1 **Physicochemical properties and adsorption of cholesterol by**
2 **okra (*Abelmoschus esculentus*) powder**
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15   **Abstract:** Okra (*Abelmoschus esculentus*) is widely used medicine purpose and as

16   functional food. In order to clarify the effects of particle size on its functional

17   properties, okra pods were subjected to superfine grinding and its properties were

18   determined using different methods. Four particle size levels of okra powders were

19   prepared- 380 to 250, 250 to 75, 75 to 40 and less than 40μm. The results showed that

20   superfine grinding technology could efficiently pulverize the particles into submicron

21   scale, whose distribution was close to a Gaussian distribution. With okra powder size

22   diminishing, specific surface area, the water holding capacity (WHC), water-retention

23   capacity (WRC), oil-binding capacity (OBC), tapped density and total flavonoids

24   extraction were increased significantly ($p < 0.05$). Moreover, the adsorption of

25   cholesterol by okra powder was improved after superfine grinding. These results

26   suggest that okra powder could be be used in the food manufacturing as a functional

27   food ingredient..

Introduction

Abelmoschus esculentus, indigenous to Africa, is usually known as okra and cultivated in many parts of the world including Middle East, the southern part of the USA and China[1]. Due to the rich amount of vitamins, dietary fiber and minerals, okra pods have been used as functional food ingredient as well as in traditional Chinese medicine for promoting diuresis and treatment of dental diseases and gastric irritations[2]. The health promoting properties of okra are suggested to reside in its non-starchy polysaccharides that are highly viscous and slimy when extracted with water.[3, 4] Chemical composition analyses of okra have reported it to be rich in K, Na, Mg, and Ca with traces of Fe, Zn, Mn, and Ni.[5] In some countries okra fruits are also dried and stored for later use.

Micronization is a process of reducing the average diameter of a solid material's particles.[6] The size has a profound effect on the physicochemical characteristics of powder. It is usually effective to grind the biomaterials into superfine powders (i.e. ultrafine powder) for efficient extraction.[7] Numerous similar unit-operations have been developed in biological powder technology over the past decades due to the rapid and steady development of superfine powder techniques.[8] Compared with the samples ground with traditional methods, ultrafine particles bear the outstanding physical properties like dispersibility and solubility.[9] With the particle size decreasing, the surface of ultrafine powder can undergo some changes, which have brought out extraordinary characteristics that crude particles do not possess, including surface effect, mini-size effect, macro quantum channel effect, quantum effect, optical

52 property, mechanical property, magnetic property, chemical and catalytic property.

53 Because of these notable characteristics, ultrafine powders have found many

54 applications in ceramics, electric materials, chemicals and papermaking fields.[10, 11]

55   Applying micronization or nanotechnology in food and pharmaceutical industries

56 has gained much attention. The fiber size of superfine grinding rice straw powder

57 promotes enzymatic hydrolysis due to the cellulose accessibility.[12] The superfine

58 ginger powder exhibits the good fluidity, water holding capacity, water solubility

59 index and protein solubility.[13] The superfine powder of mushroom (*Agrocybe*

60 *chaxingu* Huang) also exhibits good fluidity, water holding capacity and solubility,

61 and is well suited to manufacture instant and convenient foods.[14] The ultrafine

62 grinding has been suggested as a good way to fractionate biomaterials into easily

63 bio-converted and hydrolyzed part.[15]

64   To date, the information available on the effect of the particle size on

65 physical-chemical properties of okra are limited. The present research was, therefore,

66 designed to investigate the application of the superfine grinding technology on okra

67 pods and the effects of particle size on its functional properties.

68 **Materials and methods**

69 **Materials**

70   Okra pods were obtained from the Hanhua company in LuAn city, Anhui

71 Province, China. Pods were sorted, cleaned, seeds removed and cut into small pieces.

72 The pieces were dried in an electric thermostatic drying oven at 40 °C till the water

73 content was less than 10% (w/w). The water content was determined by using AACC

74   method No. 44–19.[16]

75   **Preparation of okra powders**

76   A flow diagram of the okra powder procedure is shown in Fig.1.The dried okra

77   pods were first milled to coarse particles by a disc-mill, and then screened through

78   different sized sieves (40 and 60 Mesh, GB/T6003.1-1997) to separate granules (d <

79   380μm). The superfine powders were obtained by using high pressure pulverizer

80   (Huanyatianyuan Machinery Company, Beijing, China), which were subdivided

81   through different sized sieves (200 and 350 mesh, GB/T6003.1-1997)

82   **Particle size and color difference analysis**

83   Particle size distributions were determined by laser granulometry with a Malvern

84   Mastersizer 2000 diffraction laser particle sizer (Malvern, Malvern Instruments Ltd,

85   UK). All determinations were performed in triplicate. The instrument provides

86   volume weighted size distributions and particle size parameters, such as volume

87   median diameter (d_{50}), De Brouckere mean diameter ($d_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$), and

88   Sauter mean diameter ($d_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$), where n_i is the number of droplets of

89   diameter d_i.

90   Median diameter is the value of the particle size which divides the population

91   exactly into two equal halves i.e. there is 50% of the distribution above this value and

92   50% below. Median diameter is especially important in case of a bimodal distribution.

