



HAL
open science

Arabinoxylan content and grain tissue distribution are good predictors of the dietary fibre content and their nutritional properties in wheat products

Cecile Barron, Christine Bar-L'Helgouac'H, Martine Champ, Luc Saulnier

► To cite this version:

Cecile Barron, Christine Bar-L'Helgouac'H, Martine Champ, Luc Saulnier. Arabinoxylan content and grain tissue distribution are good predictors of the dietary fibre content and their nutritional properties in wheat products. *Food Chemistry*, 2020, 328, pp.127111. 10.1016/j.foodchem.2020.127111 . hal-02887253

HAL Id: hal-02887253

<https://hal.inrae.fr/hal-02887253>

Submitted on 11 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

1 **Arabinoxylan content and grain tissue distribution are good predictors of the dietary fibre**
2 **content and their nutritional properties in wheat products**

3

4 Cécile Barron^a, Christine Bar-L'Helgouac'h^b, Martine Champ^c, Luc Saulnier^{d*}

5

6 ^a IATE, Univ Montpellier, Cirad, INRAE, Institut Agro, 34060 Montpellier, France

7 ^b ARVALIS - Institut du végétal, Station expérimentale, 91720 Boigneville, France.

8 ^c INRA UMR 1280 PhAN – F-44000 Nantes, France

9 ^d INRA UR 1268 BIA – F-44360 Nantes, France.

10

11 **Abstract**

12 Wheat millstreams and wheat-based foods (pasta, biscuits and bread) enriched or not in dietary fibre
13 with fractions extracted from wheat grains, have been characterized either for their total dietary fibre
14 content (TDF) and their arabinoxylan (AX) content. A strong correlation ($r^2 = 0.98$) is observed
15 between the AX and TDF contents indicating that AX can be used to estimate TDF content in wheat
16 products. Moreover, by adding a previous step including enzymatic hydrolysis with a xylanase, a
17 functional evaluation of DF is proposed based on the amount of AX released by the enzyme. Xylanase
18 hydrolysable AX are likely also released by microbiota's enzymes in the gut and therefore an
19 indicator for the proportion of fermentable DF in grain fractions and wheat-based foods (pasta,
20 biscuits and bread). This assay opens the door for simple characterization of qualitative attribute of
21 cereal DF.

22

23 **Keywords:**

24 bread; biscuits; pasta; dietary fibre; enzymatic hydrolysis; xylanase

25

26 **Abbreviations**

27 AX: arabinoxylan; AX_{enz}: arabinoxylan released by xylanase; DF: dietary fibre; TDF: total dietary
28 fibre; HM_wDF: high molecular weight dietary fibre; IDF: Insoluble dietary fibre; LM_wSDF: low
29 molecular weight soluble dietary fibre; HM_wSDF: high molecular weight soluble dietary fibre

30

31 * Corresponding author; Email: luc.saulnier@inra.fr

32

1. Introduction

33
34 Epidemiological studies have shown that high dietary fibre (DF) diets and whole grain consumption
35 are associated with diminished risk of coronary heart disease, colon cancers, inflammatory bowel
36 disease, and metabolic syndrome (Stephen et al., 2017). Thus, health authorities around the world
37 recommend that adults consume 25 g to 35 g of fibre per day (Stephen et al., 2017). Bread, pasta and
38 non-bread product such as wheat flour tortillas are staple foods in many countries and wheat flour is
39 the main component of many food products such as pizzas, biscuits and cakes. Obviously, an increase
40 in the DF content of these wheat-based foods might help to better meet dietary recommendations for
41 DF intake. However, DF are a complex mixture of chemically heterogeneous carbohydrate
42 components, and the quantity and nature of DF in wheat-based foods vary, in particular, depending
43 on the “refining” of the flours (white flour vs whole grain flour) and the food manufacturing processes
44 that can affect for example the amount of resistant starch. Although some of the physicochemical
45 properties of DF (solubility, viscosity, bulking) are clearly related to important nutritional outcome
46 (McRorie & McKeown, 2017), the mechanisms by which the consumption of DF modulates health
47 are multiple and not fully elucidated. However, their role in maintaining diversity and stability of the
48 microbiota probably plays a decisive role in the regulation of the immune and metabolic systems and
49 therefore on health (Sonnenburg & Bäckhed, 2016). In this respect, a key feature of DF is their
50 fermentation pattern which is related to their chemical structure and to the ability of microorganisms
51 to produce the glycosyl-hydrolases allowing their degradation in the gut (Flint, Bayer, Rincon,
52 Lamed, & White, 2008).

53 As previously stated, DF are a complex mixture of chemically heterogeneous carbohydrate
54 components and their measurements have been based on sequential enzymatic-gravimetric assays
55 mimicking human digestion. These methods have undergone numerous evolutions (Macagnan, da
56 Silva, & Hecktheuer, 2016) but the AOAC method 985.29 (Prosky, Asp., Schweizer, DeVries, &
57 Furda, 1988) was widely used for the determination of total dietary fibre (TDF) in foods. Prosky’s
58 method distinguished soluble DF from insoluble DF but did not include the low-molecular-weight
59 non-digestible oligosaccharides and neglected part of the resistant starch. Integrated methods were
60 therefore developed for the determination of TDF, including non-digestible oligosaccharides and
61 subsequently resistant starch by adopting physiological conditions for the removal of starch. The
62 AOAC method 2009-01 (McCleary et al., 2010) and its extension the AOAC method 2011.25
63 (McCleary et al., 2012) measures the fraction of high molecular weight dietary fibre (HMwDF) by
64 enzymatic–gravimetric techniques, and low molecular weight soluble dietary fibre (LMwSDF) by
65 high-performance liquid chromatography (HPLC). HMwDF are actually formed of insoluble dietary

66 fibre (IDF) and high molecular weight soluble dietary fibre (HMwSDF); $HMwDF = IDF +$
67 $HMwSDF$. An evolution of the AOAC 2009.01 method (McCleary, 2014) was introduced to correct
68 an overestimation of the LMwSDF fraction in starch-rich foods and particularly in cereal-based foods
69 (Brunt & Sanders, 2013; McCleary, Sloane, & Draga, 2015). Despite their successive improvements,
70 these methods made of several steps are time-consuming and results obtained are still under debate
71 (Hell, Kneifel, Rosenau, & Böhmdorfer, 2014). Moreover they do not take into account the chemical
72 and physico-chemical diversities of DF that is far more complex than proposed by a classification in
73 $HMwDF/LMwSDF$ and insoluble/soluble fractions (Gidley & Yakubov, 2019).

74 In view of the diversity of DF and of their mechanisms of action, reliable method to quantify their
75 amount is crucial to characterize cereal products. Such method could help to monitor the development
76 of DF enriched wheat-based foods and could also help to better assess some of their important
77 nutritional property such as their fermentation ability. The wheat grain contains about 12–14% of DF
78 (Gebruers et al., 2008) but this complex organ is composed of different tissues composed of cell wall
79 polysaccharides exhibiting various properties and composition (Barron, Surget, & Rouau, 2007;
80 Saulnier, Guillon, & Chateigner-Boutin, 2012). In wheat grain, arabinoxylans (AX) are the major
81 polymers in the cell wall and therefore a major component of DF. In the starchy endosperm cell walls
82 represent 3 to 5% of the tissue and are essentially constituted by AX with a lower proportion of
83 (1,3)(1,4)-linked beta-d-glucans (Saulnier et al., 2012) and cellulose (Gartaula et al., 2018). In this
84 tissue, AX are partly water soluble and exhibit an arabinose to xylose ratio (A/X) of about 0.6. In the
85 most outer layers cell wall polysaccharides represent up to 50% of the tissue and are mainly composed
86 of cellulose and complex AX that are mostly water insoluble and exhibit a structure more heavily
87 substituted by arabinose than in starchy endosperm (A/X close to 1) (Antoine et al., 2003; Barron et
88 al., 2007; Parker, Ng, & Waldron, 2005). In addition, hydroxycinnamic acids that are ester-linked to
89 some of the arabinose side-chains of AX (Harris & Trethewey, 2010) play an important role for
90 polymer interactions and cell wall properties, as they can dimerize under oxidizing conditions and act
91 as bridging agent interconnecting AX chains together and with lignins (de Oliveira et al., 2015). The
92 susceptibility to enzyme attack is clearly related to AX structure (Bonnin et al., 2006) and is deeply
93 impacted by the interactions of AX within the cell wall. Outer tissues of the grain (pericarp) are not
94 degraded by pure microbial xylanases (Beaugrand, Crônier, Debeire, & Chabbert, 2004; Ordaz-Ortiz,
95 Devaux, & Saulnier, 2005), whereas AX from starchy endosperm or aleurone layer are extensively
96 hydrolysed by these enzymes (Ordaz-Ortiz et al., 2005). The resistance is mainly explained by the
97 covalent interactions through ferulic acid bridges between AX chains and with lignin. These
98 interconnections are much more numerous in the outer pericarp that is resistant to enzyme attack than

99 in cell walls of endosperm tissues (including aleurone layer) (Chateigner-Boutin et al., 2018; Saulnier
100 et al., 2012) that are degraded by microbial xylanases. Actually, the ability to be degraded by
101 microbial enzymes (e.g. microbiota) differs largely between isolated polymers and polymers as
102 present in the cell wall of the different tissues (Rose, Patterson, & Hamaker, 2010). The susceptibility
103 to enzyme degradation of the different tissues of the grain has potentially important health impact as
104 isolated AX polymers and arabino-xylo-oligosaccharides (AXOS) have been shown to favour the
105 development of beneficial bacteria (Broekaert et al., 2011; Neyrinck et al., 2011) with an increase in
106 Bifidobacteria/Lactobacilli as well as in Bacteroides/Prevotella and Roseburia groups (Despres et al.,
107 2016)

108 In this work, the tissue composition of different millstreams has been measured (Hemery et al.,
109 2009) and compared to their total DF amount measured with the AOAC 2009-01 method.
110 Assuming that the most peripheral tissues are mainly constituted of DF, the tissue composition
111 could be used to estimate the total DF content in millstreams. However, such approach could not be
112 extended to wheat-based foods. Therefore, knowing that AX are the main components of cell walls
113 in the different tissue of the wheat grain, biochemical determination of AX content has been
114 investigated as a possible predictor of DF content. In addition, adding an enzymatic hydrolysis step
115 with a xylanase and quantifying the amount of AX released was carried out in order to evaluate the
116 potential fermentation capacity of various wheat millstreams.

