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Document downloaded from:

<http://hdl.handle.net/10459.1/65559>

The final publication is available at:

<https://doi.org/10.1016/j.foodcont.2018.12.014>

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1 Deoxynivalenol in cereal-based baby food production process. A 2 review

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8 **Abstract**

9 Deoxynivalenol (DON) is one of the highest occurring mycotoxin of *Fusarium* species. Its
10 presence in cereal-based infant foods is extremely undesirable, especially considering the
11 increased sensitivity of this population group to toxins. There are various studies on the
12 influence of processing operations on DON and its conjugated levels in raw materials,
13 however there is no available publication on baby food production process impact on DON.
14 According to the available studies on the occurrence of DON and its conjugates in cereal-
15 based infant food, it often occurs above the legally allowed limit, thus there is a need to study
16 the influence of production processes on its evolution from raw materials to the final product.
17 The available studies suggest that there are two production steps with a significant impact on
18 DON concentration, namely enzymatic hydrolysis, which leads to its increase due to the
19 release of the toxin from the cereal matrix, and drum drying, which might decrease DON
20 concentration thanks to the high temperatures employed and highly efficient temperature
21 penetration into the product.

22 **Keywords:** Deoxynivalenol, cereal-based baby food, processing, deoxynivalenol-3-
23 glucoside, amylases.

24 **Highlights**

- 25 - DON is the most occurring *Fusarium* mycotoxin in oats, wheat and barley.
- 26 - DON levels in several cereal-based baby food exceeding legal limits were found.
- 27 - Cereal species and nature of contamination define the efficacy of DON reduction.

- 28 - Enzymatic hydrolysis lead to an increase in DON levels.
- 29 - Drum drying may result in a decrease in DON concentration.

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45 **1. Introduction**

46 Nowadays, cereals are the most important source of energy worldwide, with approximately

47 30 % of daily calories being derived from cereals in the developed countries, reaching more

48 than 60 and 80 % in the developing and poor countries, respectively (Awika, 2011).

49 Nevertheless, cereals are not consumed directly, usually being processed to obtain various

50 foodstuff and ingredients (e.g. sweeteners). Cereal-based foods are one of the most important

51 contributors to human exposure to mycotoxins (toxic secondary fungal metabolites inducing

52 both acute and chronic exposure symptoms) (De Boevre, Di Mavungu, Landschoot, et al.,

53 2012; Zinedine, Soriano, Moltó, & Mañes, 2007), deoxynivalenol (DON) being the most

54 common contaminant. DON is produced by *Fusarium graminearum* and *F. culmorum*, both

55 implicated in FHB disease (Fusarium Head Blight) in cereal crops, especially oats, barley,

56 wheat and maize (Tanaka et al., 1988). DON was not classified as carcinogenic by IARC

57 (International Agency for Cancer Research), nevertheless studies in various animal species

58 proved its teratogenicity, cytotoxicity, genotoxicity and immunotoxicity as a results of a
59 chronic exposure, and nausea, vomiting and food rejection in the case of an acute exposure
60 (Sobrova et al., 2010). Besides all, it is highly thermostable, withstanding temperatures
61 between 170 and 350 °C (no reduction observed after 30 min at 170 °C treatment) (Wolf &
62 Bullerman, 1998) .

63 Considering the abovementioned DON toxicity, the concern regarding this toxin has
64 increased with the discovery of its major modified forms, namely deoxynivalenol-3-glucoside
65 (DON-3-Glc) and 3- and 15-acetyldeoxynivalenol (3 and 15Ac-DON) (Rychlik et al., 2014).
66 Each of these metabolites has its source of formation: DON-3-Glc is a result of plant
67 detoxification strategy (Lemmens et al., 2005) and 3- and 15Ac-DON are synthesized by the
68 *fungus* to protect itself from its own toxins (Berthiller et al., 2013). Figure 1 represents the
69 major mechanisms that cereal plants use to protect themselves from the pathogen. The
70 toxicity of the modified forms is lower compared to the parental form, however human and
71 animal digestion may lead to their conversion back into DON or to the formation of other
72 metabolised forms which proves their contribution to the exposure (Berthiller et al., 2011). A
73 co-occurrence of DON and its metabolites was repeatedly identified in cereal commodities
74 (Bryła et al., 2016; De Boevre, Mavungu et al., 2012; Uhlig et al., 2013), however the
75 existing legislation has not yet defined maximum acceptable levels for those mycotoxins in
76 food products. The available studies for cytotoxicity prove that in order of decreasing toxicity
77 DON is followed by 15-Ac-DON, 3-Ac-DON and de-epoxy-deoxynivalenol (DOM-1),
78 nonetheless more investigation is needed to better evaluate the consequences of their
79 metabolism (Dänicke & Brezina, 2013).

80 Infants and young children's diet is mainly restricted to cereal-based food. European
81 Commission defines processed cereal baby-food and baby foods as food intended for use by
82 infants (<12 months) when they are weaned and young children (from 1 to 3 years) as a

83 supplement to their diet and/or for their progressive adaptation to ordinary food (European
84 Commission, 2006a). Several recent surveys prove that the mycotoxin problem is also an
85 issue in this product category (Juan, Raiola, Manes, & Ritieni, 2014; Pereira, Fernandes, &
86 Cunha, 2015; Ul Hassan et al., 2018). Considering the increased vulnerability to
87 contaminants of this group of population, European Commission establishes the strictest
88 maximum levels of DON and other mycotoxins allowed in food intended for infants and
89 young children (200 µg/kg), which is 2.5 fold lower than the maximum level in other cereal-
90 based foodstuff and 8.75 times less than the maximum level of DON allowed in unprocessed
91 oats, wheat and maize intended for human consumption (European Commission, 2006b).
92 Although DON is one of the least lethal trichothecenes, its effects in children occur within
93 hours after the intake (Raiola, Tenore, Manyes, Meca, & Ritieni, 2015).

94 A review of the available surveys regarding the incidence of DON and its metabolites in
95 cereals and cereal-based baby foods will be performed in order to complete the study.
96 Considering the previously published research on the stability of mycotoxins during various
97 food processing operations (Scudamore, Baillie, Patel, & Edwards, 2007), this review is
98 aiming to compile the existing information on the transfer of DON and its metabolites during
99 specific cereal-based baby food production processes, focusing on both chemical and
100 biochemical changes that might be induced.

101 **2. DON occurrence in cereals intended for food production**

102 A market research on cereal-based baby foods available in the European Union (EU) showed
103 that the mainly used commodities in this sector are oats, wheat and barley followed by maize,
104 rye and triticale and, on a lower extent, millet, sorghum and spelt. Very often, these are
105 coming in formulation of three and more cereals (up to eight cereals), in order to provide a
106 more complex spectrum of nutrients needed to ensure a correct infant growth. Table 1

107 regroups the available information on the occurrence of DON in cereals intended for food
108 production worldwide.

