1 **Original research article**

2 **Dietary fibre in legumes, seeds, vegetables, fruits and mushrooms: comparing**

3 **traditional and semi-automated filtration techniques**

4 Helena Pastell[a,*], Tiina Putkonen[a1], Hannu Rita[b]

5 [a]Finnish Food Safety Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland

6 [b]Faculty of Agriculture and Forestry, P.O. Box 27 (Latokartanonkaari 7), FI-00014 University of

7 Helsinki, Finland

8 * Corresponding author: Helena Pastell, Research and Chemistry Department, Finnish Food Safety

9 Authority Evira, Mustialankatu 3, FI-00790 Helsinki, Finland, Tel. +358 50375 0968, email

10 helena.pastell@evira.fi

11 [1]Present address: Finnish Safety and Chemicals Agency, PL 66 (Opastinsilta 12 B), FI-00521

12 Helsinki, Finland

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Abstract

The method AOAC 2011.25 was used to analyze all the dietary fibre (DF) components included in the latest definition by the Codex Alimentarius Commission (2009). The traditional filtration technique, described in the method AOAC 2011.25, was compared with a new semi-automated filtration technique using Fibertec^{TM} 1023 system. For the comparisons, a statistical similarity approach was chosen to evaluate the results of six food matrixes (wheat flour, edible boletus, strawberry, tomato, green pea and horse radish). The total DF contents of the tested matrixes fit within 16% tightest data-induced similarity limit of the manual mean, with one exception (strawberry; 30%). Thus, it was concluded that both techniques are suitable for use with the method AOAC 2011.25, and therefore either technique was used to analyze a wide selection of legumes, seeds, vegetables, fruits and mushrooms (44 foods). Seeds were excellent sources of total DF, as well as water-insoluble (IDF) and water-soluble (SDFP) polysaccharides. A substantial amount of oligosaccharides (SDFS) was found in red onions. Generally, the DF contents were higher in this study than in earlier studies. Higher DF results can be partially explained by the more effective analytical method, and partly by changed varieties.

Keywords: Food analysis; Food composition; Method AOAC 2011.25; Dietary fibre (Dietary fiber); Legumes; Seeds; Vegetables; Fruits; Mushrooms; Similarity

1. Introduction

According to the latest definition by the Codex Alimentarius Commision (2009), dietary fibre (DF) is naturally occurring in food, isolated from food raw materials or synthetic health promoting carbohydrate polymers composed of ≥ 10 monomeric units, which are not hydrolyzed in the human

44 small intestine. Oligosaccharides with 3-10 monomeric units are also considered to be DF in the

45 European Union (EU). DF contains numerous of chemically divergent substances which can be

46 classified in several ways. Based on their solubility DFs can be divided as water-insoluble (e.g.

47 cellulose, linear hemicelluloses and non-carbohydrate compound lignin) and water-soluble (e.g.

48 highly substituted hemicelluloses, pectins, gums, mucilages, and oligosaccharides) components

49 (Davidson & McDonald, 1998).

50 Revising the DF definition and including non-digestible oligosaccharides as a part of DF, alongside

51 water-insoluble and water-soluble polysaccharides, gave rise to developing new analytical methods

52 capable of analyzing all of these DF components. All the methods developed prior to the latest

53 definition, are inadequate in measuring all the diverse poly- and oligosaccharides of DF. Only the

54 latest methods AOAC 2009.01 and AOAC 2011.25 are able to analyze all the components, including,

55 e.g., all types of resistant starch (RS), polydextrose, resistant maltodextrin, and non-digestible

56 oligosaccharides (McCleary et al., 2013; Westenbrink et al., 2013; McCleary, 2014). Although the

57 new methods have been available for several years, there are still limited amounts of publications

58 with DF information given using the new methods.

59 The food composition databases (FCDBs) contain dietary fibre data obtained using various analytical

60 methods, such as AOAC 985.29 (Prosky), AOAC 991.43 (Lee), Uppsala, Asp, Englyst and Southgate.

61 Depending on the methods used, the existing data may under- or overestimate the amount of dietary

62 fibre in several foods, and furthermore, the content of oligosaccharides is totally missing. However,

63 data in the food composition databases (FCDBs) is utilized by many user groups (e.g. industry,

64 dieticians, educators, consumers, risk assessors, researchers), and therefore precise dietary fibre

65 content results are required for many purposes. Hence, more analytical results are needed.

66 Traditionally, many of the gravimetric DF analysis methods include filtering that is used to separate

67 DF components based on their solubility and size. In many cases, the filtering step is time-consuming

68 and challenging because the filters get easily blocked. Because of this, new filtering techniques have

69 been introduced, such as semi-automated Fibertec 1023 utilizing traditional crucible technique with

70 possibility to use reversed pressure mode in filtration, automated Ankom Total Dietary Fibre

71 Analyzer with filter bag technology and centrifugation technique by Medallion Labs (Plank &

72 Povolny, 2015). Since fractionation is a crucial step in the DF assay, there is more need for research

73 on the impact of the filtration techniques on the content results of individual DF fractions.

74 The introduction of new technologies as part of the DF analysis requires a statistical comparison

75 between the techniques. Generally in food sciences, statistically significant differences are

76 determined using a t-test to evaluate the potential of the methods. However, in addition to representing

77 the significant differences, more attention should be focused on showing the similarity between the

78 used methods or techniques. Rita and Ekholm (2007) studied the similarity of the results given by

79 two methods used to determine algal-available phosphorus in pulp and paper mill wastewaters and

80 proposed the use of the statistical similarity approach in environmental sciences. Schuirmann

81 presented the statistical procedure in 1987, but it has not been widely used, neither in environmental

82 nor in food sciences. According to Rita and Ekholm (2007), one reason for the limited utilization of

83 the method may be the challenges in setting the similarity limit, which is required in the method.