117 2. Material and Methods

118 2.1 *Wheat grains and milling fractions*

119 Different millstreams were produced from bread wheat (Caphorn cv; *Triticum aestivum* L.) and
120 durum wheat (Miradoux cv; *Triticum durum* Desf). Fractions were obtained from conventional
121 milling technology with specific diagrams to bread wheat in ENILIA-ENSMIC pilot mill (Surgères,
122 France) or durum wheat in UMR IATE pilot plant (Montpellier, France). In addition, a PeriTech
123 debranner (Satake, UK) was used to obtain debranning fractions differing in their extraction rate.
124 Two specific fractions named F⁻ and F⁺ were also produced from Caphorn grains. Fraction F⁻
125 corresponded to a debranning level of 3.8% in weight of the grain, and fraction F⁺ was subsequently
126 isolated at a higher debranning level (recovered between 6.4% and 12.9% in weight of the grain). The
127 other debranning fractions (A to J) from both bread and durum wheats were also produced with this
128 equipment. Different debranning levels and successive debranning steps were used in order to obtain
129 fractions differing in their outer tissues content.

130 2.2 *Wheat-based foods*

131 Bread, pasta and biscuit were produced with white flour (regular product) or with flour enriched in
132 DF by incorporating F⁻ and F⁺ fractions.

133 Délifrance provided breads produced on an industrial line in Marquette-lez-Lille (France). Regular
134 bread (R-bread) was made with white flour. The same flour that incorporated fraction F⁻ (20.5%
135 weight basis) or fraction F⁺ (50.8% weight basis) was used for F⁻-bread and F⁺-bread, respectively.
136 Precooked breads (55g) were used for analysis.

137 Mondelez provided biscuits produced on a pilot line at its R & D centre (Saclay, France). Regular
138 biscuits (R-Biscuit) contained fat (15%), sugars (23%) and the rest of the formula was completed
139 with cereals (2/3 wheat flour and 1/3 oat flakes) or incorporated fraction F⁻ (10.5%) or fraction F⁺
140 (27.1%) completed with cereals for F⁻-biscuit and F⁺-biscuit, respectively.

141 Pasta were provided by Panzani. Pasta was made from 100% durum wheat semolina for regular
142 product (R-pasta) and incorporated fraction F⁻ (9%) or fraction F⁺ (25.9%) for F⁻-pasta and F⁺-pasta,
143 respectively.

144 *2.3 DF content and biochemical characterization*

145 DF content in the different samples was determined according to the AOAC 2009-01 method
146 (McCleary et al., 2010) on a dry matter basis. High molecular weight DF (soluble and insoluble
147 fractions) are reported as HMwDF and low molecular weight soluble DF as LMwSDF, and TDF =
148 HMwDF + LMwSDF. In addition, resistant starch was measured in wheat-based foods according to
149 AOAC method 2002-02. In food, the relative maximum deviation between duplicates was 14%, 30%
150 and 16% for HMwDF, LMwSDF and TDF, respectively. In wheat fractions coefficient of variation
151 between duplicates was 2%, 7% and 3% for HMwDF, LMwSDF and TDF, respectively.

152 The AX content was calculated from neutral sugar analysis. Prior to neutral sugar analysis or
153 enzymatic degradation, samples were ground in a Freezer/Mill 6700 cryogenic grinder (Spex, USA)
154 then 0.8 g were treated with 5 mL of boiling 80% (w/v) aqueous EtOH for 10 min and the insoluble
155 residue separated by centrifugation (6300 g, 10 min). The procedure was repeated once and the
156 residue finally washed with 5 mL of 95% (v/v) EtOH and again separated by centrifugation (6300 g,
157 10 min). The supernatant was discarded and the residue dried in two steps: first in an oven at 40°C
158 for 24 h and then in a vacuum-oven over P₂O₅ at 40°C for 24 h.

159 The neutral sugar composition of samples was identified, after acid hydrolysis, using gas
160 chromatography of alditol acetates (Englyst & Cummings, 1988). Acid hydrolysis of samples was
161 carried out as previously described (L Saulnier, Marot, Chanliaud, & Thibault, 1995): the samples

162 were pre-hydrolysed with 72% (w/w; 26 M) sulphuric acid for 30 min at 25°C and then hydrolysed
163 into monomers at 100°C for 2 h in 1 M sulphuric acid. Analyses were performed in duplicate and AX
164 content (AX_{tot}) was calculated by summing the amount of arabinose and xylose. The relative
165 maximum deviation between duplicates was 3% or less.

166 Samples (100 mg) were incubated in water (1 mL) with an endoxylanase (EC 3.2.1.8) from
167 *Trichoderma viride* (Xylanase M1, Megazyme, Ireland) at 40°C for 16 h under continuous stirring.
168 Xylanase amount (2.4 U/mg of AX) was adjusted according to the amount of AX determined in the
169 sample (Ordaz-Ortiz et al., 2005). The reaction mixture was centrifuged (6300 g, 10 min) and 0.7 mL
170 of supernatant was removed and boiled for 10 min to inactivate the enzymes. Neutral sugar content
171 in the supernatant was identified after acid hydrolysis (1 M H₂SO₄, 100°C, 2 h) using gas
172 chromatography of alditol acetates (Englyst & Cummings, 1988). Analyses were performed in
173 duplicate and AX amount released by the enzyme (AX_{enz}) was calculated by summing the amount of
174 arabinose and xylose in the supernatant and expressed as weight % of the starting sample. Coefficient
175 of variations between duplicates were 3% or less.

176 *2.4 Tissue composition*

177 Tissue composition was assessed by the biochemical markers methodology as previously described
178 (Hemery et al., 2009). Reference values were obtained from the chemical analysis of pure dissected
179 tissues except for starch amount in starchy endosperm, which was deduced from starch amount in
180 high purity flour. For Miradoux cultivar, separation between the outer pericarp and the so-called
181 “intermediate layer” could not be achieved by hand dissection. Therefore, both tissues were
182 monitored at the same time by looking at the amount of alkylresorcinols.

183 **3. Results & discussion**

184 *3.1 Determination of DF contents in wheat products*

185 The TDF content was measured by the AOAC 2009-01 method and the amounts of HMwDF and
186 LMwSDF are reported in Tables 1 and 2. TDF contents were determined in wheat-based foods (pasta,
187 biscuit, bread) made from white wheat flour and enriched in DF with specific wheat milling fractions
188 (Fractions F+ and F-) and over a wide range of wheat milling fractions obtained by classical milling
189 and by debranning. This set of samples provided a large range of TDF contents ranging from 3% up
190 to 70% in milling fractions. Within wheat-based foods (pasta, biscuits and bread) this range was only
191 3–15%. The higher contents (TDF > 50%) were obtained for bran and peeling fractions from common
192 wheat and durum wheat that were especially rich in HMwDF. For milling fractions the variation
193 coefficient of HMwDF values (70%) was much higher than for LMwSDF (32%), whereas the

194 variation coefficients were close for HM_wDF and LM_wSDF in wheat-based foods, 45% and 35%,
195 respectively.

196 In the literature most of TDF content were obtained by the AOAC 985-29 method that essentially
197 correspond to the HM_wDF fraction determined with the AOAC 2009-01 method (Stephen et al.,
198 2017), although resistant starch was not fully measured by the AOAC 985-29 method. Resistant
199 starch content was low in our wheat-based foods (Table 1) and was not measured in the milling
200 fractions, as it is known to be very low in unprocessed cereal samples.

201 TDF values were linearly related to HM_wDF: $TDF_{AOAC\ 2009-01} = 0.989\ HM_{w}DF + 3.26$ and very highly
202 correlated ($r^2 = 0.997$) (Figure 1). The value of the intercept is close to the average value of LM_wSDF
203 calculated from the complete set of samples (3.0%). The measurement of the LM_wSDF fraction has
204 been subjected to debate and an evolution of the AOAC 2009.01 method has been proposed to correct
205 an overestimation of the LM_wSDF fraction in starch-rich food (Brunt & Sanders, 2013; McCleary et
206 al., 2015). Using only the data from the wheat-based foods (Table 1; the richer in starch and with
207 overall lower TDF content) the intercept value of the relationship (e.g average LM_wSDF content) was
208 2.06 while this value went up to 4.05 when only data from millstreams were used (Table 2.
209 Supplementary Figure S1). Slopes and correlation coefficients of the relationships were not changed
210 compared with the complete data set. In millstream fractions, starch content varied a lot compared to
211 wheat-based foods, but the higher value of the intercept is not originating from an overestimation of
212 the LM_wSDF fraction due to starch. It is more likely due to the presence of fructans that represents 2
213 to 4% of wheat grain. These components are more abundant in bran (Haskå, Nyman, & Andersson,
214 2008; Verspreet, Dornez, Delcour, Harrison, & Courtin, 2015) than in white flour. Knowing that most
215 of the data found in the literature were obtained with the AOAC 985-29 method corresponding to the
216 values of HM_wDF in this study, the TDF contents were in good agreement with values reported
217 previously for similar wheat products and millstream fractions (Elleuch et al., 2011; Gebruers et al.,
218 2008). Nevertheless values determined for fine/coarse bran were in a high range.

