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**Inhibitory effect of quinoa and fenugreek extracts on pancreatic lipase and α -amylase under
in vitro traditional conditions or intestinal simulated conditions**

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16 **ABSTRACT**

17 Ethanol extracts (EE) from fenugreek and quinoa seeds with different total content of inhibitory
18 compounds (TIC, total saponin plus phenolic) were prepared with and without concentration of TIC
19 (CEE –concentrated EE-, and EE, respectively). Their inhibitory activity on pancreatic lipase and α -
20 amylase was assessed by traditional *in vitro* methods (with or without orbital shaking), and by
21 simulating intestinal digestion.

22 CEE contained higher contents of TIC than EE, being fenugreek superior to quinoa ($p < 0.001$). The
23 extracts inhibited enzymes in a dose-dependent manner, CEE extracts being stronger (fenugreek for
24 lipase $-p = 0.009-$, and quinoa for α -amylase $-p < 0.001-$). Shaking did not impact the activity.
25 Intestinal conditions worsened the inhibition of lipase, but slightly catalyzed the α -amylase. Longer
26 times of reaction worsened activities.

27 The importance of assessing the inhibitory activity of extracts under simulated intestinal conditions
28 is concluded, being fenugreek more interesting than quinoa, especially against pancreatic lipase.

29

30 **Keywords:** saponins, polyphenols, fenugreek, quinoa, pancreatic lipase, α -amylase, inhibition, *in*
31 *vitro* digestion

32

33

34 **Chemical compounds studied in this article:** Orlistat (PubChem CID: 3034010); acarbose
35 (PubChem CID: 70702327); bile salts (PubChem CID: 439520); soyasaponin I (PubChem CID:
36 122097); protodioscin (PubChem CID: 441891); catechin (PubChem CID: 9064); n-butanol
37 (PubChem CID: 263)

38

39 **1. Introduction**

40 The metabolic syndrome (MetS), characterized by the combination of at least three of the
41 cardiovascular risk factors (abdominal obesity, dyslipidemia, hypertension and hyperglycemia),
42 currently has a high prevalence across Europe with an age-associated increase, and is an early sign
43 for future development of chronic diseases such as obesity and/or type 2 diabetes (Scuteri et al.,
44 2015). Together with dietary and lifestyle modifications, the treatment of MetS-related risk factors
45 is mainly based on pharmacological approaches. In this way, one of the popular strategies in the
46 development of drugs against the MetS is the search for inhibitors that interfere with the digestive
47 enzymes responsible for the hydrolysis and absorption of macronutrients, such as carbohydrates and
48 triglycerides (de la Garza, Milagro, Boque, Campión, & Martínez, 2011). Thus, glycemic index
49 imbalance and glucose intolerance can be improved by inhibiting enzymes involved in carbohydrate
50 digestion, such as pancreatic α -amylase, which degrades polysaccharides into oligosaccharides and
51 disaccharides, and brush border α -glucosidase, which degrades the latter into monosaccharides.
52 However, most of the synthetic inhibitors commercially available, such as acarbose, have strong
53 amylase and glucosidase inhibitory properties, causing an excessive inhibition of amylase activity
54 and a subsequent abnormal fermentation of undigested saccharides in the colon (Ercan & El, 2016;
55 Nagmoti & Juvekar, 2013). Therefore, the preferred strategy to control the release of glucose from
56 disaccharides in the gut seems to be a moderate amylase inhibition together with strong glucosidase
57 inhibition (Ercan & El, 2016). Likewise, the inhibition of the human pancreatic lipase, the main
58 enzyme responsible for the hydrolysis of triacylglycerols into monoacylglycerols and free fatty
59 acids, is a popular therapeutic approach against overweight, obesity and obesity-related diseases.
60 Currently, the synthetic product Orlistat is clinically used as a pancreatic lipase inhibitor, although
61 side effects such as flatulence, diarrhea or dyspepsia are also commonly developed (Marrelli,
62 Conforti, Araniti, & Statti, 2016; Seyedan, Alshawsh, Alshagga, Koosha, & Mohamed, 2015). Such
63 side effects described for both commercial inhibitors are the main reasons for the continuous
64 research of novel enzyme inhibitors, preferably from natural sources versus the synthetic ones.

65 Within natural sources, ~~vegetables and~~ plants are the most popular sources containing bioactive
66 compounds of a wide diverse chemical nature that are well-known for their biological activities,
67 such as antiinflammatory, antihypertensive, antioxidant, anticarcinogenic, antidiabetic or antiobesity.
68 Within these compounds, those with inhibitory activity against digestive enzymes have been also
69 described. Specifically, the most common compounds that are found in different plant species for
70 which have been described enzyme inhibition activities are terpenes, saponins or polyphenols, being
71 the last two the widest groups of natural inhibitors against digestive enzymes (Birari & Bhutani,
72 2007; Buchholz & Melzig, 2015; Marrelli et al., 2016; Navarro del Hierro, Herrera, Fornari,
73 Reglero, & Martin, 2018; Xiao, Ni, Kai, & Chen, 2013).

74 Saponins consist of a triterpenoid or steroid non-polar aglycone attached to one or more hydrophilic
75 oligosaccharide moieties through an ether or ester glycosidic linkage. Triterpenoid saponins have
76 been identified in legumes, quinoa seeds, ginseng roots, quillaja bark or liquorice roots, whereas
77 steroid saponins have been found in fenugreek seeds, yucca, ginseng roots, asparagus or oats
78 (Güçlü-Üstündağ & Mazza, 2007; Makkar, Siddhuraju, & Becker, 2007). Although the inhibitory
79 activity of saponins from many of these sources has been intensively explored, the potential of other
80 saponin sources, such as edible seeds, has not been so extensively elucidated, especially saponin-
81 rich extracts produced from edible seeds as legumes or quinoa. **In this sense, fenugreek seeds are**
82 **legumes known to contain high levels of steroidal saponins, whereas the seeds of the pseudo-cereal**
83 **quinoa have been described as sources of triterpenoid saponins (Arivalagan, Gangopadhyay, &**
84 **Kumar, 2013; Medina-Meza, Aluwi, Saunders, & Ganjyal, 2016).**

85 Polyphenols are the most widespread and common bioactive constituents in plants, and represent
86 the most abundant antioxidants in the human diet. Polyphenols contain at least one aromatic ring
87 with one or more hydroxyl groups in addition to other substituents, and they can be divided into 15
88 major classes according to their chemical structures (Xiao et al., 2013). Together with the specific
89 activity as inhibitors of digestive enzymes, a wide range of biological effects have been described

90 for both compounds, such as hypocholesterolemic, antiinflammatory, antitumor,
91 immunomodulatory or antibacterial activities (Li et al., 2014; Singh, Singh, Singh, & Kaur, 2017).
92 Taking into consideration the potential of these two types of molecules as bioactive agents, great
93 efforts are being made to obtain saponin or polyphenol rich-extracts with inhibitory activities on the
94 main digestive enzymes. However, most of the previous studies on these inhibitors have been
95 mainly focused on the production of either saponin-rich extracts or polyphenol-rich extracts, but the
96 production of extracts with a simultaneous combination of both potential inhibitors within the same
97 extracts has been less scarcely explored and might be of interest. In this way, we have recently
98 demonstrated that the ultrasound-assisted extraction from different edible seeds (quinoa, lentil,
99 fenugreek, soybean and lupin) favors the obtaining of saponin-rich ethanol extracts with an
100 interesting content of polyphenols, although the exploration of further concentration procedures
101 might be of interest for obtaining even richer saponin extracts with stronger bioactivities (Navarro
102 del Hierro, Herrera, García-Risco, et al., 2018). Additionally, in such study, fenugreek and quinoa
103 were the richest extracts in saponins plus phenolic compounds, so the evaluation of the potential of
104 these extracts that combined both bioactive molecules on the inhibition of pancreatic lipase and α -
105 amylase might be of interest.