84 The statistical hypothesis in similarity testing is the interval $S(\theta) = [-\theta, \theta]$, where the limit θ represents

85 the largest tolerable difference between the averages of the two techniques in either direction. The

86 methods are regarded as similar if the absolute value of their difference is below a positive value θ.

87 The smaller θ is, the 'more' similar the means are. The hypothesis gains statistical support at a 5%

88 level as soon as the 90% confidence interval [L, U] for the difference (L is the lower confidence limit

89 and U the upper), is entirely within the similarity interval $[-\theta, \theta]$, i.e., $L > -\theta$ and $U < \theta$ (Rita &

90 Ekholm, 2007).

91 Although the statistical technique to show similarity is simple, an appropriate limit θ to evaluate

92 similarity is challenging for different matrixes. As the health effects of DF depend on its content and

93 type, the limit θ should be set in such a way that conclusions for, e.g., nutritional recommendations

94 or food related risk assessment remain unchanged irrespective of the technique used. It is often

95 difficult to pre-set such a value for the similarity limit θ. The statistical methodology for showing

96 similarity was originally developed in pharmaceutical drug development trials (Chow & Liu, 1999),

97 where the similarity limit for two drug products to be bioequivalent is set as 20% in the guidelines

98 given out by regulatory authorities. Such an issue-specific (pharmaceutical) limit cannot be

99 transferred as such to be used in fibre content comparisons.

100 The aim of this study was to analyze precise and up-to-date DF contents (water-insoluble DF, water-

101 soluble DF and non-digestible oligosaccharides) of legumes, seeds, vegetables, fruits and

102 mushrooms, according to the latest DF definition using the AOAC 2011.25 methodology.

103 Furthermore, the objective was to compare the DF results between the traditional and semi-automated

104 filtration techniques, and use the statistical similarity approach in the comparisons.

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106 **2. Materials and methods**

107 **2.1. Materials**

108 Pancreatic α-amylase (E-PANAA, 150,000 Ceralpha Units/g), amyloglucosidase (E-AMGDF, 3300

109 Units/mL) and purified protease (E-BSPRT, 350 tyrosine Units/mL) were purchased form

110 Megazyme (Bray, Co., Wicklow, Ireland). D-sorbitol (used as an internal standard), LC Retention

111 Time Standard (maltodextrins plus maltose, 4:1), filtration aid Celite® (acid washed, G-CEL500),

112 and ion exchange resins Amberlite® FPA53 (OH⁻; G-AMBOH) and Ambersep® 200 (H⁺; G-

113 AMBH), were all purchased from Megazyme.

Legumes: Broad bean (*Vicia faba*, 8 subsamples); common bean (*Phaseolus vulgaris,* 12); dried green pea (*Pisum sativum var. sativum*, 12); green Pea (*Pisum sativum var. sativum*, 12); sugar pea (*Pisum sativum var. macrocarpon*, 11). **Seeds:** Chia seed, whole (*Salvia hispanica*, 10); hempseed, whole and peeled (*Cannabis sativa*, 5 and 8, respectively); linseed, whole (*Linus usitasissium*, 12); pine nut, peeled (*Pinus spp.*, 12); poppy seed, whole (*Papaver somniferum*, 6); pumpkin seed, peeled (*Cucurbita pepo*, 13); sesame seed, whole and peeled (*Sesamum indicum*, 7 and 8, respectively); sunflower seed, peeled (*Helianthus annuus*, 12). **Vegetables:** Carrot (*Daucus carota*, 12); coriander (*Coriandrum sativum*, 12); horseradish (*Armoracia rusticana*, 10); lamb´s lettuce, i.e., corn salad (*Valerianelle locusta*, 7); lettuce (*Lactica sativa*, 12); pea shoot (*Pisum sativum*, 13); radicchio (*Cichorium intybus var. foliosum*, 8); red onion (*Allium cepa*, 12); romaine lettuce (*L. sativa var. longifolia (L. romana)*, 11); rucola, i.e., salad rocket (*Eruca sativa*, 12); tomato, Finnish and imported (*Solanum lycopersicum*, 12 and 9, respectively); white radish, i.e., daikon (*Raphanus sativus var. longipinnatus*, 12). **Fruits:** Apple, green, with and without skin, (*Malus pumila*, 12); apple, red, with and without skin (*Malus pumila*, 12); banana, peeled (*Musa Cavendish*, 12); blackcurrant (*Ribes nigrum*, 12); blueberry (*Vaccinium myrtillius*, 8); cloudberry (*Rubus chamaemorus*, 11); lingonberry (*Vaccinium vitis-idaea*, 12); raspberry, (*Rubus idaeus*, 13); Strawberry (*Fragaria sp.*, 12). **Mushrooms:** Chanterelle (*Cantharellus cibarius*, 13); edible boletus (*Boletus edulis*, 12); funnel chanterelle (*Craterellus tubaeformis (formerly Cantharellus tubaeformis)*, 10); northern milk-cap (*Lactarius trivialis*, 10). **Other:** Seaweed Nori (*Porphyra laciniata*, 1).

Food matrixes for internal verification: Dark wheat flour (endosperm and bran inner layer; T. aestivum L.), root vegetable mixture (containing orange and yellow carrot (*Daucus carota*), yellow turnip (*Brassica napus*), parsnip (*Pastinaca sativa*), and celery (*Apium graeveolens*)) and tomato (the scientific name given above).

Food matrixes for filtration technique comparisons: dark wheat flour, tomato, strawberry, edible boletus, pea shoot and horseradish (the scientific names given above).