219

220 *3.2 Dietary fiber content vs Arabinoxylans content*

221 DF are a complex mixture of chemically heterogeneous carbohydrate components. In wheat grains
222 arabinoxylans (AX) are the main polymers of the cell walls and therefore the main component of DF
223 in wheat grain. Total AX contents varied on a quite large range between 1% and 30%, with similar
224 trends within wheat product and millstream fractions as reported above for their TDF contents. Total
225 AX content of wheat grain, and flour were comparable to previous reports (Barron et al., 2007;

226 Gebruers et al., 2008; Henry, 1987; L Saulnier, Peneau, & Thibault, 1995) but again values for
227 coarse/fine brans were in a high range compared to literature.

228 The relationship between total AX content in the samples and TDF (or HM_wDF) content is shown on
229 Figure 2. A strong linear relationship ($r^2 > 0.97$) was established with nearly the same slope close to
230 2 between AX and TDF ($TDF = 2.15AX + 2.61$; $r^2 = 0.975$) or AX and HM_wDF ($HM_{w}DF = 2.17AX -$
231 0.57 ; $r^2 = 0.970$). Using only the data from wheat-based foods (Table 1) or milling fractions (Table 2)
232 had very little impact on the values of the slope, intercept or correlation coefficient of these equations
233 (See supplementary Figure S2). Clearly, AX content corresponds to the HM_wDF part of TDF. It is
234 remarkable to note that, despite differences in AX structure and cell wall architecture within the
235 different tissues of wheat grain, the AX content (simply measured as the sum of arabinose and xylose
236 in the sample) accurately reflects the TDF contents of very different wheat products. Similar
237 relationship between AX content of wheat samples and their dietary fibre content was previously
238 reported: $TDF_{Englyst} = 1.75AX - 0.71$ ($r^2 = 0.994$; 51 wheat samples)(Bell, 1985). In Bell's equation
239 TDF content was determined by Englyst's method, based on the determination of the non starch
240 polysaccharides and lignin content in the sample (Englyst, Wiggins, & Cummings, 1982). The use
241 of a different method for evaluating TDF content likely explains the differences in slope between the
242 equation obtained in this work and by Bell (Bell, 1985).

243 *3.3 Dietary fiber content and tissue distribution*

244 In millstreams, tissue composition was assessed through the biochemical marker methodology
245 (Hemery et al., 2009) (Table 2). Pericarp (outer and inner) is mainly constituted of secondary cell
246 walls (Chateigner-Boutin et al., 2018). The testa is also constituted of secondary cell walls surrounded
247 by a thick cuticular layer, whereas the nucellar epidermis is made of thin cuticular layer, and a tiny
248 layer of low substituted arabinoxylans (Barron et al., 2007). According to the dietary fibre definition,
249 roughly all of these tissues, recovered in the outer pericarp and the so-called "intermediate layer",
250 could be considered as dietary fibres. In the case of the endosperm tissues (the aleurone layer and the
251 starchy endosperm), the TDF content could be approximated by the amount of cell walls in these
252 tissues. In the aleurone layer approximately 45% of the dry mass is recovered in cell walls (Hemery
253 et al., 2009). Using the overall AX content in starchy endosperm or flour (around 2%) (Barron et al.,
254 2007; Gebruers et al., 2008) and the proportion of AX in starchy endosperm cell wall, the amount of
255 cell wall in this tissue was assumed to be 3%. TDF (expressed according to the dry matter) was
256 calculated from tissue composition considering the following equation assuming equal distribution
257 of water within the whole grain tissues:

$$258 \text{ TDF (\% dm)} = \%OP + \%IL + 0.45*\%AL + 0.03*\%SE$$

259 The calculated TDF was linearly correlated ($r^2=0.87$) to the measured TDF with the following
260 relationship $TDF_{\text{calculated}}=1.07 \times TDF_{\text{measured}} + 0.25$ (Figure 3). Regardless of the type of wheat or the
261 millstreams a unique relationship was obtained in the whole TDF range (4-60%).
262 For a similar TDF content, variation in the proportion of the different grain tissue between milling
263 fractions may affect their degradation by microbial enzymes and therefore their fermentation pattern
264 e.g. aleuronic layer is easily degraded whereas the most outer layers of the grain are resistant to
265 microbial attack (e.g. microbiota).

266 *3.4 AX solubilisation by xylanase in relation with DF content and tissue distribution*

267 The susceptibility to enzyme degradation of the different tissues of the grain has potentially important
268 health impact as isolated AX polymers and AXOS are known to favour the development of beneficial
269 bacteria (Broekaert et al., 2011; Neyrinck et al., 2011). Enzymatic hydrolysis with a xylanase is
270 therefore proposed as a functional evaluation of DF in wheat fractions.

271 The amount of AX released by xylanase (AX_{enz}) is reported in Table 1 and 2 and relationship with
272 AX content is displayed on Figure 4. The amount of AX_{enz} ranged from 0.7% up to 10% and was
273 poorly related to the total amount of AX ($r^2= 0.64$; Figure 4B). Especially samples with a high AX
274 content ($AX>25\%$) exhibited clearly large differences in the amount of AX_{enz} . Figure 4A shows that
275 the proportion of AX_{enz} is globally negatively correlated with AX (TDF) content ($r^2=0.57$) e.g. the
276 extent of AX solubilisation is lower for brans (see table 2; Figure 4A) and the peeling fractions and
277 the highest for pure starchy endosperm fraction (flour T55 or semolina S3; see table 2). As expected,
278 a better linear relationship ($r^2=0.73$) was observed between the proportion of starchy endosperm and
279 aleurone layer in a fraction and the proportion of AX released by the enzyme (see Figure S3).
280 Conversely, fractions very rich in outer pericarp tissue are little degraded by enzymes (e.g. fraction
281 F⁻, debranning fraction A of common wheat). Therefore, the amount of AX solubilized from different
282 fractions by xylanase treatment may be similar although these fractions may have very different AX
283 (TDF) contents (e.g. F⁻ fraction vs. F⁺ fraction). In other words, the fermentation ability of wheat
284 fractions is not simply related to AX/DF content and fractions with a high AX/DF content that have
285 a lower degree of solubilization by xylanase may have more limited prebiotic effects. Such an assay
286 can help to better understand the nutritional effects of wheat DF, which is not only related to their
287 quantity. The ability of a fraction to modulate the microbiota is probably related to its fermentability
288 (assessed by degradation with xylanase), but other characteristics such as particle size can also affect
289 the microbiota, as recently demonstrated on wheat bran in a mouse model (Suriano et al., 2018).

290

4. Conclusion

291
292 In conclusion AX content can be used to estimate TDF content in wheat milling fractions and wheat-
293 based foods. Moreover, the amount of AX released by xylanase is proposed as a functional evaluation
294 of DF. AX solubilized by xylanase (AX_{enz}) are also potentially releasable by microbiota enzymes in
295 the intestine and the AX_{enz} assay therefore indicates the proportion of fermentable DF in wheat
296 milling fraction or in wheat-based foods. This assay provides a simple characterization of an
297 important functional attribute of DF and could help to better understand the nutritional effects of
298 different wheat milling fractions. The equation developed in this work is only valid for wheat products
299 and cannot be applied to other cereal products such as corn, rye, barley, oat or rice... However we
300 anticipate that the AX content could probably be used for the estimation of DF content in rye products,
301 provided that an appropriate equation is developed. Indeed, in rye as in wheat, AX is the main
302 component of cell walls and DF. The high amount of mixed linked-glucan (MLG) in oat and barley
303 and the heterogeneity of the MLG distribution in the different parts of the grain are not favourable to
304 the development of equations based on AX content to estimate the DF content, in these cereals.

305

Acknowledgments

306 This work was funded by ANR: ANR-ALIA 2010 WHEAFI project.
307 The authors thank S. Daniel (BIA Nantes) for technical assistance in measuring AX content and
308 performing xylanase degradation on the various wheat products. They also thank K. Mouacha, A.
309 Putois and T-M Lasserre (IATE Montpellier) for tissue composition analysis and G. Maraval (IATE
310 Montpellier) for durum wheat mill streams production.