93   De Brouckere mean diameter is the volume or mass mean diameter of the particles,

94   and Sauter mean diameter is the surface area weighted mean diameter of the particles.

95   [17]

96   Color difference, as indicators being 'L', 'a' and 'b' of the okra powders, was

97   determined on a WB-2000IX A-automatic color difference meter (Shanghai Exact

98   Science Instrument Ltd., Shanghai, China).

99   **Scanning electron microscope (SEM)**

100   Morphological characterization of okra powders was performed using images

101   acquired by SEM Quanta 200FEG-SEM (FEI Co. Netherlands) at 150 kV accelerated

102   voltage and 10–15 mm working distance. The samples were coated with platinum of

103   10 nm thicknesses to make the samples conductive.

104   **Bulk density, tapped density and repose angle**

105   The bulk density (g/mL) refers to density including pores and interparticle voids.

106   The bulk density of okra powder was calculated as follows:[18]

107      $$B = \frac{W_2 - W_1}{10} \quad (1)$$

108   Where W_2 was the total weight of okra pods powder and 10 mL measuring cylinder,

109   and W_1 was the weight of the 10mL measuring cylinder. All measurements were

110   performed in triplicate.

111   The tapped density was determined according to the method of Usp32-616[19].

112   Calculate the tapped density by the formula:

113      $$T = \frac{M}{V_0 - V_1} \quad (2)$$

114   Where M was the total weight of okra pods powder, and V_0 was the unsettled

115   apparent volume, V_1 was the tapped volume. All measurements were performed in

116   triplicate.

117   Repose angle was determined according to the method of Zhang et al.[14]. A filler

118   was fixed above a glass plane so that the height (H) of the glass plane from the outlet

119   of the filler was 1 cm, and the filler was vertical to the glass plane. The okra powders

120   were poured into the filler separately until the tip of the powder cones that they

121   formed on the plane exactly touched the outlet of the filler. The radius (R) of the cone

122   was measured for each type of powder. Repose angle (α) was calculated using Eq.

123   (3)[20.]

124     $$\alpha = \tan^{-1}\frac{H}{R} \quad (3)$$

125   **Swelling capacity**

126   Swelling property is defined as the ratio of the volume occupied when the sample

127   is immersed in excess of water after equilibration to the actual weight. Accurately

128   weighed dry sample (0.2 g) was placed in a graduated test tube, around 10mL of water

129   was added and it was hydrated for 18 h. After 18 h, the final volume attained by

130   powder was measured.[21]

131     $$\text{Swelling capacity(mL/g)} = \frac{\text{volume occupied by sample}}{\text{original sample weight}} \quad (4)$$

132   **Water holding capacity and water-retention capacity**

133   The water holding capacity was determined, using the method of Anderson [22]. First,

134   the weights of empty centrifuge tubes (M) and different sized okra powders (M_1) were

135 measured. Then the powders (M_1) were dispersed in water at a ratio of 0.05:1 at 20°C

136 in centrifuge tube(M), which were placed in a water bath at 60°C. The tubes were

137 incubated for 60 min, after that moved to cold water for 30 min, followed by

138 centrifugation for 20 min at 5000 rpm. The supernatant was removed and the

139 centrifuge tubes with the powders (M_3) were weighed. The formula to calculate water

140 holding capacity (WHC) is as follows:

141 $$WHC(g/g) = \frac{M_3 - M - M_1}{M_1} \quad (5)$$

142 The water-retention capacity (WRC) was defined as the quantity of water that

143 remains bound to the hydrated fiber following the application of an external force

144 (pressure or centrifugation). The WRC was determined according to the description

145 by Raghavendra et al. (2004)[21] with modifications. The different size powders (W_1)

146 were dispersed in water with a ratio of 0.05:1 at 20°C, placed in centrifuge tubes

147 which were incubated in a shaker water bath (100rpm) maintained at 80°C, then

148 shook it for 60 min at 100r/min. At the end of incubation, mixture was centrifuged at

149 6000 rpm for 10 min, the supernatant was removed and the residue weight was

150 recorded (W_2). The samples were then weighed (W_3) again when being dried at

151 105°C.

152 $$WRC(g/g) = \frac{W_2 - W_3}{W_3} \quad (6)$$

153 **Oil-binding capacity (OBC)**

154 OBC is determined by the method of Sangnark and Noomhorm [23] with slight

155 modifications. The dried okra powders (1.0 g) were mixed with soybean oil (20 mL)

156   in centrifuge tubes and left for 1h at 37 $^{\circ}$C, respectively. The mixtures were then

157   centrifuged at 1,500g for 10min, the supernatants decanted and the pellet was

158   recovered by filtration through a nylon mesh. OBC was expressed as follows: [24]

159    $$OBC(g/g) = \frac{\text{Pellet weight} - \text{Original dryweight}}{\text{Original dryweight}} \quad (7)$$

160   **Flavonoids Extraction**

161   The extraction and determination of flavonoids were carried out, by the method of

162   Cui et al (2013)[25] with some modifications. Okra powders (3 g) were placed into an

163   Erlenmeyer flask (100 mL) with 30mL diethyl ether solvent for 12h to remove

164   lipophilic impurities. Then, the ether was removed by suction filtration. Ethanol was

165   added (95%, v/v; liquid to solid ratio 50:1, mL/g) into the Erlenmeyer flask that was

