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Idriss Miganeh Waiss, André Kimbonguila, Fatouma Mohamed Abdoul-latif, Laurette Brigelia Nkeletela, Joël Scher, et al.. Total phenolic content, antioxidant activity, shelf-life and reconstitutability of okra seeds powder: influence of milling and sieving processes. *International Journal of Food Science and Technology*, 2021, 10.1111/ijfs.15099 . hal-03330380

HAL Id: hal-03330380

<https://hal.univ-lorraine.fr/hal-03330380v1>

Submitted on 31 Aug 2021

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Total phenolic content, antioxidant activity, shelf-life, and reconstitutability of okra seeds powder: influence of milling and sieving processes.

Journal:	<i>International Journal of Food Science and Technology</i>
Manuscript ID	IJFST-2021-31332.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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Keywords:	particle size, Polyphenols, Antioxidant Activity, preservation, powder reconstitution, okra seed powder

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3 1 Total phenolic content, antioxidant activity, shelf-life, and reconstitutability of okra seeds
4 powder: influence of milling and sieving processes

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27 28 29 13 **Abstract**

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31
32 14 This study aimed at relating phytochemical properties (total phenolic content and antioxidant
33 activity), reconstitutability (water absorption capacity and water solubility index), and
34 preservation ability (water activity) of okra seed powders to physicochemical properties
35 (particle size distribution and moisture content). Okra seed powders were produced at three
36 different **milling frequencies**, then each obtained sample was sieved with 180, 315, and 500 µm
37 sieves. An increased milling frequency resulted in lower median particle size, water activity,
38 and moisture content. The maximal water activity and moisture content were 0.583 ± 0.010 and
39 12.07 ± 0.05 % (w/w), respectively, showing the good **preservation ability** of okra seed
40 powders. Total phenolic content and antioxidant activity were raised at higher milling
41 frequency. Reconstitutability was significantly enhanced at higher **milling frequency** and/or for
42 smaller median particle size. Thus, the successive milling and sieving process was successful
43 in improving physicochemical and functional properties of okra seed powders, especially for
44 smaller particles.

45
46
47 27 **Keywords:** okra seed **powder**; particle size; polyphenols; antioxidant activity; preservation;
48 powder reconstitution.

49 50 51 29 **1. Introduction**

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53
54 30 Okra fruit (*Abelmoschus esculentus* (L.) Moench) is widely consumed around the world and it
55 is cultivated in tropical and subtropical regions (Torkpo *et al.*, 2009; Jarret *et al.*, 2011). Okra
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60 31

1
2
3 32 is renowned for its nutritional potential and health benefits: chemical composition analyses of
4 33 okra revealed its richness in proteins, fat, minerals (Mn, Ca, Mg, Fe, Cu), and vitamins (B2,
5 34 B3, B6, B9, C) (Moyin-Jesu *et al.*, 2007; Xia *et al.*, 2015). Mature okra seed is a good source
6 35 of oil rich in unsaturated fatty acids such linolenic and linoleic acids, which are both essential
7 36 for humans (Ndangui *et al.*, 2010; Kumar *et al.*, 2013). It has been reported in the literature that
8 37 okra pods have high contents in polysaccharides and phenolic compounds including flavonoids
9 38 (Arapitsas, 2008). Phenolic compounds are known to possess strong antioxidant and anti-stress
10 39 effects (Ahmed *et al.*, 2016; Sabitha *et al.*, 2011). Although the high phytochemical-activity
11 40 content of okra pods has recently been proved (Hu *et al.*, 2014; Xia *et al.*, 2015, 2018), little is
12 41 known about the distribution of bioactive compounds within granulometric classes of okra
13 42 seeds powdered by milling.

14 43 Nowadays, milling is commonly used for the production of new food ingredients (Karam *et al.*,
15 44 2016; Becker *et al.*, 2017; Deli *et al.*, 2019). Powdered plant parts are used as ingredients by
16 45 many industries in the food, pharmaceutical, and cosmetics fields (Leuenberger *et al.*, 2005).
17 46 Moreover, food ingredients and pharmaceutical excipients are often manufactured in powder
18 47 form (Siliveru *et al.*, 2017; Deli *et al.*, 2019). Advantages of the powder technology resides in
19 48 the convenience offered by powdered products, especially regarding higher shelf-life and easier
20 49 transportability (Forny *et al.*, 2011). Successive drying, milling, and sieving processes have
21 50 shown a great potential in the production of food powders with enhanced functional properties
22 51 (Brewer *et al.*, 2014; Becker *et al.*, 2017; Deli *et al.*, 2019; Waiss *et al.*, 2020). It leads to the
23 52 production of microparticles from plant material (whole plant, aerial parts, stems, roots, seeds,
24 53 and/or fruits). It is worth noting that drying, milling, and sieving processes have a great impact
25 54 on physicochemical and functional characteristics of produced powders (Landillon *et al.*, 2008),
26 55 such as particle size and shape distributions, surface roughness, density, cohesion,
27 56 compressibility, water solubility, wettability, surface reactivity, and hygroscopicity (Becker *et al.*
28 57 *et al.*, 2017; Deli *et al.*, 2019). A previous study performed with the same okra powders as in the
29 58 current study showed a strong correlation between median particle size and proximate
30 59 composition: smaller granulometric classes contained more proteins and fat, but less water,
31 60 carbohydrates, and minerals (Waiss *et al.*, 2020). Also, the influence of milling frequency and
32 61 median particle size of okra seeds powder on flow properties was highlighted (Waiss *et al.*,
33 62 2020): powders with large median particle sizes were not cohesive and had better flowability.
34 63 Functional properties of powders (flowability, preservation ability, reconstitutability, etc.) are
35 64 very important for the pharmaceutical and food industries (Räsänen *et al.*, 2003), as they

1
2
3 65 determine the conditions by which they should be manufactured, handled, stored, and
4
5 66 reconstituted with a view to protect bioactive molecules (Deli *et al.*, 2019a; Meghwal *et al.*,
6
7 67 2014; Petit *et al.*, 2017). Powder physicochemical characteristics are often strongly influenced
8
9 68 by particle size and physical structure (Meghwal and Goswami, 2014; Deli *et al.*, 2019). Thus,
10
11 69 it is of great importance to determine the physical and hydration characteristics of food powders
12
13 70 in order to predict their behavior during storage and reconstitution ~~and use them efficiently for~~
14
15 71 ~~an efficient use~~ as functional food ingredients (Benković *et al.*, 2017). The current study was
16
17 72 intended to increase knowledge about the influence of particle size on phytochemical
18
19 73 ~~properties, reconstitutability, and preservation ability~~ of okra seeds powder. Okra seeds were
20
21 74 ground at three ~~milling frequencies~~ (6 000, 12 000, and 18 000 rpm); then, each powder sample
22
23 75 was fractionated in four granulometric sizes by sieving: $\leq 180 \mu\text{m}$, 180 - 315 μm , 315 - 500
24
25 76 μm , and $\geq 500 \mu\text{m}$. Last, physicochemical properties (particle size distribution and moisture
26
27 77 content), ability to interact with water (water activity, water absorption capacity, and water
28
29 78 solubility index), and phytochemical properties (total phenolic content and antioxidant activity)
30
31 79 of powders were evaluated.

80 2. Material and methods

81 2.1. Material: okra seeds

82 Okra seeds were purchased from farmers of Impfondo locality, in the north of the Republic of
83
84 the Congo (1° 32'28" N; 18°07'10" E). Then, the okra seeds were removed from the pods and
85
86 sun-dried in the open air at 30 °C. Dry seeds were stored at 10 °C until use for powder
87
88 production.

89 2.2. Drying and milling of okra seeds

90 500 g dried okra seeds were milled with an ultra-centrifugal mill ZM 200 (Retsch, France) using
91
92 a 10-cm sieve drilled with 1-mm trapezoidal holes at three ~~milling frequencies~~ (6 000, 12 000,
93
94 and 18 000 rpm). Then, obtained powder was collected in sealable polyethylene bags and stored
95
96 at 10 °C until sieving.

97 2.3. Sieving of okra seed powders

98 Each okra seed powder issued from the milling step was separated in two aliquots. ~~The first~~
99
100 ~~aliquot~~ was immediately put in sealed polyethylene bags and stored at 10 °C until analysis;
101
102 ~~these powders were not sieved, thus they will be named unsieved powders hereafter.~~ The other
103
104 aliquot was sieved with an Analyzer 3 Spartan sieve shaker (Fritsch, Idar-Oberstein, Germany)
105
106 with three superimposed sieves of 180, 315, and 500 μm mesh sizes, at 0.5 mm vibration
107
108 amplitude during 24 min. Hence, $\leq 180 \mu\text{m}$, 180 - 315 μm , 315 - 500 μm , ~~and~~ $\geq 500 \mu\text{m}$
109
110

98 granulometric classes were obtained by sieving. [These granulometric](#) classes were finally put
99 in sealed polyethylene bags and stored at 10 °C until analyses.

100 **2.4. Physical properties**

101 **2.4.1. Particle size distribution**

102 Powder granulometric characteristics define the granular state of a powder. In the
103 pharmaceutical industry, particle size analysis is of utmost importance as it partly conditions
104 the bioactivity of medicines (Carmona *et al.*, 2003). The particle size distribution of unsieved
105 powders and granulometric classes of okra seed powders was determined by laser diffraction
106 using dry dispersion (Mastersizer 3000, Malvern Instruments France, Orsay, France).
107 Dispersion conditions for all powders except for the $\leq 180 \mu\text{m}$ powder samples were as follows:
108 100 % air pressure at 4 bar, 3 mm hopper gap, and 40 % feed rate. For the $\leq 180 \mu\text{m}$ powder
109 samples, the following dispersion conditions were employed: 100 % air pressure at 4 bar, 4 mm
110 hopper gap, and 70 % feed rate. The chosen size estimator was the equivalent diameter in
111 volume. Classical granulometric parameters were determined: d10, d50, and d90, which
112 [correspond to the first decile, the median, and the ninth decile of the particle size distribution,](#)
113 and the span, which quantifies the width of the particle size distribution (equation 1):

$$114 \text{ Span} = \frac{d90 - d10}{d50} \quad (1)$$

115 **2.4.2. Moisture content and water activity**

116 Moisture content was measured by weight loss after drying 3 g powder sample at 103 °C for
117 5 h ('AFNOR, [Agence Française de Normalisation](#), 1976).

118 Water activity of okra powders was evaluated with a water activity-meter (HygroPalm23-AW-
119 A, Rotronic, France): 15 g powder was put in a WP-40 sample holder and the quick analysis
120 mode was employed at $22 \pm 3 \text{ °C}$, leading to water activity stabilization in about 5 min.

121 **2.5. Phytochemical properties**

122 **2.5.1. Preparation of hydromethanolic extracts**

123 Hydromethanolic extracts of okra seed powders were prepared by maceration at room
124 temperature (22 °C) in order to preserve the bioactivity of extracted molecules by minimizing
125 their thermal alteration (Ćujić *et al.*, 2015; Becker *et al.*, 2016). [It is worth noting that this](#)
126 [method permitted to obtain a solution of extractable phenolic compounds, but it was not able to](#)
127 [extract chemically-bound phenolic compounds.](#) 2 g powder was put in 25 mL methanol-distilled

1
2
3 128 water mixture (70/30 % (v/v)) during 24 h under stirring at 300 rpm. Then, the resulting extract
4
5 129 was centrifuged (Thermo Scientific, Heraeus Megafuge 8R Centrifuge) at $3\,460 \times g$ for 20 min
6
7 130 at 20 °C and the obtained supernatant was filtrated using Whatman filter paper n° 1 (Sigma
8
9 131 Aldrich, France). The filter paper was washed with 20 mL methanol-distilled water mixture
10
11 132 (70/30 % (v/v)) and the filtrate was also centrifuged at $3\,460 \times g$ for 20 min at 20 °C. Last, the
12
13 133 resulting supernatant was collected and stored at 4 °C until analysis.

14 134 **2.5.2. Total phenolic content**

15
16 135 In test tubes were added 30 µL okra powder hydromethanolic extract, 2.67 mL distilled water,
17
18 136 500 µL Folin-Ciocalteu reagent (1 N), and 300 µL sodium carbonate (Na_2CO_3 , 20 % (w/v)).
19
20 137 The mixture was vortexed and dark-incubated during 30 min at room temperature (22 °C).
21
22 138 Finally, absorbance of obtained solutions was measured at 750 nm with a Cary 50 Scan
23
24 139 UV/visible spectrophotometer (Agilent, Santa Clara California, USA). Standard solutions of
25
26 140 gallic acid (from 0.01 to 0.03 g/L by 0.005 mg/mL steps) were used for calibration ($R^2 = 0.99$)
27
28 141 and total phenolic content was expressed in terms of milligrams of gallic acid equivalents per
29
30 142 gram of dry matter (mg GAE/g DM).