312

References

- 313
314 Antoine, C., Peyron, S., Mabille, F., Lapierre, C., Bouchet, B., Abecassis, J., & Rouau, X. (2003).
315 Individual Contribution of Grain Outer Layers and Their Cell Wall Structure to the Mechanical
316 Properties of Wheat Bran. *Journal of Agricultural and Food Chemistry*, 51(7), 2026–2033.
317 <https://doi.org/10.1021/jf0261598>
318 Barron, C., Surget, A., & Rouau, X. (2007). Relative amounts of tissues in mature wheat (*Triticum*
319 *aestivum* L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal*
320 *Science*, 45(1), 88–96. <https://doi.org/10.1016/j.jcs.2006.07.004>
321 Beaugrand, J., Crônier, D., Debeire, P., & Chabbert, B. (2004). Arabinoxylan and
322 hydroxycinnamate content of wheat bran in relation to endoxylanase susceptibility. *Journal of*
323 *Cereal Science*, 40(3), 223–230. <https://doi.org/10.1016/j.jcs.2004.05.003>

- 324 Bell, B. M. (1985). A rapid method of dietary fibre estimation in wheat products. *Journal of the*
325 *Science of Food and Agriculture*, 36(9), 815–821. <https://doi.org/10.1002/jsfa.2740360910>
- 326 Bonnin, E., Daviet, S., Sorensen, J. F., Sibbesen, O., Goldson, A., Juge, N., & Saulnier, L. (2006).
327 Behaviour of family 10 and 11 xylanases towards arabinoxylans with varying structure.
328 *Journal of the Science of Food and Agriculture*, 86(11), 1618–1622.
329 <https://doi.org/10.1002/jsfa.2501>
- 330 Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W., & Delcour, J. A.
331 (2011). Prebiotic and Other Health-Related Effects of Cereal-Derived Arabinoxylans,
332 Arabinoxylan-Oligosaccharides, and Xylooligosaccharides. *Critical Reviews in Food Science*
333 *and Nutrition*, 51(2), 178–194. <https://doi.org/10.1080/10408390903044768>
- 334 Brunt, K., & Sanders, P. (2013). Improvement of the AOAC 2009.01 total dietary fibre method for
335 bread and other high starch containing matrices. *Food Chemistry*, 140(3), 574–580.
336 <https://doi.org/http://dx.doi.org/10.1016/j.foodchem.2012.10.109>
- 337 Chateigner-Boutin, A.-L., Lapierre, C., Alvarado, C., Yoshinaga, A., Barron, C., Bouchet, B., ...
338 Guillon, F. (2018). Ferulate and lignin cross-links increase in cell walls of wheat grain outer
339 layers during late development. *Plant Science*, 276, 199–207.
340 <https://doi.org/10.1016/j.plantsci.2018.08.022>
- 341 de Oliveira, D. M., Finger-Teixeira, A., Rodrigues Mota, T., Salvador, V. H., Moreira-Vilar, F. C.
342 F. C., Correa Molinari, H. B., ... Dantas dos Santos, W. (2015). Ferulic acid: A key
343 component in grass lignocellulose recalcitrance to hydrolysis. *Plant Biotechnology Journal*,
344 13(9), 1224–1232. <https://doi.org/10.1111/pbi.12292>
- 345 Despres, J., Forano, E., Lepercq, P., Comtet-Marre, S., Jubelin, G., Chambon, C., ... Mosoni, P.
346 (2016). Xylan degradation by the human gut *Bacteroides xylanisolvens* XB1AT involves two
347 distinct gene clusters that are linked at the transcriptional level. *BMC Genomics*, 17(1), 326.
348 <https://doi.org/10.1186/s12864-016-2680-8>
- 349 Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre
350 and fibre-rich by-products of food processing: Characterisation, technological functionality
351 and commercial applications: A review. *Food Chemistry*, 124(2), 411–421.
352 <https://doi.org/10.1016/j.foodchem.2010.06.077>
- 353 Englyst, H. N., & Cummings, J. H. (1988). Improved method for measurement of dietary fiber as
354 non-starch polysaccharides in plant foods. *Journal - Association of Official Analytical*
355 *Chemists*, 71(4), 808–814.
- 356 Englyst, H. N., Wiggins, H. S., & Cummings, J. H. (1982). Determination of the non-starch
357 polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol

358 acetates. *Analyst*, 107(1272), 307-318.

359 Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R., & White, B. A. (2008). Polysaccharide
360 utilization by gut bacteria: potential for new insights from genomic analysis. *Nature Reviews*
361 *Microbiology*, 6(2), 121–131. <https://doi.org/10.1038/nrmicro1817>

362 Gartaula, G., Dhital, S., Netzel, G., Flanagan, B. M., Yakubov, G. E., Beahan, C. T., ... Gidley, M.
363 J. (2018). Quantitative structural organisation model for wheat endosperm cell walls: Cellulose
364 as an important constituent. *Carbohydrate Polymers*, 196(May), 199–208.
365 <https://doi.org/10.1016/j.carbpol.2018.05.041>

366 Gebruers, K., Dornez, E., Boros, D., Fraś, A., Dynkowska, W., Bedo, Z., ... Courtin, C. M. (2008).
367 Variation in the Content of Dietary Fiber and Components Thereof in Wheats in the
368 HEALTHGRAIN Diversity Screen. *Journal of Agricultural and Food Chemistry*, 56(21),
369 9740–9749. <https://doi.org/10.1021/jf800975w>

370 Gidley, M. J., & Yakubov, G. E. (2019). Functional categorisation of dietary fibre in foods: Beyond
371 ‘soluble’ vs ‘insoluble.’ *Trends in Food Science & Technology*, 86(December 2018), 563–568.
372 <https://doi.org/10.1016/j.tifs.2018.12.006>

373 Harris, P., & Trethewey, J. K. (2010). The distribution of ester-linked ferulic acid in the cell walls
374 of angiosperms. *Phytochemistry Reviews*, 9(1), 19–33. [https://doi.org/10.1007/s11101-009-](https://doi.org/10.1007/s11101-009-9146-4)
375 9146-4

376 Haskå, L., Nyman, M., & Andersson, R. (2008). Distribution and characterisation of fructan in
377 wheat milling fractions. *Journal of Cereal Science*, 48(3), 768–774.
378 <https://doi.org/10.1016/j.jcs.2008.05.002>

379 Hell, J., Kneifel, W., Rosenau, T., & Böhmendorfer, S. (2014). Analytical techniques for the
380 elucidation of wheat bran constituents and their structural features with emphasis on dietary
381 fiber – A review. *Trends in Food Science & Technology*, 35(2), 102–113.
382 <https://doi.org/10.1016/j.tifs.2013.10.012>

383 Hemery, Y., Lullien-Pellerin, V., Rouau, X., Abecassis, J., Samson, M.-F., Åman, P., ... Barron, C.
384 (2009). Biochemical markers: Efficient tools for the assessment of wheat grain tissue
385 proportions in milling fractions. *Journal of Cereal Science*, 49(1), 55–64.
386 <https://doi.org/10.1016/j.jcs.2008.07.006>

387 Henry, R. J. (1987). Pentosan and (1 → 3),(1 → 4)-β-Glucan concentrations in endosperm and
388 wholegrain of wheat, barley, oats and rye. *Journal of Cereal Science*, 6(3), 253–258.
389 [https://doi.org/http://dx.doi.org/10.1016/S0733-5210\(87\)80062-0](https://doi.org/http://dx.doi.org/10.1016/S0733-5210(87)80062-0)

390 Macagnan, F. T., da Silva, L. P., & Hecktheuer, L. H. (2016). Dietary fibre: The scientific search
391 for an ideal definition and methodology of analysis, and its physiological importance as a

392 carrier of bioactive compounds. *Food Research International*, 85, 144–154.
393 <https://doi.org/10.1016/j.foodres.2016.04.032>

394 McCleary, B. V. (2014). Modification to AOAC Official Methods 2009.01 and 2011.25 to Allow
395 for Minor Overestimation of Low Molecular Weight Soluble Dietary Fiber in Samples
396 Containing Starch. *Journal of AOAC International*, 97(3), 896–901.
397 <https://doi.org/10.5740/jaoacint.13-406>

398 McCleary, B. V., De Vries, J., Rader, J., Cohen, G., Prosky, L., Mugford, D., ... Okuma, K. (2010).
399 Determination of Total Dietary Fiber (CODEX Definition) by Enzymatic-Gravimetric Method
400 and Liquid Chromatography: Collaborative Study. *Journal of AOAC International*, 93(1),
401 221–233.

402 McCleary, B. V., DeVries, J. W., Rader, J. I., Cohen, G., Prosky, L., Mugford, D. C., ... Okuma, K.
403 (2012). Determination of Insoluble, Soluble, and Total Dietary Fiber (CODEX Definition) by
404 Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study. *Journal of*
405 *AOAC International*, 95(3), 824–844. https://doi.org/10.5740/jaoacint.CS2011_25

406 McCleary, B. V., Sloane, N., & Draga, A. (2015). Determination of total dietary fibre and available
407 carbohydrates: A rapid integrated procedure that simulates in vivo digestion. *Starch - Stärke*,
408 67(9–10), 860–883. <https://doi.org/10.1002/star.201500017>

409 McRorie, J. W., & McKeown, N. M. (2017). Understanding the Physics of Functional Fibers in the
410 Gastrointestinal Tract: An Evidence-Based Approach to Resolving Enduring Misconceptions
411 about Insoluble and Soluble Fiber. *Journal of the Academy of Nutrition and Dietetics*, 117(2),
412 251–264. <https://doi.org/10.1016/j.jand.2016.09.021>

413 Neyrinck, A. M., Possemiers, S., Druart, C. C., van de Wiele, T., de Backer, F., Cani, P. D., ...
414 Delzenne, N. M. (2011). Prebiotic effects of wheat Arabinoxylan related to the increase in
415 bifidobacteria, roseburia and bacteroides/prevotella in diet-induced obese mice. *PLoS ONE*,
416 6(6), e20944. <https://doi.org/10.1371/journal.pone.0020944>

417 Ordaz-Ortiz, J. J., Devaux, M.-F., & Saulnier, L. (2005). Classification of Wheat Varieties Based on
418 Structural Features of Arabinoxylans As Revealed by Endoxylanase Treatment of Flour and
419 Grain. *Journal of Agricultural and Food Chemistry*, 53(21), 8349–8356.
420 <https://doi.org/10.1021/jf050755v>

421 Parker, M. L., Ng, A., & Waldron, K. W. (2005). The phenolic acid and polysaccharide com-
422 position of cell walls of bran layers of mature wheat (*Triticum aestivum* L . cv . Avalon)
423 grains, 2547(December 2004), 2539–2547. <https://doi.org/10.1002/jsfa.2304>

424 Prosky, L., Asp, N.-G., Schweizer, T. F., DeVries, J. W., & Furda, I. (1988). Determination of
425 insoluble, soluble, and total dietary fibre in foods and food products. *J.Assoc. Off.Anal.*

426 *Chem.*, 71, 1017.