106 Concerning the inhibitory study of compounds, it is important to remark that contradictory results
107 about the inhibitory activities from saponins and polyphenols can be found in the different studies,
108 since either positive inhibition of digestive enzymes has been shown, or either such an effect has
109 not always been clearly demonstrated. These contradictory results might be related, among other
110 factors, to differences among the methodologies used to determine such activity. Thus, most studies
111 have assessed the *in vitro* inhibitory activity by merely replicating the enzymatic reaction, that is,
112 combining substrate, enzyme and inhibitor in a proper buffer (Tucci, Boyland, & Halford, 2010).
113 However, in order to clearly understand the magnitude and potential of an inhibitor after its
114 presumable oral intake, it is essential to reproduce the environment of the gastrointestinal tract as
115 possible, as the complexity of the *in vivo* physiological conditions might affect the global enzymatic

116 result (Lee, Mohd Esa, & Loh, 2015). In agreement, Vinarova et al. (2015) carried out the *in vitro*
117 gastrointestinal digestion of several fats in presence of diverse saponin extracts and reported a lack
118 of effect on the hydrolysis of triglycerides, regardless of the saponin concentration. Ercan & El
119 (2016) evaluated the inhibitory activity of saponin extracts from chickpea and *Tribulus terrestris*
120 against glucosidase and amylase after simulation of intestinal digestion by *in vitro* conditions. Such
121 activity after the *in vitro* digestion was lower than prior to digestion, which was attributed to a likely
122 effect of the digestion process, temperature or pH of the media on the activity of the compounds.
123 Thus, when evaluating the inhibitory activity of extracts and compounds against digestives
124 enzymes, more complex factors closer to an *in vivo* situation, might also need to be considered,
125 such as temperature, time of reaction, shaking during the process, or composition of the reaction
126 media, as such activity could be either over or underestimated.

127 The present study aims to obtain different extracts with different content of potential inhibitors,
128 namely saponins and polyphenols, from two edible seeds, fenugreek (as a representative source of
129 steroid-like saponins) and quinoa (as a representative source of triterpenoid-like saponins). Then,
130 the inhibitory effect of such extracts on lipase and α -amylase enzymes was assessed by comparing
131 three *in vitro* procedures where different factors including shaking, time of digestion, and medium
132 composition, were taken into consideration. Specifically, the traditional *in vitro* method of
133 inhibition measurement, either with or without shaking, and a more complex method simulating
134 intestinal conditions, were compared.

135

136 **2. Materials and methods**

137 **2.1 Reagents and Materials**

138 Seeds of red quinoa (*Chenopodium quinoa*) as triterpenoid-like saponins, were purchased from Hijo
139 de Macario Marcos (Salamanca, Spain). Fenugreek (*Trigonella foenum-graecum*), as steroidal-like
140 saponins, were from Murciana de Herboristeria (Murcia, Spain).

141 Lipase from porcine pancreas (L3126), α -amylase from porcine pancreas (A3176), 4-
142 methylumbelliferyl oleate (4-MUO) (75164), starch from potato (S2004), lugol solution (62650),
143 acarbose (PHR1253), Orlistat (PHR1445), Dulbecco's Phosphate Buffered Saline (PBS) (59300C),
144 bile salts (B8756), phosphatidyl choline from egg yolk (61771), sodium chloride (746398), calcium
145 chloride (746495), Trizma base (T1503) and maleic acid (M0375), were from Sigma-Aldrich
146 Chemie GmbH (Steinheim, Germany). Soyasaponin I (S9951), protodioscin (G0299), catechin
147 (43412) were from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Absolute ethanol
148 (131086.1214) and n-butanol (131082.1611) were from Panreac-AppliChem (Spain), 95% n-hexane
149 (6752-25) was from Macron (Poland).

150

151 2.2 Obtention of the extracts

152 Seeds were ground in a knife mill (Grindomix GM200 RETSCH) at 10000 rpm for 1 min. The
153 resulting powder was sieved in a vertical sieve (CISA Cedacería Industrial, España) in order to
154 obtain fractions with a particle size between ≤ 250 and > 100 μm . Extraction was based on Navarro
155 del Hierro, Herrera, García-Risco, et al. (2018) with modifications. Firstly, samples were defatted
156 with hexane at a ratio of sample to solvent of 1:5 (w/v) for 2 min by direct sonication (Branson
157 SFX250 Digital Sonifier, Branson Ultrasonics, USA) with an ultrasonic probe (1/2" diameter,
158 output sonication amplitude of 60%). The mixture was then centrifuged at 4000 rpm for 5 min.
159 Supernatant was discarded and the precipitate was defatted again under the same conditions. After
160 defatting, the precipitate was extracted with ethanol at a ratio of sample to solvent of 1:10 (w/v) for
161 15 min by direct sonication (Branson SFX250 Digital Sonifier, Branson Ultrasonics, USA) with an
162 ultrasonic probe (1/2" diameter, output sonication amplitude of 60%), and the temperature during
163 the process was kept under 40 °C. The mixture was filtered under vacuum and dried using a rotary
164 evaporator. The resulting ethanol extracts (EE) were stored at -20 °C until further use. Extractions
165 were performed at least in duplicate.

166 A second extract was produced from both seeds as concentrated ethanol extract (CEE). The aim of
167 this procedure was to obtain a higher enrichment of saponins of the EE, which is frequently
168 performed by a liquid-liquid procedure with aqueous butanol (Chan, Iqbal, Khong, Ooi, & Ismail,
169 2014; Güçlü-Üstündağ & Mazza, 2007; Pham, Vuong, Bowyer, & Scarlett, 2017). Thus, starting by
170 the same procedure to obtaining the EE, an additional concentration step was performed based on
171 Chan, Iqbal, Khong, Ooi, & Ismail (2014) with modifications. Briefly, after obtaining the EE, 50
172 mL of water and 100 mL of n-butanol were added, vortexed for 1 min and centrifuged at 4500 rpm
173 for 10 min. The upper supernatant of n-butanol was filtered and the lower supernatant was extracted
174 again with water and butanol under the same conditions. The collected upper supernatants were
175 dried using a rotary evaporator and the resulting CEE were stored at -20 °C until further use.
176 Extractions were performed at least in duplicate.

177

178 **2.3 HPLC analysis of saponins and phenolic compounds**

179 Quantification of total saponins and total phenolic compounds was performed on an Agilent HPLC
180 1260 Infinity series system coupled to a photodiode-array detector (Agilent Technologies Inc.,
181 Santa Clara, CA) equipped with an ACE 3 C18-AR column (150mm x 4.6mm, 3 µm particle size)
182 protected by a guard column. Samples were dissolved in methanol at 10 mg/mL and the injection
183 volume was 20 µL. The compounds occurring in the extracts were monitored at 205 nm, and UV
184 spectra were recorded from 190 to 700 nm. Chromatographic separation was achieved at 25 °C
185 using 0.05% TFA in water (Phase A) and 0.05% TFA in acetonitrile (Phase B) at a flow rate of 0.4
186 mL/min as follows: 0 min: 95% A; 20 min: 5% A; 45 min: 5% A; 46 min 95% A; 50 min: 95% A.
187 Identification of saponins was previously confirmed in an Agilent 6120 HPLC-MS by pure
188 commercial standards or by comparison with mass spectra from the literature. The same flow and
189 gradient conditions previously described were used for the HPLC-MS analyses. The rest of the
190 conditions were as follows, according to Mad, Sterk, Mittelbach, & Rechberger (2006): MS
191 ionization mode with APcI+/- between 30 and 1500 Da range mass. Nebulizing/drying gas at a flow

rate of 5 L/min, 350 °C and 20 psi. The vaporizer temperature was set at 250 °C, the capillary voltage was set to 2000 and 4000 V, and fragmentor voltage was set at 40 V. Spectra were obtained over m/z 300-1500. On the other hand, in case of phenolic compounds, these were confirmed according to their UV spectra and compared with pure commercial standards.

Total saponin content (TSC) was calculated as g of protodioscin equivalents/100 g of extract for fenugreek, and g of soyasaponin I/100 g of extract for quinoa, each of these standards representing steroid-like or triterpenoid-like saponins, respectively. Total phenolic content (TPC) was calculated as g of catechin equivalents/100 g of extract. Finally, for comparative purposes between the extracts, a value defined as **total content of inhibitory compounds** (TIC) was estimated as the sum of TSC and TPC. **It should be remarked that, according to this given definition of TIC, this value does not consider other potential and unknown phytochemicals of the extracts with potential inhibitory activity, but only saponins and phenolic compounds.**

2.4 Enzyme inhibition assays

2.4.1 General

Both lipase and α -amilase inhibition assays were performed by incubation of the substrate, enzyme and extract at 37 °C, under three different conditions: A) traditional method, that is, static incubation, using PBS as buffer; B) traditional method with shaking incubation; and C) simulation of intestinal conditions, that is, shaking incubation using a digestion buffer (Trizma-Maleic 100 mM, pH 7.5, 0.15 M NaCl, and 5.1 mM CaCl₂), containing bile salts (7.8 mg/mL) and lecithin (3.12 mg/mL) according to the model of *in vitro* intestinal digestion of Martin et al. (2016).