31 143 **2.5.3. Antioxidant activity evaluated by DPPH scavenging**

32
33 144 The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method offers a first
34
35 145 approach for evaluating the antioxidant potential of a compound, an extract, or other biological
36
37 146 sources (Kitagaki *et al.*, 1999). The DPPH[•] scavenging method was performed according to the
38
39 147 method of Masuda *et al.* (1999). 500 µL of 0.1 mM DPPH solution was mixed with 500 µL
40
41 148 okra seed powder hydromethanolic extract. This mixture was vortexed, then dark-incubated at
42
43 149 30 °C for 30 min, and finally its absorbance was measured at 517 nm against a blank solution
44
45 150 composed of 500 µL DPPH solution mixed with 350 µL methanol and 150 µL distilled water
46
47 151 that was left 30 min at rest. The antioxidant activity was evaluated as the percentage of DPPH
48
49 152 radical inhibition and calculated from the absorbance decrease using (equation 2) (Buijnsters *et*
50
51 153 *al.*, 2001):

$$52 \quad 154 \quad \% \text{ Inhibition of DPPH radical} = \left[\frac{\text{ABS} - \text{abs}}{\text{ABS}} \right] \times 100 \quad (2)$$

53
54
55 155 with ABS (-) representing the absorbance of the blank solution and abs (-) the absorbance of
56
57 156 the solution obtained from okra seeds powder extract after the DPPH assay.

58 59 157 **2.5.4. Antioxidant activity evaluated by ABTS scavenging**

1
2
3 158 ABTS^{•+} radical was produced by a reaction between an aqueous solution of ABTS at 7 mM and
4
5 159 a potassium persulfate K₂S₂O₈ solution at 4.9 mM. This mixture was stirred for 16 h in the dark
6
7 160 at room temperature (22 °C), leading to a blue green-colored solution containing the ABTS^{•+}
8
9 161 cation radical (Cai *et al.*, 2015). Afterwards, the ABTS^{•+} solution was then diluted with ethanol
10
11 162 until reaching between 0.65 and 0.70 absorbance at 734 nm. For analysis, 100 µL powder
12
13 163 sample hydromethanolic extract was mixed with 900 µL ABTS^{•+} radical solution and the
14
15 164 mixture was vortexed. After 10 min incubation at room temperature (22 °C), the absorbance of
16
17 165 the mixture was measured at 734 nm against a blank solution (mixture of 100 µL phosphate
18
19 166 buffer at pH 7.4 and 900 µL ABTS^{•+} radical solution). The results were expressed in terms of
20
21 167 inhibition percentage according to (equation 3):

$$22 \quad 168 \quad \% \text{ Inhibition of ABTS radical} = \left[\frac{A_0 - A_1}{A_0} \right] \times 100 \quad (3)$$

23
24
25
26 169 with A₀ (-) representing the absorbance of the blank solution and A₁ (-) the absorbance of the
27
28 170 solution obtained from okra seeds powder extract after the DPPH ABTS^{•+} assay.

29 30 171 **2.6. Hydration properties**

31
32 172 Water absorption capacity (WAC) and water solubility index (WSI) were evaluated according
33
34 173 to Deli *et al.* (2019). Water absorption capacity (WAC) was determined as follows: first, 1 g
35
36 174 okra seeds powder and 10 mL distilled water were mixed in a centrifuge tube, then the mixture
37
38 175 was stirred at 300 rpm (Variomag Poly) for 30 min at 20 °C and centrifuged (Thermo Scientific,
39
40 176 Heraeus Megafuge 8R Centrifuge) at 1 500 × g for 30 min at 20 °C. The supernatant was
41
42 177 carefully removed. The pellet was weighed, dried in an oven at 103 ± 2 °C for 6 h, cooled in a
43
44 178 desiccator, and weighed again. WAC was evaluated as follows (equation 4):

$$45 \quad 179 \quad \text{WAC} = \frac{\text{mass of wet sediments}}{\text{mass of dried sediments}} \quad (4)$$

46
47
48
49 180 Where masses of wet and dry sediments were expressed in grams.

50
51 181 The following protocol was used to determine the water solubility index of powder samples:
52
53 182 2.5 g (M₁) okra powder and 20 mL distilled water were mixed in a centrifuge tube, then the
54
55 183 mixture was stirred at 300 rpm (Variomag Poly) for 30 min at 20 °C. After centrifugation
56
57 184 (Thermo Scientific, Heraeus Megafuge 8R Centrifuge) at 1 500 × g for 20 min at 20 °C, the
58
59 185 supernatant was carefully removed and the pellet was dried in an oven at 103 ± 2 °C for 6 h,
60
186 cooled in a desiccator, and weighed (M₂). WSI was calculated according to equation 5:

$$\text{WSI (\%)} = \left(\frac{M_1 - M_2}{M_1} \right) \times 100 \quad (5)$$

2.7. Statistical analysis

One-factor analysis of variance, the calculation of Pearson's correlation coefficients, and principal components analysis (PCA) were performed by using Microsoft Excel 2016 and significant differences were assessed with Tukey's HSD at a 5 % confidence level with the DSAASTAT add-on (Andrea ONOFRI, Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy). The determination of Pearson's correlation coefficients and PCA were performed to examine correlations between characterized properties of okra seed powders: median particle size, span, total phenolic content, results of DPPH and ABTS assays, water activity, moisture content, WAC, and WSI. Results were expressed as means \pm standard deviations.

3. Results and discussion

3.1. Particle size distribution

The analysis of the particle size distribution was performed in order to check if the successive milling and sieving processes were efficient in micronizing okra seeds and separating unsieved powders into sufficiently different granulometric classes. Table 1 shows that the median particle size decreased when the milling frequency was increased: for instance, for unsieved powders, median particle size was lowered value, from 723 μm at 6 000 rpm to 478 μm at 12 000 rpm and 229 μm at 18 000 rpm. The median particle size of granulometric classes was found between the mesh sizes of their respective upper and lower sieves as expected when sieving is completed, confirming the efficiency of the sieving process to separate powders in granulometric classes differing in median particle size.

Span of unsieved powders obtained at 6 000, 12 000, and 18 000 rpm were equal to 2.11 ± 0.02 , 2.03 ± 0.01 , and 2.96 ± 0.16 , respectively, showing that the width of the particle size distribution was larger at higher milling frequency. This could be explained by the higher proportion of fine particles when using a higher milling frequency, leading to increased heterogeneity of unsieved powders.

Table 1

3.2. Total phenolic content

1
2
3 216 Figure 1 presents the total phenolic content of unsieved samples and granulometric classes of
4
5 217 okra seed powders obtained at the three different milling frequencies. Higher total phenolic
6
7 218 contents were observed for samples composed of smaller particles. Moreover, a greater milling
8
9 219 frequency induced an increase in total phenolic content: for example, for unsieved powders, the
10
11 220 total phenolic content equaled 2.34, 3.44, and 7.40 mg GAE/g DM at 6 000 rpm, 12 000, and
12
13 221 18 000 rpm milling frequencies, respectively. This can be explained by the fact that a more
14
15 222 intense milling (here at higher milling frequency, but it could also result from a longer milling
16
17 223 duration) enhances the size reduction of plant material, which further facilitates the accessibility
18
19 224 of solvents for the extraction of bioactive compounds owing to the higher specific surface area
20
21 225 of smaller particles (Rosa *et al.*, 2013). This observation was confirmed by the moderate
22
23 226 negative correlation between total phenolic content and median particle size d50 evidenced by
24
25 227 a Pearson's correlation coefficient of $r = -0.71$ ($p < 0.05$). Indeed, the bioactive compounds are
26
27 228 often associated with proteic and fatty fractions in plant extracts (Becker *et al.*, 2016; Deli,
28
29 229 Baudelaire, *et al.*, 2019). As indicated in the introduction, a previous study focused on the
30
31 230 influence of granulometric characteristics of these powders on their proximate composition
32
33 231 revealed that higher protein and fat contents were found in the smaller particle size fractions of
34
35 232 okra powder seeds (Waiss *et al.*, 2020). Thus, the discriminating power of the sieving process
36
37 233 in matter of total phenolic content and proximate composition was enhanced at higher milling
38
39 234 frequency.

235 **Figure 1**

236 **3.3. Antioxidant activity**

237 The **DPPH[•]** and **ABTS^{•+}** assays are spectrophotometric techniques based on quenching of stable
238 colored radicals which are widely used for the assessment of antioxidant activities of complex
239 biological mixtures such as plant or food extracts.

240 **3.3.1 DPPH[•] radical scavenging activity assay**

241 Results of antioxidant activity of okra seed powders obtained by DPPH test are presented in
242 **Figure 2**. All extracts were able to scavenge DPPH[•] radicals. The percentage of DPPH inhibition
243 was higher for $\leq 180 \mu\text{m}$ and $\geq 500 \mu\text{m}$ granulometric classes whatever the milling frequency.
244 The $\leq 180 \mu\text{m}$ granulometric classes were richer in lipids and proteins and the $\geq 500 \mu\text{m}$
245 granulometric classes contained more carbohydrates, as shown in a previous study performed
246 with the same powders as investigated in the present study (Waiss *et al.*, 2020). It has been
247 showed that bioactive molecules such as polyphenols are often associated with lipids, proteins,

248 and polysaccharides in plant tissues (Zaiter *et al.*, 2016). This observation partially meets the
249 results obtained in the present study, as at 6 000 and 12 000 rpm milling frequencies, DPPH[•]
250 scavenging activity decreased with particle size for the ≤ 180 , 180 - 315, and 315 - 500 μm
251 granulometric classes; however, at a given milling frequency, the DPPH[•] scavenging activity
252 of the ≥ 500 μm granulometric classes were greater than for the other granulometric classes and
253 unsieved powders, indicating that bioactive compounds may be mainly associated with
254 carbohydrates, likely fibers, in okra seeds. The increasing trend of DPPH[•] scavenging activity
255 with particle size at 18 000 rpm confirms this interpretation, as a more intense milling may have
256 permitted to break fibers and decrease their particle size, increasing the DPPH[•] scavenging
257 activity of the granulometric classes of higher median particle size. Besides, micronization of
258 plant material generally leads to an increase in the antioxidant activity of plant extracts (Rosa
259 *et al.*, 2013), and this was confirmed by the present study where milling at 18 000 rpm induced
260 a marked increase in the DPPH[•] inhibition percentage. Milling is known to alter the physical
261 structure of the plant matrix, increasing its specific surface area and leading to the exposition
262 of antioxidant compounds of the plant material (Hemery *et al.*, 2010). Last, the fact that the
263 DPPH[•] scavenging activity was systematically higher for granulometric classes than for
264 unsieved powders (Figure 2) shows that hydromethanolic extraction was improved after
265 sieving, maybe by decreasing powder cohesion and then facilitating its dispersion and the
266 exposure of bioactive molecules to the solvent (Salameh *et al.*, 2016).

Figure 2

3.3.2 ABTS cation radical scavenging activity assay

269 The results of ABTS^{•+} radical scavenging activity of granulometric classes and unsieved
270 samples of okra seed powders obtained at 6 000, 12 000, and 18 000 rpm milling frequencies
271 are reported in Figure 3. High antioxidant activity of all okra seed powders was observed during
272 the ABTS assay. No great difference of ABTS^{•+} inhibition was denoted between samples, in
273 particular, no significant difference could be attributed to milling frequency, but a trend of
274 increased antioxidant activity for powder samples having a smaller median particle size was
275 apparent. This was confirmed by the Pearson's correlation coefficient between median particle
276 size d50 and ABTS^{•+} inhibition percentage of $r = -0.64$ ($p < 0.05$), which denoted a moderate
277 negative correlation between these parameters. Besides, the antioxidant activity obtained by the
278 ABTS cation radical scavenging was significantly higher than for DPPH radical scavenging for
279 all granulometric classes and unsieved powders of okra seeds (cf. figures 2 and 3). Indeed,

1
2
3 280 previous studies showed that the ABTS test leads to higher inhibition percentage than the DPPH
4
5 281 test for fruits, vegetables, and beverages (Floegel *et al.*, 2011), owing to the better reactivity of
6
7 282 high-pigmented and hydrophilic antioxidants with ABTS^{•+}. Obtained results are consistent with
8
9 283 the fact that antioxidant activity of plant products is generally attributed to radical scavenging
10
11 284 activity of phenolic compounds (Rahman *et al.*, 2007; Becker *et al.*, 2016; Deli, *et al.*, 2019).
12
13 285 Indeed, the phenolic compounds such as flavonoids, polyphenols, and tannins are the main
14
15 286 bioactive molecules that contribute to antioxidant activity of plant extracts (Rosa *et al.*, 2013;
16
17 287 Brewer *et al.*, 2014; Becker *et al.*, 2017).