427 Rose, D. J., Patterson, J. A., & Hamaker, B. R. (2010). Structural differences among alkali-soluble
428 arabinoxylans from Maize (*Zea mays*), Rice (*Oryza sativa*), and Wheat (*Triticum aestivum*)
429 brans influence human fecal fermentation profiles. *Journal of Agricultural and Food*
430 *Chemistry*, 58(1), 493–499. <https://doi.org/10.1021/jf9020416>

431 Saulnier, L, Marot, C., Chanliaud, E., & Thibault, J. F. (1995). Cell-Wall Polysaccharide
432 Interactions in Maize Bran. *Carbohydrate Polymers*, 26(4), 279–287.

433 Saulnier, L, Peneau, N., & Thibault, J.-F. (1995). Variability in grain extract viscosity and water-
434 soluble arabinoxylan content in wheat. *Journal of Cereal Science*, 22(3), 259–264.
435 <https://doi.org/10.1006/jcrs.1995.0062>

436 Saulnier, Luc, Guillon, F., & Chateigner-Boutin, A.-L. (2012). Cell wall deposition and metabolism
437 in wheat grain. *Journal of Cereal Science*, 56(1), 91–108.
438 <https://doi.org/10.1016/j.jcs.2012.02.010>

439 Sonnenburg, J. L., & Bäckhed, F. (2016). Diet–microbiota interactions as moderators of human
440 metabolism. *Nature*, 535(7610), 56–64. <https://doi.org/10.1038/nature18846>

441 Stephen, A. M., Champ, M. M.-J., Cloran, S. J., Fleith, M., van Lieshout, L., Mejbourn, H., &
442 Burley, V. J. (2017). Dietary fibre in Europe: current state of knowledge on definitions,
443 sources, recommendations, intakes and relationships to health. *Nutrition Research Reviews*, 1–
444 42. <https://doi.org/10.1017/S095442241700004X>

445 Suriano, F., Neyrinck, A. M., Verspreet, J., Olivares, M., Leclercq, S., Van de Wiele, T., ...
446 Delzenne, N. M. (2018). Particle size determines the anti-inflammatory effect of wheat bran in
447 a model of fructose over-consumption: Implication of the gut microbiota. *Journal of*
448 *Functional Foods*, 41(October 2017), 155–162. <https://doi.org/10.1016/j.jff.2017.12.035>

449 Verspreet, J., Dornez, E., Delcour, J. A., Harrison, S. J., & Courtin, C. M. (2015). Purification of
450 wheat grain fructans from wheat bran. *Journal of Cereal Science*, 65, 57–59.
451 <https://doi.org/http://dx.doi.org/10.1016/j.jcs.2015.06.013>

452

453 **Figure Captions**

454 Figure 1: TDF related to HM_wDF contents in the wheat products (Data table 1 and 2)

455

456 Figure 2: **A**) TDF content and **B**) HM_wDF content related to AX content in the wheat products
457 (Data table 1 and 2)

458

459 Figure 3: TDF content calculated from tissue composition (by the following equation $TDF = \%OP +$
460 $\%IL + 0.45x\%AL + 0.03x\%SE$) related to TDF content measured by the AOAC 2009-01 method
461 (expressed according to the dry matter) from milling fractions. .

462 %OP: proportion of outer pericarp , %IL: proportion of intermediate layer , %AL: proportion of aleurone layer , %SE:
463 proportion of starchy endosperm.

464 Figure 4: **A**) AX amount released by xylanase (AX_{enz}) and **B**) proportion of AX released by
465 xylanase (% AX_{sol}) related to AX content in the wheat products (Data table 1 and 2)

466

467 **Supplementary figures**

468 Supplementary figure S1: TDF content related to HM_wDF content **A**) in the wheat-based foods
469 (bread, pasta and biscuits; data table 1) **B**) in the milling fractions (Data table 2)

470

471 Supplementary figure S2: TDF and HM_wDF contents related to AX: **A** and **B** in wheat-based foods
472 (data Table 1); **C** and **D** in milling fractions (Data table 2).

473

474 Supplementary figure S3: proportion of AX released by xylanase (% AX_{sol}) related to the
475 proportion of aleurone (AL) and starchy endosperm (SE) (% AL+SE) in the fractions.

476

477 Table 1 : Resistant starch and dietary fibre contents of wheat food products as determined by
 478 AOAC methods 2002.02 and 2009.01, respectively.

<i>g/100g d.m.</i>	Resistant Starch	HM _w DF	LM _w SDF	TDF	AX _{tot}	AX _{enz}	% AX _{sol}
Biscuit- R	0.14	1.8	1.3	3.2	1.0	0.7	67.4
Biscuit-F ⁺	0.17	7.2	1.8	9.0	3.5	1.4	40.1
Biscuit-F ⁻	0.17	6.4	1.3	7.7	3.2	0.9	27.3
Bread-R	1.21	4.3	2.4	6.7	2.1	1.7	83.4
Bread-F ⁺	0.55	9.9	2.0	11.9	4.7	2.6	55.7
Bread-F ⁻	0.80	11.8	2.2	14.0	5.6	2.0	35.5
Dry pasta-R	0.29	4.1	3.6	7.7	2.2	1.6	73.6
Dry pasta-F ⁺	0.21	8.0	2.9	10.9	4.1	2.2	54.4
Dry pasta-F ⁻	0.27	8.5	3.2	11.7	4.2	1.9	44.2
Mean	0.42	6.9	2.3	9.2	3.4	1.7	53.5
SD	0.37	3.10	0.80	3.3	1.4	0.6	18.6
RSD	87%	45%	35%	36%	42%	37%	35%

479 d.m.: dry matter

480 R: regular bread, pasta and biscuits

481 F⁺ and F⁻: products made with flour enriched in DF with F⁺ and F⁻ fractions

482 SD : standart deviation ; RSD : relative standart deviation

483 AX_{tot}= sum of arabinose and xylose measured in the product

484 AX_{enz}= sum of arabinose and xylose released form the product after treatment with a xylanase

485 % AX_{sol} : = (AX_{enz}/AX_{tot}) x 100

486

487 Table 1 : Dietary fibre content, AX content and tissue proportion of various milling fractions from
 488 bread and durum wheats

<i>g/100g dm</i>	HM _w DF	LM _w SDF	TDF	AX _{tot}	AX _{enz}	% AX _{sol}	% OP	% IL	% AL	% SE	% EA
Grain common wheat	14.9	3.4	18.3	8.1	3.1	38.5	2.5	3.7	9.2	79.9	1.3
Coarse bran	52.4	4.6	57.0	25.0	9.9	39.6	13.3	29.4	36.0	15.5	0.5
Fine bran	47.0	4.6	51.5	25.6	8.8	34.5	11.4	27.0	29.6	18.2	2.8
Shorts	38.6	5.0	43.6	20.2	6.7	33.1	9.8	18.1	21.8	19.4	16.0
Middlings	12.2	4.4	16.5	6.2	2.3	37.3	2.0	2.7	14.3	61.7	15.4
Tail-end flour	7.6	3.8	11.4	4.0	1.6	39.3	1.2	1.3	11.5	76.7	8.7
Flour (T55)	3.7	2.0	5.7	2.2	1.5	67.4	0.2	0.1	2.0	100.7	0.2
Fibre fraction F ⁺	20.1	3.9	24.1	10.0	4.7	47.1	3.9	9.1	20.9	56.7	3.8
Fibre fraction F ⁻	57.9	1.9	59.9	25.0	4.8	19.3	26.5	12.8	18.2	16.7	3.6
Debranning fraction A	64.5	1.3	65.9	30.1	5.0	16.5	32.9	14.2	23.3	17.6	nd
Debranning fraction B	46.1	2.5	48.7	24.5	7.7	31.4	15.2	15.8	27.4	27.3	nd
Debranning fraction C	18.5	2.9	21.3	11.0	4.9	44.4	7.4	11.3	24.1	51.2	nd
Debranning fraction D	25.6	3.0	28.6	8.3	4.1	49.2	4.9	8.1	20.4	63.1	nd
Grain durum wheat	10.6	4.0	14.6	5.3	2.5	46.7	7.0	10.1	82.0	1.3	
Coarse bran	53.9	2.0	55.9	24.1	7.5	31.0	54.2	31.0	15.6	5.1	
Purified Fine bran	44.0	3.2	47.1	21.5	7.1	33.1	60.2	25.8	19.2	3.2	
Sized fine bran	49.7	2.8	52.6	25.4	5.2	20.4	51.7	27.4	18.2	2.0	
Shorts	18.0	4.1	22.2	8.7	4.7	53.7	16.9	21.8	56.1	5.1	
Tail-end flour	7.1	4.0	11.1	3.7	2.2	58.4	4.7	18.9	73.4	3.1	
Last break flour	6.7	3.8	10.5	3.4	1.8	52.1	4.0	18.3	78.7	0.8	
Semolina S6	7.4	4.2	11.7	3.8	2.3	59.6	0.8	12.0	81.9	6.6	
Semolina S3	3.1	3.6	6.7	1.8	1.5	83.0	3.6	2.4	100.0	0.0	
Debranning fraction E	69.5	0.9	70.4	28.6	2.3	8.1	67.5	12.5	10.8	nd	
Debranning fraction F	52.1	2.2	54.2	20.2	4.3	21.5	48.2	36.8	19.1	nd	
Debranning fraction G	45.5	2.6	48.0	19.4	4.6	23.5	55.4	33.7	25.6	nd	
Debranning fraction H	24.9	3.6	28.4	10.4	3.4	32.4	18.7	25.3	40.5	nd	
Debranning fraction I	15.5	3.6	18.9	8.6	3.0	35.1	11.1	17.8	62.5	nd	
mean	30.3	3.3	33.5	14.3	4.3	39.1	10.1	11.8	20.5	47.7	4.4
SD	21.1	1.1	20.6	9.5	2.3	16.6	10.0	9.2	9.3	29.5	4.7
RSD	70%	32%	61%	67%	54%	43%	99%	78%	45%	62%	106%