2.4.2 Pancreatic lipase inhibition assay

The inhibitory activity of each extract against pancreatic lipase was measured by using 4-MUO as substrate, according to Sugiyama et al. (2007) with modifications. Reaction mixture consisted of 1 mL of extract solution in buffer at different concentrations, 1 mL of freshly-prepared pancreatic

lipase at 1 mg/mL (0.01 g of lipase in 10 mL of buffer, stirred for 10 min and centrifuged at 4000 rpm for 10 min), and lastly, 2 mL of 4-MUO solution at 0.1 mM in buffer.

At least, five different concentrations of extracts were tested. Reactions at each concentration were prepared in triplicate. Control samples in absence of extracts were prepared following the same procedure, also in triplicate. Control of extracts at each concentration of each extract, in absence of lipase and substrate, were also prepared in triplicate.

The reaction mixture was placed at 37 °C in an orbital incubator, either without or with shaking (250 rpm), depending on the condition assayed. Three aliquots of 150 µL were taken at 20 and 60 min of reaction and the amount of 4-MUO hydrolyzed by lipase was measured in a 96-well microplate using a fluorescence microplate reader (Polarstar Galaxy, BMG Labtechnologies) at an excitation wavelength of 350 ±10 nm and an emission wavelength of 450 nm.

The inhibition of pancreatic lipase activity was calculated as follows:

$$Inhibition (\%) = 100 - \left[\left(\frac{F_{extract\ sample} - F_{extract\ control}}{F_{control\ sample}} \right) \times 100 \right]$$

Where $F_{extract\ sample}$ is the fluorescence of the reactions with added extract, $F_{extract\ control}$ is the fluorescence of the control of extracts, and $F_{control\ sample}$ is the fluorescence of the reactions without extract.

Finally, a logarithmic regression curve was established to calculate IC₅₀ values (mg/mL), defined as the concentration of the extract that inhibited 50% the activity of the pancreatic lipase.

Preparations using the commercial inhibitor of pancreatic lipase, namely Orlistat, were performed following the same procedure, in order to evaluate the magnitude of the results obtained for the extracts in case of the experiments performed under the conditions of simulation of intestinal digestion.

240

2.4.3 α-amylase inhibition assay

The inhibitory activity of each extract against α-amylase was measured by using starch as substrate, using the Caraway-Somogyi iodine/potassium iodide method with slight modifications, according

244 to Afiukwa, Ibiam, Edeogu, Nweke, & Chukwu (2009) and Zengin (2016). Briefly, reaction
 245 mixture consisted of 1 mL of extract solution in buffer at different concentrations and 1 mL of α -
 246 amylase solution at 0.4 mg/mL in buffer. This mixture was placed in an orbital incubator at 37 °C
 247 for 10 min. After pre-incubation, the reaction was initiated by the addition of 1 mL of potato starch
 248 solution (0.5% in water heated at 100 °C for 15 min) tempered at 37 °C.

249 At least, five different concentrations of extracts were tested. Reactions at each concentration were
 250 prepared in triplicate. Control samples in absence of extracts were prepared following the same
 251 procedure, also in triplicate. Control of extracts at each concentration of each extract, in absence of
 252 α -amylase and substrate, were also prepared in triplicate. Finally, control of substrate, in absence α -
 253 amylase and extracts were also prepared.

254 The reaction mixture was placed at 37 °C in an orbital incubator, either without or with shaking
 255 (250 rpm), depending on the condition assayed. Three aliquots of 150 μ L were taken at 20 and 60
 256 min of reaction and placed in a 96-well microplate. Then, 100 μ L of indicator (lugol) were added
 257 and absorbance was measured at 585 nm.

258 The inhibition of α -amylase activity was calculated as follows:

$$259 \quad \text{Inhibition (\%)} = 100 - \left\{ \left[\frac{A_{\text{substrate control}} - (A_{\text{extract sample}} - A_{\text{extract control}})}{A_{\text{substrate control}} - A_{\text{control sample}}} \right] \times 100 \right\}$$

260 Where $A_{\text{substrate control}}$ is the absorbance of the control of substrate, $A_{\text{extract sample}}$ is the absorbance of
 261 the reactions with added extract, $A_{\text{extract control}}$ is the absorbance of the control of extracts, and A_{control}
 262 $_{\text{sample}}$ is the absorbance of the reactions without extract.

263 Preparations using the commercial inhibitor of α -amylase, namely acarbose, were performed
 264 following the same procedure, in order to evaluate the magnitude of the results obtained for the
 265 extracts in case of the experiments performed under the conditions of simulation of intestinal
 266 digestion.

267

268 **Statistical analysis**

269 Statistical analyses were performed by means of the general linear model procedure of the SPSS
270 24.0 statistical package (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance.
271 Differences were considered significant at $P \leq 0.05$. Post-hoc Tukey's tests were performed in order
272 to establish significant differences.

273

274 **3. Results and discussion**

275 **3.1 Saponin and phenolic content of the extracts**

276 Different ethanol extracts (EE) and concentrated ethanol extracts (CEE) of fenugreek and quinoa
277 seeds were produced by ultrasound-assisted extraction, with the purpose of obtaining extracts with
278 different content of saponins and phenolic compounds and, hence, different content of potential
279 inhibitor compounds of digestive enzymes. Table 1 presents the TSC, TPC and TIC of both EE and
280 CEE from the two studied seeds. On the one hand, regardless of the procedure of extraction,
281 fenugreek granted by far higher contents of saponins and phenolic compounds than quinoa ($p <$
282 0.001). Thus, as mean value, saponin content of fenugreek extracts was around 12 g/100 g of
283 extract, compared to 0.3 g/100 g of extract of quinoa; and, as mean value, phenolic content of
284 fenugreek extracts was around 1 g/100 g of extract, compared to 0.1 g/100 g of extract of quinoa.

285 On the other hand, regardless of the seed, and although it was not significant ($p > 0.05$), the results
286 showed that CEE tended to have a slight higher content of saponins than EE, although it was more
287 noticeable in the quinoa extracts (70% increment of TSC for CEE respect to EE) than in those from
288 fenugreek (28% increment of TSC for CEE respect to EE). This trend to a concentration effect of
289 saponins with butanol was not clearly observed in case of TPC. Thus, TPC only seemed to be
290 higher in CEE than EE for quinoa (80% increment of TPC for CEE respect to EE), whereas both EE
291 and CEE from fenugreek displayed very similar values. Therefore, these results might suggest that
292 the concentration procedure of the extracts with butanol, typically performed in the literature for
293 obtaining saponin-rich extracts (Chan et al., 2014; Pham et al., 2017), might lead to extracts with an
294 effective enrichment in saponins from the assayed seeds, but with variable modifications in the

phenolic content, mainly depending on the source, and likely depending on the chemical nature of the different phenolic compounds and their affinity for butanol.

When considering the estimated value of TIC as the sum of TSC and TPC, the previously described differences between seeds reflected a final value of total inhibitors content clearly superior for fenugreek extracts than quinoa extracts (around 13 g/100 and 0.3 g/100 g, respectively). In any case, it is important to remark that, concerning the contribution of each type of inhibitor to the TIC value, it was clear that total saponins accounted for the major component, although slight differences were found between fenugreek and quinoa extracts. Thus, saponins and phenolic compounds from fenugreek extracts accounted for 91% and 9% of the TIC, respectively; whereas saponins and phenolic compounds from quinoa extracts accounted for 80% and 20% of the TIC, respectively.

As summary, the obtained results suggested that the fenugreek extracts appeared to be more interesting than the ones from quinoa as potential extracts against the activity of digestive enzymes if considering the total amount of inhibitor compounds as saponins and phenolic compounds. Furthermore, the concentration of the EE in potential inhibitors compounds was successfully achieved. Nevertheless, since the enrichment of the extracts was not remarkable, further studies might be of interest in order to explore other different conditions of extraction and/or concentration, in order to reach higher enrichments in total inhibitor compounds. Additionally, it would be also of interest to explore whether the applied procedure of concentration might also enrich the extracts in other compounds with inhibitory activity different to saponins and phenolic compounds.

3.2 Inhibitory effect against pancreatic lipase

3.2.1 Traditional method of in vitro inhibitory effect

After confirming that the different extracts from the different seeds showed interesting and diverse content of inhibitor compounds, all the extracts were tested as potential pancreatic lipase inhibitors.

Firstly, the inhibitory activity of the EE and CEE from fenugreek and quinoa was evaluated under

the traditional conditions typically described in the scientific literature. Although the traditional conditions can be variable, one of the most popular method is characterized by a simple *in vitro* incubation of the lipase enzyme with a fluorogen lipid substrate, in presence of the tested inhibitor, and in a proper phosphate buffer at 37 °C for 20 min. At the end of reaction, the fluorescence due to the released fluorogen group from the substrate is measured, and the comparison with the fluorescence measured in absence of inhibitor allows to estimate the inhibitory activity (Podsędek, Majewska, & Kucharska, 2017; Zhang et al., 2015).