166   sonicated in an ultrasonic cleaning bath for 2h at 60 °C. After the extract was filtered,

167   the evaporated filtrate was used for the determination of the flavonoids. The diluted

168   sample (1 mL) containing flavonoids was mixed with 0.3 mL of 5% (w/v) sodium

169   nitrite, 0.3 mL of 10% (w/v) aluminum nitrate and 4 mL of 4% (w/v) sodium

170   hydroxide, and the final volume was adjusted to 10 mL with 70% (v/v) ethanol. After

171   allowing the mixture to stand 15min at room temperature, the absorbance of the

172   solution at 510 nm was determined with a UV752 spectrophotometer. Flavonoid

173   concentration was calculated using rutin as standard. The calibration curve (y =

174   −0.0004+18.538x, where y is absorbance value at 510 nm of sample, x is flavonoids

175   concentration) ranged 0.0104–0.0520mg/mL ($R^2 = 0.9999$).

176   **Adsorption capacity of okra powder for cholesterol**

177   Adsorption of cholesterol by okra powder was estimated from the change in

178   cholesterol concentration on exposure of the solution to the powders. Increasing

179   concentrations of cholesterol solutions were made in glacial acetic acid. A known

180   concentration of cholesterol solution was max with and known amount of okra

181   powder and the mixture was in a shaker water bath (90 rpm) maintained at 37 $^{\circ}$C for

182   60min. A flask without any okra powder served as blank control. At the end of

183   adsorption, a 2 mL volume of the supernatant was taken for cholesterol estimation.

184   The determination on content of cholesterol in solution was detected according to

185   the national standards (GB/T 15206-94).The calibration curve (y =15.469x+0.0065,

186   where y is absorbance value at 563nm of sample, x is cholesterol concentration)

187   ranged 0.0125–0.0625mg/mL (R^2 = 0.9996).

188   The formula to calculate adsorption capacity (CAC) for cholesterol is as follows:

189     $$CAC(mg/g) = \frac{V(\rho_0 - \rho - \rho_k)}{m} \quad (8)$$

190   Where m was the total weight of okra pods powder;

191   V was the volume of the cholesterol solution;

192   ρ_0 and ρ were the concentrations of the cholesterol solution before and after

193   absorbing, respectively;

194   ρ_k was the concentration of the cholesterol solution in the blank control.

195   All measurements were performed in triplicate.

196   In vitro sorbent testing was carried out in series of single factor tests to study how

197   factors, such as the particle size of powder, temperature, absorption time and the

198   initial concentration of cholesterol influence the sorption process. In order to

199　characterize the adsorption process, the experimental data were fitted with adsorption

200　isotherms and analyzed by thematics.

Statistical analysis

202　All determinations were carried out in triplicates. The data are presented as mean+/-

203　SD and analyzed statistically using Analysis of variance. Statistical significance was

204　considered at $p < 0.05$.

Results and discussion

Surface properties

207　The different size okra powders had different surface properties (Table 1). Different

208　d_{43} values of coarse powder and superfine samples were 359.021, 273.31, 69.373 and

209　23.110 μm, respectively (Table 1). Okra powder with the smallest granule size (18.69

210　μm) was found to have the highest specific surface area as 0.824 m^2/g, lowest d_{43} and

211　d_{32}, respectively. Inversely, the okra powder with the largest particles (349.6 μm) had

212　the lowest specific surface (0.096 m^2/g), highest d_{43} and d_{32}, respectively (Table 1).

213　Therefore, superfine grinding technology could efficiently pulverize the particles to

214　submicron scale. Furthermore, the particle size distribution was close to a Gaussian

215　distribution. Finer particles tended to have a greater number of particles per unit

216　weight. It was an indication of a higher potential for achieving homogeneity when

217　mixing with the active pharmaceutical ingredient and other powder additives.[26] The

218　higher specific surface area of the finer particles also can improve the adsorption

219　capacity, which would be discussed in the following content. The specific surface area

220   and moisture adsorption tendency may have an indirect effect on drug dissolution

221   from solid dosage forms.[26]

222   **Colour differences**

223   The color is an important quality of all foods including, food powders. The previous

224   investigation indicated that the particle size had a significant effect on lightness of red

225   pepper powder.[27] The colors were expressed as L indicates lightness, while a and b are

226   chromaticity coordinates; +a is in the red direction, -a is in the green direction, +b is

227   in the yellow direction, and -b is in the blue direction[28]. The data presented in Table 2

228   show that the particle size affects the color of okra powder slightly. We observed that

229   'a' increased steadily with decreasing particle size of okra powder. And 'L' increased

230   from the grade 380-250μm to 250-75μm, while 'b' increased dramatically from the

231   grade 250-75μm to 75-40μm. As the specific surface area increased, the particles

232   become more homogeneous, with increased exposure of inside material, which

233   affected the color of the powder.

234   **Bulk density, tapped density and repose angle**

235   Because the inter-particulate interactions influence the bulking properties of powder

236   and also interferes with powder flow, a comparison of the bulk and tapped densities

237   can give a measure of the relative importance of these interactions in a given powder.

