

20 October 2017
[29–17]

Supporting Document 1

Assessment of resistant starch as dietary fibre; and suitability of AOAC 2002.02 as a regulatory method of analysis – Application A1142

Addition of a prescribed method of analysis for resistant starch

Executive summary

This Application seeks to amend the *Australia New Zealand Food Standards Code* (the Code) to include an analytical method for the determination of resistant starch as a specifically named fibre in food. It has been requested that AOAC Official Method 2002.02 (Resistant Starch in Starch and Plant Materials) is added to Schedule 11 – Calculation of values for nutrition information panel, clause S11–4 Methods of analysis for dietary fibre and other fibre content. S11–4 currently does not include a prescribed method of analysis for resistant starch for the purpose of nutrition labelling.

This assessment has concluded that resistant starch fulfils the definition of dietary fibre as defined in Standard 1.1.2 – Definitions used throughout the Code. Resistant starch satisfies the definition of dietary fibre as follows:

- Resistant starch is present in the edible parts of plant materials and can be extracted from plant materials.
- Resistant starch is resistant to digestion in the small intestine and is fermented in the large intestine.
- Replacement of digestible starch with resistant starch in a meal promotes modulation of blood glucose by reducing peak postprandial blood glucose concentration.
- Resistant starch promotes laxation.

AOAC Official Method 2002.02 (Resistant Starch in Starch and Plant Materials) is widely used internationally and is the only method of analysis for resistant starch in the Codex list of recommended methods. The method is applicable to samples containing between 1–75% resistant starch and method performance parameters including limit of quantification, repeatability, and reproducibility have been determined as being acceptable for food regulatory purposes.

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1 Introduction

This Application seeks to amend the *Australia New Zealand Food Standards Code* (the Code) to include an analytical method for the determination of resistant starch as a specifically named fibre in food. It has been requested that AOAC Official Method 2002.02 (Resistant Starch in Starch and Plant Materials) is added to Schedule 11 – Calculation of values for nutrition information panel in S11—4 Methods of analysis for dietary fibre and other fibre content. S11—4 currently does not include a prescribed method of analysis for resistant starch.

2 Assessment of resistant starch as dietary fibre as defined in the Code

In order to assess the Application it is necessary to determine if resistant starch fulfils the definition of dietary fibre in Standard 1.1.2 – Definitions used throughout the Code:

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.

2.1 Assessment against Criterion 1 of the definition of dietary fibre: a fraction of the edible part of plants or their extracts, or synthetic analogues

Starch is one of the main forms of carbohydrate in the diet. It consists of two major polymers of glucose: amylose, which exhibits a mainly linear structure, and amylopectin which is branched. Starch occurs in plants as granules which can vary markedly in size, shape and physicochemical properties (Stark and Lynn 1992). Resistant starch is the fraction of starch that is not digested when it passes through the small intestine (Raigond et al. 2015). It is at least partially fermented in the large intestine.

Five resistant starch (RS) sub-types have been defined (Englyst et al. 1992; Gelders et al. 2005). Each of these RS sub-types satisfies criterion 1 of the definition of dietary fibre. RS1 is physically inaccessible to digestion and is found in whole or partially milled grains. RS2 is granular native starch that is protected from digestion due to the conformational structure of the granule. RS3 refers to non-granular starch that is formed during retrogradation in food processing. Retrogradation occurs when starch granules are disrupted by cooking above their gelatinization temperature. Upon cooling, the starch granules re-associate into crystalline structures that resist hydrolysis by amylase. RS4 is chemically modified starch (i.e. semi-synthetic) that resists digestion (Brown 2004). Amylose can also form helical complexes with lipids in native and processed starches, thereby enhancing resistance to digestion. These complexes are referred to as RS5 (Gelders et al. 2005).

