard Deviation (RSDR) (between laboratories) | RSDR  |
| Resistant maltodextrins | RMD |
| Resistant Starch | RS |

# 1 Introduction

AOAC Official Method 2017.16 (Rapid Integrated Total Dietary Fibre Method) is a new analytical method for the determination of total dietary fibre in foods and food ingredients. This method is assessed in this Supporting Document 1 (SD1) for its suitability as a regulatory method of analysis. The assessment considered the range of particular substances measured by AOAC 2017.16 and included assessment against the Code’s definition of dietary fibre of those not previously assessed.

# 2 AOAC 2017.16 as a regulatory method of analysis

## 2.1 Background

Codex defines dietary fibre in the General Guidelines on Nutrition Labelling (Codex 2017) as:

|  |
| --- |
| ***Dietary fibre*** means carbohydrate polymers2 with ten or more monomeric units3, which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: * Edible carbohydrate polymers naturally occurring in the food as consumed
* Carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities
* Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities.

2 When derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls. These compounds also may be measured by certain analytical method(s) for dietary fibre. However, such compounds are not included in the definition of dietary fibre if extracted and re-introduced into a food. 3 Decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities. |

This definition allows Codex members to determine whether non-digestible carbohydrates with a degree of polymerisation (DP) <10 should be considered as dietary fibre. As outlined in the Australia New Zealand Food Standards Code (the Code), dietary fibre is defined as having a DP >2. AOAC methods 2009.01 and AOAC 2011.25 are the precursor methods to AOAC 2017.16 and were developed to provide a method of analysis that more comprehensively reflects the Codex definition of dietary fibre. Neither are in the Code but are accepted as methods of analysis for total dietary fibre by other comparable countries including in Canada, United States and Europe. In May 2021, CCNFSDU agreed to adopt AOAC 2017.16 in place of AOAC 2009.01 as a Type 1 method for use of all foods (applicable for determining the content of dietary fibres of higher and lower molecular weight in food that may, or may not, contain resistant starches), for adoption at CAC44 (November 2021) (CCMAS 2021). The development of AOAC 2017.16 resolves some limitations of its precursor methods (see section 2.2.2 below for more information).

AOAC granted AOAC 2017.16 first action status in 2017, and final action status in 2020 (AOAC international, 2020). The method is currently validated as first action by the International Association for Cereal Science and Technology (ICC) under ICC Standard No. 185.

AOAC 2017.16 measures, by one method, all dietary fibre fractions currently measured by other less comprehensive methods for total dietary fibre, as well as methods for specific dietary fibres, such as resistant starch, permitted by the Code. The method also measures galacto-oligosaccharides (GOS). GOS are non-digestible oligosaccharides found naturally in some plant and dairy foods, and can be synthetically produced from lactose.

The key advantage with analysing foods containing multiple dietary fibres is that AOAC 2017.16 avoids the need to address double counting of specific dietary fibre fractions. To address double counting, the amount of a specific dietary fibre measured by the older total dietary fibre method should be separately quantified (i.e. by using a specific method of analysis for that dietary fibre) and subtracted from the sum of the two methods. AOAC 2017.16 avoids the time, cost and resources of quantifying that double counted fraction.

Figure 1 below shows the range of dietary fibres measured by AOAC 2017.16 compared to older methods AOAC 985.29 and AOAC 991.43.



Figure 1 Dietary fibres measured by AOAC 2017.16[[3]](#footnote-4)

The table below sets out in more detail the scope of the various methods of analysis listed in the Code.

**Table 1** Dietary fibre components measured by AOAC methods of analysis in the Code (Fuller 2019)

| AOAC Method | NPS | RS | Inulin | FOS and GOS | RMD | PD |
| --- | --- | --- | --- | --- | --- | --- |
| 985.29 | Y | SOME | SOME | N | N | N |
| 991.43 | Y | SOME | SOME | N | N | N |
| 997.08 | N | N | Y | N | N | N |
| 999.03 | N | N | Y | FOS | N | N |
| 2001.03 | Y | SOME | Y | Y | Y | Y (product dependent) |
| 2000.11 | N | N | N | N | N | Y |
| 2002.02 | N | Y | N | N | N | N |
| 2017.16 | Y | Y | Y | Y | Y | Y |