The following foods contained samples entirely of Finnish origin: Lettuce, romaine lettuce, pea shoot, coriander, blackcurrant, lingonberry, blueberry, cloudberry, edible boletus, northern milk-cap, funnel chanterelle, (domestic) tomato, carrot, and dark wheat flour. Furthermore, the formed composite samples of dried green pea, rucola, red onion, raspberry and strawberry contained foods, of which 75% were grown in Finland, and the rest in other European countries. The origin of the seeds was multi-national, global even (Europe, Africa, Asia, Middle or South America). Imported tomatoes were Spanish with one exception (Holland), apples came from France, Italy and Austria, while bananas were Costa Rican (one sample from Brazil).

2.2. Sampling

The legume, vegetable, fruit and mushroom samples were purchased from the grocery stores and market places in Southern Finland based on availability. The seeds were purchased from Finnish grocery stores, health food shops, oriental shops and web stores. The market situation was first investigated, and the stores were chosen based on availability of the seeds. All the collected samples were the most representative brands in Finland in their category, in some categories, all the brands on the market were purchased. Most of the composite samples consisted of at least 12 subsamples, but in some cases less than 12 were accepted due to poor availability.

2.3. Sample pretreatment

An equal portion of each sample of legumes, vegetables, fruits and mushrooms were mixed individually into one composite sample by species. The composite samples were weighed prior to

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160 and after freeze-drying to determine the weight loss during drying. The samples were freeze-dried on

161 average for seven days ($< -90^{\circ}$C, $< 10^{-4}$ hPa (mbar); Scanvac CoolSafe, LaboGene ApS, Lynge,

162 Denmark). They were individually homogenized by mixing, after which an equal portion of each

163 sample was mixed into one composite sample by species. Seeds contained >10% fat, and were thus

164 de-fatted according to AOAC 985.29 by treating the samples with petroleum ether prior to milling

165 and DF analysis.

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167 **2.4. Method AOAC 2011.25**

168 All the samples were milled through a 0.5 mm sieve. The DF was analysed according to the method

169 AOAC 2011.25 (Megazyme, 2013). The sample (an exact amount of 1.000 ± 0.005 g, or in case of

170 mushroom samples 0.500 ± 0.005 g to facilitate the filtering step) was weighed in an incubation bottle.

171 An enzyme mixture of pancreatic α-amylase/amyloglucosidase (AMG) was added to each bottle to

172 remove starch, and the samples were incubated for 16 hours at 37°C in a shaking water-bath. Next,

173 protease was added (30 minutes, 60°C) to remove proteins. Water-insoluble (IDF) and water-soluble

174 polysaccharides (SDFP; DF soluble in water and precipitated by 78% aqueous ethanol) and

175 oligosaccharides (SDFS; DF soluble in water and not precipitated by 78% aqueous ethanol) were

176 analyzed as separate fractions. The enzymatically hydrolyzed samples were filtered twice to separate

177 IDF from SDF (soluble dietary fibre), and SDFP from SDFS. IDF and SDFP residues were dried,

178 weighed and corrected for protein and ash values. SDFS was hydrolyzed by AMG and analysed by

179 high performance liquid chromatography (HPLC) after deionization, as described in Rainakari et al.

180 (2016). Sorbitol was used as an internal standard for SDFS analysis. The total DF amount is the sum

181 of IDF, SDFP and SDFS. The DF contents were expressed in fresh weight (g/100 g) and therefore

182 corrected by moisture. De-fatted samples were corrected by fat. Dry weights were only used for

183 statistical treatment.

185 **2.5.Filtration techniques**

186 Two different filtration techniques, referred to as manual and semi-automated technique, were

187 compared. In the manual filtration technique, the samples were filtered through the fritted crucibles

188 with the help of an air-driven vacuum pump (PIAB, Lab Vac LVH40; Sigma-Aldrich, St. Louis, MO,

189 USA) or water-suction. Fibertec[TM] 1023 equipment (FOSS, Hillerød, Denmark) connected to water-

190 suction was used in semi-automated technique. The main differences in the techniques are collected

191 in Table 1. According to the AOAC method 2011.25, the bed of Celite® is wetted in the crucible,

192 and using suction, drawn onto the fritted glass as an even mat (Megazyme, 2013). If the sample forms

193 a viscose solution and the filtration is slow, in the manual filtration technique, the sample may be

194 stirred gently without breaking the Celite®-layer. However, when the semi-automated technique is

195 used, and if the backpressure is applied to accelerate the filtration, Celite® is mixed partially or

196 completely with the sample matrix. In both techniques, similar fritted crucibles with the same pore

197 size (coarse, ASTM 40-60 μm) are used.

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199 **2.6.Verification of the method AOAC 2011.25**

200 At Evira, an official method AOAC 2011.25 was adopted in use through the internal verification

201 procedure and accreditation. Verification was done using manual filtration technique with three

202 different food matrixes: dark wheat flour, tomato and root vegetable mixture, which were pre-treated

203 according to the AOAC 2011.25 method, and are further used as inter-laboratory control samples.

204 Also the fat removal from the samples prior to DF analysis was verified to expand the usability of the

205 method for the matrixes with fat-content over 10%. Repeatability and reproducibility were tested for

206 the gravimetric analyses of polysaccharides (IDF and SDFP) and fat removal. Limit of detection

207   (LOD) and limit of quantification (LOQ) were determined for the HPLC analysis of the

208   oligosaccharides (SDFS).

209   For implementation of semi-automated filtration technique, six different food matrixes (dark wheat

210   flour, tomato, strawberry, edible boletus, pea shoot and horseradish) were analyzed as 5-7 parallel

211   samples each. The same samples (as 3 parallels) were also analyzed using the verified method with

212   manual filtration technique, and the results were compared. The suitability of the semi-automated

213   system was evaluated based on this comparison.