288 **Figure 3**

289 **3.4. Moisture content and water activity**

290 Moisture content is an important parameter that is indirectly related to powdered food
291 ingredient stability during storage. Low levels of moisture content (up to 12 % (w/w)) are
292 known to limit microbial growth and extend food shelf-life (Aryee *et al.*, 2006; Kaur *et al.*,
293 2013). Results displayed in Table 2 show that the moisture content of granulometric classes
294 and unsieved samples of okra seed powders ranged from 8.61 ± 0.08 to 12.07 ± 0.05 (w/w),
295 presuming a good stability during storage. The moisture content was generally decreased at
296 smaller median particle size and/or at higher milling frequency (cf. Table 2), as evidenced by
297 the moderate positive correlation between moisture content and median particle size (Pearson's
298 correlation coefficient of $r = 0.73$, $p < 0.05$). Moisture content is not sufficient to characterize
299 food stability during storage, as food structure also contributes to water availability for
300 microorganisms and alteration reactions: the concept of water activity has been developed to
301 account for these combined influences of moisture content and food structure (Tapia, *et al.*,
302 2007; Bhandari *et al.*, 2013). Water activity ranged between 0.493 ± 0.010 and 0.583 ± 0.010
303 for investigated okra seed powders (cf. Table 2), which was below the threshold of 0.650
304 permitting the growth of microorganisms like xerophilic mold and bacteria (Tapia *et al.*, 2007).
305 Water activity was generally decreased at lower median particle size, similarly to water content.
306 This was expected, as a more intense milling leads to higher stress, more friction, and thus
307 higher heating of the plant material, leading to smaller particles with a lower moisture content
308 (Becker *et al.*, 2017)

309 **3.5. Water interactions**

310 The water absorption capacity (WAC) and water solubility index (WSI) results of all
311 granulometric classes and unsieved samples of okra seed powders are presented in Table 2.

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2
3 312 WSI significantly differed according to the milling frequency. When milling frequency was
4
5 313 increased, the median particle size of powders was decreased, enhancing water solubility index.
6
7 314 This was confirmed by the highly negative correlation between WSI and median particle size
8
9 315 (Pearson's correlation coefficient of $r = -0.89$, $p < 0.05$), indicating that interaction with water
10
11 316 was improved for smaller particles (cf. Table 2). This can be explained by the fact that milling
12
13 317 induces particle size reduction, leading to the improvement of material functionalities such as
14
15 318 particle surface specific area and surface energy, enhancing the interactions of plant material
16
17 319 with water (Ye *et al.*, 2016). In addition, this may result from the exposure of hydrophilic groups
18
19 320 (coming from cellulose and hemicellulose) at the surface of particles obtained by plant
20
21 321 micronization (Zhao *et al.*, 2009; Ting *et al.*, 2014). Zhao *et al.* (2009) also reported increased
22
23 322 dispersibility and solubility for ginger powders produced with a more intense milling, owing to
24
25 323 improved interactions with water evidenced by a higher WSI (Zhao *et al.*, 2009). Water
26
27 324 absorption capacity represents the ability of a product to absorb water. Table 2 shows that all
28
29 325 powders had a high WAC whatever the granulometric class and the milling frequency. This can
30
31 326 be explained by the high proportion of mucilage of okra seeds that highly contribute to water
32
33 327 absorption (Aboubakar *et al.*, 2008). No clear influence of median particle size on WAC was
34
35 328 denoted, except for a small trend of higher WAC for intermediate granulometric classes. Thus,
36
37 329 performed milling was sufficient to break cell walls and expose hydrophilic groups of all okra
38
39 330 seed components. The results of the current study are consistent with the work of (Deli, Petit,
40
41 331 *et al.*, 2019), who reported that WAC and WSI were enhanced for smaller particles of *Boschia*
42
43 332 *senegalensis* seeds, *Dichostachys glomerata* fruits, and *Hibiscus sabdariffa* calyxes. Hence, it
44
45 333 can be hypothesized that okra seed powders obtained at higher milling frequency should interact
46
47 334 more easily with water and be more soluble, which can facilitate their intestinal absorption
48
49 335 during digestion.

336 **Table 2**

337 **3.6. Principal component analysis of physicochemical, water interaction, and**
338 **phytochemical properties**

339 PCA is used to highlight similarities or oppositions between variables and identify the variables
340 that are most correlated between them. All granulometric classes and unsieved samples of okra
341 seed powders obtained at the three milling frequencies were compared using PCA on the basis
342 of their physicochemical (moisture content, median particle size), water interaction (water
343 activity, water solubility index, and water absorption capacity), and phytochemical (total
344 phenolic content, and antioxidant activity deduced from DPPH and ABTS tests) properties. The

1
2
3 345 result of this PCA is presented in Figure 4. Variables are presented in red color and principal
4 346 components are represented on F1 and F2 axes which respectively explained 43.4 % and 20.7
5 347 % variability among powder properties, allowing the PCA to explain a total variability of 64.1
6 348 % which was sufficient to outline the major links between physicochemical and phytochemical
7 349 properties of okra seed powders. F1 axis was positively correlated with ABTS⁺⁺ inhibition, WSI,
8 350 and total phenolic content, and negatively correlated with median particle size and moisture
9 351 content. F2 axis was positively correlated with span and WAC, and negatively correlated with
10 352 DPPH[•] inhibition and water activity. It is worth noting that median particle size was negatively
11 353 correlated to total phenolic content, WSI, and ABTS⁺⁺ inhibition percentage, and positively to
12 354 moisture content, confirming the previous discussion of experimental results.

13 355 The representation of okra powder samples on the F1 × F2 biplot revealed a separation of
14 356 powder samples according to median particle size (Figure 4). Smaller granulometric classes
15 357 (≤ 180 μm at 6 000, 12 000, and 18 000 rpm milling frequency and 180 - 315 μm at 18 000 rpm
16 358 milling frequency) were located at the bottom right of the F1 × F2 biplot and characterized by
17 359 their high total phenolic content and WSI, and obviously by their low median particle size.
18 360 Higher granulometric classes (≥ 500 μm at 6 000, 12 000, and 18 000 rpm milling frequency
19 361 and 315 - 500 μm at 18 000 rpm milling frequency) were found at the left of the F1 × F2 biplot
20 362 and characterized by high moisture content and median particle size, and low total phenolic
21 363 content and WSI. The other granulometric classes of intermediate median particle size and
22 364 unsieved powders were located in the top middle of F1 × F2 biplot and they were positively
23 365 correlated with span and WAC, and negatively to water activity and DPPH[•] inhibition.

24 366 **Figure 4**

25 367

26 368 **4. Conclusion**

27 369 The combination of milling and sieving processes was successful in generating granulometric
28 370 classes with significantly different physicochemical, water interaction, and phytochemical
29 371 properties. The results evidenced a considerable influence of median particle size of okra seeds
30 372 powder on its total phenolic content, antioxidant activity deduced from ABTS and DPPH tests,
31 373 moisture content, water activity, and water solubility index. Powders with smaller median
32 374 particle sizes (i.e. lower size granulometric classes and/or powders obtained at higher milling
33 375 frequency) had lower moisture content, water activity, and water absorption capacity, and
34 376 higher total phenolic content, antioxidant activity, and water solubility index. This can result

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3 377 from the higher level of disintegration of plant matrix structure induced by a more intense
4 378 milling (i.e. undergone by smaller particles and/or at higher milling frequency) which makes
5 379 its compounds more accessible to solvents, thus improving phenolic compounds extraction and
6 380 powder solubility, and decreases the number of pores of the solid matrix, thus lowering water
7 381 absorption capacity. For all samples, moisture content and water activity were sufficiently low
8 382 to avoid food spoilage. Hence, okra seed powders were biochemically and microbiologically
9 383 stable, ensuring a good shelf-life during storage. Water interaction parameters showed high
10 384 levels of water absorption and solubility, which seems to indicate that the reconstitutability of
11 385 okra powder could be improved by the successive milling and sieving process. Finally, all
12 386 obtained results suggest that okra seed powders can be used in food manufacturing as functional
13 387 ingredients enhancing antioxidant activity and water interactions.

388 5. Acknowledgments

389 The authors would like to thank Dr. Florentin MICHAUX, Dr. Jennifer BURGAIN, Pr.
390 Stephane DESOBRY, and Dr. Jordane JASNIEWSKI for their scientific advices. Special
391 thanks should be addressed to Carole PERROUD-THOMASSIN and Carole JEANDEL for
392 technical advices regarding powder production and analyses. The authors also acknowledge
393 support of the LIBio by the "Impact Biomolecules" project of the "Lorraine Université
394 excellence" (Investissements d'avenir ANR).

395 6. Conflict of interest

396 The authors declare that they have no conflict of interest.

397 7. Ethical approval

398 No experiment was performed on humans or animals: no ethics approval was not required for
399 this research.

400 8. Data availability statement

401 Research data are not shared.

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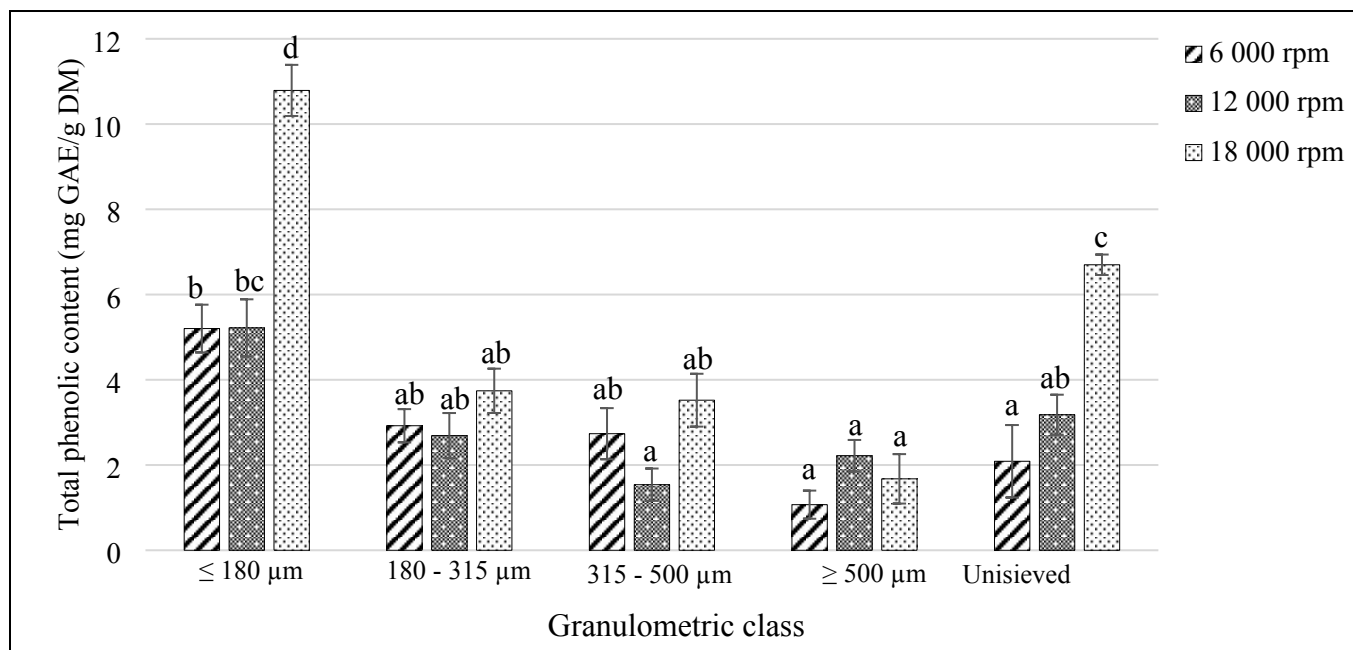
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546 Figure1: Total phenolic content of granulometric classes ($\leq 180 \mu\text{m}$; $180 - 315 \mu\text{m}$; $315 - 500$
 547 μm ; $\geq 500 \mu\text{m}$) and unsieved samples of okra seed powders. DM: dry matter, GAE: gallic acid
 548 equivalents. Bars topped by different letters were statistically different at $p < 0.05$ according to
 549 Tukey's HSD test.

