

Functional Properties of Dried *Imbrasia oyemensis* Larvae Full-fat

and Defatted Flours

3

4 Abstract

5 *Imbrasia oyemensis* is a caterpillar widely consumed in Côte d'Ivoire. Functional
6 characteristics of its full-fat and defatted flours were investigated using standard
7 methods.

8 Defatting led to a significant ($P \leq 0.05$) reduction of emulsion capacity and stability,
9 whereas

10 the soluble protein fractions (such as albumin and glutelin), dispersibility, bulk
11 density, water

12 absorption capacity, water solubility index, oil absorption capacity, foam capacity and
13 stability increased significantly ($p < 0.05$). The full-fat and defatted flours had high oil
14 and

15 water absorption capacities as desirable characteristics for use in some foods such
16 as meats,

17 sausages, breads and cakes. Furthermore, they showed high bulk density and good
18 wettability,

19 and therefore would be suitable for use as a functional ingredient in a variety of food
20 formulations.

21 **Keywords:** Functional Properties, Defatted flour, Full-fat flour, *Imbrasia oyemensis*,
22 Larvae,
23 Insects.

24 1. Introduction

25 Increases in world population will require the production of vast amount of foods in
26 the latter

27 half of the twenty-first century. However it will be difficult to increase productivity to a
28 level

29 that satisfies food demand, mainly because of limited availability of new farm land.
30 This will

31 lead to shortages of food, especially animal protein. When total food resources are
32 insufficient, it is unwise to feed livestock with grain and other foodstuffs, which can
33 be

34 consumed directly by humans [1]. Therefore, it becomes necessary to look for new
35 sources of

36 animal protein such as insects, which are rich in nutrients. Most insects
37 are edible, although

38 there are some toxic species, and they can thrive on a diet that humans cannot
39 consume. Some

40 insects are even scavengers, such as saprophagous or coprophagous. The latter
41 can contribute

42 to recycling of animal waste [2].

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28. Insects represent a significant biological resource that is still not fully utilized around the

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29 world. Eating of insects (entomophagy) has a long history as part of human diets and a large

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30 number of insect species are consumed in many parts of the world. For approximately 2.5

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31 billion people, mainly in Africa, Asia and Latin America, eating insects is part of their 32 common diets in a similar way as eating meat or fish. More than 1600 species of insects are

33 eaten worldwide [1].

34 Insects constitute high quality food for humans and animals, and there is a great potential and

35 growing global interest for utilization of insects as food resource to complement the diets of

36 continuously growing populations. Indeed, they represent an important source of proteins (20

37 to 70% of dry weight), amino acids, fat, carbohydrates, various vitamins and others micro38

39 nutrients (minerals, trace elements). Therefore insects offer an important nutritional resource

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40 for humans and are worthy of development [3, 4, 5]. Recent reports in Nigeria confirm that

41 insects are indeed excellent sources of protein and other important nutrients[6].

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Aside from

42 high quality protein, the study also found important supplements like minerals and vitamins,

43 even in dried form. Protein content can range as high as 29.8 percent for *Analeptes trifasciata*

44 F., and good quality vitamins A, B2 and C are found in *Apis mellifera* L. In the Philippines,

45 the nutritive value of *camaru* (mole cricket, *Gryllotalpa* sp.) was analysed and results showed

46 that a 150 g serving of the dish provided 28 and 74 percent of the daily protein and energy

47 requirement, respectively, of average Filipinos between the ages of 19 and 49 [7].

48 In Cote d'Ivoire, caterpillars are also prized and consumed. *Imbrasia*

49 *oyemensis* is one of the

50 most widely eaten insects in the Western and Southern Côte d'Ivoire. This caterpillar, sold at

51 high prices on Abidjan markets, is consumed by an important population fringe of the country

52 in replacement of meat and fish [8]. The present paper focuses on the functional properties of

53 full-fat and defatted flours of dried *Imbrasia oyemensis* larvae. Indeed, functional properties

52 of foods are intrinsic physicochemical characteristics, which affect the behaviour of
protein in
53 food systems during processing, manufacturing, storage and preparation [9]. This
project was
54 undertaken to assess the functional properties the flour made from *Imbrasia*
oyemensis larvae.
55 Such functional properties could be used to determine the suitability or otherwise of
this
56 larvae as food additive.