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215   2.7. Statistical testing

216   Manual and semi-automated filtration techniques were analyzed to test whether their average results

217   are close enough to each other, i.e., that the techniques give similar results. The statistical similarity

218   approach was used, but due to difficulties in pre-setting the similarity limit θ for DF contents, a data

219   exploration technique (Rita & Ekholm, 2007) was utilized. It results in the smallest similarity limit

220   value that – if this value for θ in the similarity hypothesis $S(\theta)$ could have been specified in advance

221   – would have been formally supported by the present data exactly at a specified (e.g., 5%) level. The

222   resulting estimate is called the tightest data-induced (TDI) similarity limit and denote it by θ_{TDI}, which

223   corresponds the term 'potential similarity limit' in Rita & Ekholm (2007). The TDI-value is

224   determined by taking the larger absolute value of the endpoints of the 90% two-sided confidence

225   interval for the difference. The resulting similarity hypothesis $S(\theta_{TDI}) = [-\theta_{TDI}, \theta_{TDI}]$ gains support at

226   exactly the 5% level.

227   The proportion of the used technique`s influence of the total variation of DF content was determined.

228   Coefficient of determination (R^2) corresponds to this proportion, and analysis of variance (ANOVA)

229   was used to calculate R^2 values.

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3. Results and Discussion

3.1. Verification of the method AOAC 2011.25

The method AOAC 2011.25 has been validated and evaluated through a collaborative study by McCleary et al. (2012). In internal verification of the method AOAC 2011 (using manual filtration), the standard deviation (SD) and the coefficient of variation (CV%) of total DF content were calculated to express the precision and repeatability of the assay. SD and CV%, respectively, were 0.62 and 11.2 (wheat flour, n=20), 1.81 and 8.4 (tomato, n=20) and 0.42 and 1.5 (root vegetable mixture, n=10). McCleary et al. (2012) reported that within-laboratory variability SD for TDF ranged from 0.47 to 1.41 and between-laboratory from 0.95 to 3.14 in the method validation. Thus, the analysis results are in accordance with the method requirements established in the validation. For implementing the semi-manual filtration technique, SD and CV% were determined for all six food matrices involved. SD ranged from 0.6 to 4.0 (CV% 4.0-19.0), with the highest SD for strawberry, and for other matrices less than 1.5. The results obtained by semi-automatic filtration technique, apart from strawberries, are in the same range as in initial validation and internal verification.

The limit of detection (LOD) and the limit of quantification (LOQ) in the HPLC analysis of the oligosaccharides were determined as 0.1 g/100 g and 0.2 g/100 g, respectively. Repeatability of the de-fatting was also investigated in several foods, resulting in CV percentages between 0.1 - 2.8 (SD 1.12-1.50). Low CV% indicate that de-fatting works fine. According to validation and verification guidelines (FDA, 2015), a method verified for three or more matrixes, with similar proximate composition as the foods to be analyzed, is considered to be suitable for the DF analysis for a wider range of foods as well.

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3.2. Comparison of the technique averages among six food matrixes

The means of total DF based on the semi-automated filtration technique differed upwards from those of the manual reference technique for wheat flour (by 6%) and edible boletus (7%) and downwards for strawberry (-10%), tomato (-3%), pea shoot (-2%) and horseradish (-8%). The percentages were calculated from the averages given in Table 2. The proportional differences were fairly small, and only in marginal cases may result in misclassification of, e.g., "source of fibre" (3 g of fibre per 100 g) as "high in fibre" (6 g). This could have taken place for wheat flour, as the observed total DF contents were close to the class limit 6 g.

The difference among the means of IDF obtained with the two techniques was at most 20%, but for SDFP and SDFS, the difference was larger in most cases. However, the SDFP in wheat flour was only 8% higher in manual technique, and for SDFS in edible boletus, 2% lower than in semi-automated filtration technique. In tomato, SDFS content was smallest of all six matrixes and the results varied considerably, but most of the observations were below LOQ, which impedes conclusion making.

The six food matrixes included in the technique comparison varied in their DF profiles. Cereals primarily contain hemicelluloses, cellulose and β-glucan, whereas vegetables contain proportionally more gelling fibers than cereals, e.g., pectins, mucilages and gums (Elleuch et al., 2011). Furthermore, only mushrooms contain α-glucan and chitin (Nile & Park, 2014). These differences in the DF compositions may affect the filtration step, and thus the contents of individual DF fractions. Further research is required for firmer conclusions regarding their effects and role in the similarity of the techniques' results.

3.3. Similarity and the residual variation among six food matrixes

The observed relative average differences do not, however, tell the whole truth as they bypass the effects of noise, i.e., measuring error and sampling variation. To relate the observed differences between the techniques to the amount of these uncontrolled sources of variation, analysis of variance (ANOVA) was used. It gave the proportion of the total variation that is due to the difference between the two techniques. In regression context, this proportion corresponds to the coefficient of determination, commonly denoted by R^2. It tells which proportion of the total variation of DF content can be addressed to the explanatory variable, which, in this case, is the technique. Contrary to standard regression, small values of R^2 are now desirable as they indicate the similarity of the techniques. This is because, for small R^2, most of the variation in the observations is addressed to noise and very little to the average difference between the techniques.

The proportions of total variation due to difference between the techniques (coefficients of variation R^2) were for total DF and IDF, respectively, 4.2% and 28.5% (for wheat flour), 30.6 and 5.5 (edible boletus), 1.6 and 0.1 (strawberry), 2.0 and 18.4 (tomato), 4.2 and 10.7 (pea shoot) and 59.5 and 0.2 % (horseradish). For the components SDFP and SDFS, the percentages were 2.5 and 55.4 (wheat flour), 68.6 and 1.2 (edible boletus), 33.1 and 39.2 (strawberry), 33.9 and 39.2 (tomato), 63.4 and 41.3 (pea shoot) and, finally, 86.9 and 18.4 (horseradish).