109 Usually the studies on mycotoxins incidence in commodities are not only focused on a group
110 of cereals from a country or region but also on more than one mycotoxin, yet their co-
111 occurrence was frequently proved (Bryła et al., 2016; De Boevre, Di Mavungu, Landschoot,
112 et al., 2012; Pleadin et al., 2017; Vanheule et al., 2014). The present work was oriented on
113 studying the occurrence of DON and its metabolites, over the last 25 years, in the cereal
114 species which are mainly used in baby food industry. There are very few studies searching for
115 DON metabolites in cereals. The studied metabolites are the ones which are either produced
116 by the mould itself (3Ac-DON and 15Ac-DON) or the ones which are a result of the plants
117 activity against the contamination (DON-3-Glc) (Bryła et al., 2016; De Boevre, Di Mavungu,
118 Landschoot, et al., 2012; Rasmussen et al., 2012; Uhlig et al., 2013; Vanheule et al., 2014).

119 One of the largest review on DON occurrence in cereals, after Tanaka et al. (1988b), was
120 performed by Placinta et al. (1999), regrouping the available information on DON
121 contamination in oats, wheat, barley, maize and rye from 15 countries over a decade. Its
122 concentration ranged from 4 to 62050 µg/kg, the highest levels being reported in oats from
123 Norway in 1996's harvest year.

124 According to a report published by Eurostat (2017), France is the main producer of cereals in
125 the EU, accounting for almost a fifth of total EU production in 2016 (18%), followed by
126 Germany (15.1%), Poland (9.9%) and Spain (8%). However, very limited studies are
127 available on DON occurrence in France (Tangni, Pussemier, Schneider, & Larondelle, 2013),
128 wheat presenting the highest DON incidence (145 positive samples from 169 analysed
129 samples) with a maximum of 3280 µg/kg.

130 FHB infection and DON contamination is a function of several factors such as climatic
131 factors, crop rotation, fungicide application, debris management and tillage practices applied,
132 variety resistance to FHB, etc. (Blandino et al., 2012). All the above mentioned explains the
133 differences in DON contamination among countries, different cereal varieties and among
134 years. In Norway, Finland and Sweden, for example, important spring rainfalls and relatively
135 warm temperatures during this season would promote the propagation of both *F.*
136 *graminearum* and *F. culmorum* and mycotoxin formation on cereal crops (Hope, Aldred, &
137 Magan, 2005). This explains the high DON accumulation showed in the published studies:
138 maximum 9298 µg/kg of DON in oats in Finland (Hietaniemi et al., 2016), up to 2340 and
139 22000 µg/kg in oats from Norway and Sweden, respectively (Lindblad, Börjesson,
140 Hietaniemi, & Elen, 2012); 2240 and 16000 µg/kg of DON in wheat in Finland and Norway,
141 respectively (Hietaniemi et al., 2016; Hofgaard et al., 2016); 636 and 4752 µg/kg of DON in
142 barley from Norway and Finland, respectively (Hietaniemi et al., 2016; Uhlig et al., 2013).
143 Nonetheless, no such important DON accumulation was found in other crops such as maize,
144 rye and triticale (Table 1), probably because of the differences in fungal ecology
145 characteristics of these crops (Doohan, Brennan, & Cooke, 2003).

146 Moreover, differences in DON occurrence and accumulation were observed between organic
147 and conventional agricultural systems. Nevertheless, from the available literature, it results
148 that DON accumulation is crop dependent too: in oats, maize and rye from organic
149 agriculture DON concentration is higher compared to the conventional system but it is the
150 opposite in the case of wheat and barley (Bernhoft, Clasen, Kristoffersen, & Torp, 2010;
151 Pleadin et al., 2017).

152 Many of the works listed in the Table 1 were focused on more than one mycotoxin, however
153 all of them found DON as the most prevalent product of fungal activity (in almost all the
154 studies over various years DON positive samples were found in more than 50% of the total

155 analysed cereal samples). Studies on DON-3-Glc occurrence in cereals and its fate during
156 food and feed processing are quite recent (Berthiller et al., 2007). Moreover, the concern
157 about DON-3-Glc toxic effects on human and animal health is increasing continuously,
158 which made researchers worldwide to study its occurrence (Bryła et al., 2016; De Boevre, Di
159 Mavungu, Landschoot, et al., 2012; Rasmussen et al., 2012; Uhlig et al., 2013; Vanheule et
160 al., 2014) and synthesis pathways in cereals (Berthiller et al., 2007). The levels of DON-3-
161 Glc found are usually lower or similar to DON: a mean of 18 and 51.2 µg/kg of DON-3-Glc
162 was found in wheat and oats from Belgium, respectively (de Boevre, Mavungu, et al., 2012);
163 252, 56 and 68 µg/kg in oats, wheat and barley, respectively, from Hungary (Uhlig et al.,
164 2013); 99, 102 and 138 µg/kg of DON-3-Glc in wheat, barley and triticale, respectively, from
165 Poland (Bryła et al., 2016). Two years after the study performed by De Boevre et al. (2012),
166 250 and 390 µg/kg of DON-3-Glc in wheat and barley from the same region in Belgium
167 (Flanders) were found (Vanheule et al., 2014). Interestingly, during these two years a
168 considerable increase in both average rainfall and average temperature was registered
169 (Statistica, 2018), which would explain the higher amount of both DON and DON-3-Glc
170 accumulated (Table 1).

171 Considering that oats and wheat are the primarily used cereals in baby food production and
172 that these two commodities, together with barley, are the most susceptible for DON
173 contamination, a transfer to the final product may take place, especially in the case of a high
174 contamination level, which would expose these consumers to an important health risk.

175 **3. DON occurrence in cereal-based baby food and food for young children**

176 In Europe, DON maximum allowed levels were applied in baby food, for the first time, in
177 Soviet Ukraine in 1984 (FAO, 2004; Ministry of Health of the Union of Soviet Socialist
178 Republics, 1989). The first EU harmonized regulation on mycotoxins in foodstuffs came in
179 1998, which included several limits for aflatoxins, also containing sampling and analytical

180 methods procedures (European Commission, 1998). Only with the European Regulation
181 1881/2006/EC maximum allowed limits for *Fusarium* toxins in foods were legislated
182 (European Commission, 2006b), with its updates and amendments on the following years.