238   Such a comparisons are often used as an index of the ability of the powder to flow.[19]

239   As particle size of okra powder decreased, bulk density reduced from 0.4718 to

240   0.2941 g/mL (Table 3). The bulk density of okra powder (0.4718 g/mL) with the

241   particle size of 380-250 μm was the largest. Whereas, the tapped density of okra

242   powders increased from 0.6813 to 3.3787 g/mL with the decrease of particle size

243   (Table 3). The okra particle of the higher tapped density was beneficial to fill in

244   preparing tablets or capsule products.[29]

245   The powders showed different fluidity when tested for angle of repose as shown in

246   Table 3. From the Table 3, it is obvious that the smaller the powder particle was, the

247   smaller was the angle of repose. The angle of repose ranged from 56.35° (380-250 μm)

248   to 46.58° (<40 μm) (Table 3). These results confirmed the significant effect of the

249   particle size on the angle of repose. The okra powder with least particle size had the

250   lowest angle of repose (46.58°). On the contrary, okra powder with a particle size of

251   380-250 μm had the largest angle of repose (56.35°). With decreasing angle of repose,

252   one should expect a better fluidity of granular bulk.[30]

253   **Microphotographs of okra powder**

254   The morphology of fragmented okra pods granules could be seen clearly in the

255   scanning electron microscope photos (Fig.2). Mechanical damage exhibited

256   transformation from an ordered structure to a disordered structure via the breakage of

257   intermolecular bonds.[31] We observed granular mechanical damage in broken okra

258   pods granules. Extensive milling broke the coarse particles into finer fractions, with

259   the combination of flattening, aggregation and rupture resulted in various shapes of

260   okra pods particles, as could be seen in Fig. 2. The shape of the superfine pulverous

261   particle (40 and 75 μm) by the mill was not round. It was lamellar peeling in shape.

262   The okra fiber was totally broken by the strong mechanical pressure and abrasion

263   between rubbing rings and inner bottom of pot.

264   **The hydration properties and OBC**

265   As shown in Table.4, the WHC, WRC, SC and OBC increased as the size of okra

266   particles decreased. The WHC values of okra powder with particle sizes of 380–40

267   μm ranged from 3.55 to 8.24g/g respectively (Table.4). The hydration properties

268   increased with the decrease of particle size. This may be because properties of the fine

269   were altered, e.g., increased surface area and surface energy, with grinding. Moreover,

270   grinding the dry fibrous material to fine powder may leads to large changes in the

271   fiber matrix structure.[32] Similarly, the SC of powder was 1.22, 1.53, 1.24, and 1.27 g/g,

272   respectively. The higher SC of okra powders suggests their potential use as an

273   ingredient of instant foods.

274   **The effects on total flavonoid extract**

275   The superfine milling could cause significant differences in chemical composition

276   separation of the granulometric fractions.[33] The data on solubility of flavonoids of

277   okra in different size particles are presented in Fig.3. These data show that the

278   solubility of flavonoids increased as the particle size decreased. This suggested that

279   the smaller the size, higher the content of flavonoids. The flavonoids solubility of okra

280   pods powder with particle sizes of 380–40 μm were from 3.269% to 7.251%. The

281   main factor to affect the solubility of flavonoids was shown to be the particle size and

282   the surface area of the powder.

283   **Effects on cholesterol adsorption capacity of okra powder**

284   Fig.4 showed the effect of four factors (particle size, temperature, initial concentration

285   and absorption time) on cholesterol absorption capacity of okra powder. As can be

286   seen in Fig. 4A, the adsorption capacity increased with the decrease in grain-size. The

287   higher specific surface area of the fine particles leads to the larger contact area, which

288   shortened the path distance for absorbing. It is well-known that the temperature

289   usually has great influence on the adsorption because it affects the dispersion of the

290   powder, concentration of the cholesterol which lies on the functional groups of the

291   adsorbents in the adsorption process. Lower temperature was conducive to adsorption

292   powder (Fig. 4B). The initial concentration of cholesterol has been proved as a key

293   factor which can be illustrated in Fig. 4C, exhibiting a dose-dependence tendency. The

294   experimental results in Fig. 4D displayed that the adsorption increased with the

295   contact time from10 to 60 min reaching a plateau over the next of 60–90 min. It

296   should be pointed out that the adsorption of cholesterol is a fast process that can

297   remove more than 80% cholesterol from aqueous solution in the short time interval of

298   less than 15 min.

299   **Adsorption isotherms and thermotics analysis**

300   To examine the relationship between the sorption capacity (qe) and the concentration

301   of cholesterol at equilibrium (Ce), experimental data are fitted to Langmuir,

302   Freundlich and Dubinin-Radushkevish (D-R) isotherms models. These three models

303   are widely used for adsorption data analysis, since they have the ability to describe

304   experimental results for a wide range of initial concentrations. Langmuir[34] and

305   Freundlich[35] adsorption isotherms are also classical models for describing equilibrium

306   between adsorbate adsorbed onto the sorbent and adsorbate remaining in solution at

307   equilibrium at a constant temperature.

308   The theoretical Langmuir isotherm relies on the chemical or physical interaction (or

309   both) postulated to occur between the solute and the available vacant sites on the

310   sorbent surface, which may be described as below:

311    $$\frac{1}{q_e} = \frac{1}{Q_m K_L} \times \frac{1}{C_e} + \frac{1}{Q_m} \quad (9)$$

312   where Q_m is the maximum concentration of the sorbent (mg/g), Ce is the equilibrium

313   concentration (mg/mL), and K_L is a coefficient related to the affinity between the

314   adsorbate and the sorbent.