## 2.2 Method of analysis AOAC 2017.16

### 2.2.1 Analytical process

AOAC 2017.16 quantifies total dietary fibre by using enzymatic-gravimetric techniques and high performance liquid chromatography. AOAC International (2020) provide a summary of the analytical method at AOAC final action stages. The first phase involves enzymatic digestion with pancreatic α-amylase plus amyloglucosidase at 37°C for 4 hours to align with human digestion in the small intestine. The second phase is the analysis of the enzymatic digest. Total dietary fibre is calculated as the sum of two parts:

1. High molecular weight dietary fibre (HMWDF) is measured gravimetrically and includes as a single value for dietary fibre insoluble in water (i.e. insoluble dietary fibre (IDF)), and dietary fibre soluble in water and precipitated by 78% ethanol (i.e. soluble dietary fibre precipitate in ethanol (SDFP)). The HMWDF fraction includes cellulose, resistant starch (RS); cereal β-glucan, galactomannan; arabinoxylan; pectin; arabinogalactan.
2. Low molecular weight dietary fibre (LMWDF) is measured by high performance liquid chromatography (HPLC) and measures dietary fibre soluble in water and also soluble in 78% ethanol (i.e. soluble dietary fibre soluble in ethanol (SDFS)). This fraction includes non-digestible oligosaccharides such as FOS, inulin, polydextrose, GOS, and resistant maltodextrins.

A simplified schematic of the analytical process is shown below in Figure 2.



Figure 2. Simplified analytical steps for AOAC 2017.16[[4]](#footnote-5).

The total dietary fibre result is the sum of HMWDF and SDFS. AOAC 2017.16 cannot further determine individual values for LMWDF fractions (B McCleary, pers. com.)[[5]](#footnote-6). FSANZ understands Megazyme is currently developing a modification to AOAC 2017.16 that further separates HMWDF into two fractions (IDF and SDFP). This is the same as AOAC 991.43 is to AOAC 985.29 and AOAC 2011.25 is to AOAC 2009.01.

### 2.2.2 Methodological processes

AOAC 2017.16 has an incubation time and incubation temperature aligning it with *in vivo* physiological conditions for the digestion of dietary fibre in the small intestine. Compared to AOAC 985.29, 991.43 and 2009.01, AOAC 2017.16 contains substantially increased enzyme levels which remove:

* the underestimation for resistant starch RS2, RS3 and RS4 to bring all RS values closer to those obtained by AOAC 2002.02 (specific method for resistant starch)
* the underestimation of fructo-oligosaccharide (FOS) and overestimation of resistant maltodextrin seen in AOAC 2009.01

Additionally, AOAC 2017.16 introduces the use of some safer reagents which mitigates the risks of using hazardous chemicals seen in previous methods.

#### 2.2.2.1 Recovery

Recovery data indicate the ability of a method to measure the entire amount present in a matrix. The percentage of recovery is a measure of analytical method accuracy. The recovery data for LMWDF presented in Table 2 (McCleary and Cox 2017) show the proportion recovered by AOAC 2017.16 from seven commercially produced dietary fibre mixtures. Based on data specific to oligosaccharides and allowing for normal batch variance, AOAC 2017.16 has a mean recovery of 97.4% (93–100%), which is a good result.

**Table 2** Recovery of LMWDF DP >2 in original samples and AOAC 2017.16 incubation samples (g/100 g)

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Original sample | AOAC 2017.16  | Recovery (%) |
| Neosugars (FOS) | 93.0 | 92.8 | 100 |
| Raffinose P95 (FOS + inulin)  | 89.0 | 88.2 | 99 |
| Polydextrose | 84.3 | 82.5 | 98 |
| Fibersol 2 (RMD) | 88.5 | 82.4 | 93 |
| GOS powder | 76.0 | 72.0 | 95 |
| Xylo-oligosaccharides | 78.0 | 76.2 | 98 |
| Raffinose | 99.0 | 98.0 | 99 |

The limit of detection (LOD) and the limit of quantification (LOQ) are key parameters that determine how a food analytical method performs at low concentrations (JRC Technical Reports 2016). These parameters were not presented for assessment at first action, however based on communication with an Australian laboratory (H. Salman, pers.com)[[6]](#footnote-7), AOAC 2017.16 should have similar LOD and LOQ values for the same sample conditions (e.g. dry matter content) as the existing total dietary fibre methods in the Code.