183 There is a limited number of studies available on the occurrence of DON in cereal-based
184 baby food, even considering that the EU regulation of DON in this product category is
185 particularly strict (maximum allowed DON level is 200 µg/kg). Nowadays, available reliable
186 methods for DON analysis using liquid chromatography coupled with diode array detectors
187 (DAD) have relatively high limits of detection and quantification (usually around 100 µg/L)
188 (Sugita-Konishi et al., 2006), and more sensitive techniques are necessary such as mass
189 spectrometry to increase the accuracy of the analysis, particularly for this product category
190 (Habler & Rychlik, 2016; Mats Lindblad et al., 2013; A. Malachova, Varga, Schwartz, Krska,
191 & Berthiller, 2012). Table 2 regroups the available published surveys on DON occurrence in
192 infant foodstuffs. Maize, wheat, barley and oats are the cereals most susceptible for *Fusarium*
193 infestation and DON accumulation in the commodity and the final product (European Food
194 Safety Authority, 2013). In the present review, it was observed that maize and wheat-based
195 cereals show not only higher contamination incidence but also present higher levels of
196 mycotoxins, and in the case of DON incidence, often being close to or overcoming legal
197 limits: mean DON of 260 µg/kg in barley based cereals from Canada (Lombaert et al., 2003),
198 mean DON of 103 µg/kg in wheat based cereals from Italy (Juan et al., 2014); mean DON of
199 131 µg/kg in cereal-based baby food from Spain (Cano-Sancho, Gauchi, Sanchis, Marín, &
200 Ramos, 2011).

201 Even considering the abovementioned strict legislation implemented in 2006, there are
202 studies performed on the following years which found DON contaminated commercial
203 products overcoming the stipulated limits (Cano-Sancho et al., 2011; Juan et al., 2014;
204 Pereira et al., 2015; Zhang, Wong, Krynitsky, & Trucksess, 2014). Interestingly, multi-cereal

205 infant and young children foods are the ones most frequently encountered as contaminated
206 above the regulation, moreover in the ones where barley, wheat or maize were the
207 predominant cereal in the formulation, the highest maximum levels were observed (Juan et
208 al., 2014; Lombaert et al., 2003). On the contrary, Lu, Ruiz Leal, Míguez, & Fernández-
209 Franzón (2013) did not found any DON contamination in the 57 analysed baby food samples
210 from the Spanish market. Also, very few surveys encountered or analysed DON-3-Glc, 3- or
211 15-Ac-DON (Juan et al., 2014; Oueslati, Berrada, Mañes, & Juan, 2018). These studies prove
212 again the heterogeneity in mycotoxins contamination in cereals over different harvest seasons
213 (Doohan et al., 2003), and justify the need for a deeper investigation on the effect of
214 processing on the evolution of grains contamination with DON and its metabolites.

215 **4. Changes of DON levels during cereal-based baby food production process**

216 The production of cereal-based baby foods and food for young children implies the following
217 main process operations: flour roasting, pre-gelatinization, enzymatic hydrolysis, enzymes
218 inactivation, drying and packaging (Figure 2). The objective is to obtain a food product
219 adapted to the particularities of the digestive system of an infant or young child, which has a
220 limited ability to digest starch. The most frequently used cereals are oats, barley, wheat, rice,
221 rye, maize and, on a lower extent, millet, sorghum, triticale and spelt. Also, adjuncts might be
222 added, namely barley or rye malt extracts, maize extracts, quinoa flour, fructo-
223 oligosaccharides (FOS), vitamins and minerals etc., in order to complete or enrich product's
224 nutritional value.

225 **4.1 Flour roasting**

226 Roasting consists in increasing flour temperature to the range of 105-120 °C and maintaining
227 it from 20 to 40 min. This stage is important in the modulation of organoleptic characteristics
228 of the flour, leading to caramelization and Maillard reactions. Also, it helps improving flour
229 dispersibility in water during the next stage (Fernández-Artigas, Guerra-Hernández, &

230 García-Villanova, 1999). An important aspect to consider is the change in colour of the flour
231 under this production step, browning being an undesirable result for cereal-based baby food
232 products. It is also crucial not to overcome these physical production conditions, yet that a
233 longer time above 120 °C might lead to the formation of a carcinogenic compound,
234 acrylamide, which is proven to be particularly dangerous for children under 2 years old
235 (Erkekoğlu & Baydar, 2010).

236 Only a few studies are available on the occurrence of DON in cereal flour, mainly being
237 focused on wheat or rye flours used in bakery products industry (Malachova et al., 2011;
238 Rasmussen, Ghorbani, & Berg, 2003; Schollenberger, Jara, Suchy, Drochner, & Muller,
239 2002; Škrbić, Živančev, Durišić-Mladenović, & Godula, 2012). Moreover, those studies are
240 limited in terms of geographical origin of the analysed samples and present a high variability
241 of the results. One of the largest occurrence study in cereal flours was performed by
242 Rasmussen et al. (2003). They collected samples of wheat and rye flour from Denmark over 4
243 years (1998-2001), finding a high incidence of DON contamination with an average level of
244 114 (85 % positive samples) and 42.5 µg/kg (59 % positive samples) in wheat and rye flour,
245 respectively. A more recent study in Czech Republic, identified a range of 13 to 320 µg/kg of
246 DON and 11 to 94 µg/kg of DON-3-Glc in wheat flour (Malachova et al., 2011). In the
247 abovementioned studies, very few samples were found exceeding the maximum allowed level
248 of DON in cereal flour (750 µg/kg). Thus, considering the specificity of the contamination
249 with DON mainly in the outer layers of the kernel and the trend in using whole-grain flours
250 for its better nutritional properties compared to the white flour, requires more investigation on
251 mycotoxin occurrence, especially for the products in which flour is used as raw material.

252 Studies showed a very low DON reduction in roasting cereal flour at temperatures below 180
253 °C. Yumbe-Guevara, Imoto, & Yoshizawa (2003) investigated the impact of various
254 temperature levels between 140 and 220 °C on DON in standard solution and contaminated

255 wheat and barley (whole grains and powder). They found that 8 and 11 min at 220 °C were
256 necessary to reduce 50 and 90 % of DON in barley powder, respectively. The first significant
257 reduction was observed at 180 °C. Also, toxin reduction in these conditions was higher in
258 grain powder than in kernels, which led them to the conclusion that heat penetration is an
259 important factor in the effectiveness of thermal treatment. A similar study performed later
260 reached quite controversial results, with only 1.7 to 7.6 % reduction after applying a 230 °C
261 treatment to wheat flour (Israel-Roming & Avram, 2010). Nonetheless, the authors of the
262 mentioned study did not specify the duration of the treatment. Considering the parameters for
263 flour roasting, there is a chance that although lower temperatures are applied, the longer
264 treatment time could compensate for it, at least, slightly decreasing the level of DON,
265 however there are no available studies that could prove this statement.