489 d.m.: dry matter

490 SD : standart deviation ; RSD : relative standart deviation

491 AX_{tot}= sum of arabinose and xylose measured in the product

492 AX_{enz}= sum of arabinose and xylose released form the product after treatment with a xylanase

493 % AX_{sol} : = (AX_{enz}/AX_{tot}) x 100

494 OP: outer pericarp; IL: intermediate layers; AL: aleurone layer; SE: starchy endosperm; EA:
 495 embryonic axis

496

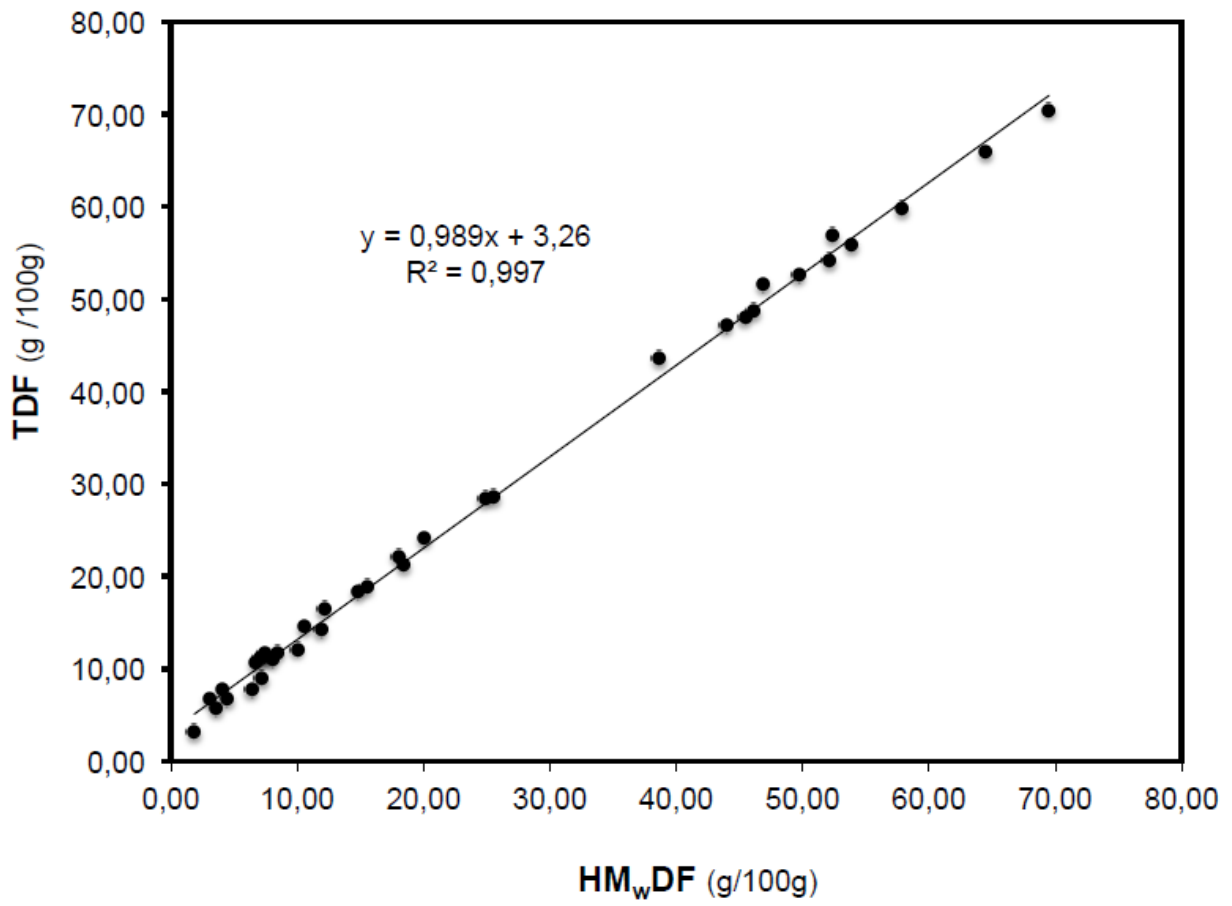


Figure 1

497

498

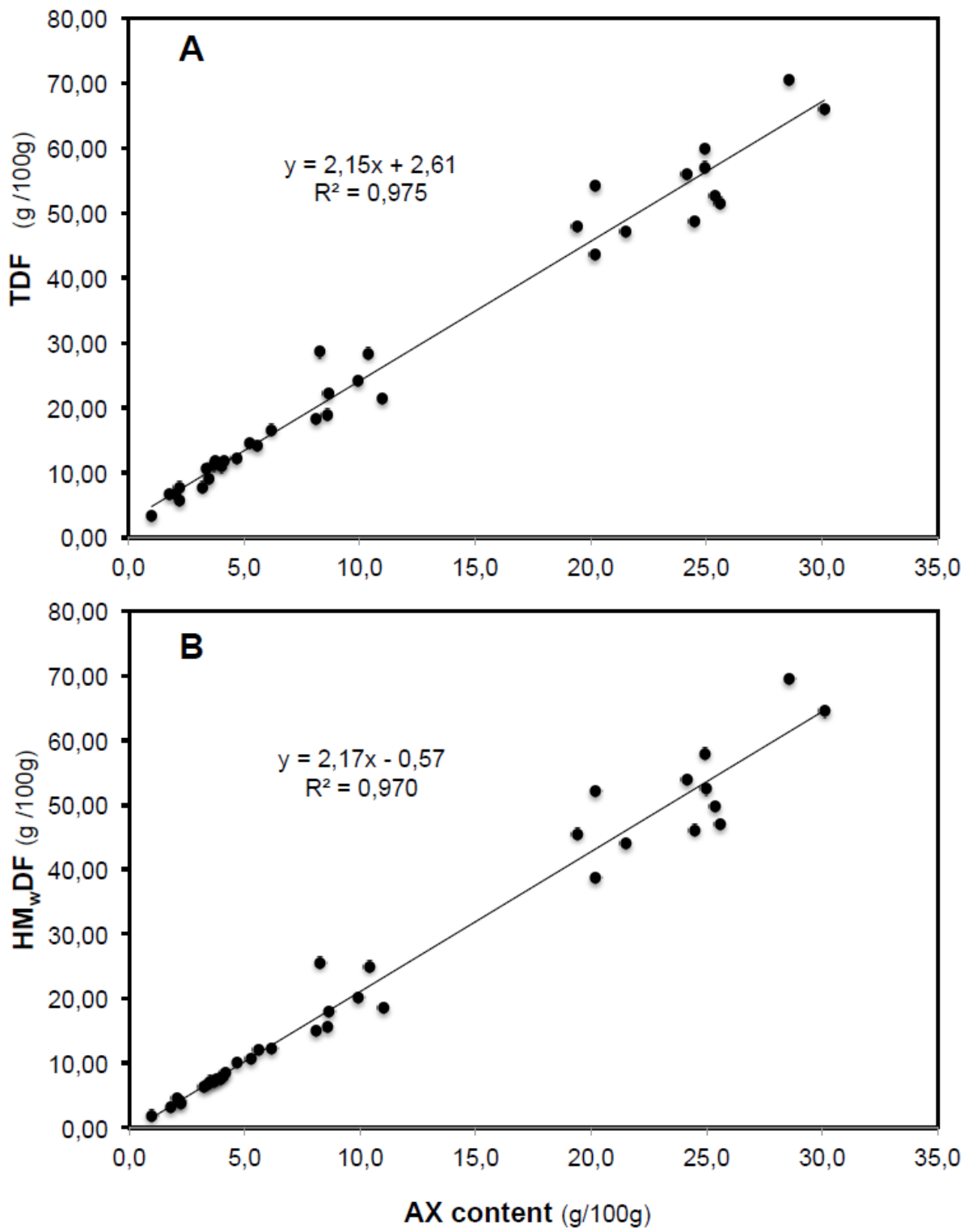


Figure 2

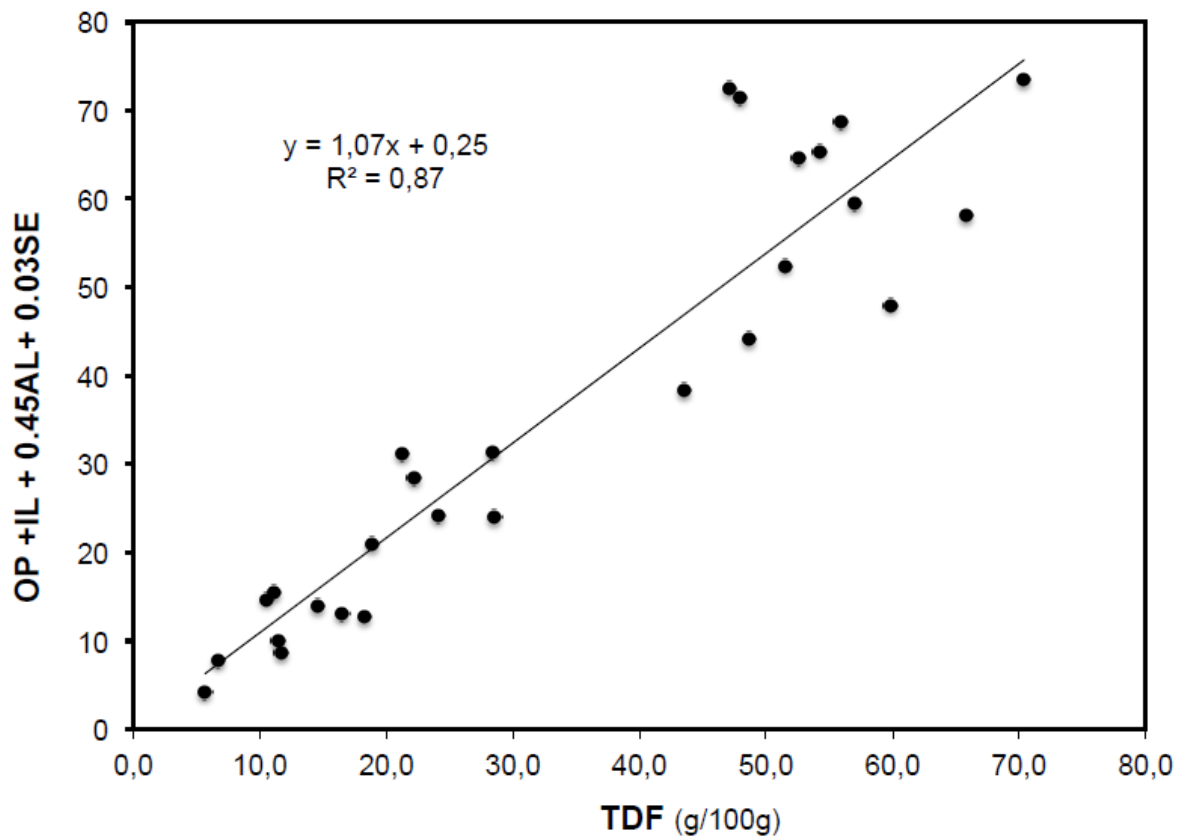


Figure 3

501

502

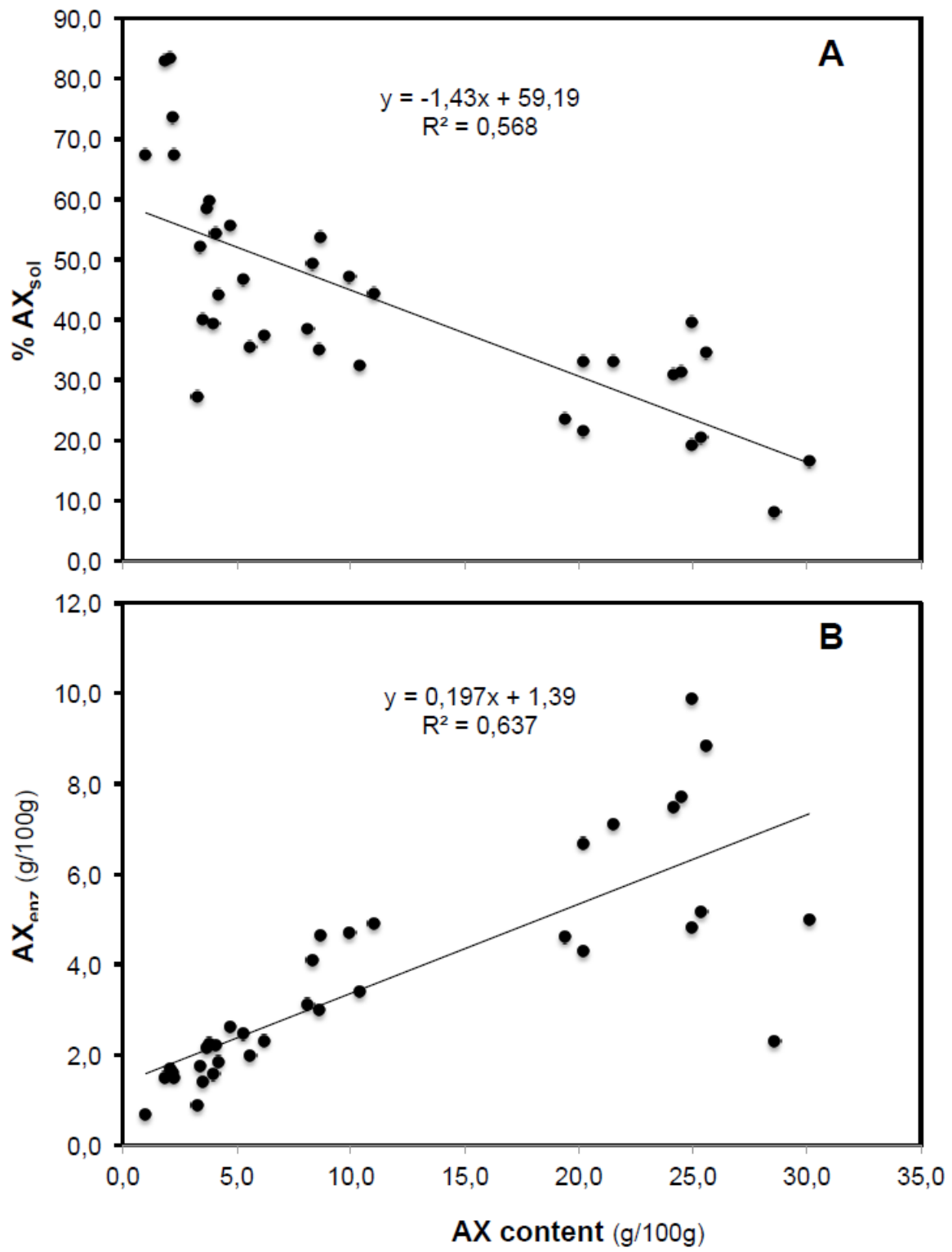


Figure 4

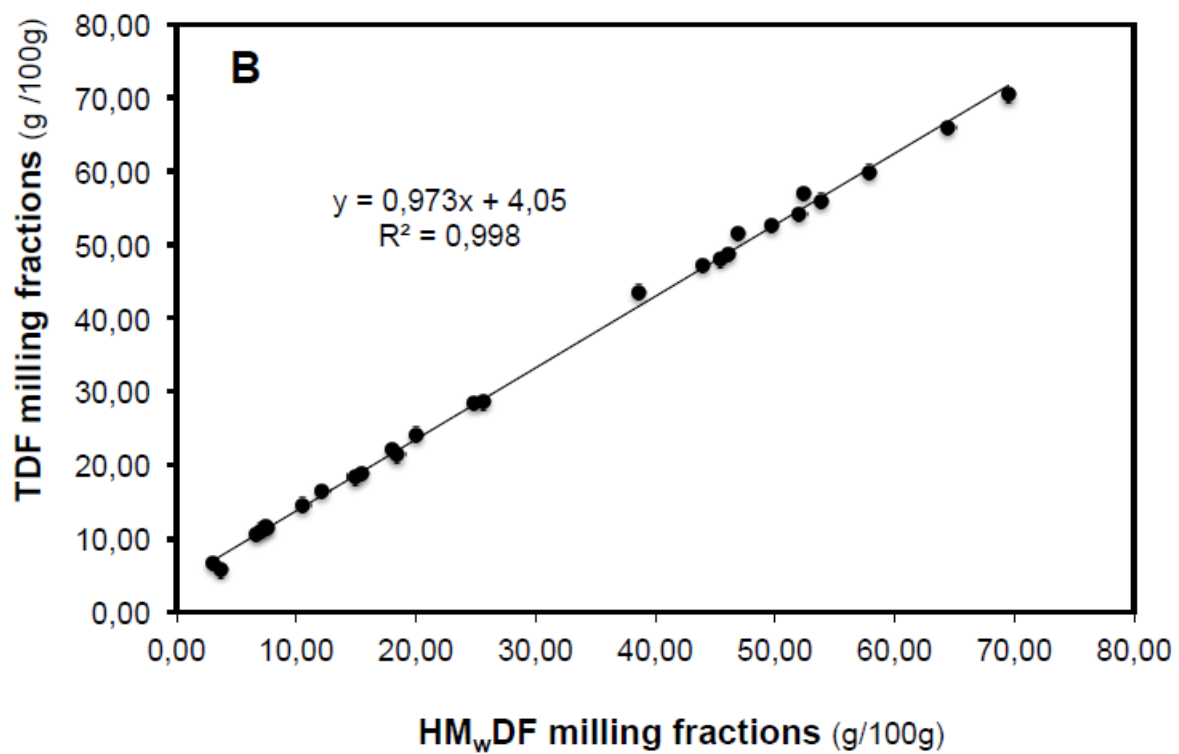
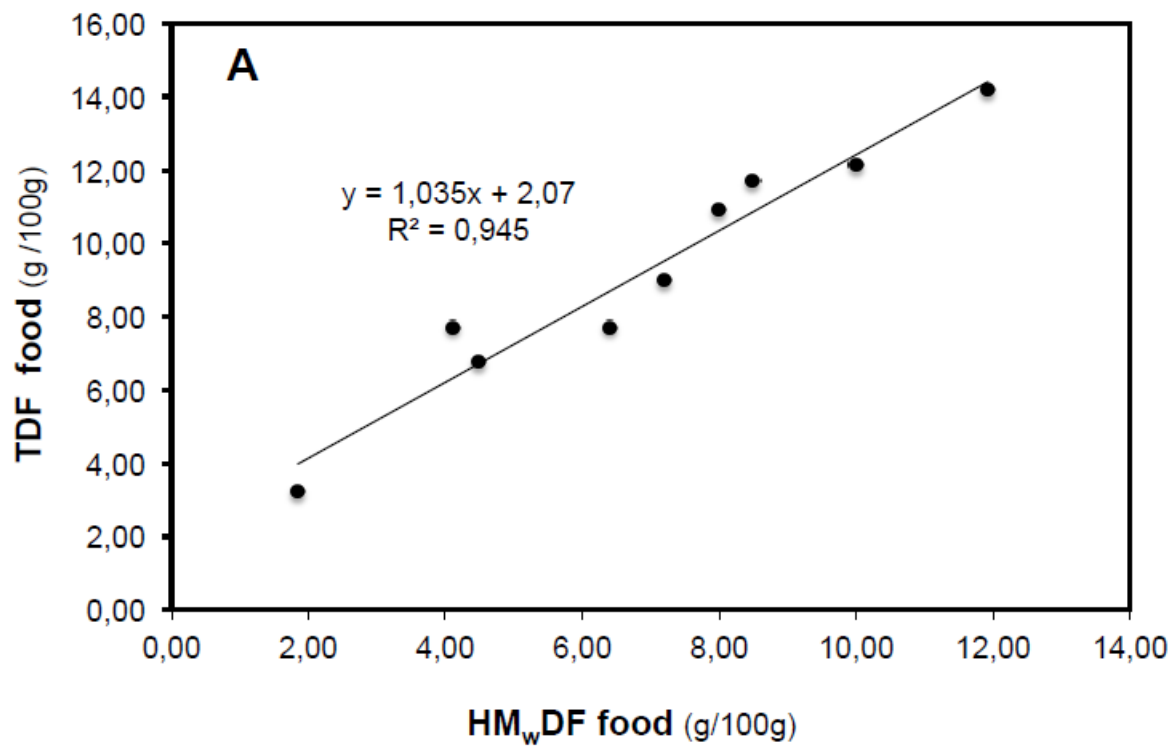