As first and important finding, all the assayed extracts showed the ability to inhibit the pancreatic lipase activity, and in a dose-dependent manner, allowing to estimate an inhibition of 50% of the activity, as shown in Figure 1. However, interesting differences in this bioactivity were found between seeds and procedure of extraction. On the one hand, regardless of the procedure of extraction, a significant effect of the seed factor on the IC_{50} value was obtained ($p = 0.003$). Thus, the mean IC_{50} value for fenugreek extracts was significantly lower than the mean IC_{50} value of quinoa. Therefore, the inhibitory activity from fenugreek extracts was, as mean, 10 folds higher than that of quinoa, indicating a strongest inhibitory activity for fenugreek extracts.

Concerning the effect of the extraction/concentration procedure of the extracts, a significant effect was also found on the IC_{50} values ($p = 0.009$). Thus, regardless of the seed, the CEE extracts showed significant lower IC_{50} values than the EE extracts. Therefore, in general, the inhibitory activity from CEE extracts was around 20 folds higher than that of the EE extracts, indicating a strongest inhibitory activity for CEE. Therefore, taking into account the effect of both factors on the IC_{50} value, namely the seed and the extraction/concentration procedure, it was concluded that the most efficient inhibitory extract of pancreatic lipase was the CEE from fenugreek ($IC_{50} = 0.07$ mg/mL), whereas the less efficient inhibitory extract was the EE from quinoa ($IC_{50} = 2.81$ mg/mL) (Figure 1).

These differences obtained due to both the seeds and the extraction/concentration procedure could be related to the content of saponins and phenolic compounds of the extracts. In fact, as shown in

Figure 2, a noticeable trend was observed to lower IC_{50} values as the value of TIC increased, for both fenugreek and quinoa extracts (Figure 2.a and 2.b, respectively). Therefore, this would suggest that a higher value of TIC of the CEE extracts from both seeds might be one of the factors that would cause a better inhibitory activity of these extracts against pancreatic lipase. Nevertheless, further studies would be necessary in order to confirm these results, since the concentration procedure of the extracts might also cause the potential concentration of other compounds of different chemical nature whose contribution to the inhibitory activity would need to be elucidated. In any case, in agreement with the present results, Oishi et al. (2007) also found that the butanol fraction (saponin fraction) of an aqueous methanol extract of *Momordica charantia* displayed a higher lipase inhibition than the water fraction due to the richness of saponins in the first fraction. Additionally, these authors attributed the inhibitory effect of saponins to their ability to bind to the substrate, causing a possible reduction in the contact area between the substrate and lipase and indirectly inhibiting the action of the enzyme. In the case of the seeds evaluated in this work, the effect of fenugreek and quinoa extracts on lipase inhibition has been very scarcely explored to date. Chaubey et al. (2018) have recently observed that the IC_{50} value of an ethanolic extract of fenugreek was 2.4 mg/mL. As difference, p-nitrophenol was used as substrate and absorbance was measured after 10 min of incubation. Tang et al. (2016) evaluated the effect of an alkaline-hydrolyzable bound phenolic fraction obtained from red quinoa seeds and found a IC_{50} value of 10 mg/mL. These authors used 4-MUO as substrate, although the reaction was prolonged for 1 hour. The abovementioned values in both seeds widely differ from the ones obtained in this work, probably due to factors such as the different composition of the extracts or the different method conditions.

3.2.2 Traditional method of *in vitro* inhibitory effect under shaking

The traditional method of *in vitro* measurement of inhibition of pancreatic lipase is typically performed under static conditions, according to most of the scientific literature reviewed. Therefore,

373 this was the procedure performed in the previous section 3.2.1. However, in order to simulate an *in*
374 *vivo* enzymatic reaction as the intestinal lipolysis, and to test the potential inhibitory activity of
375 presumably orally ingested compounds, we consider that, at least, some kind of movement of the
376 media should be performed, since the intestinal tract is not a static environment, and the impact of
377 this movement on the enzymatic reactions or inhibitory activities should be considered.
378 Additionally, we consider that is especially relevant when complex extracts of difficult aqueous
379 solubility as the assayed are tested. In these cases, it is frequent to find different procedures in the
380 literature to enhance the solubility of the extracts in the reaction media, mainly by the help of
381 addition of minor amounts of organic solvents that favors the perfect solubility of the extracts.
382 However, in the present study, we preferred to avoid the use of any solvent different to the aqueous
383 buffer, and dispersion of the extracts by mechanical procedures might be used instead, closer to a
384 real physiological situation. Thus, the exploration of the effect of a simple shaking movement on
385 the inhibitory activity of the extracts on pancreatic lipase was carried out. Due to the stronger
386 inhibitory effect observed and to the higher content in TIC detected in both CEE of fenugreek and
387 quinoa, the following assays were performed with those extracts only.

388 As shown in Figure 3, the application of shaking to the samples did not lead to significant
389 differences in neither of the IC_{50} values when compared to the assays in absence of shaking.
390 However, it was observed a less variability in the IC_{50} values when shaking was applied. This result
391 might be likely due to either a more adequate dispersion of the extracts in the buffer that did not
392 occur in static conditions, as well to a better homogeneous interaction of all the components of the
393 reaction, in general. Therefore, according to the obtained results, and in order to reach more
394 reproducible results of *in vitro* measurement of inhibitory activities, the application of shaking
395 procedures during the whole reaction might be recommended. Additionally, it was considered an
396 interesting finding that the mechanical shaking of the aqueous environment of the enzymatic
397 reaction did not impact on the magnitude of the inhibitory effects. Whether this lack of effect would

398 take place in a real *in vivo* situation under the peristaltic movements would be worthy for further
399 evaluation.

400

401 3.2.3 *In vitro* inhibitory effect under simulation of intestinal digestion

402 Together with the movement of the enzymatic reaction environment, the simulation of other
403 intestinal *in vivo* parameters should be considered essential in order to elucidate the potential
404 inhibitory activity of compounds on digestive enzymes, especially presumable orally ingested
405 compounds. Thus, a simple simulation of the *in vitro* intestinal digestion of the substrate by
406 pancreatic lipase was performed. The major modification of the method was the inclusion of
407 lecithin and bile salts in the enzymatic reaction, in order to simulate the components of the biliary
408 secretion necessary during the *in vivo* intestinal digestion of lipids.

409 According to Figure 3, the simulation of an *in vitro* intestinal digestion did cause a significant
410 increase ($p < 0.001$) in the IC₅₀ values of the extracts from both seeds (around 0.7 mg/mL for
411 fenugreek extract and 2 mg/mL for quinoa extract), that is, a decrease in the lipase inhibition
412 activity. Therefore, regardless of the seed, the inhibitory efficiency of the extracts against the
413 pancreatic lipase activity was worse after simulation of intestinal conditions than by performing the
414 traditional assay of inhibitory activity.

415 The explanation of these results is complex, because either the activity of the lipase itself, the
416 interaction between the components of the reaction, the modification of the components of the
417 extracts, as well as a sum of all these reasons, might be involved in the observed effect. Lee et al.
418 (2015) recently performed a similar approach as the present study, by comparing traditional method
419 of inhibitory measurement, with the simulation of intestinal conditions for inhibitory measurement.
420 In agreement with the findings of the present study, these authors also showed that, under
421 simulation of intestinal conditions, extracts of black soya, black eye pea and red bean had a lower
422 inhibitory activity against pancreatic lipase when compared to the inhibitory activity under *in vitro*
423 traditional conditions. Only in the case of chickpea, the inhibitory activity did not differ between

both conditions. These authors attributed the results to the degradation of the bioactive compounds during the simulation of the intestinal digestion. On the other hand, Ercan & El (2016) evaluated the inhibitory activity of saponin extracts from chickpea and *Tribulus terrestris* against lipase after simulation of intestinal digestion by *in vitro* conditions. These authors found that such activity after the *in vitro* digestion was lower than the activity prior to digestion, which was associated to the pH of the media or the digestion process itself. Together with these previous reasons, the composition of the digestion buffer used in the present study might have also caused an increase in the lipase activity, acting the components of the buffer, such as bile salts, lecithin and calcium chloride as cofactors (McClements & Li, 2010). In this way, surface active materials are desorbed from the surfaces of emulsified lipids together with free fatty acids by bile salts and lecithin. This situation enables the lipase to keep adsorbed onto the surface of the emulsified lipids, enhancing its activity (Gargouri, Julien, Bois, Verger, & Sarda, 1983; Lee et al., 2015; Scow, 1988).