266 Taking into account the risk of acrylamide formation during flour roasting, this stage may not
267 be considered as crucial in the case of adoption of a mitigation strategy against DON
268 contamination, however its possible impact, although small, need to be considered in the
269 evaluation of DON transfer to the final product.

270 **4.2 Pre-gelatinization and enzymatic hydrolysis**

271 **4.2.1 Process description**

272 One of the most important stages in cereal-based baby food production process is enzymatic
273 hydrolysis. It allows a partial break down of the starch contained in the cereals and facilitates
274 its assimilation by infants digestion system (Fernández-Artigas et al., 1999). Starch is the
275 most abundant carbohydrate found in plants, used as source of carbon and energy (Alcázar-
276 Alay & Meireles, 2015). It is formed by two types of polymers: (i) amylose, composed of a
277 linear chain of α -1,4-glucose units representing from 15 to 30 % of the total starch and (ii)
278 amylopectin, composed of α -1,4-glycosidic bonds intensively branched in α -1,6 positions
279 with glucose units (70 to 85% of the starch structure) (Fig. 3) (Martinez & Gómez, 2016).

280 The arrangement of these two polymers form a matrix of starch granules alternating
281 crystalline (amylopectin) and amorphous (amylose) phases. Cereal starches may have
282 associated other molecules to their structure, such as phospholipids and free fatty acids (0.15
283 to 0.55%) and proteins (up to 0.6%), which increase the nutritional value of the starch
284 (Alcázar-Alay & Meireles, 2015). Starch granules have microscopic sizes and a great shape
285 variability depending on their biological origin (Tester & Somerville, 2001). Cereal
286 starches are mainly characterized by the A-type structure (polymerization degree <15), and
287 B1-type structure (polymerization degree between 15 and 25) (Tester, Karkalas, & Qi, 2004).

288 Starch is not available to the enzymes without undergoing a pre-gelatinization process (loss
289 of swelling and crystallinity phase which result in a formation of a gel) (Tako, Tamaki,
290 Teruya, & Takeda, 2014). The pre-gelatinization would serve two purposes: (i) increase in
291 starch swelling capacity, solubility and cold water dispersion (Alcázar-Alay & Meireles,
292 2015) and (ii) facilitate the access of α - and β -amylases to the polymer chains (Martinez &
293 Gómez, 2016). The process is determined by transition temperatures (the higher the amount
294 of crystalline structures, the higher the temperatures required for the gelatinization to take
295 place) and gelatinization enthalpies, specific for each botanical source (Tester & Morrison,
296 1990). Knowing those characteristics together with the temperature for the optimum
297 enzymatic activity allow the producer to identify the best setup of technological conditions in
298 order to minimize energy losses and ensure a more sustainable production. Table 3 regroups
299 the morphology and transition temperatures for native starches from different common cereal
300 sources. In baby food production, pre-gelatinization consists of mixing the roasted cereal
301 flour with a high amount of water (between 60 and 80%, w/w) and heating the mix between
302 40 and 70 °C.

303 When gelatinization is achieved a mix of α - and β - amylases is added, followed by a
304 continuous stirring from 10 to 90 min. Amylases are enzymes hydrolysing starch into smaller

305 molecules to produce dextrans and other small polymers composed of glucose units. They can
306 be synthesized from plants, animals or microorganisms. The last source completely replaced
307 chemical starch hydrolysis in the starch processing industry due to their cost effectiveness,
308 higher stability, great production yields and ease of process modifications (Gupta, Gigras,
309 Mohapatra, Goswami, & Chauhan, 2003). Microbial enzymes include fungal and bacterial
310 amylases. There are two groups of amylases: endoamylases (α -amylase) and exoamylases (β -
311 hydrolases). The α -amylase can be obtained from several bacteria or fungi, bacterial enzyme
312 being preferred due to a larger spectrum of optimum conditions of temperature (30-50 °C)
313 and pH (2 to 12, most of the α -amylases being active within the neutral range) (Martínez &
314 Gómez, 2016). The mechanism of their action allows to rapidly fragment the poly-glucoside
315 chain and decrease the viscosity of the paste. The products of their activity (mono- and oligo-
316 saccharides) are very numerous, depending on the nature of the α -amylase active site and on
317 the biological origin of the starch (Martínez & Gómez, 2016). There are two types of exo-
318 acting hydrolases: β -amylases and glucoamylases. β -amylase activity leads to the formation
319 of β -maltose and β -limit dextrans of high molecular weight (the distribution of hydrolysis
320 products of amylopectin is half maltose and half limit dextrans). Glucoamylases are able to
321 hydrolyse both α -1,4 and α -1,6 bonds of the starch molecule into D-glucose. β -amylases can
322 be obtained from microbial origins, however the most cost-effective are β -amylases from
323 plant sources (sweet potatoes, soybean, barley and wheat). Glucoamylases can be also
324 derived from animal sources besides plants and microorganisms. Their optimum temperature
325 and pH for activity are 40-60 °C and 4.9-6.6, respectively (Martínez & Gómez, 2016).

326 Considering the high diversity and complexity of the product, each producer would establish
327 the technological parameters in accordance to the cereal species used and the desired degree
328 of hydrolysis.

329 4.2.2 Impact on deoxynivalenol

330 There are no available studies on the impact of enzymatic hydrolysis on DON during infant
331 food production process, however the addition of enzymes is common in breadmaking and
332 brewing processes. Garda-Buffon, Baraj, & Badiale-Furlong (2010) studied the effect of
333 DON on malt amylase activity (both α - and β -amylases). They found not only an increase in
334 DON content during mashing but also an impact of its concentration by inhibiting the
335 enzymatic activity. While observing those effects, DON concentration varied between 146
336 and 854 ng/g. Moreover, an increase in the enzymatic activity outside of this range (both
337 below and above) was registered. Simsek, Burgess, Whitney, Gu, & Qian (2012) studied the
338 evolution of DON concentration in wheat flour, dough and bread, with the addition of α -
339 amylase, cellulase, protease and xylanase during breadmaking. The increase of DON level in
340 bread compared to the wheat flour was registered together with the decrease of DON-3-Glc
341 level after baking. Enzymatic activity was found to be the main cause of the increase,
342 especially the protease and xylanase activity, suggesting the release of DON from the grain
343 wall matrix. α -amylase was not found to have a significant impact on DON, probably
344 because of the higher exposure to the toxin of the outer layers of the seed compared to the
345 endosperm, where starch is stored. This result is not confirmed by the work performed by
346 Vidal, Ambrosio, Sanchis, Ramos, & Marín (2016), where they identified a significant
347 increase in DON concentration under amylases activity (10 %), nevertheless confirming that
348 xylanase activity had a slightly higher impact on the increase in DON concentrations during
349 the process (15 % increase). Zachariasova, Vaclavikova, Lacina, Vaclavik, & Hajslova
350 (2012) also suggested a release of DON and its conjugates from starch and dextrins during
351 the hydrolytic enzymatic activity, however the extent of this release depended on wheat
352 variety and the mechanism of *Fusarium* infection.