2.2 Assessment against Criterion 2 of the definition of dietary fibre: are resistant to digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine

Englyst et al. (2002) outlined a method for the isolation and measurement of non-starch polysaccharides in plant based food. It describes a fraction of starch, referred to as resistant starch, that is resistant to digestion by α -amylase following food processing. Englyst et al. describes the requirement of an additional step in the method to remove the resistant starch from plant based food (Englyst et al. 1982).

Englyst and colleagues continued this work by studying the effect of three starch-containing foods (oats, cornflakes and white bread) in seven ileostomy subjects who had an average of 10 cm of terminal ileum removed (Englyst and Cummings 1985). Cornflakes and white bread contain RS3, while negligible amounts are present in oats. Following 24 hours of standardised plant-polysaccharide free diet and overnight fast, subjects consumed test foods as a breakfast meal. Ileostomy effluent was collected at two hourly intervals for 10 hours after the meal. Transit through the small intestine was tracked by xylose that was present in the test foods in order to ensure an adequate washout period. A mean transit time of 6–8 hours for each test food was observed. Resistant starch levels were tested in foods prior to consumption and in the resulting effluent (Table 1).

Table 1: Resistant starch levels identified in foods and ileostomy effluent of seven subjects (Englyst and Cummings 1985)

| Test food (g) | Resistant starch per serving (g) | Resistant starch recovered in effluent (g) | % Recovery |
|-------------------|----------------------------------|--|--------------|
| Cornflakes (100) | 3 | 1.8 | 60 |
| White bread (150) | 1.1 | 0.9 | 82 |
| Oats (100) | Trace | 0.04 | Not provided |

Note: Standard deviations were not provided.

Although recovery was incomplete for both foods, this study indicates that resistant starch resists digestion in the small intestine in vivo.

Four publications were identified by FSANZ that studied the fermentation of resistant starch in the large intestine, thereby addressing the second part of the criterion – ***usually with complete or partial fermentation in the large intestine*** (Phillips et al. 1995; Jenkins et al. 1998; Hylla et al. 1998; Muir et al. 2004).

Phillips and colleagues studied the effect of two intake levels of RS2 consumed over three weeks on eleven healthy adults in a randomised controlled trial in which macronutrient intake remained constant. RS levels were based on normal energy intake determined from food diaries prior to commencement of the study and ranged from 26–50 g/day for high RS and 3–8 g/day for low RS. Subjects that consumed a diet high in resistant starch had significant increases in the daily faecal excretion of acetate ($72\% \pm 3.7\%$, $p < 0.05$), butyrate ($100\% \pm 1.4\%$, $p < 0.05$) and total short chain fatty acids (SCFAs) ($67\% \pm 5.9$, $p < 0.05$) compared to the low RS group (Phillips et al. 1995).

Jenkins et al. (1998) studied the effect of two sources of resistant cornstarch on faecal bulk, SCFA production, blood lipids and glycaemic index. Twenty-four healthy adults were studied in a randomised crossover trial that tested the effect of supplementing breakfast cereals and muffins with wheat bran, RS2, RS3 or low-fibre control for two weeks each with a two week washout period between trial foods. Daily resistant starch intakes were estimated to be 21.5 g, 27.9 g, 2.3 g and 1.5 g for RS2, RS3, low-fibre control and wheat bran containing

foods, respectively. The mean increase in faecal butyrate concentration compared to low-fibre control was $56\% \pm 18\%$ ($p = 0.006$). However no significant difference in breath methane or hydrogen was found.

A non-randomised crossover study of 12 healthy adults by Hylla et al compared the effect of a high-RS2 standardised diet (55.2 g/day) to a low-RS2 diet (7.7 g/day). A significant increase in breath hydrogen concentration area under the curve was reported on day 7 (209 ± 20 ppm versus 139 ± 27 ppm, $p \leq 0.05$) and day 28 (195 ± 28 ppm versus 131 ± 31 ppm, $p \leq 0.05$) but not on day 14 or day 21 in the high-RS group compared to the low-RS group (Hylla et al. 1998).