57 2. Materials and methods

58 2.1. Collection and preparation of samples

59 Dried caterpillars *I. oyemensis* were obtained from the “Gouro” market of Adjamé
(Abidjan,
60 Côte d'Ivoire). After collection, they were sorted and free from any kind of waste.
Then, dried
61 caterpillars (2 kg) were ground using a porcelain mortar for get the full-fat flour. For
defatted
62 flour, cold extraction method was used. Full-fat flour was placed in a conical flask
and mixed
63 with hexane; the mixture was stirred by mechanical shaker for 16 hours and then
filtered. The
64 filtrate was washed again with hexane and filtered. The resulting filtrate was dried in
an open
65 air at room temperature. The dried flours were then stored at 0°C for further
analysis.

66 2.2. Protein Fractionation

67 Extraction and fractionation of coconut proteins were carried out according to the
modified
68 method of Hu and Essen [10]. Four different solvents deionized distilled water, 0.5 M
NaCl
69 (30 mL: 1 g), 70% Ethanol (w/v) and 0.1 M NaOH (25 mL: 1 g) were used in
sequence to
70 extract virtually all of the proteins in *Imbrasia oyemensis* flours. Full-fat or defatted
flour was
extracted by simply stirring, using a meal to solvent ratio of 1:10 (71 w/v), for 14-16 h at
4 °C.
72 Insoluble residue was removed by centrifugation at 6000 rpm for 30 min. Extraction
with
73 each solvent was repeated three times, and all supernatants for each solvent were
pooled to
74 obtain a representative of each solubility fraction. Each fraction then was dialyzed
against its
75 own solvent followed by deionized water. Dialysates were stored in a freezer at
about -20 °C.
76 Distilled water-, NaCl, Ethanol and NaOH soluble proteins were designated
“albumin”,

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77 “globulin”, “prolamine”, “glutelin”, respectively.

78 2.3. Protein Content

79 Each solubility fraction was assayed for protein content according to the method of Lowry et

80 al. [11] using bovine serum albumin (BSA) as the protein standard. In addition, nitrogen

81 content of unsoluble fraction (residue) was determined by micro-Kjeldahl method [12].

82 2.4. Dispersibility

83 The dispersibility of flours at selected protein concentration (2, 4, 6, 8 and 10 %) was

84 measured according to the method of Mora-Escobedo *et al.* [13]. One gram of the flour was

85 dispersed in distilled water in a 10 mL stoppered measuring cylinder. Then distilled water was

86 added to reach a volume of 30 mL, the mixture was stirred vigorously and allowed to settle

87 for three hours, the volume of settled particles was subtracted from 30 and multiplied by 100

88 and reported as percentage dispersibility.

89 2.5. Volume and bulk density measurement

90 The **volume** and bulk density were determined according to the modified method of Okezie

91 and Bello [14], by pouring 2 g of flour into a 10 mL measuring **cylinder**, and then holding the

92 **cylinder** on a vortex vibrator for 1 min to obtain a constant **volume** of the sample.

The **volume**

93 of the sample was recorded against the scale on the **cylinder**. The bulk density value was

94 calculated as the ratio of mass of the powder and the **volume** occupied in the **cylinder**.

95

2.6. Determination

96 of wettability

97 The method described by Onwuka [15] was adopted. One gram (1 g) of each flour sample was

98 measured into a 10 cm³ measuring **cylinder**. The **cylinder** was inverted at 10 cm above the

99 water contained in 600 mL beaker. The finger was used to close the **cylinder** disallowing the

100 flour sample from falling. By removing the finger and giving the **cylinder** a gentle tap, the

101 flour sample was discharged into the water surface. The time taken by the sample to get

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102 completely wet was recorded as the time of wettability.

103 **2.7. Water absorption capacity and water solubility index**

104 The water absorption capacity and solubility index of flours from *Imbrasia oyemensis* larvae

105 were evaluated according to Phillips *et al.* [16] and Anderson *et al.* [17] methods, respectively.