353 In conclusion, previous studies show an increase of DON and DON-3-Glc levels due to
354 amylase activity, however these studies are limited to certain cereal species (mainly wheat
355 and barley). Considering the complexity of food matrices and the differences in their
356 composition, which result in differences in *Fusarium* infection and, as a result, DON
357 contamination, more studies are needed in order to complete the knowledge regarding the
358 evolution of DON levels under hydrolases activity, especially taking into account that infant
359 food production usually implies the use of a mix of cereals. Also, an interesting object for
360 study would be identifying if there is a correlation between the progress of starch hydrolysis
361 by amylases and the change of mycotoxins level.

362 **4.3 Enzymes inactivation**

363 Enzymes inactivation is performed by heating the mass until 105-135 °C, from 2 to 120 s.
364 The applied temperature will depend on the biological origin of the enzymes: bacterial are the
365 most thermostable, then fungal ones and then the enzymes coming from plant sources
366 (Martinez & Gómez, 2016). The heating would take place in a recirculation circuit in order to
367 ensure a minimal exposure of the mass to the high temperature (browning reactions are
368 undesirable at this stage) and to the environmental factors.

369 According to the studies on the impact of heat operations on DON, this stage might not be of
370 a special concern for toxin reduction, especially because of the time of action: research works
371 suggest the need of at least 180 °C temperature to be applied in order to observe significant
372 modifications in DON levels (Yumbe-Guevara et al., 2003). Nevertheless, considering the
373 efficient temperature penetration, it has to be considered for further studies.

374 **4.4 Drying and packaging**

375 Drying is performed using a drum drying system which consists of applying the paste as a
376 thin layer (approximately 1.5-2 mm) on the outer face of an internally steam-heated revolving
377 drum. The applied internal steam temperature is up to 200 °C. The product becomes dry after

378 three quarters of a drum revolution, with a residence time ranging from few seconds to
379 dozens of seconds, being then removed with a static scraper with a final humidity <5%, on a
380 wet basis. The obtained dry material is then ground into flakes or powder and packaged for
381 delivering (Heldman, 2003). Similar to the roasting stage, an important safety factor to
382 control is browning reactions (Maillard or caramelisation reactions), which, as mentioned
383 above, are highly undesirable, and thus the duration of the treatment has to be strictly
384 controlled.

385 Regarding DON contamination and the impact of drum drying process, there are no studies
386 available in baby-food or flour processing. Nevertheless, a study on cereal kernels drum
387 drying indicates this process as highly efficient, leading to a 25 and 50 % reduction of DON
388 at 160 and 185 °C, respectively (initial concentration of DON was $15.8 \pm 1.5 \mu\text{g/g}$ (Pronyk,
389 Cenkowski, & Abramson, 2006)). Thus, considering the better potential for heat penetrability
390 in the case of a flour material obtained during cereal-based infant food production process, a
391 greater decrease in DON and its conjugates might be expected, nonetheless experimental
392 studies have to be performed in order to confirm that.

393 **5. Further considerations**

394 Cereals are the most important source of **energy** worldwide and, thus, might represent an
395 important contributor to mycotoxins exposure through food. DON is the most occurring
396 *Fusarium* mycotoxin **within** cereal production chain. Nowadays, researchers are concerned
397 about the rates of transfer of these mycotoxins through food production chains. Cereal-based
398 infant food production process includes flour roasting, pre-gelatinization, enzymatic
399 hydrolysis, enzymes inactivation, drying and packaging steps. From the revised **scientific**
400 **literature in the** present work two process operations can be highlighted as significantly
401 affecting DON levels, mainly enzymatic hydrolysis (increase in DON **level from 10 to 15 %**
402 **compared to the initial contamination by its release from the food matrix**) and drum drying

403 (decrease in DON due to highly effective thermal treatment). Considering the absence of
404 available studies specifically designed in mycotoxin transfer through the production of
405 cereal-based infant foods, a deeper understanding of the production process in combination
406 with the applied physical and biochemical parameters is required to better assess the nature of
407 changes in DON and its conjugates levels while processing. Also, taking into account the
408 strict European regulation concerning the maximum content of DON in infant foods (200
409 $\mu\text{g}/\text{kg}$), there is an increased need in controlling raw material contamination levels and
410 designing mitigation strategies against the problem. The main limitation that might be
411 encountered while designing these mitigation strategies against mycotoxin contamination is
412 the sensitivity of the raw materials to undergo undesirable chemical changes, mainly
413 browning reactions, which could imply other health risks for the consumer.

414 6. Acknowledgements

415 The authors are grateful to the University of Lleida (grant JADE Plus 218/2016), and to the
416 Spanish Ministry of Economy and Competitiveness (MINECO, Project AGL2017-87755-R)
417 for funding this work.