According to all these previous evidences, and in order to evaluate whether the activity of the extracts might result modified during the time of reaction, the IC₅₀ value of the extracts was also assessed at 60 min of reaction time, either under simulation of intestinal digestion and by the traditional method of inhibitory measurement. A significant effect of the time of reaction was found on the IC₅₀ values ($p = 0.028$). Thus, in general, 60 min of reaction time led to higher IC₅₀ values than 20 min of reaction time, that is, a worse inhibitory activity (Supplementary material). Therefore, longer reaction times hindered the inhibitory potential of the extracts, possibly due to a progressive degradation of the compounds responsible for such activity, as previously explained. Interestingly, this effect was independent on the used conditions to measure inhibition, as well as on the extract or seed.

Finally, in order to evaluate the magnitude of the obtained IC₅₀ values of the extracts, comparative assays with the commercial inhibitor Orlistat were performed and used as positive control under simulated intestinal conditions. The IC₅₀ value was found to be 0.1 µg/mL. These extremely low IC₅₀ values for Orlistat are usually found when this compound is tested by the traditional method.

Therefore, it is worthy to mention that finding such extremely low value for Orlistat when it is assayed under the method of simulating intestinal conditions would validate this last method as a correct procedure to test inhibitors of pancreatic lipase. This validation would allow to conclude that any result observed for the extracts during the simulation of intestinal digestion, would be due to the extracts, and that the generation of artifacts due to the method itself could be discarded. Comparing with the extracts, the IC₅₀ value of Orlistat was considerably lower. Nevertheless, this was a reasonable result taking into account that Orlistat is a pure compound, whereas the assayed extracts consisted of a complex mixture of compounds, where only a minor part are potential inhibitors, and even potential enzymatic catalysts might not be discarded in the extracts. This is a typical situation observed when complex extracts are compared with pure inhibitors. In any case, and as general summary, it can be clearly concluded that all the assayed extracts from fenugreek and quinoa were inhibitors of the pancreatic lipase, in a dose-dependent manner, although fenugreek was superior, and that intestinal simulated conditions and long times of reaction may worsen the inhibitory activity of the extracts. Further *in vivo* studies would be necessary in order to effectively confirm that the oral intake of the extracts might interfere the digestion of dietary lipids and lead to a subsequent systemic health effect.

466

3.3 Inhibitory effect against α -amylase

3.3.1 Traditional method of *in vitro* inhibitory effect

Similar to the pancreatic lipase assays, the inhibitory activity of the EE and CEE from fenugreek and quinoa against α -amylase was firstly evaluated under the traditional conditions (Figure 4). Although the traditional conditions can be variable, one of the most popular method is characterized by a simple *in vitro* incubation of the α -amylase enzyme with starch as substrate, in presence of the tested inhibitor, and in a proper phosphate buffer at 37°C for 20 min. At the end of reaction, the absorbance due to the residual non-hydrolyzed starch is measured, and the comparison with the

absorbance measured in absence of inhibitor allows estimating the inhibitory activity (Ercan & El, 2016; Podsędek et al., 2017).

As shown in Figure 4, all the assayed extracts showed the ability to inhibit the α -amylase activity in a dose-dependent mode, but up to a maximum inhibitory dose. Thus, contrary to the lipase assays, a maximum inhibitory activity around 25% was reached in the best cases (EE and CEE fenugreek extracts and CEE quinoa extract), being not possible to estimate an inhibition of 50% of the activity for any of the extracts. Thus, for fenugreek, the highest inhibition was found at 3.3 mg/mL in both EE and CEE ($25.6 \pm 1\%$ and $27.3 \pm 2.9\%$ inhibition, respectively), with no significant differences due to the extraction/concentration process. Additionally, in case of fenugreek extracts, even a decrease of the inhibitory activity was observed as the concentration of the extract increased. On the contrary, in the case of quinoa, the highest inhibition was found for the CEE extracts at 0.6 mg/mL ($24.8 \pm 1.7\%$), whereas at that same concentration, the EE exhibited almost no inhibition ($0.29 \pm 0.1\%$) ($p < 0.001$). According to these results, it was clear that the ability to inhibit α -amylase activity of the extracts was much lower than the ability to inhibit pancreatic lipase. Furthermore, those extracts that were more effective for the inhibition of the lipase activity were not the same than those for the inhibition of α -amylase activity (fenugreek CEE and quinoa CEE, respectively).

The inhibitory effect of fenugreek and quinoa extracts on α -amylase has been barely described in the literature. Similarly, Funke & Melzig (2006) described an inhibitory activity of amylase lower than 20% for fenugreek extracts obtained with boiling water. A water-soluble extract of fenugreek with an optimized phenolic content displayed an amylase inhibition index of approximately 1.15, that is, a very moderate inhibitory activity (McCue, Kwon, & Shetty, 2005). In the case of quinoa, Ranilla, Apostolidis, Genovese, Lajolo, & Shetty (2009) did not detect any inhibitory activity for aqueous extracts from either this seed or the other pseudocereals, cereals and legumes studied. Although Hemalatha, Bomzan, Sathyendra Rao, & Sreerama (2016) described a IC_{50} value of 0.16 mg/mL for aqueous methanol extracts from whole grains of quinoa, which correlated with the phenolic content.

501 It is important to remark that the mild inhibitory activity of the assayed extracts on the α -amylase
502 activity would not be a reason to consider this extracts as not worthy. As previously explained, a
503 preferred strategy to control the release of glucose from disaccharides in the gut seems to be a
504 moderate amylase inhibition together with a strong glucosidase inhibition (Ercan & El, 2016). This
505 is because strong amylase inhibitors cause a subsequent abnormal fermentation of undigested
506 saccharides in the colon, leading to gastrointestinal problems (Ercan & El, 2016; Nagmoti &
507 Juvekar, 2013). Thus, it could be hypothesized that the moderate inhibitory activity of the tested
508 extracts against amylase might be beneficial if stronger glucosidase inhibitory effects are
509 demonstrated. In this sense, further studies would be necessary to analyze the effect of the extracts
510 on glucosidase activity in order to evaluate the real potential of the extracts against carbohydrates
511 digestion and absorption.

512

513 *3.3.2 Traditional method of in vitro inhibitory effect under shaking*

514 Due to the same reasons as stated previously for the lipase inhibition assays, the following two
515 methods of inhibitory activity measurement were performed with the CEE only. Concerning the
516 traditional method under shaking, and similar to the lipase assays, no significant differences were
517 observed when applying shaking in neither of the seeds ($p = 0.349$ for fenugreek and $p = 0.492$ for
518 quinoa) (Figure 5). Therefore, the movement of the aqueous environment of the enzymatic reaction
519 did not impact on the magnitude of the inhibitory effects, as already observed for pancreatic lipase
520 activity.

521

522 *3.3.3 In vitro inhibitory effect under simulation of intestinal digestion*

523 Surprisingly, under simulated intestinal conditions, the extracts from both seeds not only did not
524 display any inhibitory activity against α -amylase, but even a slight catalysis of the enzyme was
525 observed in a dose-dependent mode (Figure 5). The explanation of this result is complex, since in
526 this case, the presence of lecithin and bile salts, as used in the method, is not relevant for the activity

527 of α -amylase. Further studies would be necessary in order to find and confirm an explanation to this
528 result. In any case, it seems clear that under simulation of intestinal digestion, the inhibitory activity
529 of extracts is more complicated to be evidenced, and even, contrary catalytic effects might appear.
530 Concerning the effect of the time of reaction on the inhibitory activity of the extracts, the same
531 trend as previously described for pancreatic lipase was also observed after 60 min of reaction time
532 for amylase (data not shown), possibly due to the degradation of the inhibitor after a longer period
533 of time.
534 In the case of α -amylase, the commercial inhibitor acarbose was used as the positive control under
535 simulated intestinal conditions. Contrarily to the extracts, the IC_{50} value for acarbose was
536 effectively found, showing a value of 25 $\mu\text{g/mL}$. Similar to the previous explanation given for
537 Orlistat in the case of lipase, finding an extremely low IC_{50} value for acarbose, similar to those
538 found for the traditional method of measurement, would validate the method of simulation of
539 intestinal digestion as a correct procedure to test inhibitors of α -amylase. Again, it would be
540 possible to conclude that the poor results observed for the extracts during the simulation of
541 intestinal digestion, would be due to the extracts, and that the generation of artifacts due to the
542 method itself could be ruled out.
543 As summary, it can be concluded that the inhibitory activity against α -amylase of both fenugreek
544 and quinoa extracts was inexistent, and even slightly catalytic, when submitting them to simulated
545 intestinal conditions, and that only mild inhibitory effect were observed in traditional assays.
546 Further *in vivo* studies would be necessary in order to effectively confirm the real effect after oral
547 intake of the extracts on the digestion of dietary carbohydrates and the subsequent systemic health
548 effect.