106 The full-fat and defatted flours from *Imbrasia oyemensis* larvae (2 g) were each weighed into

107 a centrifuge tube and 50 mL distilled water added. The content of the centrifuge tube was

108 shaken for 30 min in a KS 10 agitator. The mixture was kept in a water-bath (37°C) for 30

109 min and centrifuged (Ditton LAB centrifuge, UK) at 5000 rpm for 15 min. The resulting

110 sediment (M2) was weighed and then dried at 105°C to constant weight (M1). The WAC was

111 then calculated as follows:

120 **2.8. Determination of oil absorption capacity**

121 For the oil absorption capacity, the method of Beuchat [18] was used. One gram of each flour

122 sample was mixed with 10 mL of oil for 30 min in a mixer (Vari-whirl-mixing control set at

123 fast speed). The sample was then allowed to stand at room temperature for 30 min. It was then

124 centrifuged at 5000 rpm for 30 min, using a magnetic stirrer and the volume of the 125 supernatant noted in a 10 mL graduated cylinder. The density of the oil was determined too.

126 The volume of oil absorbed was multiplied by the density of the oil to determine the weight of

127 oil so absorbed.

128

129 Where:

130 V = Initial volume of oil used 1

131 V = Volume remaining (not absorbed) 2

132 P = density of the oil used

133 W = Weight of sample

134 **2.9. Foam capacity and foam stability:**

135 The foam capacity (FC) and stability (FS) of flours from *Imbrasia oyemensis* larvae were

136 studied according the method of Coffman and Garcia [19]. Three (3) g of each flour were

137 transferred into clean, dry and graduated (50 mL) cylinders. The flour samples were gently

138 leveled and the volumes noted. Distilled water (30 mL) was added to each sample; the

139 cylinder was swirled and allowed to stand for 120 min while the change in volume was

140 recorded every 15 min.

$(V_1 - V_2) \times P$

W

OAC (%) = $\frac{W}{V_1 - V_2} \times 100$

148

Where V_0 is the original volume of sample (mL), V_t is the total volume after 149 different times

150 (mL) and FC_0 is the foam capacity (FC) at 0 min.

151 **2.10. Determination of emulsion capacity and stability**

152 The method of Beuchat [18] was used. 2 g of each flour sample and 50 ml distilled water were

153 blended at room temperature for 30 sec in Philips blender at 1600 rpm. After complete

154 dispersion, vegetable oil (Gino) was added continuously in 10 mL portions from a burette.

155 Blending continued until the emulsion breakpoint (where a separation into two layers/phases)

156 was observed. The emulsion capacity was expressed as mL of oil emulsified per gram of

157 sample and was expressed as %:

158

159

160 Where:

161 W = weight of sample

$V_t - V_0$

V_0

$FC (\%) = \frac{FC_0}{V_t - V_0} \times 100$

FC

FC_0

$FS (\%) = \frac{V_E}{V} \times 100$

$V_E \times 100$

$V \times W$

EC (%) =

V_E = Volume 162 of emulsion layer E

163 V = Total volume of mixture

164 For the emulsion stability, the emulsion so prepared was then allowed to stand in a 250 mL

165 graduated cylinder over time and the volume of the emulsion layer read. The stability was

166 measured in terms of the amount of oil that was retained in the emulsion layer and given by:

167

168

169

170 V_{ET} = Emulsion volume at Time (T)

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171 V = total volume of the mixture

172

173 2.11. Determination of swelling index

174 The swelling capacity of the samples was determined using the method of Lin *et al.* [20], with

175 slight modification. One gram (1 g) of the flour sample was dispersed in 10 mL of cold

176 distilled water in a graduated centrifuge tube. The suspension was left at room temperature for

177 5 min to absorb water but not to swell. After 5 min the mixture was centrifuged at 2000 rpm

178 for 30 min and the volume of the sediment recorded as initial volume. Another 1 g of the

179 sample was dispersed in a centrifuge tube of known weight and the suspensions heated in

180 boiling water for 30 min. The suspension was cooled to room temperature under the tap water

181 and then centrifuged at 2000 rpm for 30 min- using a magnetic stirrer. The volume of the

182 heated sediment was recorded as final volume:

$V_{ETX} 100$

V

ES (%) =

Swelling index = Final Vol. after heating / 183 Initial Vol. before heating

184 2.12. Statistical analysis

185 All experiments in this study are reported as means of three replicate analyses.

One-way

186 analysis of variance (ANOVA) was carried out to compare the mean values of the two flours.