#### ***2.2.2.2 Precision***

Precision is a quantitative measurement for `scatter’ of replicated analyses of a food sample (within and between laboratories; under the same analytical conditions) (Greenfield 2002). AOAC International (2002) provides guidelines about the precision parameters from inter-laboratory collaborative analytical studies for calculation:

* relative repeatability SD (RSDr) (within a laboratory), or
* relative reproducibility SD (RSDR) (between laboratories).

Precision was specifically validated and quantified for AOAC’s assessment of first action status (AOAC International, 2019). The parameters for AOAC 2017.16 are given in Table 3. Generally, the lower the parameter value, the more precise the method. In general, AOAC 2017.16 has a similar level of precision compared to AOAC 985.29, 991.43, and 2001.03. However, AOAC 985.29 is less precise compared to AOAC 2017.16 for certain foods e.g. soy isolate with a high protein content and physical attributes of starch in rice combined with a low total dietary fibre value (i.e. ≤~1%).

**Table 3** Precision parameters for AOAC 2017.16 and older total dietary fibre methods (McCleary et al. 2019; McCleary & Cox 2017)

|  |  |  |
| --- | --- | --- |
| AOAC method | Relative repeatability SD (RSDr) | Relative reproducibility SD (RSDR) |
| 985.29 | 0.56–66.25 | 1.58–66.25 |
| 991.43 | 1.50–-6.62 | 1.58–12.17 |
| 2001.03 | 1.33–6.10 | 1.79–9.39 |
| 2017.16 | 1.22–6.52 | 2.14–10.62 |

### 2.2.3 Comparative scope of AOAC 2017.16 and AOAC 985.29

As shown in Figure 1 and Table 1, older total dietary fibre methods such as AOAC 985.29 do not analyse all or part of some dietary fibre components compared to more recent total dietary fibre methods.

For labelling purposes and for determining total dietary fibre for the purpose of Nutrient Profile Scoring Calculator (NPSC), manufacturers have the option of calculating the dietary fibre content of their food from the dietary fibre values for their ingredients in the [Australian Food Composition database](https://www.foodstandards.gov.au/science/monitoringnutrients/afcd/Pages/foods-in-release-1.aspx) (FSANZ 2019). These dietary fibre values are mostly analysed by AOAC 985.29 and, depending on the content and type of LMWDF, they may underestimate the total dietary fibre content according to the Code’s definition of dietary fibre. AOAC 2017.16 enables manufacturers of relevant foods or products to declare the dietary fibre content more in line with the Code’s definition of dietary fibre, without the need to use multiple methods.

Table 4 provides total dietary fibre values for nine Irish cereal and vegetable foods, analysed by AOAC 2017.16 and AOAC 985.29 at different times and from different samples of food. The differences are also due to the analysis of all resistant starch, and LMWDF by AOAC 2017.16 compared to only partial or no analysis of specific fibres by the older total dietary fibre methods.

The authors’ food descriptions are as reported and the original dietary fibre values are converted from dry matter to an approximate wet weight value using the water content given in the latest Australian Food Composition Database. From Table 4, AOAC 2017.16 measures about 2 g/100 g or about 22% more dietary fibre than AOAC 985.29 in this group of cereals and vegetables due mostly to measurement of the LMWDF.

**Table 4** Comparison of total dietary fibre measured by AOAC 2017.16 and AOAC 985.29 (g/100g edible portion) (McCleary et al. 2013; McCleary & Cox 2017)

|  |  |  |  |
| --- | --- | --- | --- |
| Food samples  | AOAC 985.29^ | AOAC 2017.16#  | Difference representing increase in TDF value between 2 methods |
| Wholemeal bread  | 6.0 | 8.2 | 2.2 |
| Oat bran | 16.8 | 19.6 | 2.8 |
| WeetaBix | 8.7 | 10.9 | 2.2 |
| Kellogg All Bran  | 24.6 | 30.6 | 6.0 |
| Whole wheat pasta  | 8.4 | 11.1 | 2.7 |
| Sweet corn (tinned) | 3.1 | 2.9 | -0.2 |
| Garden peas (tinned)  | 4.3 | 5.4 | 1.1 |
| Broccoli  | 2.8 | 3.1 | 0.3 |
| Carrot | 2.5 | 2.7 | 0.2 |
| Mean  | 8.6 | 10.5 | 1.9 |

## 2.3 Conclusion

Based on FSANZ’s quantitative and qualitative assessment, AOAC 2017.16:

* is more comprehensive than older methods in the Code for measuring total dietary fibre
* has a similar level of precision to older methods in the Code for total dietary fibre (AOAC 985.29, 991.43 and 2001.03)
* has good recovery (mean recovery of 97.4% from seven samples)
* avoids the need to account for the double counting of specific dietary fibre fractions if total dietary fibre is measured by two or more methods
* has an incubation temperature that matches physiological conditions (37°C) and incubation time (4 h) that, compared with existing methods, aligns more closely to conditions for the digestion of dietary fibre in the small intestine
* has substantially increased enzyme levels (compared to AOAC 985.29, 991.43 and 2009.01) so that more closely measures resistant starch values in line with those seen in AOAC 2002.02, resolves the underestimation of fructo-oligosaccharide and overestimation of resistant maltodextrin and seen in AOAC 2009.01.

The method is therefore a suitable regulatory alternative for manufacturers seeking to analyse more complex foods and food ingredients containing specific dietary fibres, particularly those including RS and LMWDF.

# 3 Assessment of galacto-oligosaccharides against the Code’s definition of dietary fibre

As shown in Figure 1 and Table 1, AOAC 2017.16 measures GOS as part of total dietary fibre according to Codex and some overseas regulatory definitions of dietary fibre but it is not measured by the older total dietary fibre methods AOAC 985.29 and 991.43. FSANZ has not considered GOS in its previous assessments for dietary fibre methods of analysis, and therefore it has not been assessed against the Code’s definition of dietary fibre.

FSANZ has since become aware that an existing method in the Code AOAC 2001.03 for analysis of total dietary fibre and resistant maltodextrin (Gordon and Okuma, 2002) also measures naturally occurring and synthetic analogues of GOS (B McCleary, pers. com.)4.

Standard 1.1.2 – *Definitions used throughout the Code* defines dietary fibre as:

|  |
| --- |
| ***Dietary fibre*** means that fraction of the edible part of plants or their extracts, or synthetic analogues that:(a) are resistant to digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine; and(b) promote one or more of the following beneficial physiological effects:(i) laxation;(ii) reduction in blood cholesterol;(iii) modulation of blood glucose;and includes:(c) polysaccharides or oligosaccharides that have a degree of polymerisation greater than 2; and(d) lignins. |

The following sections assess GOS against the five elements of the Code’s definition of dietary fibre.

## 3.1 Fraction of the edible part of plants or their extracts, or synthetic analogues

GOS is naturally present in small amounts in certain plant foods such as legumes, cereals and vegetables as alpha (α)-GOS (Choct, 2010), and in even smaller amounts in dairy products as beta (β)-GOS (Otieno, 2010; Austin, 2014). Naturally occurring forms of GOS that have a DP >2 and contain at least one galactose moiety include:

* Raffinose (DP 3): glucose-galactose-fructose.
* Stachyose (DP 4): glucose-2(galactose)-fructose.
* Verbacose (DP 5): glucose-3(galactose)-fructose.
* Ajugose (DP6): glucose-4(galactose)-fructose.

Synthetic analogues of GOS, (also known as trans-GOS or β-GOS) are generally produced by enzymatic treatment of lactose and has an average DP <8. Although disaccharides are included in the Code’s definition of (synthetic) GOS in Standard 1.1.2—2, they are not measured by AOAC 2017.16.

Naturally occurring GOS from dairy does not meet this element of the Code’s definition of dietary fibre.

## 3.2 Resistant to digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine

GOS is recognised as part of the Fermentable Oligo- Di- and Mono-Saccharides and Polyols group (FODMAPs) (Gibson 2017) and as a ‘prebiotic’ (Gibson et al. 2004). FODMAPs and prebiotics are well recognised as non-digestible carbohydrates and absorption in the small intestine is incomplete and they are therefore partially fermented by colonic bacteria in the large intestine. The by-products of their fermentation can include hydrogen, methane, carbon dioxide, short chain fatty acids (mainly acetate, propionate and butyrate) and lactate (Slavin 2013). Certain short chain fatty acids and lactate are generally understood to provide energy to the host’s epithelial cells of the large intestine. It appears that the degree of GOS fermentation is dose dependent and relative to the diversity of colonic bacteria within the host prior to GOS consumption (Poeker et al. 2018). As GOS can be fermented in the large intestine, it fulfils this part of the Code’s definition of dietary fibre.