Muir and colleagues studied the effects of wheat bran with added high amylose maize (21.6 g RS3) or wheat bran alone (1.8 g RS3) on a standardised diet in 20 healthy volunteers in a randomised crossover design with 3 week test periods and one week washout (Muir et al. 2004). Faecal matter was collected for five consecutive days in the third week of each test period. Dietary intake was controlled for the study period and macronutrient composition was closely matched. Intake of wheat bran with added RS3 resulted in an increase in the daily excretion of acetate (50%, $p = 0.001$), butyrate (77%, $p = 0.001$) and total SCFAs (49%, $p = 0.001$) compared to the wheat bran group.

Evaluation of evidence for criterion 2

The reviewed evidence relevant to the first part of criterion 2 – ***are resistant to the digestion and absorption in the small intestine*** indicates from data in ileostomy patients that resistant starch transits through the small intestine without undergoing digestion and absorption.

The reviewed evidence relating to the second part of criterion 2 – ***usually with complete or partial fermentation in the large intestine*** showed that resistant starch is at least partially fermented in the large intestine. Foods supplemented with resistant starch resulted in increases in at least one marker of colonic fermentation including SCFAs, hydrogen and methane. It should be noted that these markers do not accurately measure the levels of fermentation products but indicate an increase in fermentation levels among subjects with increased resistant starch intake; SCFAs are absorbed in the colon and therefore levels in faecal samples will be lower than levels produced by colonic microbiota.

Conclusion for criterion 2

It is concluded that there is sufficient evidence that resistant starch meets the requirements of criterion 2 of the definition of dietary fibre.

2.3 Assessment against Criterion 3 of the definition of dietary fibre – promote one or more of the following beneficial physiological effects: (i) laxation, (ii) reduction in blood cholesterol, (iii) modulation of blood glucose

The definition of dietary fibre in Standard 1.2.8 currently has no formal requirements for the assessment of laxation, reduction in blood cholesterol, or modulation of blood glucose.

The Applicant provided evidence in support of modulation of blood glucose. FSANZ also considered studies investigating the effect of resistant starch on laxation.

2.3.1 Laxation

A benchmark of a greater than 1 g increase in faecal wet weight per gram of test fibre consumed was used in previous dietary fibre Applications assessed by FSANZ including A491 – *Resistant maltodextrin as dietary fibre* and A277– *Inulin and fructo-oligosaccharides as dietary fibre*. For consistency with past assessments this benchmark was used for assessment of this Application.

FSANZ identified two suitably designed studies reporting an effect of resistant starch intake on laxation. Muir et al. (2004) studied the effect of bran or bran with RS2 supplements on 20 healthy volunteers in a randomised controlled crossover design with 3 week test periods and one week washout. Faecal matter was collected for five consecutive days in the third week of each test period. Dietary intake was controlled for the study period and macronutrient composition, including that of wheat bran, was closely matched. An increase of 19.8 g in daily resistant starch intake resulted in a 2.2 g increase in faecal wet output per gram of resistant starch consumed ($p < 0.001$).

Maki et al. (2009) describes a randomised double blinded crossover trial in which 14 healthy subjects consumed 25 g RS3, wheat bran fibre or control for 14 days following a 14-day baseline period in which subjects consumed a low-fibre test product. A seven day washout followed each treatment. Subjects were instructed to maintain their habitual diets, smoking and physical activity patterns. Faecal samples were then collected for the last 4 days in each treatment phase. Faecal output during the resistant starch phase was 164 ± 23 g/day compared to 128 ± 18 g/day during the control phase; an increase of 35 g/day ($p < 0.02$), equivalent to a 1.4 g increase per gram of resistant starch.

These two studies were considered to be suitably designed to provide evidence for an increase in faecal bulk on consumption of resistant starch. Both studies fulfilled the benchmark criterion of an increase of at least 1 g faecal bulk per day per gram of resistant starch consumed. Therefore, for the purpose of this Application, FSANZ considers that there is sufficient evidence to consider that resistant starch promotes laxation.