3.4. Evaluation of TDI similarity limits of six food matrixes

Next, the tightest data induced similarity limits (TDI) were related to the reference (manual filtering) averages (Table 2). As the limit is based on a confidence interval, it takes into account variation among the parallel samples, and gives a realistic view of the degree of similarity that potentially gains statistical support from the present data (which is not available by only inspecting the averages). Two averages may be very close to each other, yet, if there is a lot of residual variation, this could be just a coincidence.

For total DF contents, TDI was within 16% of the manual mean for the tested food matrixes, except for strawberry, where θ_{TDI} was 30% of the reference mean. For IDF, 44% covers θ_{TDI} of all six foods, strawberry being again responsible for the highest limit, as the other five studied foods fall within 31% of the reference. For the remaining two fractions, TDI limits reflect the high relative variability (mostly due to several observations below LOQ). This seriously counsels to abstain from overly assertive opinions regarding the similarity of the techniques for these two DF components.

Since the exploration (TDI-technique) was used in evaluating the similarity, and there is no actual regulation of the magnitude concerning the accepted difference between the techniques, the magnitude of the results can be estimated by comparing them with the internal variation of the manual technique in verification. In wheat flour, CV% for total DF, IDF, SDFP and SDFS were 12%, 13%, 22% and 33%, respectively. For tomato, the corresponding values were 10%, 11%, 19% and 187% (Table 2). Despite some of the foods having had higher θ_{TDI}, the results were in the order of the variation observed within the manual technique. The most important is that the total DF results are similar. It is more difficult to determine what should be the absolute amounts of IDF, SDFP and SDFS, and which technique gives results that are closest to the correct content. For that, further research is required. Thus, perhaps, at least for the time being, it can be stated that similarity of the techniques is adequate for using either one.

3.5. Similarity approach versus testing for difference

Standard t-test is commonly used in food science to determine whether there is statistically a significant difference between data sets. In practice, if large p-values (>0.05) are obtained, the data sets are often argued to be similar. However, strawberry in the data gives an outstanding example of how fatally erroneous conclusions 'large p-value argument' for similarity may lead. For it, difference between the two techniques for the total DF contents was not statistically significant: t-test, $p = 0.54$

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325   (or 0.67, if one does not assume equal variances within the techniques). As 0.54 > 0.05, similarity of

326   the two techniques would have been concluded based on t-test. However, for strawberry, the tightest

327   data induced total DF similarity limit θ_{TDI} was the largest of all six matrixes, i.e., 7.09 g/100 g; 30%

328   (Table 2).

329   On the other hand, for horseradish, a smaller difference in the total DF content (-2.07 g/100 g; Table

330   2) resulted in statistical significance ($p < 0.01$); yet θ_{TDI} was only 12% of the reference mean. For

331   strawberry, the large p-value is due to large standard error of the difference (2.48, largest among the

332   six matrixes), whereas, for horseradish, standard error was only 0.60.

333   It is worth noting that similarity and existence of a difference do not exclude each other. Even a small

334   difference can be statistically significant when testing for difference (especially when the number of

335   replicates is large) and, at the same time, within the similarity tolerance limits, especially for large

336   values of the tolerance θ. Which results are relevant, depends on the objective(s) of the study. In

337   addition, this emphasizes the fact that testing for similarity calls for quantitative specification of the

338   limit θ whereas testing for difference does not.

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340   **3.6.Dietary fibre contents in legumes, seeds, vegetables, fruits, and mushrooms**

341   The analyzed total DF contents as fresh weights varied from 1.2 g/100 g in lamb´s lettuce to 36.3

342   g/100 g in whole chia seeds (Table 3). In addition to chia seeds, many other seeds, such as whole

343   linseeds and whole hempseeds contained substantial amounts of DF (35.0 g/100 g, fresh weight).

344   Although most seeds contained DF abundantly, peeled hemp seeds and peeled pine nuts are not

345   considered to be high fibre foods. The limits for nutritional claims "high in fibre" and "source of

346   fibre" are 6 g of fibre per 100 g and 3 g of fibre per 100 g, respectively (EC Regulation, 2006). Most

347   of the studied berries can be considered as good sources of fibre, as their DF content were 3.0-6.5

348   g/100 g (fresh weight). Generally, the largest component of DF was IDF in the analyzed foods, as

349   expected. The foods contained 58-95% IDF of the total DF, only in red onions less than that, as the

350   corresponding ratio was 17%. Proportionally the lowest amount of IDF was found in pine nuts and

351   the highest in imported tomatoes. The best sources for IDF were linseeds, chia seeds and hempseeds,

352   consumed as whole (ca. 30 g/100 g, fresh weight). The range of SDFP in foods was 4-37% of total

353   DF. Proportionally most SDFP was found in seaweed Nori, green apples, peeled hemp seeds, peeled

354   pine nuts and carrots. Absolut amounts for SDFP are the highest in seeds. Apart from red onion (4.4

355   g/100g, fresh weight), the oligosaccharides (SDFS) were only found in small amounts from seeds,

356   which contained 0.7-1.8 g/100 g (fresh weight) SDFS. Only traces (mostly under LOQ) of SDFS was

357   detected in other vegetables, fruits, and mushrooms.