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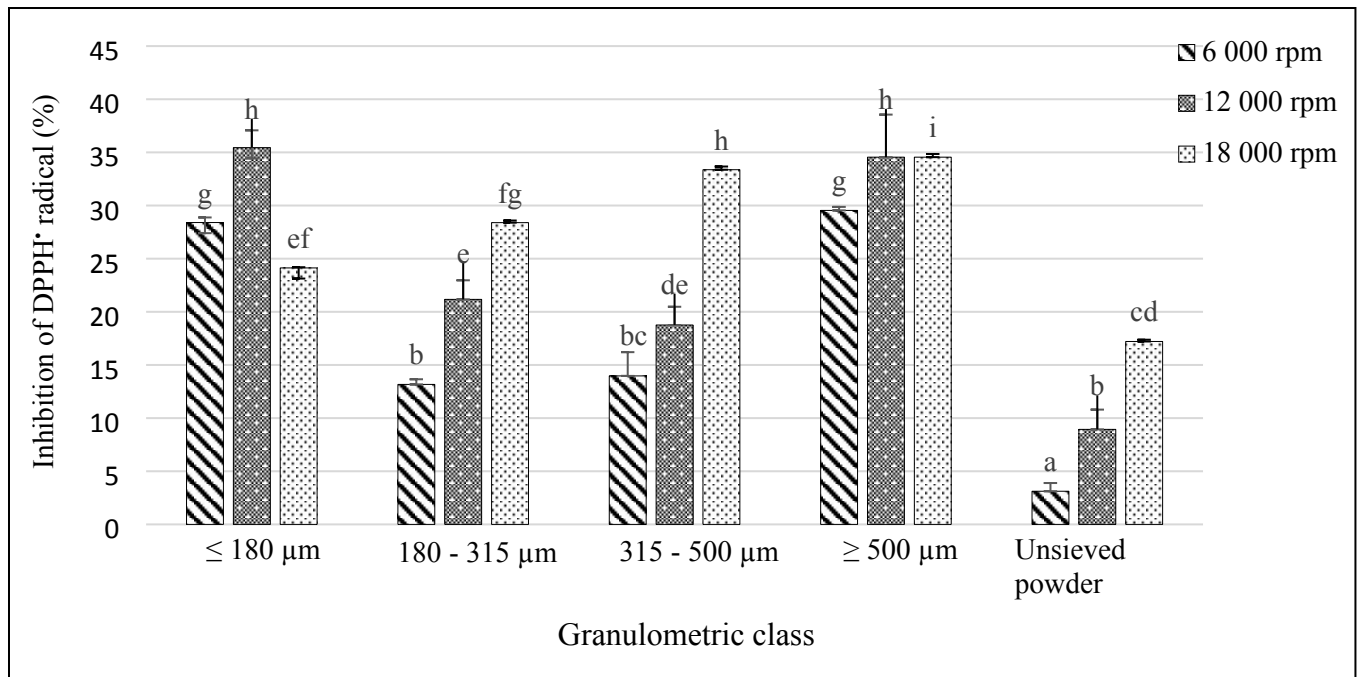
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562 Figure 2: Inhibition of DPPH radical by hydromethanolic extracts of granulometric classes (\leq
 563 $180 \mu\text{m}$; $180 - 315 \mu\text{m}$; $315 - 500 \mu\text{m}$; $\geq 500 \mu\text{m}$) and unsieved samples of okra seed powders.
 564 Bars topped by different letters were statistically different at $p < 0.05$ according to Tukey's
 565 HSD test.

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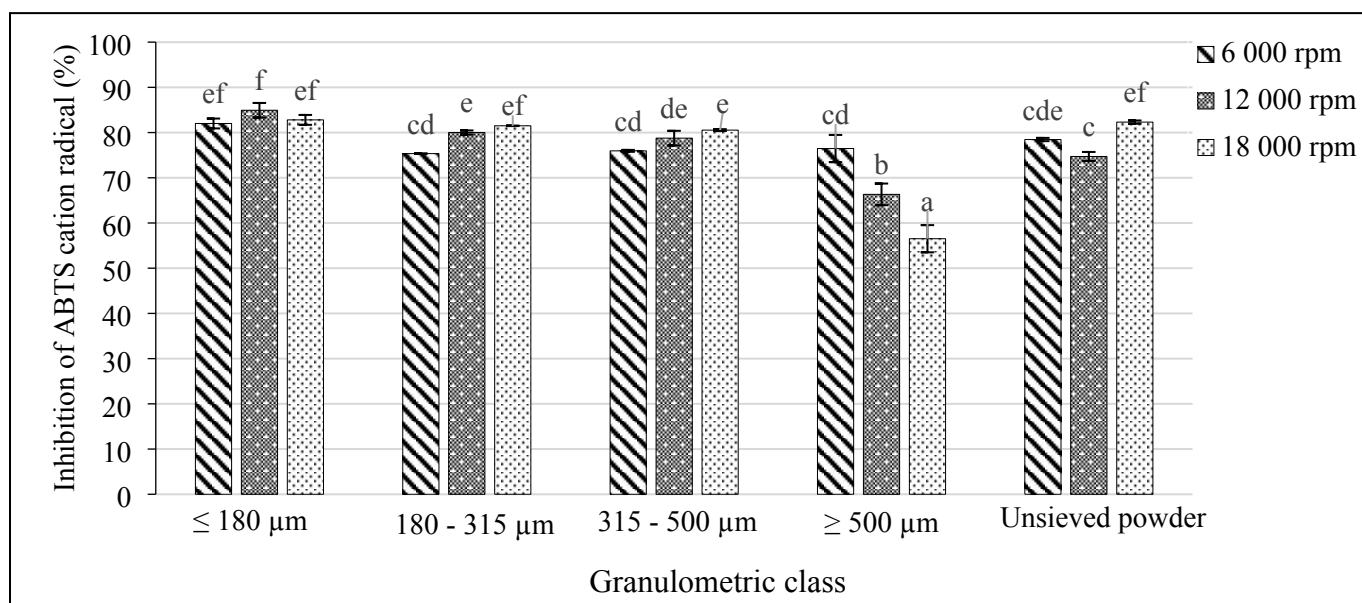
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579 Figure 3: Inhibition of ABTS cation radical by hydromethanolic extracts of granulometric
 580 classes ($\leq 180 \mu\text{m}$; $180 - 315 \mu\text{m}$; $315 - 500 \mu\text{m}$; $\geq 500 \mu\text{m}$) and unsieved samples of okra seed
 581 powders. Bars topped by different letters were statistically different at $p < 0.05$ according to
 582 Tukey's HSD test.

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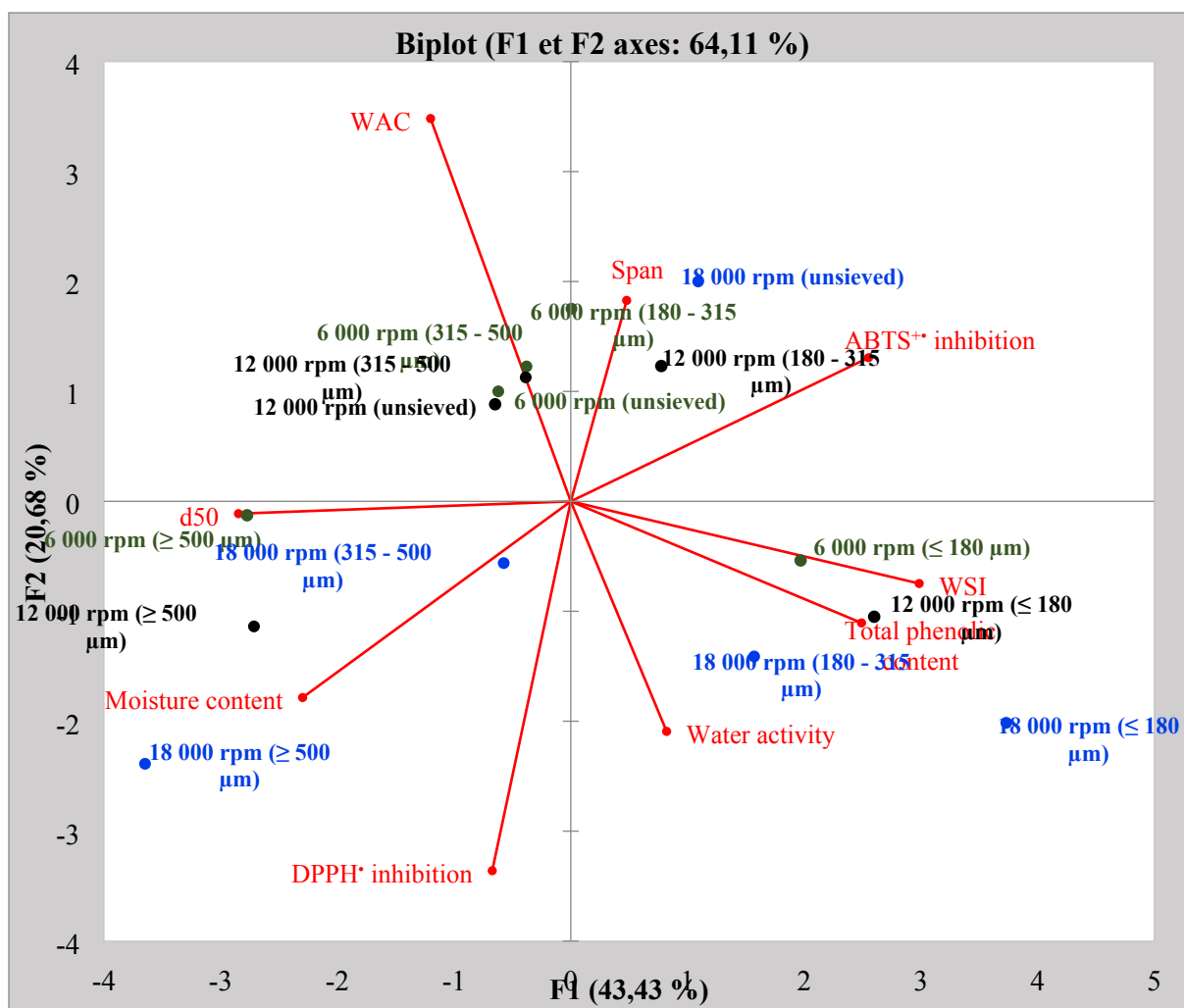
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594 Figure 4: Representation of all granulometric classes (≤ 180 ; $180 - 315$; $315 - 500$; $\geq 500 \mu\text{m}$)
 595 and unsieved samples of okra seed powders obtained at 6 000 rpm (green), 12 000 rpm (black),
 596 and 18 000 rpm (blue) milling frequencies and their physicochemical and functional
 597 characteristics (red) on the principal components F1 and F2 deduced from PCA.

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For Peer Review

608 Table 1: Granulometric parameters of granulometric classes ($\leq 180 \mu\text{m}$, $180 - 315 \mu\text{m}$, $315 - 500 \mu\text{m}$, $\geq 500 \mu\text{m}$) and unsieved samples of okra
609 seed powders obtained at 6 000, 12 000, and 18 000 rpm milling frequencies (Waiss *et al.*, 2020).

Milling speed (rpm)	Granulometric classes	d10 (μm)	d50 (μm)	d90 (μm)	Span (-)
6 000	$\leq 180 \mu\text{m}$	$23.1 \pm 0.2^{\text{ab}}$	$85.3 \pm 0.6^{\text{a}}$	$181.3 \pm 0.5^{\text{a}}$	$1.87 \pm 0.01^{\text{f}}$
	$180 - 315 \mu\text{m}$	$152.0 \pm 1.0^{\text{de}}$	$257.0 \pm 1.0^{\text{c}}$	$406.0 \pm 1.0^{\text{b}}$	$0.97 \pm 0.01^{\text{d}}$
	$315 - 500 \mu\text{m}$	$326.6 \pm 6.0^{\text{g}}$	$498.3 \pm 2.5^{\text{e}}$	$745.3 \pm 2.5^{\text{c}}$	$0.87 \pm 0.03^{\text{bcd}}$
	$\geq 500 \mu\text{m}$	$589.3 \pm 18.5^{\text{i}}$	$1100.0 \pm 43.5^{\text{h}}$	$2173.3 \pm 73.7^{\text{g}}$	$1.44 \pm 0.02^{\text{e}}$
	Unsieved powder	$141.0 \pm 7.8^{\text{d}}$	$723.3 \pm 29.9^{\text{f}}$	$1670.0 \pm 65.5^{\text{f}}$	$2.11 \pm 0.02^{\text{h}}$
12 000	$\leq 180 \mu\text{m}$	$21.9 \pm 0.1^{\text{ab}}$	$75.6 \pm 2.8^{\text{a}}$	$167.3 \pm 6.3^{\text{a}}$	$1.95 \pm 0.06^{\text{fg}}$
	$180 - 315 \mu\text{m}$	$165.6 \pm 1.5^{\text{e}}$	$264.6 \pm 1.5^{\text{c}}$	$409.3 \pm 9.8^{\text{b}}$	$0.92 \pm 0.03^{\text{cd}}$
	$315 - 500 \mu\text{m}$	$312.0 \pm 1.0^{\text{g}}$	$463.3 \pm 1.1^{\text{de}}$	$671.6 \pm 4.9^{\text{c}}$	$0.78 \pm 0.01^{\text{bc}}$
	$\geq 500 \mu\text{m}$	$542.6 \pm 5.6^{\text{h}}$	$801.3 \pm 25.0^{\text{g}}$	$1243.3 \pm 75.0^{\text{e}}$	$0.90 \pm 0.05^{\text{bcd}}$
	Unsieved powder	$52.9 \pm 0.5^{\text{c}}$	$457.6 \pm 5.1^{\text{de}}$	$983.0 \pm 7.8^{\text{d}}$	$2.03 \pm 0.01^{\text{gh}}$
18 000	$\leq 180 \mu\text{m}$	$17.8 \pm 0.2^{\text{a}}$	$52.1 \pm 0.6^{\text{a}}$	$149.3 \pm 1.5^{\text{a}}$	$0.77 \pm 0.01^{\text{b}}$
	$180 - 315 \mu\text{m}$	$37.4 \pm 0.1^{\text{bc}}$	$181.0 \pm 0.0^{\text{b}}$	$332.6 \pm 2.0^{\text{b}}$	$0.48 \pm 0.01^{\text{a}}$
	$315 - 500 \mu\text{m}$	$290.0 \pm 4.3^{\text{f}}$	$442.6 \pm 12.8^{\text{d}}$	$644.6 \pm 25.9^{\text{c}}$	$0.80 \pm 0.02^{\text{bc}}$
	$\geq 500 \mu\text{m}$	$527.6 \pm 4.7^{\text{h}}$	$781.0 \pm 14.1^{\text{g}}$	$1186.6 \pm 37.8^{\text{e}}$	$0.84 \pm 0.02^{\text{bcd}}$
	Unsieved powder	$25.7 \pm 0.3^{\text{ab}}$	$228.6 \pm 7.5^{\text{bc}}$	$702.67 \pm 55.7^{\text{c}}$	$2.96 \pm 0.16^{\text{i}}$

610 Means \pm standard deviations followed by the same superscripted letters in the same column were not significantly different at $p < 0.05$ according
611 to Tukey's HSD test.