2.3.2 Modulation of blood glucose

In support of this criterion, the Applicant submitted a report from the European Food Safety Authority (EFSA) that assessed the health claim that resistant starch reduces post-prandial glycaemic response (EFSA 2011). EFSA considered 14 publications as suitable evidence for assessing the claim. The studies typically compared the effect of a food/meal containing either maize or wheat flour to one with high amylose corn flour on postprandial blood glucose and insulin concentrations at 30 minute intervals up to two hours post-meal, using a crossover or Latin square study design. Most studies reported the difference in the area under the concentration-time curve (AUC) for the two foods.

EFSA noted that most studies reported statistically significant decreases in post-prandial glycaemic responses after consumption of RS2 when it partially replaced digestible starch in baked goods but not when RS2 was added to foods so that available carbohydrate levels were maintained. The decreases were only observed when at least 14% of total starch was replaced with resistant starch. EFSA determined that the observed effect of replacement is due to the decrease in available carbohydrate (EFSA 2011). It was concluded that this effect is expected to be observed for any type of resistant starch.

FSANZ identified three additional studies in which the acute effect of resistant starch on post-prandial blood glucose concentration was studied (Seal et al. 2003; Li et al. 2010; Luhovvy et al. 2014). These studies tested the effect of replacing digestible starch with RS2 on postprandial blood glucose in healthy adults using a randomised crossover design (Table 2). The number of subjects in each study ranged from 8 to 30. Test foods included meals (rice, cookie) or starch gel/suspensions. Venous blood samples were taken at a minimum of 30 minute intervals for 3 hours after consumption of test foods.

All of the studies demonstrated statistically significant ($p < 0.05$) decreases in peak postprandial blood glucose concentration on substitution of RS2 for digestible starch.

Table 2: Randomised controlled trials measuring plasma glucose concentrations after intake of resistant starch

| Reference | Study design | Mean RS2 dose (gram) | Peak plasma glucose concentration \pm SEM (mmol/L) | Peak change from baseline plasma glucose concentration \pm SEM (mmol/L) | |
|----------------|--|---|--|---|-----------------|
| Seal (2003) | Randomised, double-blind crossover design in healthy (n = 8) and type-2 diabetic (n = 13) subjects | | | | |
| | Healthy subjects | Control: raw waxy maize starch suspension | 5.6 | NR | 2.31 \pm 0.25 |
| | | Test: raw tapioca and maize starch blend suspension | 15.9 | NR | 1.17 \pm 0.13 |
| | Type-2 Diabetic subjects | Control: raw waxy maize starch suspension | 5.6 | NR | 2.90 \pm 0.24 |
| | | Test: raw tapioca and maize starch blend suspension | 15.9 | NR | 1.46 \pm 0.23 |
| | Li (2009) | Randomised, crossover design in healthy subjects (n = 16) | | | |
| | | Control: wild type rice | 1.0 | 7.2 \pm 0.6 | NR |
| | | Test: RS Rice | 8.1 | 6.8 \pm 0.4 | NR |
| Luhovyy (2014) | Randomised, crossover design in healthy subjects (n = 30) | | | | |
| | | Control: cookie with all-purpose starch | 11.1 | 7.95 \pm 0.22* | NR |
| | | Test: cookie with Hi-maize starch | 22.2 | 7.24 \pm 0.17* | NR |

NR: not reported, * Whole blood tested

Note: only relevant arms from each study are shown.

In each study the differences between the test and control peak glucose responses were statistically significant ($p < 0.05$).

2.3.3 Reduction in blood cholesterol

Because resistant starch satisfies two of the three beneficial physiological effects defined for dietary fibre, it was not necessary to consider whether resistant starch promotes reduction in blood cholesterol.