## 3.3 Promote one or more beneficial physiological effects

As advocated in Australia and New Zealand’s dietary guidelines, consuming foods high in dietary fibre has an important role in contributing to lowering the risk of many non-communicable diseases. Many consumers perceive fibre as being ‘good for health’, and it is common for processed foods to be manufactured with added dietary fibre, whether extracted or synthetically produced. The evidence supporting fibre’s role in preventing non-communicable disease is based primarily on intake of fibre from whole grains, fruits, and vegetables.

The benefits of dietary fibres are often assessed according to their effects on physiological or biochemical outcomes associated with non-communicable disease risk; in this case the three outcomes listed in the Code: laxation; reduction in blood cholesterol; and, modulation of blood glucose. FSANZ’s assessment described in Supporting Document 2 (SD2) considers whether GOS meets the three beneficial physiological effects and the Code’s definition of dietary fibre.

FSANZ’s assessment evaluates parallel or crossover controlled trials in humans, where the intervention group consumed additional GOS in isolation to other non-digestible carbohydrates, and that reported an outcome related to the three beneficial physiological effects listed in the Code. The eligible studies were mainly randomised trials with a placebo control. Naturally occurring GOS were not used in any trial but the outcome of physiological effects assessment on laxation, blood cholesterol or blood glucose are inferred on the basis of structural similarities to synthetic analogues and extend from an indirect body of evidence (i.e. synthetic analogues). We conducted a meta-analysis if the outcome was assessed by at least two studies. A narrative review was completed for other outcomes.

The results of our meta-analyses indicate that GOS intake does not alter total or LDL cholesterol, fasting glucose, or stool weight (summary effect sizes are provided in Table 5). The results from the trials also show that GOS do not affect other outcomes related to blood lipids, glycaemic control, or laxation, including: HDL-cholesterol; fasting triglycerides; glycosylated haemoglobin; postprandial glycaemic response; fasting insulin; HOMA-IR; five additional insulin-related outcomes; relative stool weight; bowel movement or stool frequency; and, intestinal transit time (summary effects of some outcomes are provided in Table 5, Appendix Section 2.1, SD2).

**Table 5** Galacto-oligosaccharide intake has no overall mean effect on outcomes related to laxation, blood cholesterol, and blood glucose.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Outcome | Units | Mean difference (95% CI)1 | *P*2 | Number of included studies and pair-wise comparisons |
| Stool weight | gram | 6.34 (-6.21, 18.9) | 0.32 | 3, 6 |
| Total cholesterol | mmol/L | -0.13 (-0.34, 0.08) | 0.21 | 4, 4 |
| LDL-cholesterol | mmol/L | -0.11 (-0.29, 0.08) | 0.25 | 3, 3 |
| Fasting glucose3 | mmol/L | -0.09 (-0.19, 0.00) | 0.06 | 4, 4 |

CI, confidence interval; L, litre; LDL, low density lipoprotein. 1Mean difference is calculated as intervention minus placebo. 2*P-value* pertains to overall mean difference (i.e. effect size). 3Fasting status confirmed by three of four studies and assumed for one study (Vulevic et al. 2013).

Most of this assessment’s evidence is derived from seven controlled studies, involving a total of 223 participants. These studies collectively assessed a wide range of intakes of added GOS, up to very high intakes of 15 g per day. All seven trials had links with industry, whether through declared interests or funding; however, the small number of trials and lack of studies without industry associations precluded any subgroup analysis to examine potential bias in the findings. The chemical composition of GOS mixtures, which can include large proportions of non-GOS components, is highly variable and this is likely to contribute to variability in physiological effects reported in human trials.

Overall, the mean effect estimates show that GOS do not exert a beneficial effect on stool weight, total or LDL-cholesterol, or fasting blood glucose. All mean effect sizes for the relevant physiological outcomes were small (e.g. a decrease of ~0.1 mmol/L for the blood markers) and 95% confidence intervals spanned the null (i.e. zero effect). Although the mean effect sizes are small, the lower limits of the confidence intervals include a possible desirable outcome, meaning a small beneficial effect cannot be excluded entirely. However, the likelihood of this is low because the results of future research would need to differ considerably from the current body of evidence to shift the mean effect size enough to show a clinically meaningful benefit. In general, as evidence accrues, summary estimates of effect tend to be attenuated rather than increased (Strazzullo et al. 2009). A summary of the strengths and limitations of our assessment is provided in Table 6 (see Appendix Section 2.1, SD2).