315   The Freundlich isotherm is an empirical equation that is based on the sorption of a

316   sorbate on a heterogeneous surface of a sorbent as given by the equation:[36]

317    $$\ln q_e = \ln K_f + \frac{1}{n} C_e \quad (10)$$

318   where K_f and n are the Freundlich empirical constants indicative of sorption capacity

319   and sorption intensity, respectively.

320   The Dubinin-Radushkevich (D-R) model, which does not assume a homogeneous

321   surface or a constant biosorption potential as the Langmuir model, was also used to

322   test the experimental data. The D-R isotherm can be written as: [37]

323    $$\ln q_e = \ln q_m - k\xi^2 \quad (11)$$

324   where k is a coefficient related to the mean free energy of adsorption (mol^2/kJ^2), qm is

325   the maximum adsorption capacity and ξ is the Polanyi potential (kJ/mol) that can be

326   written as

327    $\xi = RT \ln(1 + \frac{1}{c_e})$ (12)

328   The mean free energy of biosorption, E, can be estimated using the following

329   equation:

330    $E = -\frac{1}{\sqrt{2k}}$ (13)

331   Experimental data from curves given in Table 5 were used Eq. (9),(10) and (11),

332   allowing us to determine kinetic parameters. According to the regression coefficient

333   values R^2 in Table 5, it can be pointed out that the adsorption isotherms can be fairly

334   described by the Langmuir equation rather than the Freundlich one. And the parameter

335 |E| from the D-R isotherm model is 3.595< 8kJ/mol, which demonstrate the energy of

336   activation. It illustrate the physical adsorption might be a dominant mechanism

337   involved in adsorption by okra powder. [38,39]

338   The character of Langmuir isotherm can also be described by the balance coefficient

339   R_L,[40] which may be described as below:

340    $R_L = \frac{1}{1 + K_e C_0}$ (14)

341   where Ke and C0 are the Langmuir empirical constants indicative of sorption capacity

342   and the initial concentration of cholesterol, respectively.

343   Fig.5 present the change of balance coefficient R_L with the initial concentration of

344   cholesterol. As can be seen, the value of R_L is between 0 and 1, which shows the

345   adsorption property of okra powder is considered to be favorable.[41] In other words,

346   okra superfine powder is a suitable biosorbent .

347   The thermotics properties of adsorption is analyzed with Gibbs free energy *ΔG*,

348   adsorption enthalpy variation ΔH, adsorption entropy variation ΔS.

349   The absorption balance coefficient K_e, which be described as below:

350    $K_e = \frac{C_e}{Q_e}$ (15)

351   The ΔG can be estimated using the equation according to the classical Van't Hoff

352   equation:

353    $\Delta G = -RT \ln K_e$ (16)

354   The mutual relation among ΔG, ΔH and ΔS is showed as bellow: [42]

355    $\Delta H = \Delta G + \Delta S \times T$ (17)

356   Where R and T are ideal gas constant (8.314×10^{-3}kJ/(mol·K)) and adsorption

357   temperature (K).

358   Eq. (16) and (17) can derive to the following:

359    $\ln K_e = \frac{\Delta S}{R} - \frac{\Delta H}{RT}$ (18)

360   Fit the experiment data to the equation (18), and the values of ΔG, ΔH and ΔS

361   obtained are showed in Table. 6. Based on the results, the Gibbs free energy ΔG

362   increases with the temperature rise, which indicate that the lower temperature is

363   conducive to the sorption process. Besides, when the temperature is less than 323.15K,

364   the ΔG is less than zero, which means the adsorption is spontaneous.[43] The adsorption

365   is exothermic, which could be confirmed by the adsorption enthalpy variation ΔH

366   being less than zero.

367   **Conclusion**

368 In this study, the physicochemical properties and adsorption of cholesterol by four

369 sizes of okra powder obtained by micronization were investigated. The results showed

370 the particle size significantly influenced the properties of okra powder. With the size

371 decreasing, the specific surface area, bulk density, tapped density, repose angle,

372 hydration properties, oil-binding capacity performed better and the extraction of

373 flavonoid increased notably. What's more, the powder showed a better binding

374 capacity for cholesterol with the particle size diminishing. The adsorption equilibrium

375 data followed the Langmuir adsorption isotherm. The results indicated the promising

376 potential of the superfine okra powder to be a cholesterol sorbent or an alternative

377 source with additional benefits in function food. The effects of particle size on the

378 physical-chemical properties provide a theoretical base and reference for potential

379 using of okra in the food industry.

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449

Figure captions

451   **Fig.1** Flow diagram of the okra powder procedure

452   **Fig.2** SEM images of different particle size okra powders. (A) Particle size 380-250

453   μm, 50× ; (B) Particle size 250-75 μm, 50× ; (C) Particle size 75-40 μm, 50× ; (D)

454   Particle size <40 μm, 500× ; (E) Particle size 75-40 μm, 1,000× ; (F) Particle size

455   <40 μm, 2,500×.

456   **Fig.3** The change of the solubility percentage of flavonoids of different particle size

457   okra powders.