Conclusion for Criterion 3

FSANZ concludes that resistant starch has a positive effect on laxation as defined in previous FSANZ assessments of dietary fibres. Also, evidence provided by the Applicant is consistent with evidence reviewed by FSANZ that supports the conclusion that resistant starch modulates blood glucose. Therefore it was concluded that there is sufficient evidence to support criterion 3.

2.4 Conclusion

It has been demonstrated from the evidence provided by the Applicant and other scientific literature identified by FSANZ that resistant starch satisfies the definition of dietary fibre as follows:

- Resistant starch is present in the edible parts of plant materials and can be extracted from plant materials.
- Resistant starch is resistant to digestion in the small intestine and is fermented in the large intestine.
- Replacement of digestible starch with resistant starch in a meal promotes modulation of blood glucose by reducing peak postprandial blood glucose concentration.
- Resistant starch promotes laxation.

3 Suitability of AOAC 2002.02 as a regulatory method of analysis for resistant starch

3.1 Dietary fibre and resistant starch

Three of the five AOAC methods presented in Schedule S11—4 describe measurement of 'total' dietary fibre. These official methods of AOAC International are AOAC 985.29¹ and its derivative AOAC 991.43², and AOAC 2001.03³ which is a derivative of AOAC 991.43. AOAC International is a globally recognised, independent association that develops consensus standards in the area of analytical chemistry.

Resistant starch (RS) is described in this report as the fraction of starch that is not digested when it passes through the small intestine; it is also at least partially fermented in the large intestine. Five subtypes (RS1 – RS5) are now classified as described in Table 3.

¹ AOAC 985.29 Total Dietary Fiber in Foods

² AOAC 991.43 Total, Soluble and Insoluble Dietary Fiber in Foods

³ AOAC 2001.03 Dietary Fiber containing Supplemented Resistant Maltodextrin

Table 3: Subtypes of resistant starch

| RS subtype | Description |
|------------|--|
| 1 | Physically inaccessible to digestion |
| 2 | Native starch granules protected from digestion due to the conformational structure of the granule |
| 3 | Non-granular starch formed during retrogradation of starch granules in food processing |
| 4 | Chemically modified starch to decrease digestibility |
| 5 | Amylose-lipid complex found in native starch granules and processed starch |

3.2 AOAC 2002.02 – resistant starch

The design of this method aims to accurately measure RS using enzymes and incubation conditions that simulate physiological conditions to produce results as close as possible to in vivo RS results from ileostomy patients. AOAC 2002.02 can also measure non-resistant starch and total starch.

McCleary and Rossiter (2004) compared the analytical performance of AOAC 2002.02 with RS analysed as the difference between AOAC 991.43 before and after a modification to completely remove all RS. The results are shown in Table 4. Only a small range of ingredients and food matrices have been assessed in this way in the literature.

These data also indicate that the older ‘total’ dietary fibre methods measure some but not all RS; also AOAC 2002.02 correctly does not measure pectin as RS.

Table 4: RS (g/100 g) measured by AOAC 2002.02, AOAC 991.43 and by difference in AOAC 991.43 before and after modification

| Samples | AOAC 2002.02 | AOAC 991.43 – | AOAC 991.43 – difference |
|------------------------------------|--------------|---------------|-----------------------------|
| High amylose maize starches | | | |
| Hylon VII | 53.7 | 25.9 | — |
| Hi-maize 1043 | 45.7 | 54.5 | — |
| Novelose 240 | 46.9 | 52.3 | — |
| CrystaLean | 40.9 | 34.0 | — |
| Actistar | 58.0 | <0.1 | — |
| Native potato starch | 78.1 | <0.1 | — |
| Food | | | |
| Wheat bran | 0.42 | 38.7 | 0.7 |
| Hi-maize bread | 5.1 | 9.2 | 5.7 |
| Rye crispbread | 1.2 | 15.0 | 1.4 |
| Kidney beans | 5.3 | 21.5 | 5.0 |
| Corn flakes | 2.8 | 3.3 | 2.3 |
| Cooked/cooled potato | 3.8 | 7.1 | 1.7 |
| Pectin | 0 | 83.5 | 11.7 |

— Difference not quantified by authors. FSANZ notes that <1.0 g dietary fibre per 100 g of food remains after analysis by modified AOAC 991.43 (see Table 6)

3.2.1 Subtypes of resistant starch

AOAC 2002.02 more accurately measures RS1–3 subtypes than the Code’s three methods as qualitatively shown in Table 5. RS4 is underestimated due to its incomplete hydrolysis to glucose (Megazyme 2016).