358   Most of the results obtained by the method AOAC 2011.25 in this study are bigger than the values

359   presented in Finnish Food Composition Database Fineli earlier (Table 3). As big as two- or even

360   threefold differences were found in the total DF content in red onions, broad beans, green peas and

361   bananas. Red onions contained considerable amounts of oligosaccharides, which were not measured

362   by the earlier methods. Legumes are known to contain naturally occurring resistant starch types 1 and

363   2 (RS1, RS2), while green bananas are a source of RS2 (Fuentes-Zaragoza et al., 2010). The classical

364   analytical methods for DF are able to measure only retrograded starch/amylose (RS3), which is

365   formed when starch containing foods are cooked and cooled, and is predominant RS in most food

366   products. In addition to RS3, other RS types (RS1, RS2 and RS4) are also analyzed by the method

367   AOAC 2011.25 (Westenbrink et al., 2013). RS concentrations in legumes and unripe bananas are

368   high (mean 25% and 52%, respectively), which may partially explain the observed differences in their

369   DF content (Fuentes-Zaragoza et al., 2010). Brummer et al. (2014) reported that cooked pulses

370   contained 3.75-4.66 g/100 g resistant starch and 1.5-4.5 g/100 g non-digestible oligosaccharides

371   (raffinose, verbascose and stachyose) on a dry weight basis. The amount of oligosaccharides varied

372   also in the studied legumes with the highest content measured in dried green peas (2.45 g/100 g)

373   which is in the same range with the above mentioned study. Although the resistant starch was not

374   separately measured, it surely explains, together with oligosaccharides, at least partially the

375   differences in total DF contents between this study and Fineli database. The degree of ripeness of the

376   measured banana samples has a high impact on the amount of RS2, and hence on the total DF content.

377   Garcia-Amezquita et al. (2018) reported the DF content in banana as 26% higher by the method

378   AOAC 2011.25 compared to AOAC 991.43 due to presence of RS2. The ripeness of the banana

379   samples was not defined in this study, but it can be one of the influential factors together with the

380   method used, since the total DF content of bananas was analyzed to be 50% higher compared to the

381   previous value in Fineli database.

382   Other observed differences can partially be explained by the distinctions in the analyzed food

383   samples. Those are, e.g., the variation in plant genetics and growth-influencing aspects, such as soil

384   and weather conditions of the growing season. The influence of different analytical methods was

385   investigated in the article of McCleary et al. (2013), where the methods AOAC 985.29, 2009.01 and

386   2011.25 were compared. The method AOAC 985.29 has been widely used to analyze the total DF

387   content, and the AOAC 2009.01 method is an improved version of it, with considerable changes in

388   the analytical conditions (enzymes, temperatures). The difference between the new methods AOAC

389   2011.25 and 2009.01 is, that the former analyzes IDF, SDFP and SDFS separately, while the latter

390   gives the results as HMWDF (high molecular weight DF) and SDFS. According to McCleary et al.

391   (2013), the content of HMWDF in green bananas was 5.0 times bigger, and in ripe banana still 4.7

392   times higher measured by AOAC 2009.01 than by AOAC 985.29, representing the earlier mentioned

393   underestimation of RS by the older methods. In beans and peas, the total DF content was 16-21%

394   higher, measured by 2009.01 than 985.29. Carrots were analyzed using all three methods, and all the

395   results were almost identical (McCleary et al., 2013). The SDFS amounts of the present study are

396   well in line with the ones obtained by McCleary et al. (2013). The DF composition of the food

397   determinates the amount of the total DF analyzed by the various methods. Currently, the safest

398   solution is to use the methods AOAC 2009.01 and 2011.25 to ensure the analysis of all the

399   components. The total DF results are similar enough for one to choose either filtering technique,

400   manual or semi-automated.

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4. Conclusions

403   Two different filtering techniques, manual and semi-automated, were evaluated with respect to the

404   similarity of DF content measurements. Showing similarity calls for a different statistical technique

405   than testing of difference. The very definition of similarity requires pre-setting of tolerance, i.e., the

406   largest acceptable difference θ between the means. In problematic cases, where pre-setting of θ is not

407   possible, the tightest data induced limit, θ_{TDI}, is useful in evaluating the degree of similarity. It takes

408   the effects of sample size and noise into account, as any statistically valid method shall. Based on

409   similarity approach, it can be stated, that similarity of the techniques is adequate for using either one.

410   However, some foods, such as strawberries, may well behave aberrantly during filtration as several

411   DF components (such as pectin, hemicelluloses and lignin) and their countless combinations

412   presumably affect the filtration. Today, the mechanisms remain opaque and call for further research

413   on the topic. Finally, the DF content of legumes, seeds, vegetables, fruits and mushrooms was

414   analyzed by the method AOAC 2011.25 with varying filtering technique due to similarity evaluation

415   results above. The measured total DF results were mostly bigger than the previous values obtained

416   by the older methods, because when the method AOAC 2011.25 is used, e.g., oligosaccharides and

417   RS are included in DF as well, in contrast to the preceding methods.

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504   Table 1. Comparison of the manual and semi-automated technique in dietary fibre analysis.

Variable	Manual technique	Semi-automated technique
Instrumentation	Water-suction or air-driven vacuum pump	FOSS Fibertec™ 1023 connected to water-suction
Incubation bottles	250 mL Fisherbrand® soda glass bottle or similar	600 mL bottle with removable bottom (FOSS)
Fritted crucibles	Gooch, fritted disk, 50 mL, pore size coarse, ASTM 40-60 μm	Gooch, fritted disk, 30 mL, pore size coarse, ASTM 40-60 μm
Procedure if filter is clogged	Gentle manual stirring without breaking the Celite®-layer	Reversed pressure to open the pores resulting in mixing up of Celite® and the sample matrix
Filtering time/sample*	5-30 min	1-5 min
Sample processing	One by one	Six samples at a time

505   * Filtration times can be much longer if the samples form strong viscose solutions.

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516 Table 2. Similarity of the results of dietary fibre (DF) composition analysis based on the filtration techniques 'semi-automated' (Semi-auto) and 'manual' (Manu). Tightest

517 data-induced similarity limits θ_{TDI} corresponding to 5% risk in similarity testing are given for each matrix (see text). Means are expressed as g/100 g (dry weight).