187 Differences in the mean values were determined using Duncan's multiple range tests (SAS,

188 1990).

189 3. Results and Discussion

190 3.1. Soluble and insoluble fractions

191 The functional properties of flours play important role in the manufacturing of products. In

192 present study the functional properties of full-fat and defatted *Imbrasia oyemensis* flours were

193 evaluated. Table 1 presents the Protein content of the soluble and insoluble fractions of the

194 caterpillar flours. As regards these results, glutelin was found to be the major component in

195 both full-fat (31.20 ± 1.47 %) and defatted (32.21 ± 0.59 %) flours. It is well known that

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196 glutelin is generally associated to elasticity and toughness properties in flours [21].

Albumin

197 was the second component with content of 11.96 ± 0.09 % in crude flour and 18.51 ± 0.24 %

198 in defatted flour. These results suggest that defatting of *Imbrasia oyemensis* flour significantly

199 increase its glutelin and albumin contents. The statistical analysis showed no significant

200 difference in globulin (11.96 ± 0.09 % in crude flour and 18.51 ± 0.24 % in defatted flour)

201 and prolamin (11.96 ± 0.09 % in crude flour and 18.51 ± 0.24 % in defatted flour) contents.

204

3.2. Nitrogen

205 solubility profile

206 The solubility of a protein is the thermodynamic manifestation of the equilibrium between

207 protein–protein and protein solvent interactions [22]. Solubility of a protein is one of the

208 critical functional attributes required for use as food ingredient, because it greatly influences

209 other properties such as emulsification, gelation and foaming [23]. Thus, it is an important

210 property governing the functional behavior of proteins and their potential application to food

211 processing. The pH effect on protein solubility is shown in Fig.1. It shows lower solubility in

212 alkaline media. Higher solubility values were obtained in acid media and the isoelectric points

213 (IEP) values are 4, and 7.6. At the isoelectric point, there is no net charge on the protein;

214 resulting in no repulsive interactions and the protein–protein interactions disfavoring

215 solubility [24]. The minimum solubility was found to be 0.11 ± 0.008 and 0.13 ± 0.02 mg/mL

216 (at pH 4) and 0.0 ± 0.0 and 0.03 ± 0.0 mg/mL (at pH 7.6) for full-fat and defatted flours,

217 respectively, indicating that the isoelectric points of the flour proteins were pHs 4.0 and 7.6.

218 On either sides of this pH, the solubility was increased up to maximum values at the extreme

219 levels of pH. Generally, the dependency of the solubility on pH has been attributed to the

220 change in the net charges carried by the flour protein as the pH changes [25]. The high net

Comment [SPCA5]: Look at the table values: 4,18 and 4,19

Comment [SPCA6]: Look at the table values: 4,44 and 4,16

Comment [SPCA7]: Compare with other studies

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221 charge acquired at both acid and alkaline pH's caused arise in solubility due to
unfolding of
222 the flour protein with the degree of unfolding being greater at acidic than the
alkaline pH [22].

223 The possession of 2 isoelectric points (~~IEP 4 and 7.6~~) suggests that *Imbrasia*
oyemensis has
224 more than one major protein constituent.
227

228 3.3. Dispersibility and wettability

229 The dispersibility of a mixture in water indicates its reconstitutability [26]. The better
230 temperature, ionic composition, pH and ~~degree of agitation~~ degree of the solvent
are major factors

231 affecting dispersibility[23]. The protein dispersibility index is a means of comparing
the
232 solubility of a protein in water, and this property is widely used in the flour and
powder

233 studies. The dispersibility of full-fat and defatted *I. oyemensis* flours is shown in

Table 2. The

234 results indicated that defatted flour had higher dispersibility than full-fat flour. Also,
the

235 dispersibility for both was higher at low concentration (2%) than high. ~~As shown in~~
Table 2