Table 5: Measurement of RS subtypes by ‘total’ dietary fibre methods in the Code and AOAC 2002.02

| AOAC Method | RS1 | RS2 | RS3 | RS4 | RS5 |
|-------------|----------------------|----------------------|----------------------|---------------|----------------------|
| 985.29 | underestimate | underestimate | underestimate | overestimate | not accurate measure |
| 991.43 | underestimate | underestimate | underestimate | overestimate | not accurate measure |
| 2001.03 | underestimate | underestimate | underestimate | overestimate | not accurate measure |
| 2002.02 | satisfactory measure | slight underestimate | satisfactory measure | underestimate | underestimate |

3.3 Suitability of AOAC 2002.02 as a regulatory method

3.3.1 International analytical organisations

Of the 12 methods developed to measure RS between 1992 and 2006, AOAC 2002.02 is the only one to successfully pass an evaluation of its interlaboratory performance statistics by three organisations as follows:

- AOAC as method 2002.02 (McCleary et al. 2002)
- AACCI as method 32–40.01
- Codex (Codex STAN 234–1999, Method of analysis for individual components of dietary fibre, Type II).

3.3.2 Usage

The Australian Export Grains Innovation Centre, Sydney (AEGIC) offers AOAC 2002.02 as the method of choice for resistant starch measurement in food (H. Salman, pers.com.)⁴. FSANZ understands the Australian National Measurement Institute refers requests for government analysis of RS to AEGIC. In New Zealand,ASUREQuality Ltd, a government-owned enterprise serving the food and agricultural sector, also offers food analysis using this method⁵. The method is widely used internationally and is the only method for RS in the Codex list of recommended methods.

3.3.3 Analytical parameters

AOAC 2002.02 takes about 24 hours to complete and is applicable to samples containing between 1–75% RS. For samples containing >2% RS, the limit of quantification (LOQ) = 0.5%; repeatability relative standard deviation (RSD_r) <2%; reproducibility relative standard deviation (RSD_R) <4%; (McCleary et al. 2002) and standard error (SE) ± 5% (Megazyme 2015).

⁴ H. Salman, Business Manager, Sydney analytical laboratory, Export Grains Innovation Centre, Sydney, personal communication 19 July 2017

⁵ J. Thompson, Team Leader, General Chemistry, ASUREQuality Limited, Auckland, personal communication 13 October 2017

Variance factors are higher when the samples contain either very low or very high RS (<1% RS with relatively high total starch and non-resistant starch contents; or 64–75% RS) (McCleary et al. 2002).

3.3.4 Limitations

The most obvious limitation of this method is the underestimation of RS4. As a consequence of etherising, esterifying or cross-bonding starch with particular chemicals (to reduce digestibility) these steps also interfere with the enzymatic hydrolysis of the recovered alkali RS fraction and therefore the RS4 is underestimated (Megazyme 2016).

Other limitations in the literature refer to digestion time, incubation temperatures and use of enzymes as indicated by the following. The use of purified porcine pancreatic α -amylase and prolonged 16-hour digestion period may not accurately mimic the conditions of human digestion. The procedure is lengthy and has numerous opportunities for human error during the decanting steps, thus rendering it impractical for rapid analysis of RS-containing samples (Moore 2013). The method still employs incubation temperatures (50°C) not normally encountered in vivo (Haugabrooks 2013). The analytical procedure uses an enzyme for removing digestible starch which can generate high variability compared to gas-liquid chromatography, where errors can be corrected by using an internal standard (Ang 2011).