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618 Table 2: Moisture content, water activity, water absorption capacity, and water solubility index of granulometric classes ($\leq 180 \mu\text{m}$, $180 - 315$
 619 μm , $315 - 500 \mu\text{m}$, $\geq 500 \mu\text{m}$) and unsieved samples of okra seed powders obtained at 6 000, 12 000, and 18 000 rpm milling frequencies.

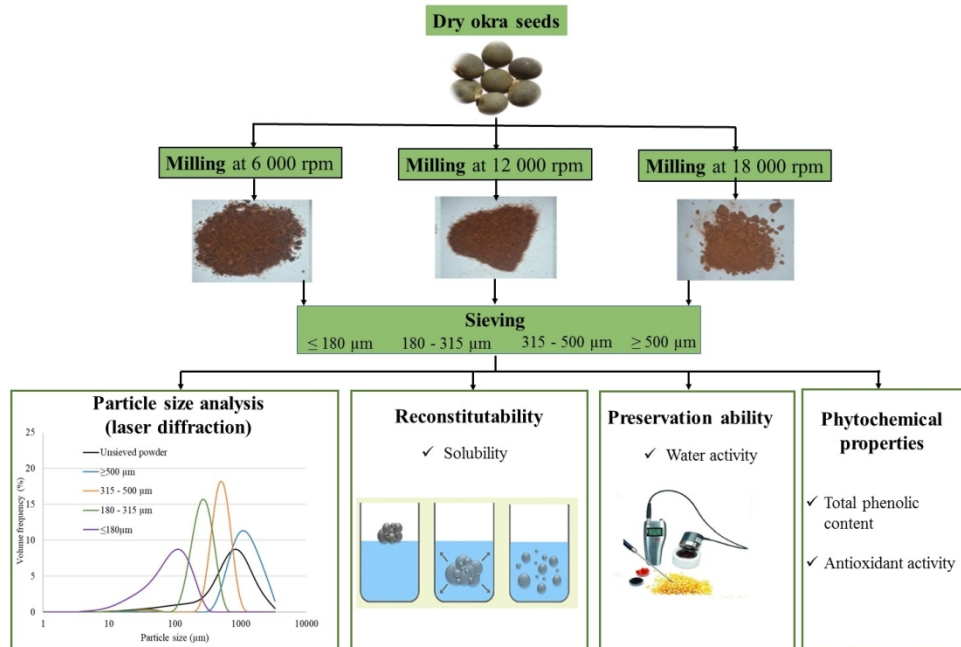
Milling frequency (rpm)	Powder samples	Moisture content (g/g) (Waiss <i>et al.</i> , 2020)	Water activity (-)	Water absorption capacity, WAC (g/g)	Water solubility index, WSI (g/100 g)
6 000	$\leq 180 \mu\text{m}$	$9.59 \pm 0.12^{\text{cd}}$	$0.555 \pm 0.001^{\text{ef}}$	$4.16 \pm 0.46^{\text{abc}}$	$32.71 \pm 0.53^{\text{f}}$
	$180 - 315 \mu\text{m}$	$9.01 \pm 0.67^{\text{ab}}$	$0.556 \pm 0.002^{\text{ef}}$	$6.46 \pm 0.20^{\text{e}}$	$24.06 \pm 0.10^{\text{cd}}$
	$315 - 500 \mu\text{m}$	$9.21 \pm 0.11^{\text{bc}}$	$0.540 \pm 0.001^{\text{cd}}$	$5.58 \pm 0.25^{\text{cde}}$	$22.74 \pm 0.74^{\text{bc}}$
	$\geq 500 \mu\text{m}$	$12.07 \pm 0.05^{\text{i}}$	$0.524 \pm 0.003^{\text{b}}$	$4.76 \pm 0.24^{\text{abcd}}$	$15.98 \pm 0.23^{\text{a}}$
	Unsieved powder	$11.10 \pm 0.07^{\text{fg}}$	$0.583 \pm 0.007^{\text{h}}$	$5.07 \pm 0.75^{\text{cde}}$	$22.75 \pm 0.37^{\text{bc}}$
12 000	$\leq 180 \mu\text{m}$	$9.15 \pm 0.10^{\text{abc}}$	$0.565 \pm 0.008^{\text{efg}}$	$4.09 \pm 0.28^{\text{ab}}$	$37.75 \pm 0.46^{\text{g}}$
	$180 - 315 \mu\text{m}$	$8.61 \pm 0.08^{\text{a}}$	$0.524 \pm 0.002^{\text{b}}$	$5.77 \pm 0.41^{\text{de}}$	$32.53 \pm 1.06^{\text{f}}$
	$315 - 500 \mu\text{m}$	$9.22 \pm 0.05^{\text{bc}}$	$0.503 \pm 0.004^{\text{a}}$	$5.12 \pm 0.30^{\text{bcde}}$	$25.05 \pm 0.57^{\text{d}}$
	$\geq 500 \mu\text{m}$	$11.79 \pm 0.06^{\text{hi}}$	$0.531 \pm 0.004^{\text{bc}}$	$4.98 \pm 0.23^{\text{bcde}}$	$17.64 \pm 0.16^{\text{a}}$
	Unsieved powder	$11.27 \pm 0.08^{\text{fgh}}$	$0.550 \pm 0.001^{\text{de}}$	$5.19 \pm 0.52^{\text{bcd}}$	$22.00 \pm 0.78^{\text{b}}$
18 000	$\geq 500 \mu\text{m}$	$9.56 \pm 0.08^{\text{bc}}$	$0.572 \pm 0.002^{\text{gh}}$	$3.52 \pm 0.12^{\text{a}}$	$41.12 \pm 1.16^{\text{h}}$
	$315 - 500 \mu\text{m}$	$10.15 \pm 0.02^{\text{de}}$	$0.564 \pm 0.005^{\text{fgh}}$	$4.06 \pm 0.87^{\text{abc}}$	$36.77 \pm 0.66^{\text{g}}$
	$180 - 315 \mu\text{m}$	$11.32 \pm 0.06^{\text{gh}}$	$0.560 \pm 0.002^{\text{efg}}$	$5.60 \pm 0.50^{\text{cde}}$	$23.50 \pm 0.07^{\text{bc}}$
	$\leq 180 \mu\text{m}$	$13.20 \pm 0.17^{\text{j}}$	$0.550 \pm 0.010^{\text{ef}}$	$4.67 \pm 0.12^{\text{abc}}$	$17.00 \pm 0.14^{\text{a}}$
	Unsieved powder	$10.69 \pm 0.09^{\text{ef}}$	$0.493 \pm 0.009^{\text{a}}$	$5.55 \pm 0.55^{\text{bcde}}$	$30.58 \pm 0.80^{\text{e}}$

620 Means \pm standard deviations followed by the same superscripted letters in the same column were not significantly different at $p < 0.05$ according to Tukey's
 621 HSD test.

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3 **Conflict of interest**
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5 The authors declare that they have no conflict of interest.
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3 **Data availability statement**
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5 Research data are not shared.
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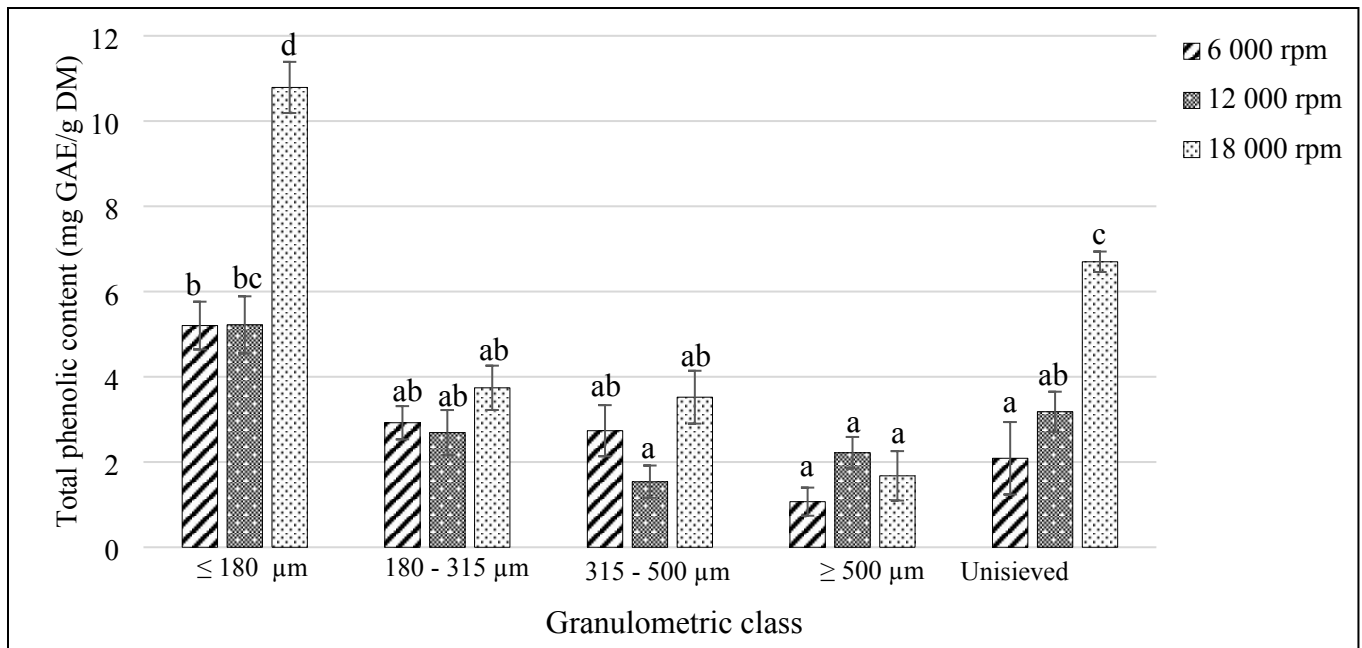


Figure 1: Total phenolic content of granulometric classes ($\leq 180 \mu\text{m}$; 180 - 315 μm ; 315 - 500 μm ; $\geq 500 \mu\text{m}$) and unsieved okra seed powders. DM: dry matter, GAE: gallic acid equivalents. Bars topped by different letters were statistically different at $p < 0.05$ according to Tukey's HSD test.

Figure 2

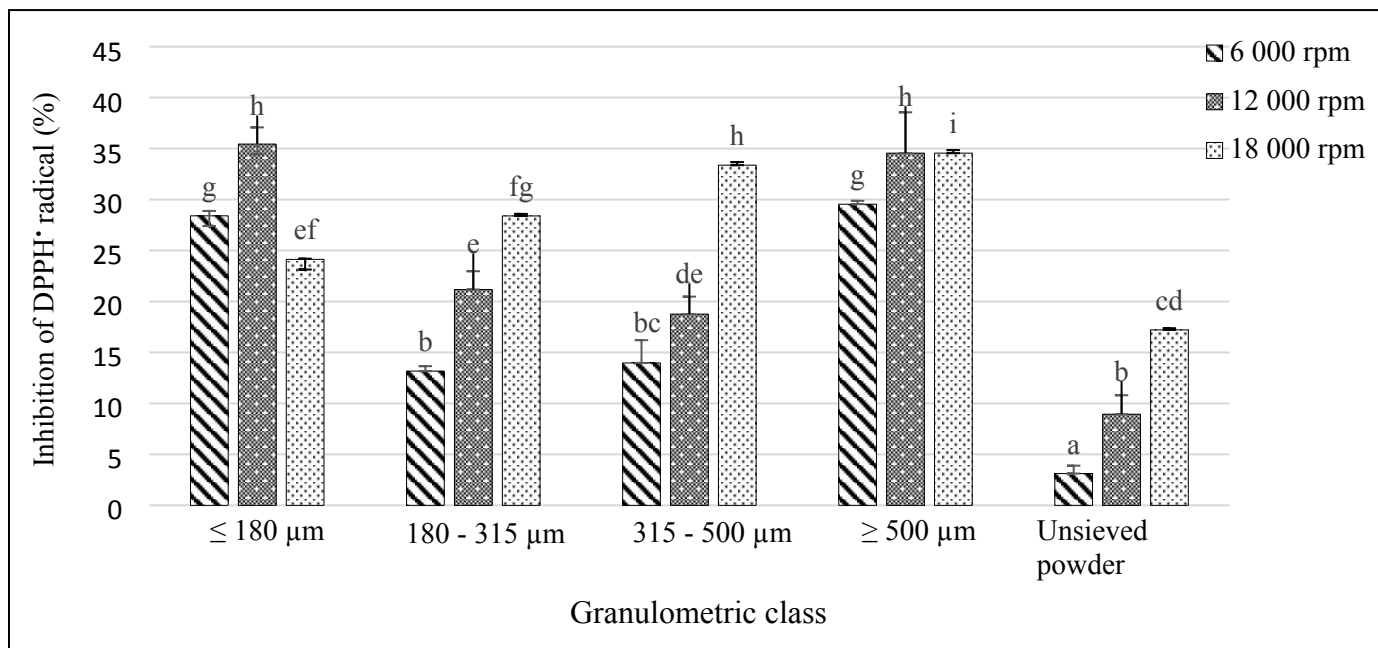


Figure 2: Inhibition of DPPH• radical by hydromethanolic extracts of granulometric classes ($\leq 180 \mu\text{m}$; 180 - 315 μm ; 315 - 500 μm ; $\geq 500 \mu\text{m}$) and unsieved okra seed. Bars topped by different letters were statistically different at $p < 0.05$ according to Tukey's HSD.