	N		Mean		SD		SE		Mean difference (Semi-auto – Manu)	SE of difference	CI (90%) for difference	Tightest data-induced limit	
Matrix	Semi-auto	Manu	Semi-auto	Manu	Semi-auto	Manu	Semi-auto	Manu				θ_{TDI}	%[2]
Wheat flour													
Total DF	6	20	5.87	5.56	0.617	0.654	0.252	0.146	0.310	0.301	(-0.205, 0.825)	0.83	15
IDF			1.98	2.47	0.399	0.328	0.163	0.0734	-0.492	0.160	(-0.766, -0.217)	0.77	31[1]
SDFP			1.73	1.88	0.384	0.415	0.157	0.0929	-0.149	0.190	(-0.475, 0.177)	0.48	25
SDFS			2.16	1.21	0.229	0.402	0.0934	0.0898	0.946	0.173	(0.649, 1.24)	1.24	103[1]
Edible boletus													
Total DF	5	3	25.2	23.5	1.53	1.29	0.682	0.742	1.72	1.06	(-0.337, 3.78)	3.78	16
IDF			17.7	18.8	2.55	2.08	1.14	1.20	-1.04	1.75	(-4.45, 2.37)	4.45	24
SDFP			6.61	3.83	1.14	0.83	0.511	0.478	2.77	0.766	(1.28, 4.26)	4.26	111[1]
SDFS			0.866	0.880	0.0882	0.0173	0.0395	0.0100	-0.0140	0.0531	(-0.117, 0.0890)	0.12	13
Strawberry													
Total DF	6	3	20.9	23.3	3.96	1.98	1.62	1.14	-2.38	2.48	(-7.09, 2.32)	7.09	30
IDF			16.6	20.6	4.14	2.27	1.69	1.31	-4.02	2.62	(-8.98, 0.932)	8.98	44
SDFP			3.43	2.36	2.22	0.45	0.91	0.26	1.07	1.34	(-1.47, 3.61)	3.61	153
SDFS			0.873	0.300	0.456	0.125	0.186	0.0721	0.573	0.277	(0.0490, 1.10)	1.10	366
Tomato													
Total DF	6	20	21.0	21.7	1.52	2.17	0.622	0.485	-0.671	0.955	(-2.30, 0.963)	2.30	11
IDF			18.5	18.6	1.02	2.09	0.415	0.468	-0.133	0.893	(-1.66, 1.37)	1.66	9
SDFP			1.97	2.91	0.680	0.548	0.278	0.123	-0.945	0.269	(-2.17, -0.681)	2.17	48[1]
SDFS			0.543	0.132	0.100	0.247	0.0408	0.0553	0.412	0.105	(0.233, 0.591)	0.59	449[1]
Green pea													
Total DF	6	3	30.1	30.7	1.20	2.43	0.49	1.40	-0.643	1.16	(-2.85, 1.56)	2.85	9
IDF			27.4	26.4	0.684	2.51	0.279	1.45	0.945	1.03	(-1.01, 2.90)	2.90	11
SDFP			2.27	3.69	0.649	0.142	0.265	0.0818	-1.42	0.392	(-2.17, -0.681)	2.17	59[1]
SDFS			0.417	0.580	0.117	0.061	0.0478	0.0351	-0.163	0.0737	(-0.303, -0.0240)	0.30	52
Horseradish													
Total DF	7	3	23.8	25.8	1.01	0.120	0.381	0.0693	-2.07	0.604	(-3.19, -0.946)	3.19	12[1]
IDF			22.5	22.6	0.852	0.170	0.322	0.0984	-0.0695	0.512	(-1.02, 0.883)	1.02	5
SDFP			0.620	2.71	0.477	0.0737	0.180	0.0426	-2.09	0.286	(-2.62, -1.55)	2.62	97[1]
SDFS			0.611	0.530	0.100	0.0265	0.0380	0.0153	0.0814	0.0607	(-0.0310, 0.194)	0.19	37

518 [1] Significance in 'normal' t-test (p<0.05), where alternative hypothesis is 'the means differ'.

519 [2] Percentages are calculated with Manu-mean as reference.

520 N = amount of samples, SD = standard deviation, SE = standard error mean, CI = confidence interval

521 Table 3. The dietary fibre results from the Finnish vegetables, seeds, berries, mushrooms and fruits expressed as fresh
522 weights (g/100 g), n = 2-3 of each composite food sample. The values provided using earlier methods are presented in
523 the column 'Fineli', as they were obtained from the Finnish food composition database (Fineli).

524 DF = dietary fibre; IDF = water-insoluble DF; SDFP = DF soluble in water but precipitated by 78% aqueous ethanol (water-
525 soluble polysaccharides); SDFS = DF soluble in water and not precipitated by 78% aqueous ethanol (oligosaccharides)