236 The dispersibility of full-fat flour decreased significantly ($P \leq 0.05$) with increasing
the level

Full fat flour Defatted flour

off. *oyemensis* protein concentration (2%, 4%, 6%, 8% and 10%). As regards
237 dispersibility of

238 defatted flour, it also decreased significantly ($P \leq 0.05$) but until concentration of
6%. These

239 results indicated that increasing of protein concentration wasn't favourable for a
better

240 dispersibility of *I. oyemensis* flours. On the over side, defatting increased
significantly this

241 dispersibility suggesting that fats might play a negative role on this functional
property. The

242 dispersibility of a mix in water indicates its reconstitutionability. The higher the
dispersibility,

243 the better the reconstitution property [26]. Higher dispersibility enhances the
emulsifying and

244 foaming properties of proteins, which was observed during making of bread,
macaroni and

245 cookies [27].

246 As regards wettability, it is an important property when protein powders are
dispersed to

Comment [SPCA9]: Extend the idea

Comment [SPCA10]: Compare with results
of other studies

Comment [SPCA11]: It is necessary to
reduce o split the Table 2, it is so big

247 produce aqueous beverages and batters [28]. Wettability of proteins is affected by
248 surface
249 polarity, topography, texture, area and by the size and microstructure of the protein
250 particles

249 [29]. The results presented in **Table 2**, shows that full-fat flour wet slowly ($50.33 \pm$
250 0.58 min)
251 while defatted sample wet very quickly (9.67 ± 0.58 min). This might be due to
252 presence or
253 absence of fat. So, defatting greatly influences the wettability.

252 3.4. Bulk density

253 Bulk density is a measure of heaviness of a flour sample. It is important for
254 determining
255 packaging requirements, material handling and application in wet processing in the
256 food

255 industry. The bulk density of full-fat and defatted *I. oyemensis* flour was found to be
256 $1.00 \pm$

256 0.0005 and 1.04 ± 0.02 g/mL (**Table 2**). It worth to note that the bulk density
257 reported in the
258 present study for the two samples is higher than the previously reported values for
259 *I. belina*

258 ($0.67\text{--}0.71$ g/mL) [30]. Higher bulk density is desirable for the greater ease of
259 dispersibility

259 and reduction of paste thickness which is an important factor in convalescent and
260 child

260 feeding [31]. Thus, the full-fat and defatted *I. oyemensis* flour could possibly be
261 used in food

261 formulation as supplement for child food. The high bulk density 261 of *I. oyemensis*
262 flours

262 indicates that they would serve as good thickeners in food products.

263 3.5. Water and oil absorption capacity

264 Hydration or rehydration is the first and perhaps most critical step in imparting
265 desirable

265 functional properties to proteins in a food system. Interactions of water and oil with
266 flours are

266 very important in food systems because of their effects on the flavour and texture
267 of foods.

267 Intrinsic factors affecting water binding properties of food flours with relatively high
268 protein

268 contents include amino acid composition, protein conformation and surface

269 polarity/hydrophobicity [32]. Water absorption capacity describes flour – water
270 association

270 ability under limited water supply. The results of water and oil absorption capacity,
271 foaming

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271 and emulsifying properties of the two samples are shown in **Table 2**. The defatted
272 *I.*

272 *oyemensis* flour (65.44 ± 5.81 to 86.89 ± 1.38 %) had higher water absorption
capacity

273 (WAC) than the full-fat flour (56.90 ± 1.39 to 66.07 ± 0.55 %). These values are
higher than

274 those reported for the Jackfruit seed flour (25 %) [33] and for African yam bean (17
%) [34].

275 The results obtained are however lower than those of *Cirina forda* (300.00 ± 0.15
%) reported

276 by Omotoso [35] and than those of mushroom flours reported by Aremu et al. [36].
This shows

277 that *I. oyemensis* is slowly hydrophilic. The difference in protein structure and the
presence of

278 different hydrophilic carbohydrates might be responsible for variation in the WAC of
the

279 flours. Flours with high WAC have more hydrophilic constituents such as
polysaccharides.