549

550 **4. Conclusions**

551 Fenugreek extracts obtained by ethanol ultrasound assisted extraction, with a subsequent
552 concentration procedure with butanol, are preferred than the ones from quinoa, considering the total

553 amount of ~~potential inhibitor compounds of digestive enzymes, that is,~~ saponins and phenolic
554 compounds. Thus, all the assayed extracts from fenugreek and quinoa inhibit the digestive enzyme
555 pancreatic lipase, but fenugreek extracts display a stronger inhibition ~~likely due to their higher total~~
556 ~~inhibitors content~~. In any case, intestinal simulated conditions and longer times of reaction worsen
557 the inhibitory activity of both extracts when compared to traditional methods of measurement.
558 Concerning the inhibitory activity against α -amylase, both fenugreek and quinoa extracts display a
559 mild inhibition by traditional methods of measurement, but a slightly catalytic effect is evidenced
560 when submitting them to simulated intestinal conditions.
561 These findings would confirm the importance of assessing the inhibitory activity of extracts against
562 digestive enzymes under enzymatic conditions similar to those found *in vivo* in order to more
563 realistically evaluate the potential of natural extracts.

564

565 **Abbreviations used**

4-MUO	4-Methylumbelliferyl Oleate
CEE	Concentrated Ethanol Extract(s)
EE	Ethanol Extract(s)
MetS	Metabolic Syndrome
TFA	Trifluoroacetic Acid
TPC	Total phenolic content
TSC	Total saponin content
TIC	Total content of inhibitory compounds

566

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575

576 **Notes**

577 Declaration of interests: none.

578

579 **References**

- 580 Afiukwa, C., Ibiam, U., Edeogu, C., Nweke, F., & Chukwu, U. (2009). Determination of amylase
581 activity of crude extract from partially germinated mango seeds (*Mangifera oraphila*). *African*
582 *Journal of Biotechnology*, 8(14), 3294–3296.
- 583 Arivalagan, M., Gangopadhyay, K. K., & Kumar, G. (2013). Determination of steroidal saponins
584 and fixed oil content in fenugreek (*Trigonella foenum-graecum*) genotypes. *Indian Journal of*
585 *Pharmaceutical Sciences*, 75(1), 110–113.
- 586 Birari, R. B., & Bhutani, K. K. (2007). Pancreatic lipase inhibitors from natural sources: unexplored
587 potential. *Drug Discovery Today*, 12(19), 879–889.
- 588 Buchholz, T., & Melzig, M. (2015). Polyphenolic Compounds as Pancreatic Lipase Inhibitors.
589 *Planta Medica*, 81(10), 771–783.
- 590 Chan, K. W., Iqbal, S., Khong, N. M. H., Ooi, D.-J., & Ismail, M. (2014). Antioxidant activity of
591 phenolics–saponins rich fraction prepared from defatted kenaf seed meal. *LWT - Food Science*
592 *and Technology*, 56(1), 181–186.
- 593 Chaubey, P. S., Somani, G., Kanchan, D., Sathaye, S., Varakumar, S., & Singhal, R. S. (2018).
594 Evaluation of debittered and germinated fenugreek (*Trigonella foenum graecum* L.) seed flour
595 on the chemical characteristics, biological activities, and sensory profile of fortified bread.
596 *Journal of Food Processing and Preservation*, 42(1), e13395.

597 de la Garza, A., Milagro, F., Boque, N., Campión, J., & Martínez, J. (2011). Natural inhibitors of
 598 pancreatic lipase as new players in obesity treatment. *Planta Medica*, 77(08), 773–785.

599 Ercan, P., & El, S. N. (2016). Inhibitory effects of chickpea and *Tribulus terrestris* on lipase, α -
 600 amylase and α -glucosidase. *Food Chemistry*, 205, 163–169.

601 Funke, I., & Melzig, M. F. (2006). Traditionally used plants in diabetes therapy: phytotherapeutics
 602 as inhibitors of alpha-amylase activity. *Revista Brasileira de Farmacognosia*, 16(1), 1–5.

603 Gargouri, Y., Julien, R., Bois, A. G., Verger, R., & Sarda, L. (1983). Studies on the detergent
 604 inhibition of pancreatic lipase activity. *Journal of Lipid Research*, 24(10), 1336–42.

605 Güçlü-Üstündağ, Ö., & Mazza, G. (2007). Saponins: Properties, applications and processing.
 606 *Critical Reviews in Food Science and Nutrition*, 47(3), 231–258.

607 Hemalatha, P., Bomzan, D. P., Sathyendra Rao, B. V., & Sreerama, Y. N. (2016). Distribution of
 608 phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α -
 609 amylase and α -glucosidase activities. *Food Chemistry*, 199, 330–338.

610 Lee, S. S., Mohd Esa, N., & Loh, S. P. (2015). In vitro inhibitory activity of selected legumes
 611 against pancreatic lipase. *Journal of Food Biochemistry*, 39(4), 485–490.

612 Li, A. N., Li, S., Zhang, Y. J., Xu, X. R., Chen, Y. M., & Li, H. Bin. (2014). Resources and
 613 Biological Activities of Natural Polyphenols. *Nutrients*, 6(12), 6020–6047.

614 Mad, T., Sterk, H., Mittelbach, M., & Rechberger, G. N. (2006). Tandem mass spectrometric
 615 analysis of a complex triterpene saponin mixture of *Chenopodium quinoa*. *Journal of the*
 616 *American Society for Mass Spectrometry*, 17(6), 795–806.

617 Makkar, H. P. S., Siddhuraju, P., & Becker, K. (2007). Saponins. In *Plant Secondary Metabolites*
 618 (pp. 93–100). Totowa, NJ: Humana Press.

619 Marrelli, M., Conforti, F., Araniti, F., & Statti, G. (2016). Effects of Saponins on Lipid Metabolism:
 620 A Review of Potential Health Benefits in the Treatment of Obesity. *Molecules*, 21(10), 1404.

621 Martin, D., Navarro del Hierro, J., Villanueva Bermejo, D., Fernández-Ruiz, R., Fornari, T., &
 622 Reglero, G. (2016). Bioaccessibility and antioxidant activity of *Calendula officinalis*

623 supercritical extract as affected by in vitro codigestion with olive oil. *Journal of Agricultural*
624 *and Food Chemistry*, 64(46), 8828–8837.

625 McClements, D. J., & Li, Y. (2010). Review of in vitro digestion models for rapid screening of
626 emulsion-based systems. *Food & Function*, 1(1), 32.

627 McCue, P., Kwon, Y.-I., & Shetty, K. (2005). Anti-amylase, anti-glucosidase and anti-angiotensin
628 I-converting enzyme potential of selected foods. *Journal of Food Biochemistry*, 29(3), 278–
629 294.

630 Medina-Meza, I. G., Aluwi, N. A., Saunders, S. R., & Ganjyal, G. M. (2016). GC–MS Profiling of
631 Triterpenoid Saponins from 28 Quinoa Varieties (*Chenopodium quinoa* Willd.) Grown in
632 Washington State. *Journal of Agricultural and Food Chemistry*, 64(45), 8583–8591.

633 Nagmoti, D. M., & Juvekar, A. R. (2013). In vitro inhibitory effects of *Pithecellobium dulce*
634 (Roxb.) Benth. seeds on intestinal α -glucosidase and pancreatic α -amylase. *Journal of*
635 *Biochemical Technology*, 4(3), 616–621.

636 Navarro del Hierro, J., Herrera, T., Fornari, T., Reglero, G., & Martin, D. (2018). The
637 gastrointestinal behavior of saponins and its significance for their bioavailability and
638 bioactivities. *Journal of Functional Foods*, 40, 484–497.

639 Navarro del Hierro, J., Herrera, T., García-Risco, M. R., Fornari, T., Reglero, G., & Martin, D.
640 (2018). Ultrasound-assisted extraction and bioaccessibility of saponins from edible seeds:
641 quinoa, lentil, fenugreek, soybean and lupin. *Food Research International*, 109, 440–447.