We conclude, based on the best available scientific evidence, that GOS intake does not exert clinically meaningful or beneficial effects on laxation, blood cholesterol, or blood glucose. Thus, these carbohydrates do not promote any of the three beneficial physiological effects listed in the Code’s definition of dietary fibre. This is consistent with international assessments of GOS by the United States Food and Drug Administration (FDA 2016) and the United Kingdom Food Standards Agency (SACN 2008).

## 3.4 Polysaccharides or oligosaccharides with a degree of polymerisation greater than 2

Naturally occurring and synthetic analogues of GOS, with a DP >2, are measured by AOAC 2017.16 and therefore they meet this definitional element.

## 3.5 Conclusion

Naturally occurring GOS in plant foods meets elements of the Code’s definition of dietary fibre in relation to origin, digestive aspects, and DP >2. Naturally occurring GOS in dairy foods and synthetic analogues of GOS meet the elements of the Code’s definition of dietary fibre in relation to digestive aspects and DP >2, although naturally occurring GOS from dairy is not of plant origin so cannot meet the Code’s definition of dietary fibre.

Synthetic analogues of GOS were found to not promote one or more of the three beneficial physiological effects in the Code’s dietary fibre definition. Trials assessed only include results from clinical trials which used synthetic analogues, not the natural forms. Therefore, insofar as naturally occurring GOS is concerned, the physiological effects are inferred on the basis of structural similarities to synthetic analogues and extend from an indirect body of evidence (i.e. synthetic analogues). FSANZ concludes that GOS in any form does not meet all criterion for the Code’s definition of dietary fibre.

# 4 Galacto-oligosaccharides in food

Given GOS does not meet all criterion for the Code’s definition of dietary fibre, this assessment includes a review of data estimating the proportion of GOS in total dietary fibre measured by AOAC 2017.16 to indicate the relative extent of a potential overestimation of dietary fibre content (as defined by the Code) when a food containing GOS is measured by AOAC 2017.16.

## 4.1 Naturally occurring galacto-oligosaccharides in food

The GOS content of food is rarely presented in national Food Composition Databases. However, as a FODMAP, naturally occurring GOS has been measured in Australian plant foods by high performance liquid chromatography in recent years (Biesiekierski et al. 2011; Muir et al. 2009). Tables 6 and 7 present these data supplemented by data from the Indian food composition database (Longvah et al. 2017). All food descriptions and data are reported as published. Many analysed foods in the Australian references did not detect GOS.

**Table 6** Naturally occurring content of galacto-oligosaccharides in 32 plant foods

| Food source (by food group) | GOS (g/100 g, as consumed) |
| --- | --- |
| Pistachio nuts | 4.34\* |
| Cashew nuts | 2.62\* |
| Almond | 0.12\* |
| Safflower seeds | 3.53\* |
| Linseeds | 3.52\* |
| Cumin seeds | 0.37\* |
| Coriander seeds | 0.37\* |
| Sunflower seeds | 0.09\* |
| Split peas, soaked, boiled | 1.88^ |
| Red kidney beans, boiled | 1.44^ |
| Chickpeas, soaked, boiled/canned | 1.25#/0.19^ |
| Soya bean, soaked, boiled | 0.79^ |
| Lentils, soaked, boiled/canned | 0.46^/0.22^ |
| Butter beans, canned | 0.42^ |
| Bread, wheat, wholemeal | 0.50^ |
| Bread, wheat, wholegrain  | 0.59^ |
| Bread, rye | 0.24^ |
| Bread, wheat, multigrain | 0.38^ |
| Bread, wheat, white | 0.20^ |
| Brown rice, | ND^/0.08\* |
| Wheat flour, refined | 0.35\* |
| Kellogg All-bran | 1.32^ |
| Wholegrain wheat biscuit, Weet-bix | 0.31^ |
| Muesli | 0.34^ |
| Oats, dry | 0.34^ |
| Apricot, dried | 0.24\* |
| Garlic clove, small | ND\*/0.39\* |
| Onion, white, raw | 0.19# |
| Beetroot, raw | 0.14# |
| Broccoli, raw | 0.13# |
| Lettuce, radicchio, raw | 0.11# |
| Fennel bulb, raw | 0.10# |

^Biesiekierski et al. (2011); #Muir et al. (2009); \*Longvah et al. (2017)