458   **Fig.4** The effect of particle size (A), temperature (B), initial concentration (C) and

459   absorption time (D) on cholesterol absorption capacity of okra powder

460   **Fig.5** The balance coefficient R_L for Langmuir isotherm

461

462   **Table 1** Physical characteristics of okra powder particles obtained from the laser
463   diffraction method

Powder (μm)	Equivalent diameter particles accounted for by measuring the proportion (μm)					Specific surface area (m²/g)
	D_{10}	D_{50}	D_{90}	D(4,3)	D(3,2)	
380-250	48.419	349.6	646	359.021	34.403	0.096
250-75	15.847	243.770	562.426	273.310	25.615	0.234
75-40	9.30	65.679	128.079	69.373	16.474	0.364
<40	3.61	18.690	49.273	23.110	7.285	0.824

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465

466 **Table 2** Color difference of okra powders

Okra pods particle (μm)	L	a	b
380-250	60.21±0.7891	6.58±0.7670	14.93±0.5749
250-75	64.9±1.1223	7.58±0.5908	18.05±0.8741
75-40	65.21±1.0729	8.25±0.8757	24.98±0.4588
<40	64.95±0.8821	8.93±1.7165	23.12±0.2557

467 The data are mean± standard deviation of triplicate samples.

468

469

470 **Table 3** Bulk density, tapped density and repose angle of different particle size okra
471 pods powders

Okra pods particle (μm)	Bulk density (g/mL)	Tapped density (g/mL)	Repose angle (°)
380-250	0.4718±0.06	0.6813±1.13	56.3582±2.76
250-75	0.3562±0.03	1.0374±0.44	53.9147±3.25
75-40	0.3042±0.03	1.6669±0.23	48.2771±1.36
<40	0.2941±0.01	3.3787±0.01	46.5808±1.30

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Table 4 Hydration properties and OBC of okra powders

Okra pods particle (μm)	WHC (g/g)	WRC (g/g)	Swelling capacity (mL/g)	OBC (g/g)
380-250	3.55±0.7423	0.56±0.0134	1.28±0.0047	1.22±0.0124
250-75	4.86±0.5671	0.59±0.0109	1.59±0.0339	1.23±0.1048
75-40	6.42±0.0114	0.71±0.0252	2.42±0.0206	1.24±0.0251
<40	8.24±0.0907	0.77±0.0271	2.79±0.0072	1.27±0.2126

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476

477   **Table 5** Fitted isothermal adsorption models and their parameters

Isotherm model	Parameters		equation	R^2
Langmuir isotherm	Q_m	0.891±0.0570	$1/q_e = 0.318/C_e + 1.122$	0.95
	K_e	3.528±0.479		
Freundlich isotherm	$1/n$	0.460±0.1039	$\ln Q_e = 0.46C_e - 0.854$	0.805
	K_f	0.426±0.1088		
D-R isotherm	q_m	1.295±0.06311	$\ln q_e = -0.0387\xi^2 - 0.2588$	0.969
	K	0.0387±0.0235		
	E	-3.595		

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479

480   **Table 6** Thermodynamic parameters for cholesterol adsorption

Temperature/K	ΔG/(kJ/mol)	ΔH/(kJ/mol)	ΔS/(J/(mol·K))
293.15	-6.236		
303.15	-2.984		
313.15	-0.614	-62.765	-195.454
323.15	0.328		
333.15	1.713		

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482

483   **Fig.1** Flow diagram of the okra powder procedure

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485
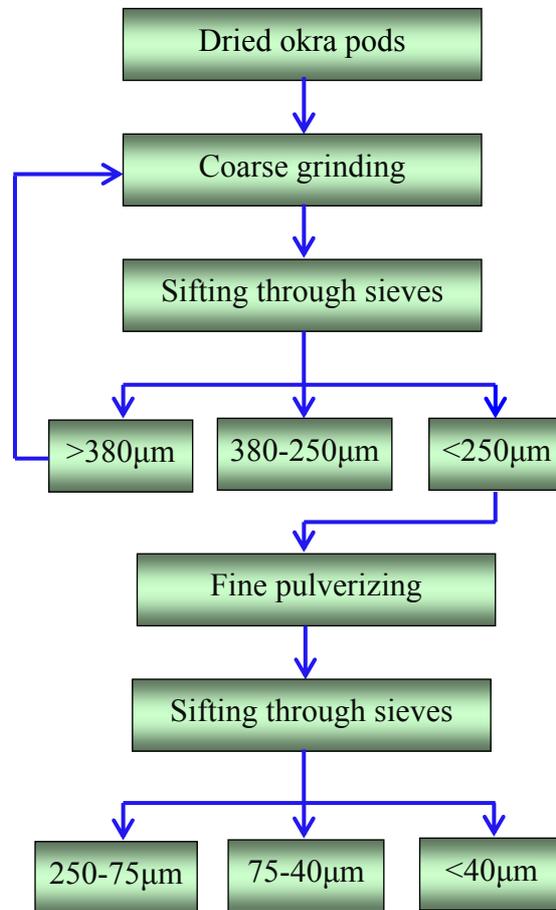
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Fig.2 SEM images of different particle size okra powders. (A) Particle size 380-250
μm, 50× ; (B) Particle size 250-75 μm, 50× ; (C) Particle size 75-40 μm, 50× ; (D)
Particle size <40 μm, 500× ; (E) Particle size 75-40 μm, 1,000× ; (F) Particle size <40
μm, 2,500×.