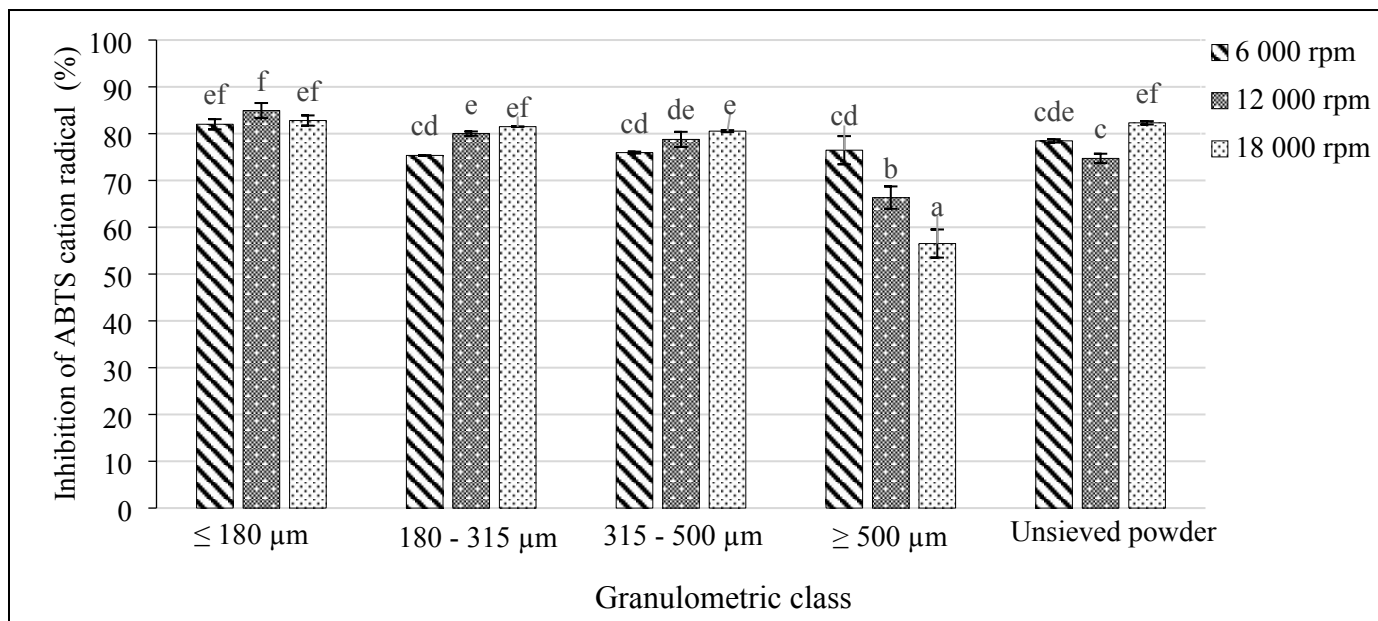


Figure 3: Inhibition of ABTS•+ by hydromethanolic extracts of granulometric classes (≤ 180 μm ; 180 - 315 μm ; 315 - 500 μm ; ≥ 500 μm) and unsieved powders of okra seed. Bars topped by different letters were statistically different at $p < 0.05$ according to Tukey's.

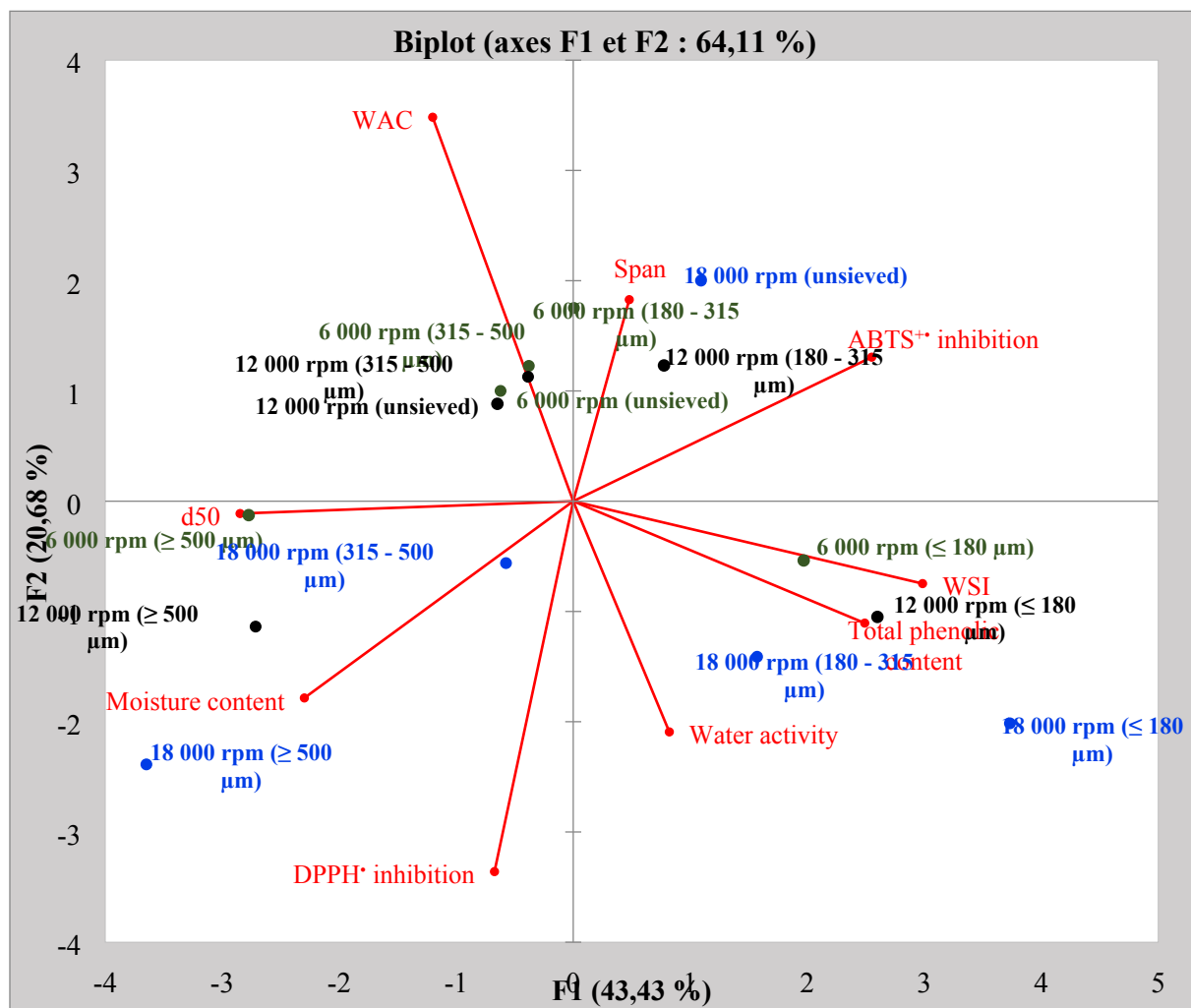


Figure 4: Representation of all granulometric classes (≤ 180 ; $180 - 315$; $315 - 500$; $\geq 500 \mu\text{m}$) and unsieved samples of okra seed powders on the principal components F1 and F2 deduced from PCA.

Table 1: Granulometric parameters of granulometric classes ($\leq 180 \mu\text{m}$, $180 - 315 \mu\text{m}$, $315 - 500 \mu\text{m}$, $\geq 500 \mu\text{m}$) and unsieved samples of okra seed powders obtained at 6 000, 12 000, and 18 000 rpm milling speed (Waiss et al., 2020).

Milling speed (rpm)	Granulometric classes	d10 (μm)	d50 (μm)	d90 (μm)	Span (-)
6 000	$\leq 180 \mu\text{m}$	$23.1 \pm 0.2^{\text{ab}}$	$85.3 \pm 0.6^{\text{a}}$	$181.3 \pm 0.5^{\text{a}}$	$1.87 \pm 0.01^{\text{f}}$
	$180 - 315 \mu\text{m}$	$152.0 \pm 1.0^{\text{de}}$	$257.0 \pm 1.0^{\text{c}}$	$406.0 \pm 1.0^{\text{b}}$	$0.97 \pm 0.01^{\text{d}}$
	$315 - 500 \mu\text{m}$	$326.6 \pm 6.0^{\text{g}}$	$498.3 \pm 2.5^{\text{e}}$	$745.3 \pm 2.5^{\text{c}}$	$0.87 \pm 0.03^{\text{bcd}}$
	$\geq 500 \mu\text{m}$	$589.3 \pm 18.5^{\text{i}}$	$1100.0 \pm 43.5^{\text{h}}$	$2173.3 \pm 73.7^{\text{g}}$	$1.44 \pm 0.02^{\text{e}}$
	Unsieved powder	$141.0 \pm 7.8^{\text{d}}$	$723.3 \pm 29.9^{\text{f}}$	$1670.0 \pm 65.5^{\text{f}}$	$2.11 \pm 0.02^{\text{h}}$
12 000	$\leq 180 \mu\text{m}$	$21.9 \pm 0.1^{\text{ab}}$	$75.6 \pm 2.8^{\text{a}}$	$167.3 \pm 6.3^{\text{a}}$	$1.95 \pm 0.06^{\text{fg}}$
	$180 - 315 \mu\text{m}$	$165.6 \pm 1.5^{\text{e}}$	$264.6 \pm 1.5^{\text{c}}$	$409.3 \pm 9.8^{\text{b}}$	$0.92 \pm 0.03^{\text{cd}}$
	$315 - 500 \mu\text{m}$	$312.0 \pm 1.0^{\text{g}}$	$463.3 \pm 1.1^{\text{de}}$	$671.6 \pm 4.9^{\text{c}}$	$0.78 \pm 0.01^{\text{bc}}$
	$\geq 500 \mu\text{m}$	$542.6 \pm 5.6^{\text{h}}$	$801.3 \pm 25.0^{\text{g}}$	$1243.3 \pm 75.0^{\text{e}}$	$0.90 \pm 0.05^{\text{bcd}}$
	Unsieved powder	$52.9 \pm 0.5^{\text{c}}$	$457.6 \pm 5.1^{\text{de}}$	$983.0 \pm 7.8^{\text{d}}$	$2.03 \pm 0.01^{\text{gh}}$
18 000	$\leq 180 \mu\text{m}$	$17.8 \pm 0.2^{\text{a}}$	$52.1 \pm 0.6^{\text{a}}$	$149.3 \pm 1.5^{\text{a}}$	$0.77 \pm 0.01^{\text{b}}$
	$180 - 315 \mu\text{m}$	$37.4 \pm 0.1^{\text{bc}}$	$181.0 \pm 0.0^{\text{b}}$	$332.6 \pm 2.0^{\text{b}}$	$0.48 \pm 0.01^{\text{a}}$
	$315 - 500 \mu\text{m}$	$290.0 \pm 4.3^{\text{f}}$	$442.6 \pm 12.8^{\text{d}}$	$644.6 \pm 25.9^{\text{c}}$	$0.80 \pm 0.02^{\text{bc}}$
	$\geq 500 \mu\text{m}$	$527.6 \pm 4.7^{\text{h}}$	$781.0 \pm 14.1^{\text{g}}$	$1186.6 \pm 37.8^{\text{e}}$	$0.84 \pm 0.02^{\text{bcd}}$
	Unsieved powder	$25.7 \pm 0.3^{\text{ab}}$	$228.6 \pm 7.5^{\text{bc}}$	$702.67 \pm 55.7^{\text{c}}$	$2.96 \pm 0.16^{\text{i}}$

For each milling speed separately, means \pm standard deviations followed by the same superscripted letters in the same column were not significantly different at $p < 0.05$ according to Tukey's HSD test.

Table 2: Moisture content, aw (-): water activity, WAC: Water Absorption Capacity(g/) and WSI: Water Solubility Index (g/100g) of $\geq 500 \mu\text{m}$; $500\text{--}315\mu\text{m}$, $315\text{--}180 \mu\text{m}$, $180 \mu\text{m} \geq$ and for unsieved seeds. Means \pm standard deviations followed by the same superscripted letters in the same row were not significantly different at $P < 0.05$ according to Tukey's HSD test.