**Table 7** Naturally occurring content of galacto-oligosaccharides in 6 dairy foods

|  |  |
| --- | --- |
| Food source  | GOS (g/100 g food as consumed) |
| Commercial plain yoghurt (containing L. casei) | 0.20-0.44\* |
| Commercial plain yoghurt (containing Bifidobacteria) | 0.36-0.58\* |
| Commercial UHT milk | 0.09-0.44^ |
| Goats milk | 0.01# |
| Lactose free UHT milk | 0.095-0.435^ |
| Lactose free UHT dairy drink | 0.06-0.226^ |

^Ruiz‐Matute, A. et. al (2012); #Oliveira, D et al. (2015); \*Martínez‐Villaluenga et al. (2008)

From Table 6, some nuts, seeds and legumes generally contain higher amounts of GOS than cereals and vegetables per 100 g edible portion. Similarly, from Table 7, yoghurts contain higher amounts of naturally occurring GOS than liquid milk. Measurement of GOS could increase naturally occurring total dietary fibre values in plant foods on average by 0.85 g/100 g and up to 4 g/100 g and in dairy products by up to 0.6 g/100 g.

## 4.2 Proportion of naturally occurring GOS in total dietary fibre measured by AOAC 2017.16

Very limited data are available for naturally occurring GOS, and total dietary fibre measured by AOAC 2017.16, in different samples of the same foods. These data are shown in Table 8. FSANZ converted the AOAC 2017.16 dietary fibre values in Table 8 from dry matter to an approximate wet weight value using the water content given in the latest Australian Food Composition Database. GOS content has then been expressed as a proportion of AOAC 2017.16 total dietary fibre.

In their comprehensive analyses of Australian plant foods, Biesiekierski et al. (2011) and Muir et al. (2009) analysed a total of 140 commonly eaten grains and pasta, bread, breakfast cereals, biscuits, pulses, vegetables and fruit of which 35 foods, mostly pulses and wheat products, contained quantifiable GOS (raffinose and stachyose) (25% foods). Few vegetables and no fruit contained GOS. Dairy products or individual types of nuts and seeds were not analysed by these authors. The analysed foods contain more fructans than GOS in all categories except pulses which indicate the reverse (data not shown).

From the seven foods in Table 8, GOS in cereals, pulses and vegetables contribute about 3–6% of total dietary fibre analysed by AOAC 2017.16.

**Table 8** Naturally occurring galacto-oligosaccharide in plant foods as a proxy for overestimation of total dietary fibre by AOAC 2017.16 in relevant foods

| Food Category  | Total dietary fibre (AOAC 2017.16) g/100 g as consumed | Naturally occurring GOS g/100 g as consumed | GOS as proportion of total dietary fibre (%) |
| --- | --- | --- | --- |
| Weetabix\*/Weet-bix^ | 10.9\* | 0.31^ | 2.8 |
| Broccoli  | 2.8\* | 0.13# | 4.6 |
| Kellogg All Bran  | 30.6\* | 1.32^ | 4.3 |
| White bread | 3.7\* | 0.20^ | 5.4 |
| Wholemeal bread  | 8.2\* | 0.50^ | 6.1 |
| Chickpeas, tinned\*/canned^ | 6.5\* | 0.19^ | 2.9  |
| Butter beans, tinned\*/canned^ | 6.9\* | 0.42^ | 6.1 |

\*McCleary et al. (2015); ^Biesiekierski et al. (2011)

## 4.3 Australian intake of naturally occurring galacto-oligosaccharides

Data on GOS intake is not reported in national nutrition surveys. Halmos et al. (2015) estimated adult naturally occurring GOS consumption at 1.1 g/day based on a ‘typical Australian diet’ developed by the author from a food frequency questionnaire. No GOS intakes appear to be reported for New Zealand.

## 4.4 Synthetic analogues of Galacto-oligosaccharides in food in Australia and New Zealand

GOS can be synthesised in different ways, however commercial GOS is usually produced by enzymatic catalysis from lactose using glycosyltransferases (EC 2.4) or glycoside hydrolases (EC 3.2.1) (Torres et al. 2010; De Roode et al. 2003). Commercial GOS has variable amounts of monosaccharides (glucose, galactose) and lactose and GOS DP >2. This variability affects physicochemical properties such as sweetness, solubility, osmolality, crystal formation ability, and reactivity (maillard reactions) (Torres et al. 2010; Playne and Crittenden 2009). FSANZ is not aware of extracted or isolated forms of naturally occurring GOS used in foods.