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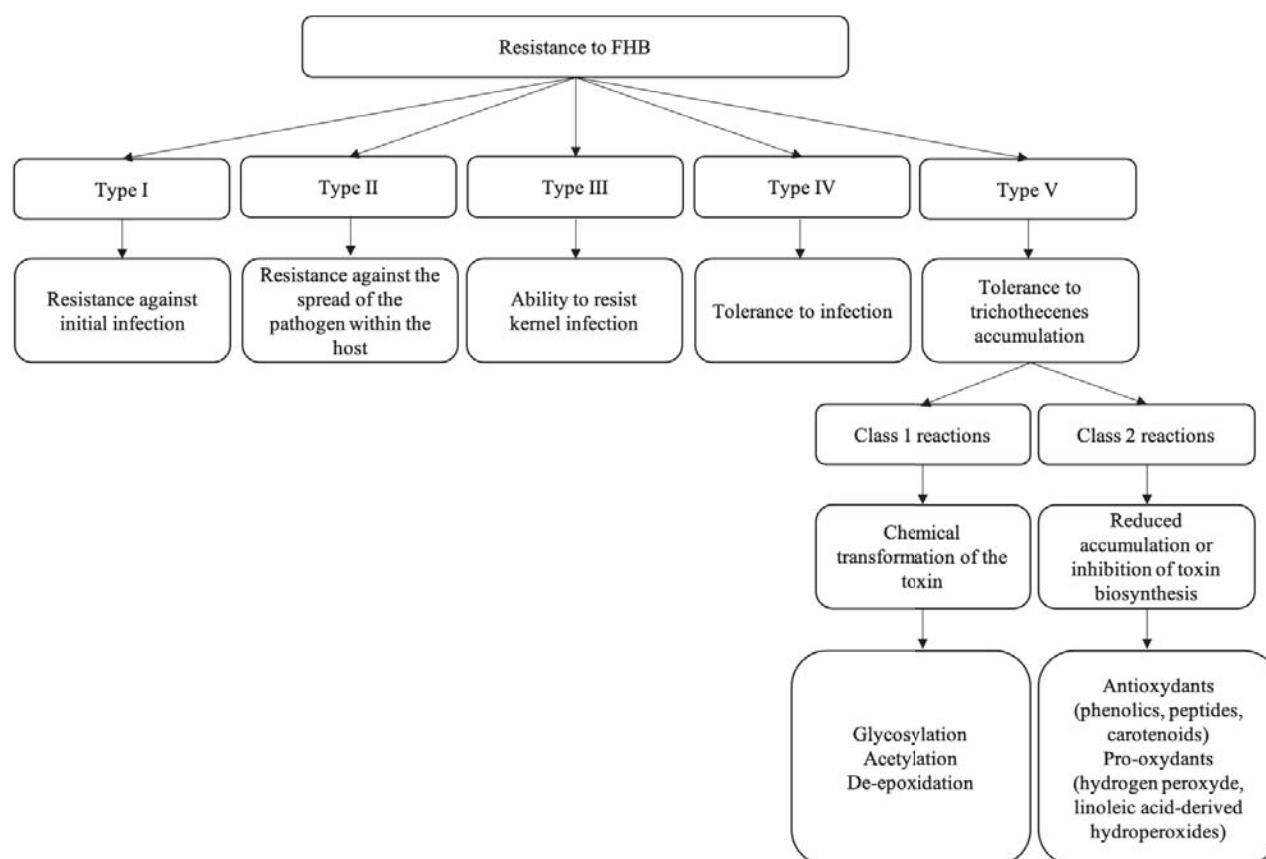


Figure 1: Natural mechanisms for cereal resistance to Fusarium Head Blight (FHB) and trichothecenes accumulation (adapted from Boutigny, Richard-Forget, & Barreau, 2008).