With respect to the slight underestimate of RS2 by AOAC 2002.02, updates are planned modelled on improvements made to analysis of this component in the *rapid integrated method for total dietary fiber* which is currently undergoing AOAC accreditation. This improvement increases pancreatic alpha-amylase and amyloglucosidase concentrations and reduces incubation time from 16 hours to 4 hours. (Megazyme 2016; McCleary et al. 2015).

3.4 Potential for double counting of dietary fibre

The extent to which the Code's three 'total' dietary fibre methods and AOAC 2002.02 measure RS will be markedly influenced by the distribution of (non-resistant) starch and RS in the food. Many diagrammatic representations in the literature of the two older methods indicate about 75% RS is overall measured as dietary fibre.

When comparing results from any of the Code's three methods with AOAC 2002.02, the dietary fibre content of most general food samples is the same or higher than the RS content. This occurs when the cellulose, beta-glucan, guar gum and certain xylan content is relatively high and the RS content is relatively low. However, for some high amylose starch ingredients and foods containing added RS ingredients, the 'total' dietary fibre content measured is less than total RS. This is occurs when the cellulose, beta-glucan, guar gum and certain xylan content is relatively low and the RS content (e.g. RS2, RS3) is relatively high (Megazyme 2016).

Therefore, RS quantified by AOAC 2002.02 should not be summed with the results of any of the 'total' dietary fibre methods to obtain a better estimate of dietary fibre (DeVries 2010) without adjustment for double counting (Champ et al. 2003).

The Code already anticipates this situation in S11—4(3) as shown.

- (3) If the dietary fibre content of a food has been determined by more than 1 method of analysis, the total dietary fibre content is calculated by:
 - (a) adding together the results from each method of analysis; and
 - (b) subtracting any portion of dietary fibre which has been included in the results of more than one method of analysis.

As previously discussed above, McCleary and Rossiter 2004 obtained quantitative estimates of how much RS could be double counted, by measuring dietary fibre by AOAC 991.43

before and after total removal of RS with treatment with hot dimethyl sulphoxide The difference in results of the two procedures was attributed to RS. Table 6 shows the results.

Nearly all total dietary fibre measured by AOAC 991.43 is measured as RS in high amylose maize starches whereas in the foods sampled, 2–70% dietary fibre is measured as RS (i.e. for Hi-maize bread, % total dietary fibre measured as RS = $(9.2-3.5)100/9.2$ i.e. 62%.

Table 6: 'Total' dietary fibre measured as RS by AOAC 991.43

| Food | AOAC 991.43 (g/100 g) | Modified AOAC 991.43 (g/100 g) | % 'Total' dietary fibre measured as RS |
|------------------------------------|--------------------------|--------------------------------------|---|
| High amylose maize starches | | | |
| Hylon VII | 25.9 | 1.0 | Nearly all |
| Hi-maize 1043 | 54.5 | 0.5 | Nearly all |
| Novelose 240 | 52.3 | 0.3 | Nearly all |
| CrystalLean | 34.0 | 0.3 | Nearly all |
| Food | | | |
| Wheat bran | 38.7 | 38.0 | 2 |
| Hi-maize bread | 9.2 | 3.5 | 62 |
| Rye crispbread | 15 | 13.6 | 9 |
| Kidney beans | 21.5 | 16.5 | 23 |
| Corn flakes | 3.3 | 1.0 | 70 |
| Cooked/cooled potato | 7.1 | 5.4 | 24 |

3.5 Conclusion

AOAC Official Method 2002.02 is widely used internationally and is the only method of analysis for resistant starch in the Codex list of recommended methods. The method is applicable to samples containing between 1–75% resistant starch and method performance parameters including limit of quantification, repeatability, and reproducibility have been determined as being acceptable for food regulatory purposes.

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