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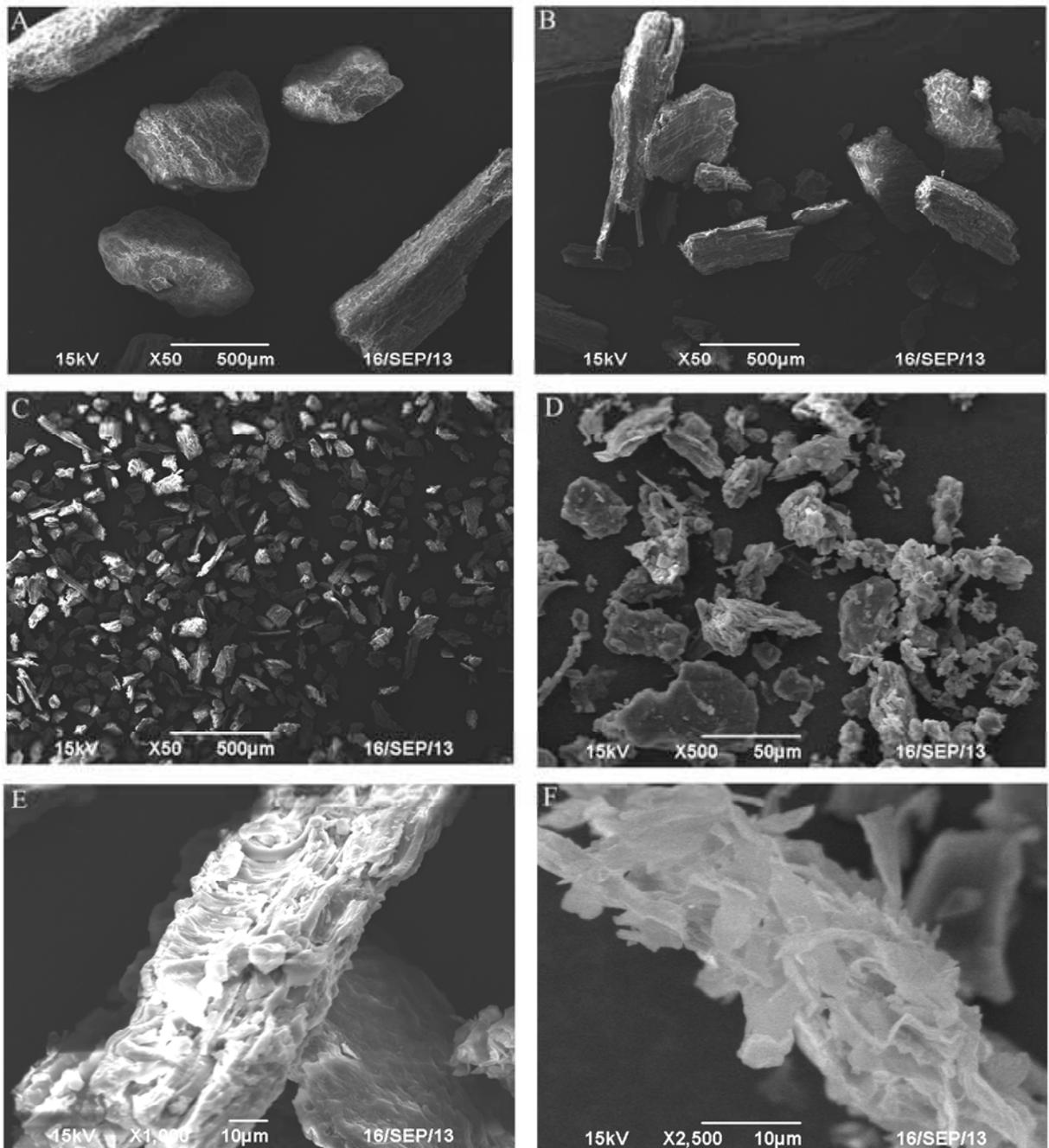
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495   **Fig.3** The change of the solubility percentage of flavonoids of different particle size
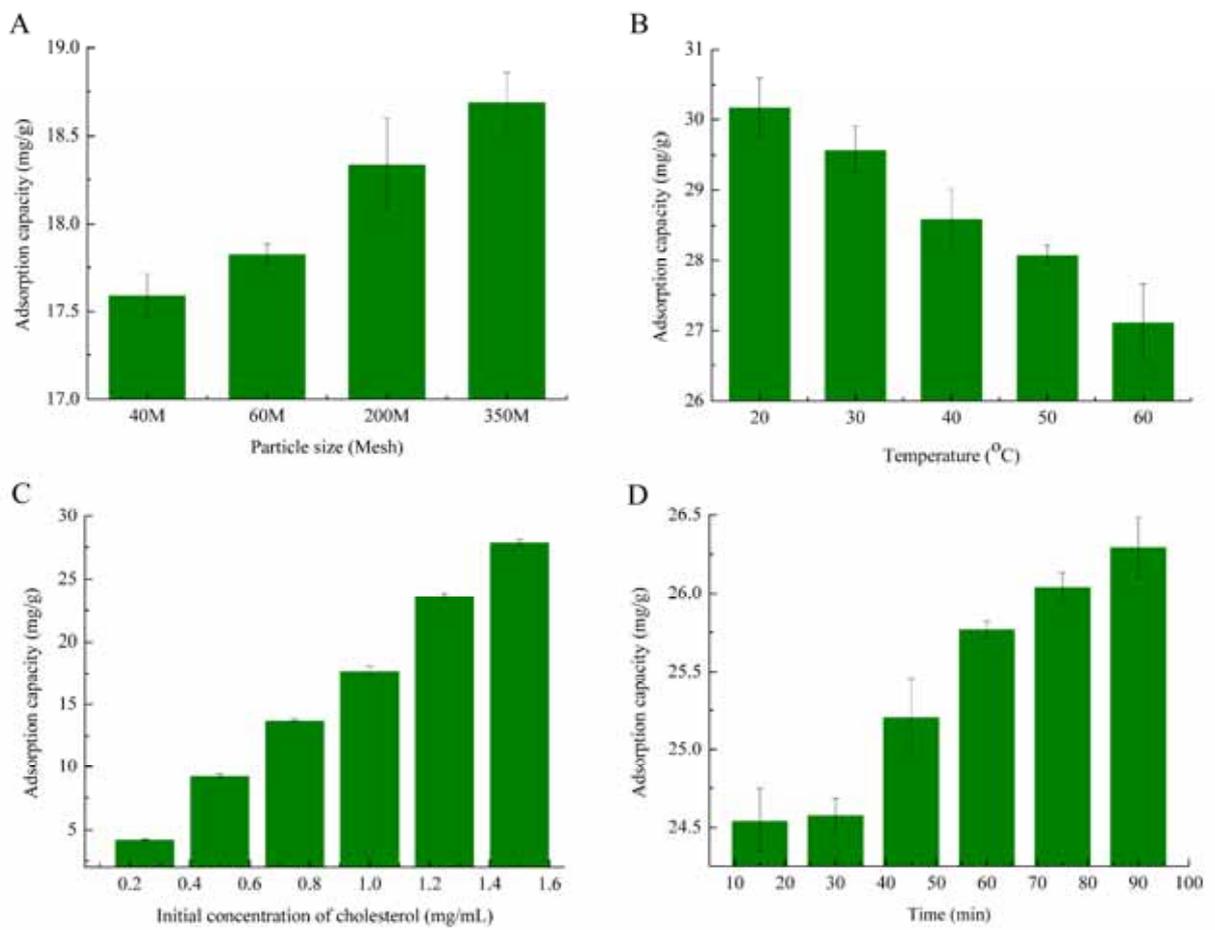496   okra powders.