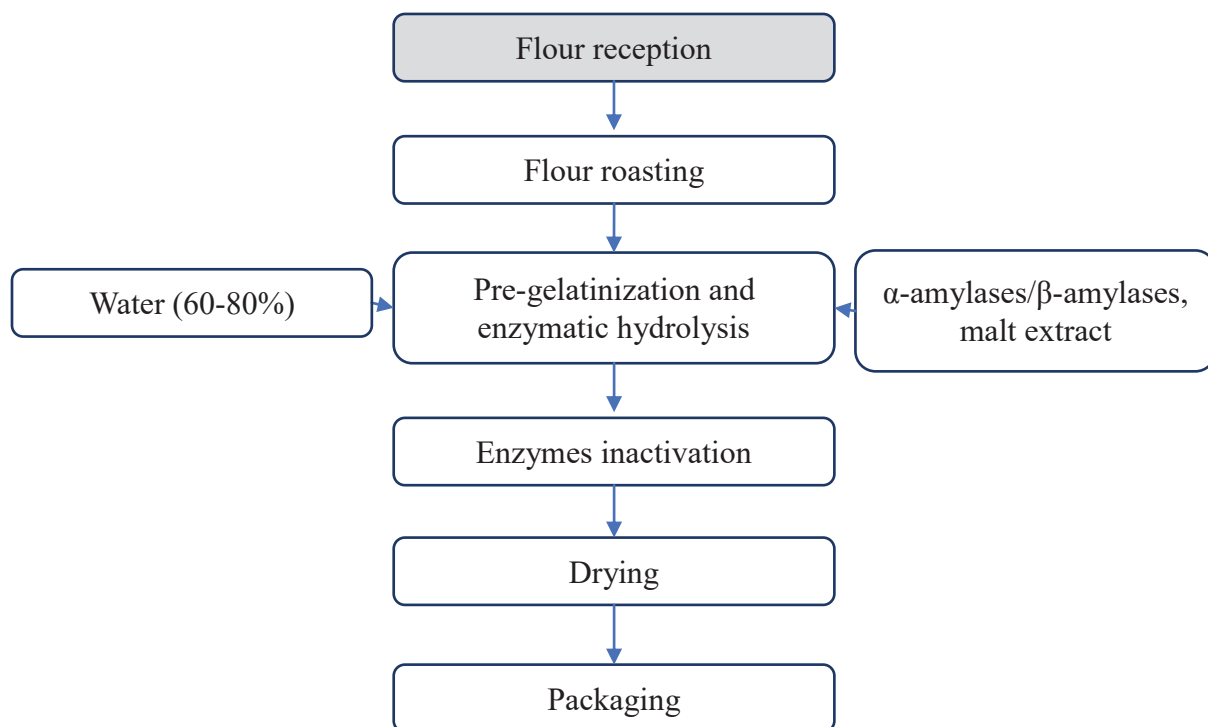


Figure 2: Infant and young children food production scheme

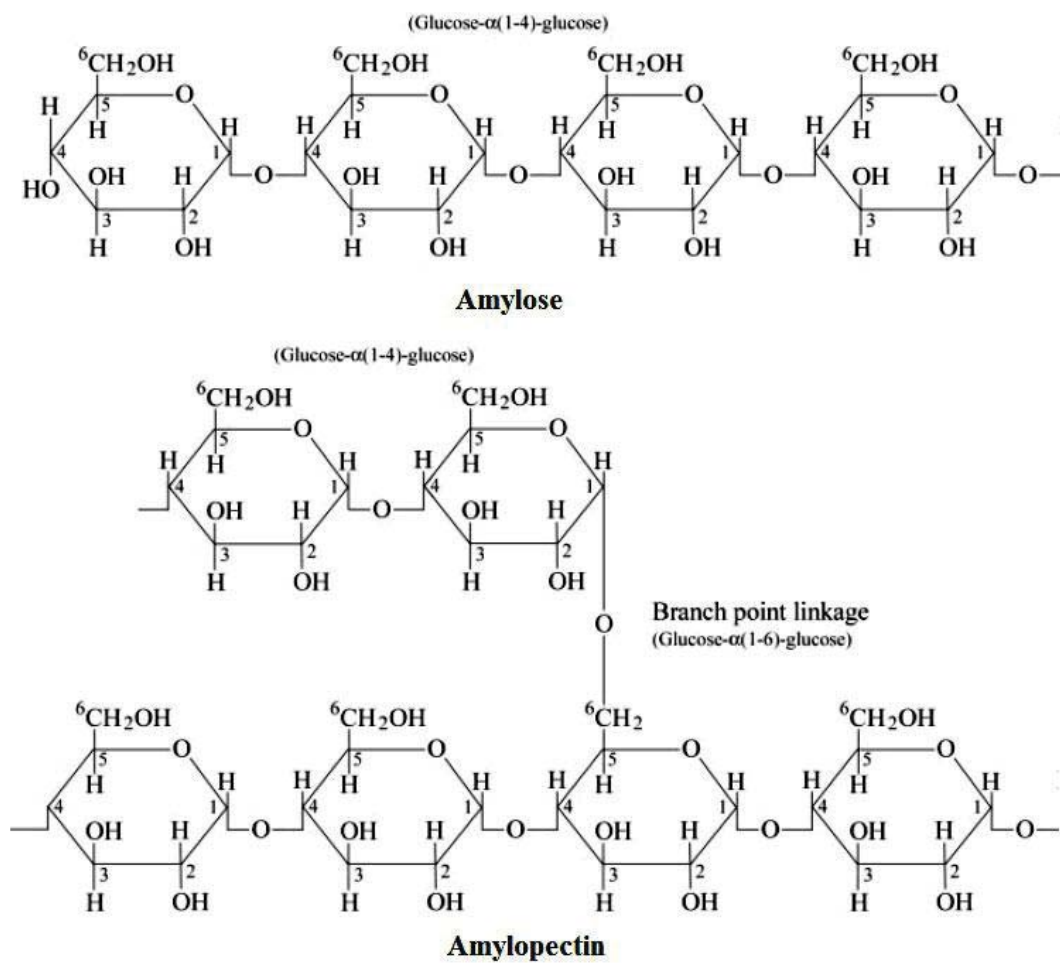


Figure 3: Chemical structure of amylose and amylopectin (Ghanbarzadeh & Almasi, 2013)

Cereal	Country/region	Positive/total samples analysed	Concentration ($\mu\text{g}/\text{kg}$)		Reference
			Mean	Range	
Oats	Belgium	1/6	46	-	(De Boevre et al., 2012)
		9/11	810.1	62-2216	(Rasmussen et al., 2012)
	Canada	34/73	330	<100-1200	(Campbell, Choo, Vigier, & Underhill, 2000)
		63/82	190	-	(Clear, Patrick, & Gaba, 2000)
		6/10	42.5	22-71	(Martos, Thompson, & Diaz, 2010)
		4/4	700	200-1200	(Tamburic-Ilincic, 2010)
	Croatia	7/33	145	34-201	(Pleadin et al., 2013)
		8/13	O: 119	O: 32-377	(Pleadin et al., 2017)
		8/14	C: 207	C: 33-546	
	Denmark	-/22	44	-	(Nielsen et al., 2011)
	Finland	ND	-	1300-2600	(Placinta, D'Mello, & MacDonald, 1999)
		35/51	154.8	25-896	(Hietaniemi et al., 2004)
		-/470	237.4	Max:8800	(M. Lindblad, Börjesson, Hietaniemi, & Elen, 2012)
		129/137	618	0-9298	(Hietaniemi et al., 2016)
	Hungary	8/29	272	222-359	(Tima, Brückner, Mohácsi-Farkas, & Kiskó, 2016)
	Norway	ND	-	7200-62500	(Placinta et al., 1999)
		-/101	O: 111 C: 404	O: <20-447 C:<20-2056	(Bernhoft, Clasen, Kristoffersen, & Torp, 2010)
		-/171	476.4	Max: 22000	(M. Lindblad et al., 2012)
		28/28	M: 2070	Max: 7230	(Uhlig et al., 2013)
		260/289	-	50-30000	(Hofgaard et al., 2016)
	Poland	6/6	28	1-48	(Krysinska-Traczyk, Perkowski, & Dutkiewicz, 2007)
		34/35 14/18	O: 636 C: 697	O:250-1700 C:220-2150	(Kuzdraliński, Solarska, & Mazurkiewicz, 2013)
		7/34 20/24	O: 33.1 C: 28.7	O: 5-107 C: 5-189	(Twaruzek, Błajet-Kosicka, Wenda-Piesik, Pałubicki, & Grajewski, 2013)
		4/4	113	67-149	(Bryła et al., 2016)
	Russia	2/8	92	90-94	(Tutelyan et al., 2013)
	Scandinavia	4/4	628	-	(Scudamore, Baillie, Patel, & Edwards, 2007)
Sweden	-/33	199.5	Max: 2340	(M. Lindblad et al., 2012)	
	93/93	M: 172	Max: 5544	(Mats Lindblad et al., 2013)	
UK and Ireland	8/8	13	-	(Scudamore et al., 2007)	
UK	147/458	11	0-282	(Edwards, 2009)	

Wheat	Argentina	ND	-	100-9250	(Placinta et al., 1999)
	Belgium	4/6	58.5	16-150	(De Boevre et al., 2012)
		6/6	910.5	46-2638	(Rasmussen et al., 2012)
		81/93	1053	-	(Vanheule et al., 2014)
	Brazil	ND	-	470-590	(Placinta et al., 1999)
	Bulgaria	ND	-	<1800	
	Canada	ND	-	10-10500	(Placinta et al., 1999)
		40/40	2732	54-8792	(Martos et al., 2010)
	Croatia	O: 14/25 C: 16/27	O: 256 C: 252	O: 62.4-678 C: 27-1220	(Pleadin et al., 2017)
	Finland	28/61	420	Max: 2224	(Hietaniemi et al., 2016)
	Germany	ND	-	4-20500	(Placinta et al., 1999)
	Hungary	2/2	383	<1400	(Uhligh et al., 2013)
		21/29	478	230-1880	(Tima et al., 2016)
	Japan	ND	-	30-1280	(Placinta et al., 1999)
	Netherlands	ND	-	20-231	(Placinta et al., 1999)
	Norway	ND	-	450-4300	(Placinta et al., 1999)
		92/92	O: 91.7 C: 180	O: <20-358 C: <20-797	(Bernhoft et al., 2010)
163/178		-	Max: 16000	(Hofgaard et al., 2016)	
Poland	ND	-	2000-40000	(Placinta et al., 1999)	
	45/45	770.7	82-2975	(Bryła et al., 2016)	
USA	ND	-	<9300	(Placinta et al., 1999)	
Barley	Belgium	64/65	2029	-	(Vanheule et al., 2014)
	Canada	84/116	1370	Max: 9110	(Campbell et al., 2000)
		20/20	816.4	78-2449	(Martos et al., 2010)
	Croatia	18/34	342	74-228	(Pleadin et al., 2013)
		O: 6/11 C: 8/13	O: 71.8 C: 140	O: 32.3-157 C: 42.6-389	(Pleadin et al., 2017)
	Finland	59/86	308.8	Max: 4752	(Hietaniemi et al., 2016)
	Hungary	14/29	339	240-429	(Tima et al., 2016)
	Korea	ND	-	5-361	(Placinta et al., 1999)
	Netherlands	ND	-	4-152	(Placinta et al., 1999)
	Norway	ND	-	2200-13300	(Placinta et al., 1999)
		108	O: 43.7 C: 44	O: <20-154 C: <20-207	(Bernhoft et al., 2010)
		20/20	M: 150	Max: 636	(Uhligh et al., 2013)
	Poland	5/5	22	Max: 40	(Krysinska-Traczyk et al., 2007)
20/24		370	54-1602	(Bryła et al., 2016)	
Russia	10/214	370.2	60-1280	(Tutelyan et al., 2013)	
USA	ND	-	<500-26000	(Placinta et al., 1999)	
Maize	Belgium	6/6	2036	411-5245	(Boevre, Mavungu, Maene, & Audenaert, 2012)
	Canada	ND	-	20-4090	(Placinta et al., 1999)
		14/15	1513.5	574-4865	(Martos et al., 2010)
	China	ND	-	490-3100	(Placinta et al., 1999)
	Croatia	45/63	1565	215-1942	(Pleadin et al., 2013)
		O: 31/33 C: 30/37	O: 564 C: 350	O: 35-2260 C: 28-1430	(Pleadin et al., 2017)
Hungary	25/29	1872	225-2963	(Tima et al., 2016)	