642 Oishi, Y., Sakamoto, T., Udagawa, H., Taniguchi, H., Kobayashi-Hattori, K., Ozawa, Y., & Takita,
643 T. (2007). Inhibition of increases in blood glucose and serum neutral fat by *Momordica*
644 *charantia* saponin fraction. *Bioscience, Biotechnology, and Biochemistry*, 71(3), 735–740.

645 Pham, H. N. T., Vuong, Q. V., Bowyer, M. C., & Scarlett, C. J. (2017). Phytochemical profiles and
646 antioxidant capacity of the crude extracts, aqueous- and saponin-enriched butanol fractions of
647 *Helicteres hirsuta* Lour. leaves and stems. *Chemical Papers*, 71(11), 2233–2242.

648 Podsędek, A., Majewska, I., & Kucharska, A. Z. (2017). Inhibitory potential of red cabbage against

649 digestive enzymes linked to obesity and type 2 diabetes. *Journal of Agricultural and Food*
650 *Chemistry*, 65(33), 7192–7199.

651 Ranilla, L. G., Apostolidis, E., Genovese, M. I., Lajolo, F. M., & Shetty, K. (2009). Evaluation of
652 Indigenous Grains from the Peruvian Andean Region for Antidiabetes and Antihypertension
653 Potential Using *In Vitro* Methods. *Journal of Medicinal Food*, 12(4), 704–713.

654 Scow, R. O. (1988). Effect of sodium taurodeoxycholate, CaCl₂ and albumin on the action of
655 pancreatic lipase on droplets of trioleoylglycerol and the release of lipolytic products into
656 aqueous media. *Biochimie*, 70(9), 1251–61.

657 Scuteri, A., Laurent, S., Cucca, F., Cockcroft, J., Cunha, P. G., Mañas, L. R., ... Nilsson, P. M.
658 (2015). Metabolic syndrome across Europe: Different clusters of risk factors. *European*
659 *Journal of Preventive Cardiology*, 22(4), 486–491.

660 Seyedan, A., Alshawsh, M. A., Alshagga, M. A., Koosha, S., & Mohamed, Z. (2015). Medicinal
661 plants and their inhibitory activities against pancreatic lipase: A review. *Evidence-Based*
662 *Complementary and Alternative Medicine*, 2015, 1–13.

663 Singh, B., Singh, J. P., Singh, N., & Kaur, A. (2017). Saponins in pulses and their health promoting
664 activities: A review. *Food Chemistry*, 233, 540–549.

665 Sugiyama, H., Akazome, Y., Shoji, T., Yamaguchi, A., Yasue, M., Kanda, T., & Ohtake, Y. (2007).
666 Oligomeric Procyanidins in Apple Polyphenol Are Main Active Components for Inhibition of
667 Pancreatic Lipase and Triglyceride Absorption. *Journal of Agricultural and Food Chemistry*,
668 55(11), 4604–4609.

669 Tang, Y., Zhang, B., Li, X., Chen, P. X., Zhang, H., Liu, R., & Tsao, R. (2016). Bound Phenolics of
670 Quinoa Seeds Released by Acid, Alkaline, and Enzymatic Treatments and Their Antioxidant
671 and α -Glucosidase and Pancreatic Lipase Inhibitory Effects. *Journal of Agricultural and Food*
672 *Chemistry*, 64(8), 1712–1719.

673 Tucci, S. A., Boyland, E. J., & Halford, J. C. (2010). The role of lipid and carbohydrate digestive
674 enzyme inhibitors in the management of obesity: a review of current and emerging therapeutic

675 agents. *Diabetes, Metabolic Syndrome and Obesity : Targets and Therapy*, 3, 125–43.

676 Vinarova, L., Vinarov, Z., Atanasov, V., Pantcheva, I., Tcholakova, S., Denkov, N., & Stoyanov, S.

677 (2015). Lowering of cholesterol bioaccessibility and serum concentrations by saponins: in vitro

678 and in vivo studies. *Food & Function*, 6(2), 501–512.

679 Xiao, J., Ni, X., Kai, G., & Chen, X. (2013). A Review on Structure–Activity Relationship of

680 Dietary Polyphenols Inhibiting α -Amylase. *Critical Reviews in Food Science and Nutrition*,

681 53(5), 497–506.

682 Zengin, G. (2016). A study on in vitro enzyme inhibitory properties of *Asphodeline anatolica*: New

683 sources of natural inhibitors for public health problems. *Industrial Crops and Products*, 83,

684 39–43.

685 Zhang, B., Deng, Z., Ramdath, D. D., Tang, Y., Chen, P. X., Liu, R., ... Tsao, R. (2015). Phenolic

686 profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and

687 inhibitory effects on α -glucosidase and pancreatic lipase. *Food Chemistry*, 172, 862–872.

688

689 **Figure Captions**

690

691 **Figure 1.** IC₅₀ value (mg/mL) of ethanol extracts (EE) (lined bars) and concentrated ethanol extracts
692 (CEE) (dotted bars) from fenugreek and quinoa against pancreatic lipase assayed under traditional
693 conditions. (*) Values of IC₅₀ for EE and CEE within each seed extract are significantly different (p
694 ≤ 0.05).

695

696 **Figure 2.** Total inhibitors content (g/100 g) and IC₅₀ value (mg/mL) against pancreatic lipase of
697 ethanol extracts (EE) and concentrated ethanol extracts (CEE) from fenugreek (A) and quinoa (B).

698

699 **Figure 3.** IC₅₀ value (mg/mL) of CEE extracts of fenugreek and quinoa against pancreatic lipase
700 assayed under traditional (horizontal lines), traditional with stirring (oblique lines) and simulated
701 intestinal digestion (dots) conditions. ^{a,b} Different letters within a same seed are significantly
702 different (p ≤ 0.05).

703

704 **Figure 4.** Effect of concentration (mg/mL) of ethanol extracts (EE) (▲) and concentrated ethanol
705 extracts (CEE) (■) from fenugreek and quinoa on the inhibition (%) of α-amylase assayed under
706 traditional conditions.

707

708 **Figure 5.** Effect of concentration (mg/mL) of CEE extracts from fenugreek (A) and quinoa (B) on
709 the inhibition (%) of α-amylase assayed under traditional (▲), traditional with stirring (■) and
710 simulated intestinal (●) conditions during 60 min.

711

712

713

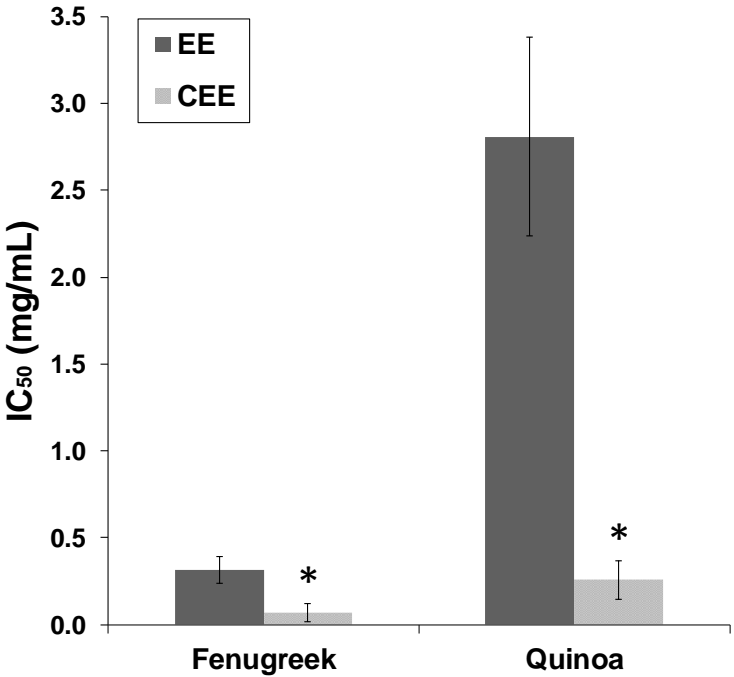
714

Table 1. Total saponin content (TSC), total phenolic content (TPC) and total inhibitor content (TIC) expressed as g per 100 g of extract.