497
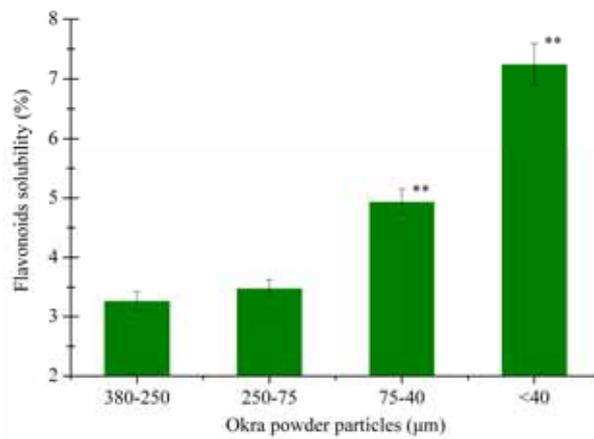
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500   Values are expressed as means ± SD (n = 3). Statistical analysis was performed using ANOVA. **
501   means significantly different from the value of 380-250μm. (p < 0.01).
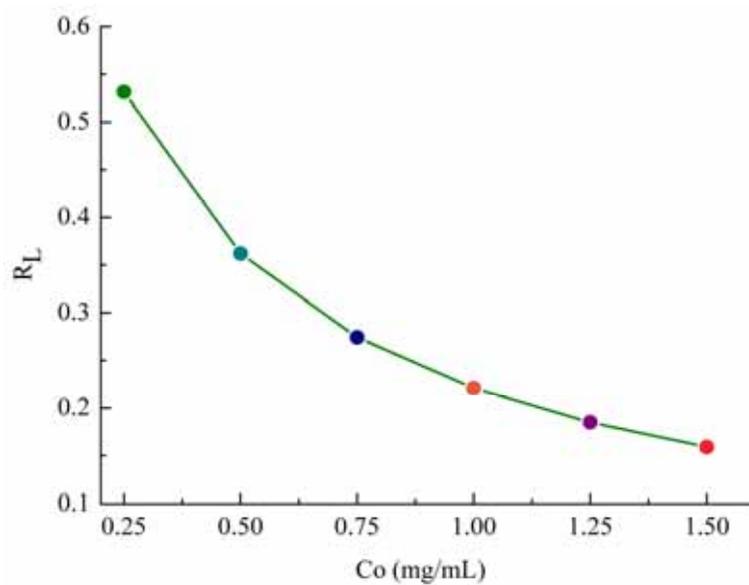
502   **Fig. 4** The effect of particle size(A), temperature(B), initial concentration(C) and

503   absorption time(D) on cholesterol absorption capacity of okra powder

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506

507 **Fig. 5** The balance coefficient R_L for Langmuir isotherm
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