Milling speed (r.p.m.)	granulometric classes	Moisture content (g/g) (Waiss et al., 2020)	aw (-)	WAC (g/g)	WSI (g/100 g)
6 000	$\geq 500 \mu\text{m}$	12.07 ± 0.05^c	$0,524 \pm 0,00^a$	$4,76 \pm 0,24^{ab}$	$15,98 \pm 0,23^b$
	$315 - 500 \mu\text{m}$	9.21 ± 0.11^a	$0,540 \pm 0,00^b$	$5,58 \pm 0,25^b$	$22,74 \pm 0,74^b$
	$180 - 315 \mu\text{m}$	9.01 ± 0.67^a	$0,556 \pm 0,00^c$	$6,46 \pm 0,20^c$	$24,06 \pm 0,10^b$
	$\leq 180 \mu\text{m}$	9.59 ± 0.12^a	$0,555 \pm 0,00^c$	$4,16 \pm 0,46^a$	$32,71 \pm 0,53^c$
	Unsieved powder	11.10 ± 0.07^b	$0,583 \pm 0,01^d$	$5,07 \pm 0,75^b$	$22,75 \pm 0,37^b$
12 000	$\geq 500 \mu\text{m}$	11.79 ± 0.06^d	$0,531 \pm 0,00^b$	$4,98 \pm 0,23^b$	$17,64 \pm 0,16^a$
	$315 - 500 \mu\text{m}$	9.22 ± 0.05^b	$0,503 \pm 0,00^a$	$5,12 \pm 0,30^b$	$25,05 \pm 0,57^b$
	$180 - 315 \mu\text{m}$	8.61 ± 0.08^a	$0,524 \pm 0,01^b$	$5,77 \pm 0,41^b$	$32,53 \pm 1,06^c$
	$\leq 180 \mu\text{m}$	9.15 ± 0.10^b	$0,565 \pm 0,00^d$	$4,09 \pm 0,28^a$	$37,75 \pm 0,46^d$
	Unsieved powder	11.27 ± 0.08^c	$0,550 \pm 0,00^c$	$5,19 \pm 0,52^b$	$22.00 \pm 0,78^{ab}$
18 000	$\geq 500 \mu\text{m}$	13.20 ± 0.17^e	$0,550 \pm 0,01^b$	$4,67 \pm 0,12^{ab}$	$17,00 \pm 0,14^a$
	$315 - 500 \mu\text{m}$	11.32 ± 0.06^d	$0,560 \pm 0,00^c$	$5,60 \pm 0,50^b$	$23,50 \pm 0,07^b$
	$180 - 315 \mu\text{m}$	10.15 ± 0.02^b	$0,564 \pm 0,00^{cd}$	$4,06 \pm 0,87^{ab}$	$36,77 \pm 0,66^d$
	$\leq 180 \mu\text{m}$	9.56 ± 0.08^a	$0,572 \pm 0,00^d$	$3,52 \pm 0,12^a$	$41,12 \pm 1,16^e$
	Unsieved powder	10.69 ± 0.09^c	$0,493 \pm 0,01^a$	$5,55 \pm 0,55^b$	$30,58 \pm 0,80^c$

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17 Dear Editor-in-chief of International Journal of Food science and Technology,
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19 Please find the following manuscript 'Total phenolic content, antioxidant activity, shelf-life,
20 and reconstitutability of okra seeds powder: influence of milling and sieving processes.', by
21 'Idriss MIGANEH WAISS, André KIMBONGUILA, FATOUMA MOHAMED ABDOUL-
22 LATIF, Laurette Brigelia NKELETELA, Joël SCHER, Jérémie PETIT which we wish to submit
23 for publication as an original article in 'International Journal of Food science and Technology'.
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26
27 The objective of this work is to determine the impact of the particle size of okra seed powders
28 on physicochemical, water interaction, and phytochemical properties. To our knowledge, this
29 is the first time that a study focuses on characterization of okra seed powders with granulometric
30 method.
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34 The results of this study permitted to identify a granulometric class achieving a compromise
35 between particle size of powders and bioactive compound content, powders reconstitution with
36 interaction of water.
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40 We confirmed that the manuscript or its contents in some other form have not been published
41 previously by any of the authors and/or is not under consideration for publication in another
42 journal at the time of submission.
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50 Kind regards,
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52 Jérémie PETIT
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5 **Becker, L. et al. (2017)** ‘How do grinding and sieving impact on physicochemical properties,
6 polyphenol content, and antioxidant activity of Hieracium pilosella L. powders?’, *Journal of*
7 *Functional Foods*, 35, pp. 666–672. doi: 10.1016/j.jff.2017.06.043.
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11 This reference was cited in the introduction as matters the impact of grinding and sieving on
12 physicochemical and phytochemical properties of resulting powders. It was useful to discuss
13 results of chemical composition and antioxidant activity.
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16 **Deli, et al. (2019)** ‘Effect of sieved fractionation on the physical, flow and hydration properties
17 of *Boscia senegalensis* Lam., *Dichostachys glomerata* Forssk. and *Hibiscus sabdariffa* L.
18 powders’, *Food Science and Biotechnology*. Springer Singapore, 22, pp. 1–15. doi:
19 10.1007/s10068-019-00597-6.
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23 This reference was cited because it reports information on the influence of sieving and grinding
24 on the physical properties (particle size, shape, and specific surface area), aptitude for
25 preservation (water activity), and reconstitutability (solubility, water absorption capacity) of
26 plant powders. Similar grinding and sieving conditions and methods for the determination of
27 proximal composition were employed.
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31 **Arapitsas, P. (2008)** ‘Identification and quantification of polyphenolic compounds from okra
32 seeds and skins’, *Food Chemistry*, 110(4), pp. 1041–1045. doi:
33 10.1016/j.foodchem.2008.03.014.
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37 This reference, cited in introduction, reports information regarding contents of phenolic
38 compounds in Ours seed.
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44 **Tapia MS, et al. (2007)** ‘Effects of water activity (aw) on microbial stability: as a hurdle in
45 food preservation’, in *Fundamentals and Applications*. Woodhead Publishing Limited, pp. 599–
46 630. doi: 10.1533/9781845690243.3.599.
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50 This article was useful in the discussion section. It was cited for interpreting okra seed powders
51 stability during storage from water activity values.
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The authors wish to thank the reviewers for their helpful comments. Detailed point-by-point response can be found hereafter, with reviewers' comments in black (line numbers associated with reviewers' comments correspond to submitted manuscript) and answers in blue (line numbers related to manuscript revision correspond to revised manuscript). In revised manuscript, additions were performed in blue and deletions in red crossed words.

Editor comments

Please pay particular attention to:

- The Graphical Abstract should be designed to be read in conjunction with the text abstract, it should be approximately square and contain a high impact Figure, Graph or Photograph that summarises the key findings of your research.

The graphical abstract was corrected.

- UK spelling should be used.

This was made in whole the manuscript.

- Tables & Figures must be in separate files, at the end of the manuscript.

This was made in the manuscript.

- References must follow the Harvard system of referencing and should be shown at the end of the paper, listed in alphabetical order.

This was made in the manuscript.

- Annotated References: authors are asked to annotate approximately 4 references indicating why the article was cited.

This was included in the submission file.

- Author statements on Conflict of Interest; Ethics and Data Availability.

This was included in the submission file.

- Figures and Tables should be uploaded as separate files. Tables should be in editable format and Figures. Scans (TIFF only) should have a resolution of at least 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size

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3 Ø Printing charges: Printing up to 7 journals pages is free to authors. Articles longer than
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5 prepared to pay excess charges you should aim for approximately 4,500 words and 6 display
6 items. Extra items may be assigned to Supplementary Files.
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10 11 12 **Reviewer #1**

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14 This is an interesting work that contributes with important results considering the influence
15 of the particle size of milling in relation to the antioxidant activity and extractable phenolic
16 levels. This is then related to the accessibility of these important compounds as well as of
17 proteins and polysaccharides into the gut. This work complements a previous one of these
18 authors where a complete determination of the chemical composition of these fractions with
19 determined particle size ranges was performed.
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24 This manuscript is acceptable for publication after correction according to the following
25 revision.
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28 Please, have the manuscript grammar and writing revised.
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31 Amendments can be also found in the pdf file of the manuscript.

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33 When a Figure number and a Table number is mentioned along the text of the manuscript,
34 they also should begin written in Capital letter:
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37 Examples: "as shown in Figure 2....." "as summarized in Table 2,"

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39 This should be corrected along the manuscript.

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41 [This was corrected in the whole manuscript.](#)

42
43 ABSTRACT.

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45 The Abstract was in general well written.

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47 Please, better write: "The influence of the particle size derived by milling of okra seeds at
48 three different speed velocities (6,000; 12,000 and 18,000 rpm) on functionality (total
49 phenolic content and antioxidant activity), reconstitutability (water absorption capacity,
50 water solubility index), and preservation (water activity) of powders was studied. Okra seed
51 powders were sieved with 180, 315, and 500 µm sieves. An increased"
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55 [The abstract was clear, complete and grammatically sound, thus the authors preferred to](#)
56 [keep it unchanged. In particular, 6 000, 12 000, and 18 000 rpm are values of milling](#)
57 [frequencies, not milling velocities. Also, reconstitutability and preservation ability are powder](#)
58 [functionalities.](#)
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3 Please, could you express the water activity value here and along the manuscript with three
4 figures after the decimal point? Water activity meters are supposed to measure the water
5 activity with three figures after the decimal point. It is important this complete value.
6
7

8 This was corrected in the whole manuscript.
9

10 "preservation" (not preservability)

11 "Preservability" was replaced by "preservation ability" in L21.
12

13 KEYWORDS.
14

15 Please, replace "okra seeds" by "okra seed powder".
16

17 This was corrected in L27.
18

19 INTRODUCTION.
20

21 It was well written indeed.
22

23 L38. Please, correct to: "of oil rich in unsaturated fatty acids including linolenic and linoleic
24 acids, which are both essential for humans"
25

26 Concerned sentence was corrected (cf. L35).
27

28 L40. Please, correct to: "contents in polysaccharides and phenolic compounds including
29 flavonoids (Arapitsas, 2008)."
30

31 Concerned sentence was corrected (cf. L37-38).
32

33 L41. "Phenolic compounds are known to"
34

35 Concerned sentence was corrected (cf. L38).
36

37 L42. Please, correct to: "Although the high phytochemical content of ..."
38

39 What is phytochemical "activity"?"
40

41 Concerned sentence was corrected (cf. L39-40).
42

43 All compounds in a plant are "phytochemicals", many of which are not precisely healthy (e.g.
44 curare, valerian, alkaloids, etc., etc.).
45

46 L64. Please, correct to: "okra seed powder" ("seed" in singular because here it functions as
47 an adjective).
48

49 Concerned sentence was corrected (cf. L61).
50

51 L73. Please, change to: "predict their powder behavior during storage and reconstitution for
52 an efficient use as functional"
53
54
55
56
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1
2
3 Concerned sentence was corrected (cf. L70-71).

4
5 L76. "as well as on preservation"

6
7 Concerned sentence was corrected (cf. L73).

8
9 L92. "The powder obtained was then collected...."

10
11 As concerned sentence appeared clear and grammatically sound, it was kept unchanged.

12
13 L104-106. These two sentences should be passed to the Results and Discussion section.

14
15 Sentence in L102-104 was used to introduce the interest of analysing particle size
16 distribution of powders. So, authors judged preferable to keep it at the beginning of the
17 method subsection related to granulometry.

18
19 L106. Please, define clearly here in the text the meaning of 'unsieved' powders. It is really
20 not understandable.

21
22 This was added in L92-94 when defining how unsieved powders were prepared.

23
24 L113-115. Please, re-write to: "d10, d50, and d90, which signifies the point in the size
25 distribution, up to and including which, 90% or 50% or 10% of the total volume of material
26 in the sample is 'contained'. For example, if the d90 is 315 micrometres, this means that 90%
27 of the sample has a size of 315 micrometres or smaller. The span of a volume-based size
28 distribution is defined as indicated by equation (1), which quantifies the...."

29
30 The authors preferred to redefine granulometric parameters from a statistical point of view
31 for accuracy purposes.

32
33 L120. Please, correct to: "5 h (AFNOR, Agence Française de Normalisation, 1976)."

34
35 Concerned sentence was corrected (cf. L117)

36
37 L121. The water activity meter reported gave the water activity value with three decimal
38 figures after the decimal point. Please, report the water activity values in the manuscript with
39 three decimal figures after the decimal point, including in the Abstract.

40
41 Indeed, an additional significant digit was added to water activity values in the abstract and
42 in the results and discussion sections.

43
44 L125. With this method only the extractable phenolics are being obtained, but not the
45 chemically bound phenolics. Please, indicate this fact under 'Results and Discussion' section.

46
47 This was added in L125-127.