Figure S1

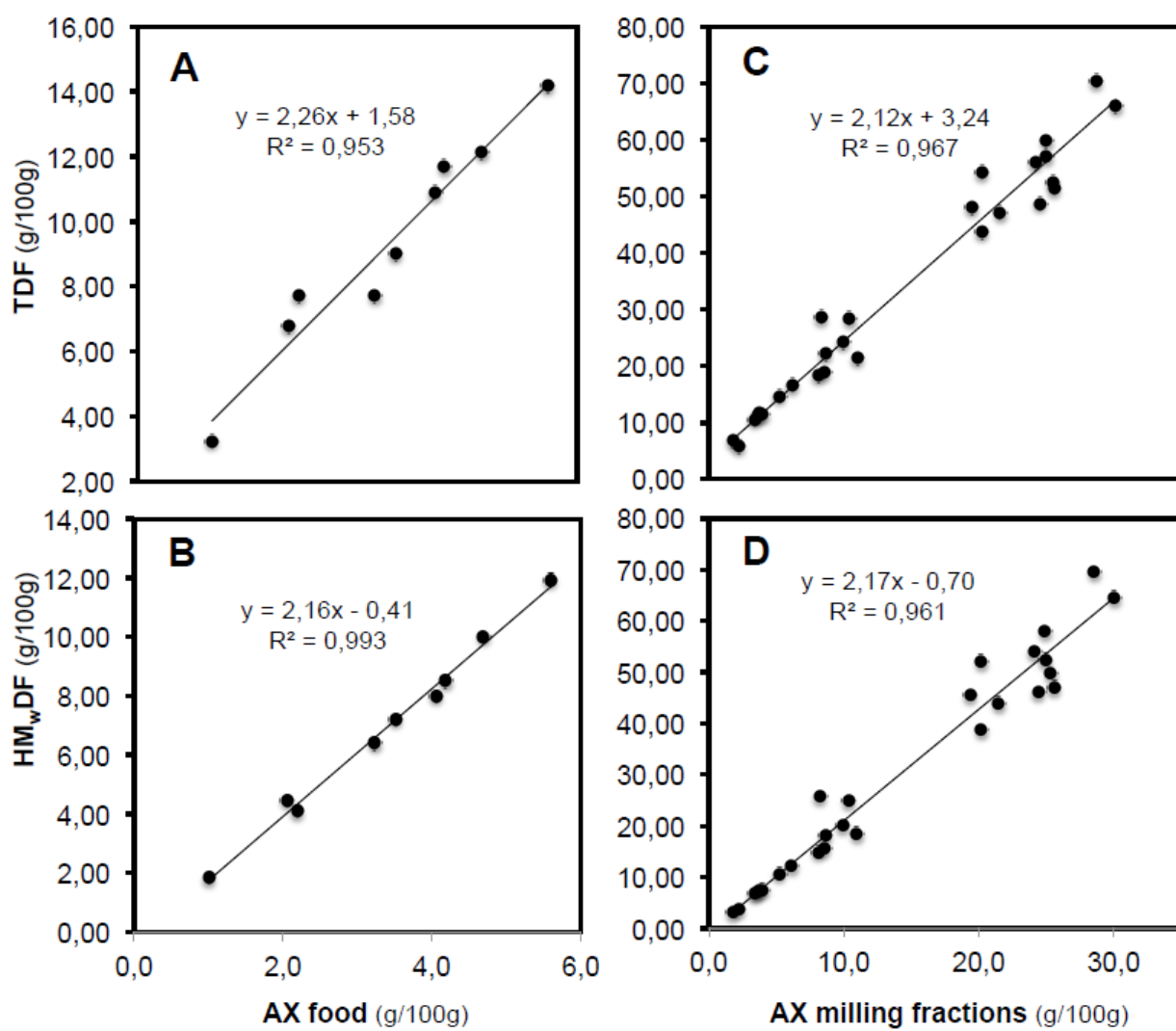


Figure S2

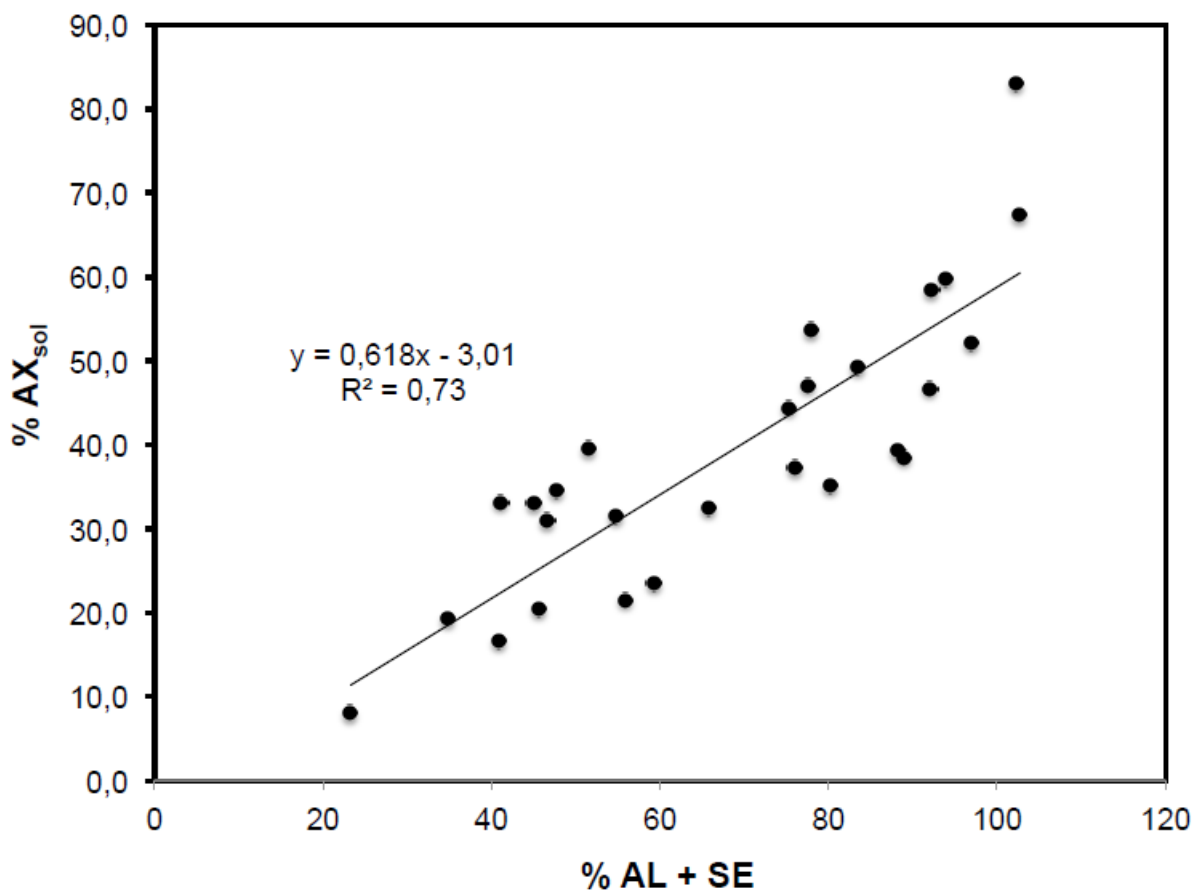


Figure S3