Seed Extract	TSC	TPC	TIC
Fenugreek			
EE	10.187 ± 0.796^a	1.105 ± 0.247^a	11.293 ± 0.549^a
CEE	13.076 ± 3.248^a	1.071 ± 0.227^a	14.147 ± 3.475^a
Quinoa			
EE	0.203 ± 0.024^b	0.050 ± 0.004^b	0.253 ± 0.019^b
CEE	0.342 ± 0.035^b	0.088 ± 0.021^b	0.430 ± 0.055^b

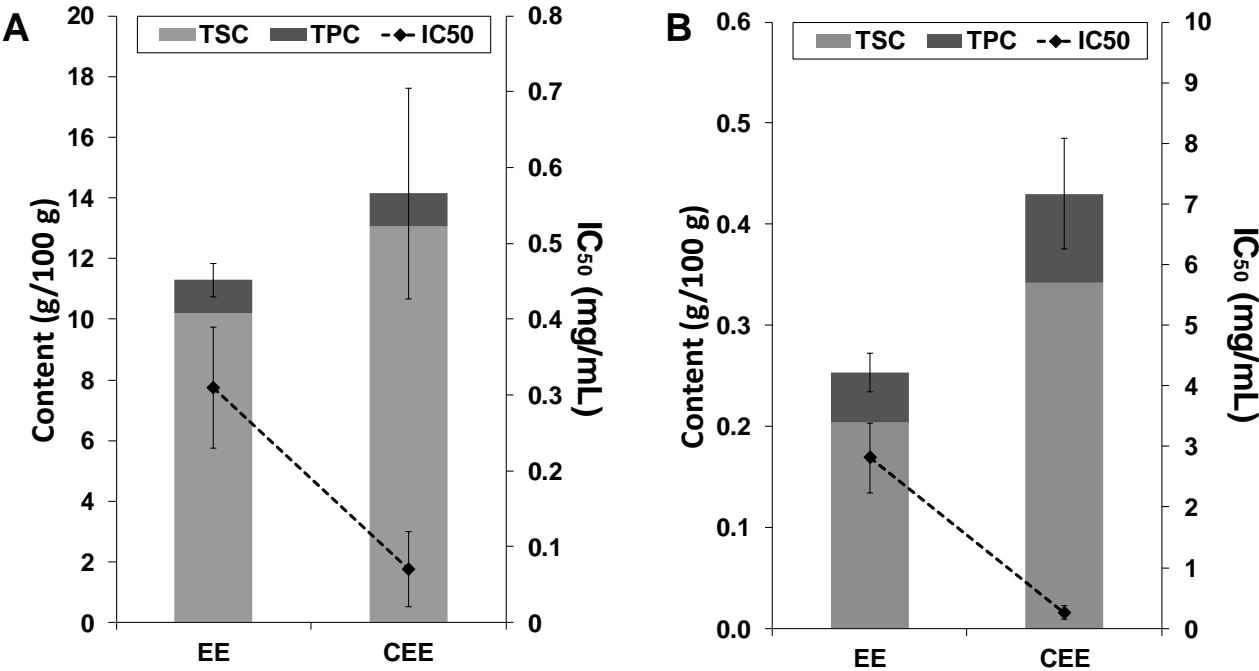
^{a,b} Different letters within the same column denote statistically significant differences ($P \leq 0.05$)

726 **Figure 1.**



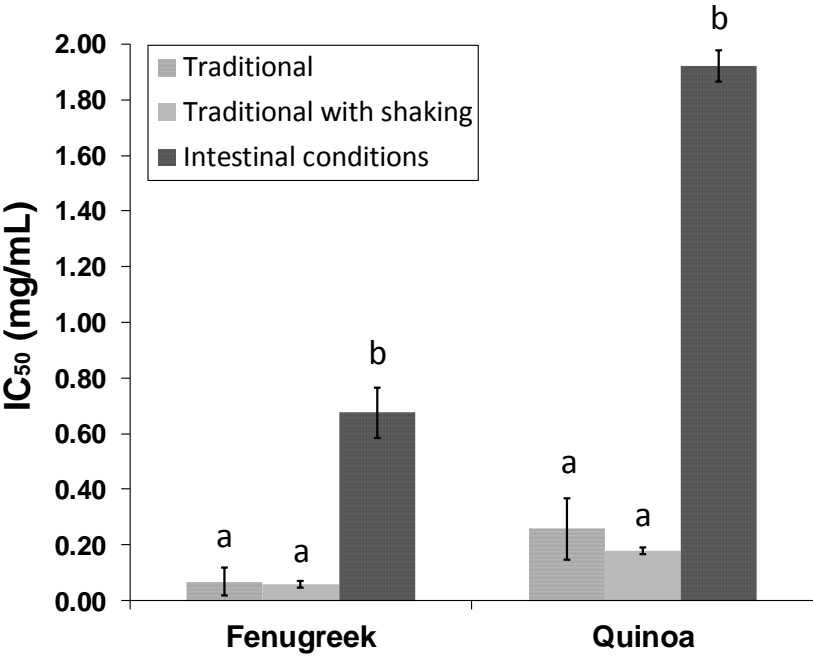
727
728

729 **Figure 2.**



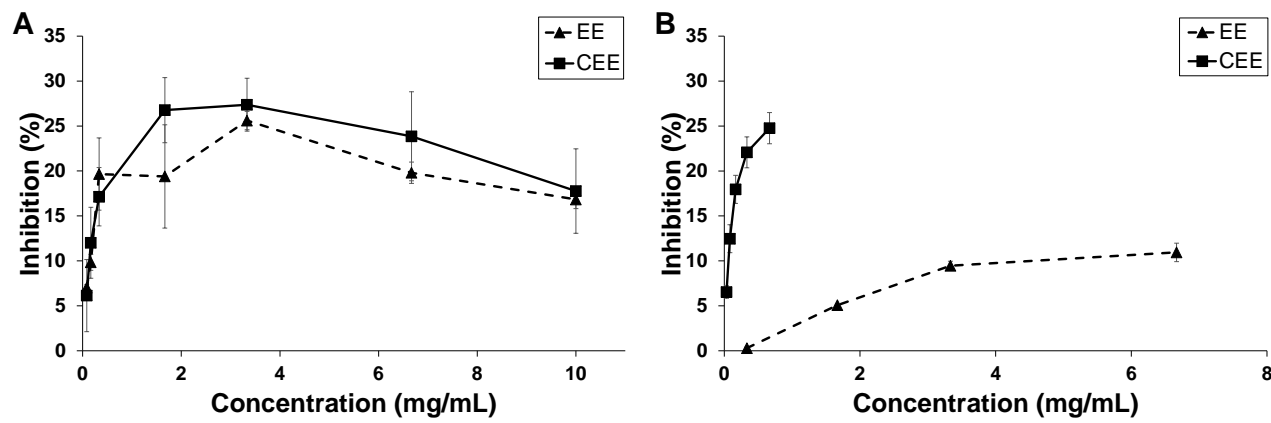
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732 **Figure 3.**

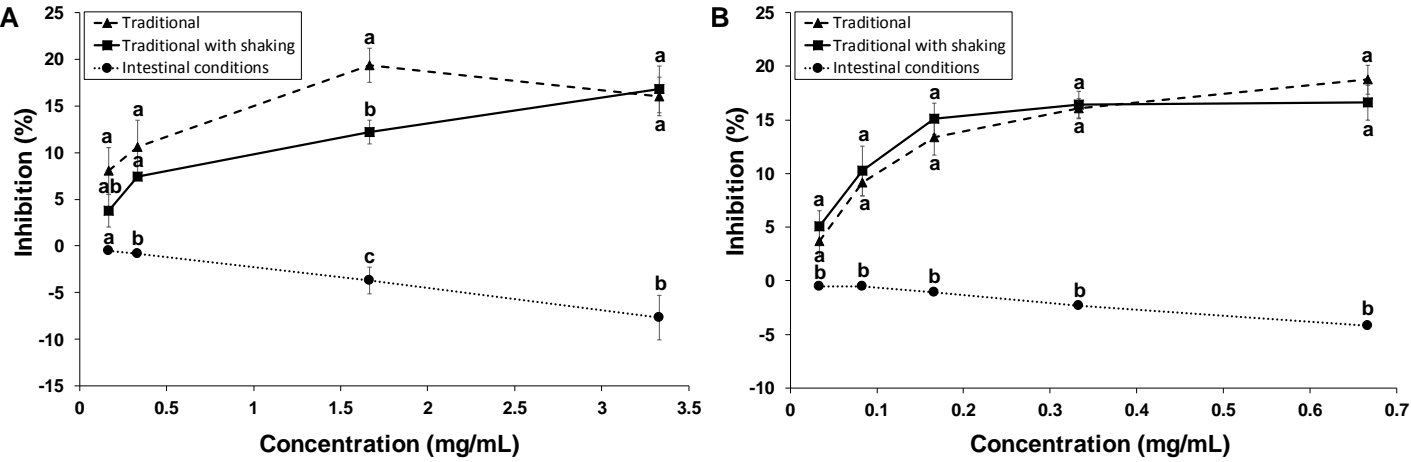


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735 **Figure 4.**



738 **Figure 5.**



739

Supplementary Material

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