Food	Moisture	IDF	SDFP	SDFS	Total DF	Fineli
Legumes						
Broad bean	69	8.61 ± 0.3	0.67 ± 0.1	0.70 ± 0.0	9.98 ± 0.2	4.2[1]
Common bean	88	3.63 ± 0.1	0.34 ± 0.0	<0.2	4.01 ± 0.1	2.4*
Dried green pea	13	15.4 ± 0.4	1.59 ± 0.1	2.45 ± 0.3	19.5 ± 0.6	20.0*
Green Pea	72	5.99 ± 0.0	1.27 ± 0.1	0.21 ± 0.0	7.47 ± 0.0	3.1*
Sugar pea	90	2.00 ± 0.1	0.23 ± 0.0	<0.2	2.23 ± 0.0	2.2[1]
Seeds						
Chia seed, whole	7.0	33.2 ± 2.1	1.54 ± 0.3	1.75 ± 0.2	36.3 ± 2.2	-
Hempseed, whole	7.1	30.2 ± 0.0	4.14 ± 0.2	0.71 ± 0.1	35.0 ± 0.3	27.6[2]
Hempseed, peeled	5.5	3.26 ± 0.2	1.58 ± 0.3	0.48 ± 0.0	5.40 ± 0.1	-
Linseed, whole	6.3	25.0 ± 0.6	4.78 ± 0.2	0.62 ± 0.0	30.4 ± 0.7	26.0[3]
Pine nut, peeled	1.4	3.19 ± 0.1	1.46 ± 0.0	0.77 ± 0.0	5.50 ± 0.0	3.7[1]
Poppy seed, whole	5.3	16.0 ± 0.7	2.28 ± 0.1	0.70 ± 0.1	19.0 ± 0.9	-
Pumpkin seed, peeled	5.8	5.93 ± 1.0	1.94 ± 0.1	1.40 ± 0.0	9.20 ± 1.0	6.0[1]
Sesame seed, whole	4.0	8.56 ± 0.0	2.42 ± 0.2	1.03 ± 0.0	12.0 ± 0.2	12.3[4]
Sesame seed, peeled	3.4	7.22 ± 0.2	1.55 ± 0.0	1.73 ± 0.1	10.4 ± 0.1	11.3*
Sunflower seed, peeled	4.4	9.51 ± 0.4	1.34 ± 0.5	0.72 ± 0.0	11.5 ± 0.8	6.0[4]
Vegetables						
Carrot	88	1.76 ± 0.1	0.61 ± 0.0	<0.2	2.37 ± 0.0	2.5[5]
Coriander	93	1.85 ± 0.3	0.46 ± 0.1	<0.2	2.31 ± 0.2	2.8[1]
Horseradish	75	5.97 ± 0.0	0.72 ± 0.0	<0.2	6.69 ± 0.0	7.5[6]
Lamb´s lettuce (corn salad)	96	0.97 ± 0.1	0.17 ± 0.0	<0.2	1.15 ± 0.1	1.8[4]
Lettuce	95	1.44 ± 0.0	0.16 ± 0.0	<0.2	1.60 ± 0.0	1.0[5]
Peashoot	92	2.16 ± 0.2	0.30 ± 0.0	<0.2	2.46 ± 0.2	1.6*
Radicchio	96	1.09 ± 0.0	0.20 ± 0.0	<0.2	1.29 ± 0.0	1.1[7]
Red onion	87	1.13 ± 0.0	0.85 ± 0.0	4.37 ± 0.1	6.35 ± 0.1	2.0[4]
Romaine lettuce	96	1.24 ± 0.1	0.17 ± 0.0	<0.2	1.41 ± 0.1	1.8[4]
Rucola (salad rocket)	94	1.76 ± 0.0	0.30 ± 0.0	<0.2	2.06 ± 0.0	1.6[1]
Tomato (domestic, winter)	94	1.11 ± 0.1	0.18 ± 0.0	<0.2	1.29 ± 0.1	1.4[5]
Tomato (imported)	92	2.42 ± 0.0	0.11 ± 0.0	<0.2	2.53 ± 0.0	1.4[5]
White radish (daikon)	95	1.21 ± 0.0	0.09 ± 0.0	<0.2	1.30 ± 0.0	1.6[1]
Fruits						
Apple (green), with skin	85	1.73 ± 0.0	0.68 ± 0.0	<0.2	2.41 ± 0.0	1.5[5]
Apple (green), pealed	86	1.21 ± 0.0	0.60 ± 0.0	<0.2	1.81 ± 0.0	1.8[8]
Apple (red), with skin	86	1.50 ± 0.0	0.18 ± 0.1	<0.2	1.68 ± 0.1	1.5[5]
Apple (red), pealed	85	1.26 ± 0.0	0.22 ± 0.1	<0.2	1.48 ± 0.1	1.8[8]
Banana, pealed	75	3.09 ± 0.2	0.30 ± 0.0	0.20 ± 0.1	3.59 ± 0.1	1.8[5]
Blackcurrant	76	5.44 ± 0.8	1.02 ± 0.1	<0.2	6.46 ± 0.8	5.8[5]
Blueberry	85	4.21 ± 0.1	0.25 ± 0.1	<0.2	4.46 ± 0.1	3.3[3]
Cloudberry	85	5.44 ± 0.3	0.30 ± 0.0	<0.2	5.74 ± 0.4	6.3*
Lingonberry	85	2.32 ± 0.2	0.63 ± 0.1	<0.2	2.95 ± 0.2	2.6[3]
Raspberry	87	3.92 ± 0.2	0.32 ± 0.1	<0.2	4.24 ± 0.2	3.8*
Strawberry	89	2.44 ± 0.3	0.28 ± 0.1	<0.2	2.76 ± 0.2	1.9[5]
Mushrooms						
Chanterelle	89	3.51 ± 0.2	0.31 ± 0.0	<0.2	3.82 ± 0.2	3.2[4]
Edible boletus	91	1.86 ± 0.2	0.38 ± 0.1	<0.2	2.24 ± 0.1	3.0*
Funnel chanterelle	90	4.20 ± 0.2	0.34 ± 0.1	<0.2	4.54 ± 0.2	3.2*
Northern milk-cap	95	2.43 ± 0.3	0.18 ± 0.0	<0.2	2.61 ± 0.3	1.5[9]
Other						
Seaweed Nori	6	21.9 ± 0.2	13.1 ± 0.4	<0.2	35.0 ± 0.2	44.4[6]

526

527   References behind the DF values in Fineli Food Composition Database (release 17): *value created within host-system,

528   [1]Borrowed value from USDA, [2]Callaway (2004), [3]Independent laboratory, [4]Borrowed value from Livsmedelsverket,

529   [5]Plaami et al. (1992), [6]McCance & Widdowson (1960), The composition of foods, [7]Danish Food Composition Databank,

530   [8]Moller & Saxholt (1996), [9]Mattila et al. (2002)

531   Limit of quantification (LOQ) for SDFS was 0.2 g/100 g (LOD 0.1 g/100 g), results under LOQ are expressed as <0.2.

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