48
49 L136. "In test tubes, 30 μ L of okra powder"

1
2
3 This was kept unchanged, as it was grammatically sound (cf. the numerous occurrences of
4 "X mL distilled water" for instance : [https://www.linguee.com/english-](https://www.linguee.com/english-french/search?source=auto&query=ml+distilled+water)
5 [french/search?source=auto&query=ml+distilled+water](https://www.linguee.com/english-french/search?source=auto&query=ml+distilled+water)).
6
7

8 L136. "2.67 mL of distilled"
9

10 *Idem.*
11

12 L137. "500 μ L of Folin-Ciocalteu"
13

14 *Idem.*
15

16 L137. "and 300 μ L of sodium carbonate (Na_2CO_3 , 20 % w/v) were added."
17
18

19 *Idem.*
20

21 L141. "for calibration curve ($R^2 = 0.99$)"
22

23 The initial sentence was grammatically sound; thus, it was kept unchanged. Also, the "2"
24 should be in exponent to mean "power 2", it was also kept unchanged.
25
26

27 L145-148. "The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method was
28 used according to Matsuda et al. (1999). Five hundred microlitres of 0.1 mM DPPH"
29

30 Concerned sentence was corrected (cf. L144-146).
31

32 L148. "with 500 μ L of okra seed powder"
33

34 As previously indicated, this was kept unchanged, as it was grammatically sound.
35
36

37 L151. "of DPPH solution"
38

39 *Idem.*
40

41 L151. "and 150 μ L of distilled water"
42

43 *Idem.*
44

45 L155. "with ABS"
46

47 The authors did not understand this comment. "(-)" means dimensionless and unit should
48 be given in brackets for parameters used in presented equations.
49
50

51 L171. "after the ABTS+• assay" (no "DPPH")
52

53 This was corrected in L170.
54

55 L173-174. "(WAC) was determined by addition of 10 mL of distilled water to 1 g of okra
56 seed powder contained into a centrifuge tube. The mixture was stirred at 300 rpm"
57
58
59
60

1
2
3 Concerned sentences are grammatically sound, thus authors preferred to keep it
4 unchanged.
5

6
7 L192. "at a 5 % of confidence level"

8
9 "X % confidence level" can be employed: cf. <https://www.linguee.fr/anglais-francais/traduction/confidence+level.html>
10
11

12
13 L193. "Please, report the version, trade mark and country of origin of DSAASTAT add-on
14 software of Excel."
15

16
17 It was added in L192-193.
18

19 L201. Please, also clarified here what is the meaning of "unsieved"

20
21 Unsieved is now defined in L91-94.
22

23 L202-203. Please, clarify the meaning of d50, at least between parentheses to remember it.

24
25 "d50" was replaced by "median particle size" in L202-203.
26

27 L205-206. Please, indicate here the mesh size of the 'upper' and the mesh size of the 'lower'
28 sieves used.
29

30
31 "their respective" was added in L206 to indicate that upper and lower mesh sizes depend
32 on the considered granulometric class.
33

34
35 L206. It was written: "confirming the efficiency of the sieving process." Why? Please, these
36 explanations and conclusions are not understandable. Please, explain first these facts, based
37 on the literature too.
38

39
40 The efficiency of the sieving process concerns its ability to separate powders into
41 granulometric classes differing in median particle size (this was added L207-208). The fact
42 that median particle size is found between upper and lower mesh sizes indicates that sieving
43 was completed, i.e. particles were separated in granulometric classes according to their size
44 comprised between the upper and lower mesh sizes ("sieves as expected when sieving is
45 completed" was added in L203-204 to improve clarity).
46
47
48
49

50 L208. "in Table 1."
51

52 Concerned sentence was corrected (cf. L202).
53

54 L208. Nothing has been here reported and explained about the span (equation 1) results
55 obtained and shown in Table 1. The values of span obtained are high, low, (????).
56
57

58 A discussion about span results was added in L209 -213.
59
60

1
2
3 L234. "The DPPH^{•+} and ABTS^{•+} assays"

4
5 The order was inverted in L237.

6
7 L239. "Figure 2."

8
9 Figure was capitalised in L242.

10
11 L239. "All extracts were able to scavenge DPPH[•] radicals and this capacity increased with
12 the speed of milling from 6,000 to 18,000 rpm, that is, with the decrease in particle sizes."

13
14 Concerned sentence was kept unchanged because it was grammatically sound, and the
15 influence of particle size was discussed in L251,254.

16
17 L248-249. As observed in Figure 2, this is not true when comparing ≥ 500 microns
18 granulometry and ≤ 180 microns for 6,000 and 12,000 rpm.

19
20 "at a given milling frequency" was added in L251 to remind that this observation concerns
21 granulometric classes and unsieved powder obtained at the same milling frequency.

22
23 L251. "The general increase in DPPH[•] scavenging"

24
25 Concerned sentence was corrected (cf. L 254).

26
27 L260. "unsieved powders (Fig. 2) shows"

28
29 "(Figure 2)" was added in concerned sentence (cf. L264).

30
31 L269. "in Figure 3."

32
33 F of figure was capitalised in L271.

34
35 L290-291. It was written: "Moisture content is an important parameter that determines
36 powdered food ingredient stability during storage." This is not true!!! It is a wrong
37 physicochemical concept. The 'water activity' is which determines the stability and NOT the
38 water content or moisture content. Please, correct it.

39
40 Indeed, moisture content is indirectly related to food stability during storage, as moisture
41 content and food structure both impact food water activity, and the latter parameter is the
42 one that is determinant for food stability during storage. Thus, it is not false to write that
43 "moisture content is an important parameter that determines powdered food ingredient
44 stability during storage" but it can be misleading. Concerned sentence was reformulated (cf.
45 L290, 293), and additions were made in L300-302.

46
47 Please, begin this part (3.4) with the information of the water activity results obtained.
48 Afterwards, report the moisture contents.
49
50
51
52
53
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1
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3 The authors preferred to keep the original order: moisture content results first, and water
4 activity results after. Indeed, this permitted to remind that water activity is the good
5 parameter to evaluate water availability for microorganisms and alteration reactions.
6
7

8 L291-292. It was written: "are known to limit microbial growth and extend food shelf-life"
9 Again, this is the same mistake. The 'water activity' is which determines the growth of
10 bacteria, yeasts and molds. Please, see the stability map of Labuza (1977). Be careful, this is
11 a physicochemical concept. Labuza (1977) - Figure 10: [https://doi.org/10.1111/j.1745-](https://doi.org/10.1111/j.1745-4549.1977.tb00321.x)
12 4549.1977.tb00321.x Indeed, as indicated in a previous answer, water activity is the good
13 parameter to evaluate water availability for microorganisms and alteration reactions.
14 However, it is true that "low levels of moisture content are known to limit microbial growth
15 and extend shelf-life". The threshold of 12 % (w/w) is often reported in the literature as a
16 global limit for all types of food. Thus, it was decided to keep concerned sentence
17 unchanged, as additions were made in L290 and 299-302 to better define the link between
18 water moisture, water activity, and food preservability (Tapia, *et al.*, 2007; Bhandari *et al.*,
19 2013).
20
21
22
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25
26

27 L293-295. Please, re-analyzed all results with the water activity of each granulometry.

28
29 The influence of median particle size (directly linked to granulometry) on water activity was
30 already discussed in L290-293, and the influence of particle size was similar for all milling
31 frequencies. Thus, no addition was made in the previous discussion of the influence of
32 median particle size on water activity.
33
34
35

36 The moisture content is a function of the water activity of equilibration and the sorption
37 capacity of the solid material, where the latter depends on the chemical composition and
38 porosity.
39
40

41 Indeed, this was briefly added with the concept of "food structure" in L308-311.

42
43 L293. "in Table 2"

44
45 Concerned sentence was corrected (cf. L293).

46
47 L309. "in Table 2."

48
49 Concerned sentence was corrected (cf. L296).

50
51 L310-312. WAC and WSI results are not reported in Table 2. Please, complete the Table. It
52 is then not possible to revise this part.
53
54

55
56 The result of WAC and WSI were added in table 2. The comments were added in L323-335.

57
58 L312. "high" (not 'highly', an adverb)
59
60

1
2
3 Concerned sentence was corrected (cf. L314).

4
5 L314. "smaller particles (Table 2)."

6
7 Table was capitalized (cf. L316).

8
9 L339. "in Figure 4."

10
11 Figure 4 was deleted. The comments were added in L345-349.

12
13 L340-341. The total explained varianza obtained is certainly lower than that recommended
14 by the PCA method (80% to 90%).

15
16 It was added that this was sufficient to outline the main links between parameters in L348-
17 349.

18
19 CONCLUSIONS.

20
21 L371-372. It was written: "Powders with smaller median particle sizes had lower moisture
22 content and water activity," I'm thinking about: If powders with smaller median particle
23 sizes had lower moisture content and also lower water activity, hence the conclusion is not
24 that 'as the particle size was reduced bellow XXX microns, the aDsorption capacity of the
25 solid matrix of the powder decreased.' I believe that the true conclusion is that the porous
26 were in part collapsed by the milling process. Please, treat to indicate possible explanations
27 for the results observed.

28
29 This was described in more details in L374-382.

30
31 L374. "food spoilage by microbial"

32
33 Concerned sentence was corrected (cf. L382).

34
35 L375. Why biochemically???

36
37 Biochemically was indicated here to refer to enzymatic reactions.

38
39 TABLES.

40
41 Footnotes: Please, delete "according to Tukey's HSD test" since this was already explained
42 under 'Statistical analyses' section.

43
44 This information must be indicated for each table where samples were sorted by Tukey's
45 HSD test, as it explains the meaning of superscripted letters.

46
47 Table 2. It was written in the legend: "and water solubility index of granulometric classes"
48 Indeed, the water solubility index was not reported in the Table. Please, add a column with
49 these results. Please, also comment and discuss them in the text.

1
2
3 WAC and WSI were added in table 2 and discussed in L323-330 and 333-335.
4
5
6
7
8

9
10 Referee # 2

11
12 Comments to the Author

13
14 I think this is an interesting and well-written study.
15
16
17
18

19 A few questions:

20
21
22 - what is the definition of superfine milling in terms of particle size?
23

24 Superfine milling permits to reach particle sizes up to tens of micrometers. This term has
25 been removed from the manuscript.
26
27

28
29 - why didn't you repeat with more than one okra sample? How general are the results?
30

31 Okra seeds were imported from the Republic of Congo by the first author of the article
32 and only a limited amount of material was allowed to cross borders. It appeared important
33 to work with a single lot of okra seeds in order to limit the impact of proximal composition
34 variability among lots. Thus, the authors chose to work with a single lot and to produce a
35 single replicate of each sample, but to replicate analyses three times to ensure result
36 accuracy.
37
38
39
40
41
42

43
44 - How did you control and measure moisture content during milling and sieving? It must have
45 a very big effect on results.
46

47 Equipments employed for milling and sieving were not equipped to control moisture
48 content of product. This was achieved after milling and sieving steps by a
49 thermogravimetric method (cf. L122-123). Milling and sieving steps were performed the
50 same day for all powders in a close room where air thermohygrometry was controlled (20
51 °C, 40 - 50 % relative humidity). This permitted to limit the influence of surrounding air
52 relative humidity on moisture content of produced powders.
53
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3 - It is very much preferable, if you report total phenolics, to also measure and report the
4 chemical composition of the phenolics.
5

6
7 The laboratory is not equipped with HPLC able to separate and quantify phenolic
8 compounds, thus it was chosen to determine the total phenolic content only.
9

10
11 Please format every reference accurately and consistently –

12
13 - Scientific names in italics, and words like ‘in vitro’. Genus name – upper case initial
14 letter, species name, lower case initial letter.
15

16
17 This was corrected in the whole manuscript.
18

19
20 - Upper and lower case initial letters as required for article title, and for journal name.
21

22
23 This was corrected in the whole manuscript.
24

25
26 - Pay attention to accurate format for book or chapter citation
27

28
29 This was corrected in the whole manuscript.
30

31 Referee #3

32 Comments to the Author

33
34 The scientific data is relevant and should be shared with others. Nonetheless, the manuscript
35 has several problems.
36

37
38 1 . All the affiliation of authors should be in English.
39

40
41 Affiliations are standardised by the universities of authors and should be kept in their
42 native language.
43

44
45 2. In “3.4. Moisture content and water activity”, $\geq 500 \mu\text{m}$ granulometric classes at 6 000, 12
46 000, and 18 000 rpm milling speed have higher moisture content, but why did the powders
47 have lower water activity in table 2?
48

49
50 Indeed, $> 500 \mu\text{m}$ granulometric classes have the highest moisture contents but intermediate
51 values of water activity. This may be due to the fact that components of $> 500 \mu\text{m}$ granulometric
52 classes interact more with water and decrease its bioavailability. This is consistent with the
53 hypothesis that these higher size granulometric classes may contain more fibres, which are
54 known to strongly retain water.
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1
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3. In Line 308-309, “The water absorption capacity (WAC) and water solubility index (WSI)
4 results of all granulometric classes and unsieved samples of okra seed powders are presented
5 in table 2.”. However, no WAC and WSI in table 2. The data was not found in the manuscript.
6
7

8
9 WAC and WSI were added in table 2 and discussed in L323-335.
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