280 The highest oil absorption capacity was found in defatted flour at 2 % of sample
(84.08 ± 0.43

281 %) and the lowest in full-fat flour at 10 % of sample (56.71 ± 0.74 %). Oil
absorption capacity

282 is important since oil acts as flavour retainer and increases the palatability of foods
[23]. The

283 result obtained shows that *I. oyemensis* flours are high flavour retainer and may
therefore find

284 useful application in food systems such as ground meat formulations.

3.6. 285 Emulsifying properties

286 The emulsifying properties are usually attributed to the flexibility of solutes and
exposure of

287 hydrophobic domains. Food emulsions are thermodynamically unstable mixtures of
288 immiscible liquids. The formation and stability of emulsion is very important in food
systems

289 such as salad dressing. As shown in Table 2 the emulsion capacity was higher for
defatted

290 flour than full-fat flour at 2 % proteins concentration and, also for both, it was higher
at low

291 concentration (2%) than high. The emulsion capacity of full-fat as defatted flour
decreased

292 significantly ($P \leq 0.05$) with increasing the level of *I. oyemensis* proteins
concentration (2%,

293 4%, 6%, 8% and 10%). These values were higher than those reported for defatted
groundnut

294 cake flour (28.10 %) [37] and for roasted peanut [38]. The emulsifying activity
results were

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295 80.85 ± 0.98 and 86.46 ± 1.38 % respectively for full-fat and defatted flour,
whereas the
296 emulsion stability was 84.76 ± 0.34 and 87.21 ± 0.31 % for the two samples,
respectively.

297 The results obtained were much higher than those reported by Fekria et al. [37] for
defatted
298 groundnut cake flour and Omotoso [35] for *Cirina forda* larva protein. The capacity
of
299 proteins to enhance the formation and stabilization of emulsion is important for
many
300 applications in cakes, coffee whiteners, and frozen desserts. In these products
varying
301 emulsifying and stabilizing capacities are required because of different
compositions and
302 stresses to which these products are subjected [39].

303 **3.7. Foaming capacity and foam stability**

304 The foaming capacity (FC) of a protein refers to the amount of interfacial area that
can be
305 created by the protein and foam stability (FS) refers to the ability of protein to
stabilize
306 against gravitational and mechanical stresses [40]. Foam formation and foam
stability are a
307 function of the type of protein, pH, processing methods, viscosity and surface
tension. The
308 full-fat *I. oyemensis* flour showed no foam whereas the defatted flour had foaming
capacity of
 18.006 ± 2.37 % and foaming stability of 27.19 ± 0.98 %. These values 309 are greater
than some
310 insect flours as reported by some workers: *I. belina*, 11.8 ± 1.15 % (Ekpo et al.,
2008) and
311 *Rhyncophorus phoenicis*, 10 % [41]. Akubor and Chukwu [42] reported that foams
are used to
312 improve the texture, consistency and appearance of foods. Graham and Phillips
[43] linked
313 good foamability with flexible protein molecules, which reduces surface tension.
Low
314 foamability on the other hand can be related to highly ordered globular proteins,
which resists
315 surface denaturation. The basic requirements of proteins as good foaming agents
are the
316 ability to (i) adsorb rapidly at airwater interface during bubbling, (ii) undergo rapid
317 conformational change and rearrangement at the interface, and (iii) form a
cohesive
318 viscoelastic film via intermolecular interactions. The first two factors are essential
for better

Comment [SPCA18]: You can see this results in the table 2, it is not necessary to repeat it.

319 foamability whereas the third is important for the stability of the foam.
320

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344 **4. Conclusion**

345 The data obtained in this study showed that defatting have significant effect on
functional
346 properties of dried *I. oyemensis* flours. The high protein content of both cultivars
indicated
347 that they could be a valuable protein. Moreover, high solubility of both flours at acid
pH
348 makes them suitable in beverages. Furthermore, full-fat and defatted flours had
high oil and
349 water absorption capacities as desirable characteristics for use in some foods such
as meats,
350 sausages, breads and cakes. Both the full-fat and defatted flours showed high bulk
density and
351 would be suitable for use in weaning food. The good wettability of the two flours
makes them
352 suitable for use in textured meats and baked products.
353

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