Synthetic analogues of GOS are considered a stable, soluble ingredient that can be used for low-energy sweetness, as a bulking agent, for increasing shelf life by reducing microbial contamination and as a prebiotic. It is suitable for addition to a wide variety of food products such as baked goods, sweeteners, yoghurts, nutrition bars, meal replacement shakes, fruit drinks and water quenches (Affertsholt‐Allen 2007, Torres et al. 2010, Research and markets 2019, Stephen 2017).

Based on publically available market share data (Research and Markets 2019), Europe holds the largest market share followed by the Asia Pacific regions and the USA. GOS can be used as an ingredient in food in Australia and New Zealand, and is permitted in controlled amounts in infant formula products, infant foods and formulated supplementary foods for young children.

FSANZ requested the applicant provide an estimate of the current use of synthetic analogues of GOS in foods in Australia New Zealand. The applicant surveyed members of the respective Australian and New Zealand Food and Grocery Councils by mailing list. In the Australian part of the survey, 11 of 74 respondents indicated GOS is added to their products:

* infant formula (n=2): 3–9 g/100 g
* milk products (n=2): 1 g/100 mL[[7]](#footnote-8)
* ice cream products (n=1): 5.5 g/100 g
* dairy alternative and cereal products (n=1): no value provided
* cereal products (n=1): 10 g/100 g.

In the New Zealand part of the survey, 5 of 15 respondents indicated GOS is added to their products - all of these were infant formula products.

The survey data suggests synthetic analogues of GOS are added to only a small number of general foods in the Australian and New Zealand food supply. Since these surveys were sent by mailing list, the results are indicative only and cannot be considered to represent all manufacturers in Australia and New Zealand.

FSANZ sought to further clarify the findings of the applicants survey through targeted consultation. Confidential data on GOS content in food products across Australia and New Zealand provided by members and representatives of industry and the applicant confirmed the findings from the applicants survey.

Additionally, cost data provided by the applicant indicated GOS is significantly more expensive than other dietary fibre options. For example, from $19 per kg for GOS compared to FOS which is the next most expensive fibre ranging from $7 to $12 per kg.

## 4.5 Conclusion

Based on the available data of 140 analysed Australian plant-based foods, naturally occurring GOS was found in pulses, wheat products and some other cereals, nuts, seeds and few vegetables. GOS Levels were on average 0.85 g/100 g and up to 4 g/100 g for a small number of foods (7 out of 32 foods analysed had a GOS value greater than 1 g/100 g), and dairy products up to 0.6 g/100 g. GOS was present in about 25% of the surveyed plant foods. Likely due to its cost, GOS is not added to many foods in Australia and New Zealand beyond infant formula products, infant food and formulated supplementary foods for young children. Halmos et al. (2015) estimated a daily GOS consumption of 1.1 g/day. Based on the Code’s definition of dietary fibre, GOS-containing foods measured with AOAC 2017.16 will have a slight overestimation of dietary fibre values.

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1. All references in this report to ‘dietary fibre’, which are made in relation to requirements in the Code, are references to ‘dietary fibre’ as defined by the Code (unless specified otherwise). ‘Total dietary fibre’ refers to the value measured by one or more specified method of analysis, values may be higher or lower depending on method used. [↑](#footnote-ref-2)
2. <http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-715-41%252FFinal%252520Report%252FREP21_MASe.pdf> [↑](#footnote-ref-3)
3. Available from: <https://www.megazyme.com/focus-areas/dietary-fiber-portal/megazyme-and-dietary-fiber> [↑](#footnote-ref-4)
4. <https://prod-media.megazyme.com/media/pdf/86/b3/f2/df-brochure-full-brochure.pdf> [↑](#footnote-ref-5)
5. B McCleary, Strategic Advisor, Megazyme Ltd, personal communication, 8 December, 2020 [↑](#footnote-ref-6)
6. H. Salman, Business Manager – Analytical Services, Australian Export Grains Innovation Centre (AEGIC), personal communication, 19 June 2020 [↑](#footnote-ref-7)
7. FSANZ identified one milk product containing added GOS at 1.0 g/100 mL, however GOS was declared on the label as a prebiotic and declared as GOS in the NIP, and not as dietary fibre. [↑](#footnote-ref-8)