	New Zealand	ND	-	3400-8500	(Placinta et al., 1999)
	Poland	1/2	180	180	(Krysinska-Traczyk et al., 2007)
	Russia	1/53	70	-	(Tutelyan et al., 2013)
	South Africa	ND	-	Max: 1830	(Placinta et al., 1999)
Rye	Canada	15/15	269.8	87-500	(Martos et al., 2010)
	Croatia	O: 4/7 C: 2/9	O: 68.6 C: 35.4	O: 34-113 C: 31-40.2	(Pleadin et al., 2017)
	Denmark	17/17	56	-	(Nielsen et al., 2011)
	Finland	38/43	-	5-100	(Eskola, Parikka, & Rizzo, 2001)
		2/13	25	12-40	(Hietaniemi et al., 2016)
	Netherlands	ND	-	8-384	(Placinta et al., 1999)
	Poland	5/5	19	-	(Krysinska-Traczyk et al., 2007)
Russia	1/63	60	-	(Tutelyan et al., 2013)	
Triticale	Denmark	4/5	306	43-737	(Rasmussen et al., 2012)
	Poland	15/20	573	196-1326	(Bryła et al., 2016)

O=organic; C=conventional, M=median; ND= not defined

1 Table 2: Occurrence of deoxynivalenol (DON) in cereal-based baby food and food for young
 2 children

Baby and young children foods	Country	DON, µg/kg		Reference
		Mean (+ve/total samples)	Range	
Cereal-based food	Germany	61(15/25)	15-314	(Schollenberger, Suchy, Jara, Drochner, & Müller, 1999)
Biscuits and pasta	Italy	35 (7/12)	7-166	(Cirillo, Ritieni, Galvano, & Cocchieri, 2003)
Oat based cereals	Canada	52 (33/53)	Max: 90	(Lombaert et al., 2003)
Barley based cereals		260 (29/50)	Max: 980	
Soy based cereals		116 (8/8)	Max: 240	
Rice based		(0/9)	-	
Multigrain cereals		116 (62/86)	Max: 400	
Cereal-based food	Italy	(16/44)	ND	(Romagnoli, Ferrari, & Bergamini, 2010)
Barley based cereals	USA	63.5 (10/11)	ND	(DOMBRINK-KURTZMAN, POLING, & KENDRA, 2010)
Oat based cereals		13 (8/18)		
Mixed cereals		35.1 (15/23)		
Cereal-based food	Spain	131 (12/30)	Max: 286	(Cano-Sancho et al., 2011)
Wheat-based cereals	Italy	(11/11)	Max: 245	(Juan et al., 2014)
3 cereals (rice >70 %)		(1/2)	Max: 40.2	
3 cereals (maize > 65%)		(2/2)	Max: 103.8	
4-5 cereals (wheat >50%)		(3/7)	Max: 268	
5 cereals (barley >50%)		(2/3)	Max: 108	
Rice/corn-based meals	ND	(4/7)	46-877	(Zhang et al., 2014)
Cereal-based food	Portugal	160.6(4/9)	29-271	(Pereira et al., 2015)
Breakfast cereals	Tunisia	13 (5/10)	5-47	(Oueslati et al., 2018)
Infant cereals		46 (6/6)	10-110	
Baby mix		61 (7/9)	12-109	

3 ND=not defined.

Table 3: Morphology and transition temperatures for native starches from different cereal sources

Species	Granule shape	Diameter (μm)	Amylose (% w/w)	Crystalline type	Gelatinization temperatures ¹			Reference
					T _o (°C)	T _p (°C)	T _c (°C)	
Wheat	Spherical and lenticular*	<30*	25.6±1*	A*	52**	57**	63**	*Alcázar-Alay & Meireles, 2015 **Sasaki & Matsuki, 1998
Barley	Lenticular (A-type), spherical (B-type)*	15-25, 2-5*	19-22.1*	A, B*	46.7**	56.5**	73.7**	*Tester et al., 2004 **Tester & Morrison, 1990
Oats	Polyhedral*	3-10 (single) 80 (compound)*	28.4±0.8**	Do not fall into discrete size distribution***	45**	57**	72**	*Tester et al., 2004 **Tester & Karkalas, 1996 ***Zhou, Robards, Glennie-Holmes, & Helliwell, 1998
Maize	Angular*	11.5±0.3*	23-28*	A*	67.3**	72.9**	82.7**	*Alcázar-Alay & Meireles, 2015 **Chung, Liu, & Hoover, 2009
Rye	Lenticular (A-type), spherical (B-type)*	10-40 5-10*	29.7**	A**	51**	56**	62**	*Tester et al., 2004 **Verwimp, Vandeputte, Marrant, & Delcour, 2004
Sorghum	Polygonal, dented, round*	0.8*	23.7-27.6*	A*	67.9**	70.7**	75.7**	*Alcázar-Alay & Meireles, 2015 **Sang, Bean, Seib, Pedersen, & Shi, 2008
Triticale	Disc-shaped, Spherical*	1-30*	26.9**	A, B**	43.8**	53.6**	60.1**	*Tester et al., 2004 **Ao & Jane, 2007
Rice	Angular, polygonal*	<20*	21-25*		72**	76.6**	89.2**	*Alcázar-Alay & Meireles, 2015 **Liu, Shao, & Tseng, 1995

¹ T_o, T_p and T_c are onset, peak and conclusion temperatures, respectively; the stars in a line correspond to the references the data was cited from.