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Protein Fractions and Functional Properties of Dried Imbrasia oyemensis Larvae Full-Fat and Defatted Flours

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EAD and PLK designed the study, wrote the protocol and supervised the work. Authors RAA, YDD and MDK carried out all laboratories work and performed the statistical analysis. Authors JPENK and RAA managed the analyses of the study. Author EAD wrote the first draft of the manuscript, managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To assess the functional properties of flours made from *Imbrasia oyemensis* larvae, a caterpillar widely consumed in Côte d'Ivoire, for industrial purposes. **Methodology:** Full-fat and defatted flours were obtained from dried *Imbrasia oyemensis* larvae collected on the "Gouro" market of Adjamé (Abidjan, Côte d'Ivoire). Protein fractionation, protein content and functional properties were investigated using standard methods. All results were statistically analysed.

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Results: Defatting led to a significant ($P \le 0.05$) reduction of emulsion capacity and stability, whereas the soluble protein fractions (such as albumin and glutelin), dispersibility, bulk density, water absorption capacity, water solubility index, oil absorption capacity, foam capacity and stability increased significantly (p < 0.05). The full-fat and defatted flours had high oil (78.12 and 84.08% respectively) and water absorption capacities (86.89% for full-fat and 66.07% for defatted flour) as desirable characteristics for use in some foods such as meats, sausages, breads and cakes. Fevermore, they showed high bulk density (1.00 and 1.04g/mL respectively for full-fat and defatted) and good wettability and therefore would be suitable for use as a functional ingredient in a variety of food formulations.

Conclusion: Defatting has significant effect on functional properties of dried *I. oyemensis* flours. Full-fat as well as defatted flour show good functional characteristics for use in many food industries.

Keywords: Functional properties; protein fractions; defatted flour; Full-fat flour; Imbrasia oyemensis; larvae; insects.

1. INTRODUCTION

Increases in world population will require the production of vast amount of foods in the latter half of the twenty-first century. However it will be difficult to increase productivity to a level that satisfies food demand, mainly because of limited availability of new farm land. This will lead to shortages of food, especially animal protein. When total food resources are insufficient, it is unwise to feed livestock with grain and other foodstuffs, which can be consumed directly by humans [1]. Therefore, it becomes necessary to look for new sources of animal protein such as insects, which are rich in nutrients. Most insects are edible, although there are some toxic species, and they can thrive on a diet that humans cannot consume. Some insects are even scavengers, such as saprophagers or coprophagers. The latter can contribute to recycling animal waste [2].

Insects represent a significant biological resource that is still not fully utilized around the world. Entomophagy (eating of insects) has a long history as part of human diets and a large number of insect species are consumed in many parts of the world. For approximately 2.5 billion people, mainly in Africa, Asia and Latin America, eating insects is part of their common diets in a similar way as eating meat or fish. More than 1600 species of insects are eaten worldwide [1].

Insects constitute high quality food for humans and animals, and there is a great potential and growing global interest for utilization of insects as food resource to complement the diets of continuously growing populations. Indeed, they represent an important source of proteins (20 to 70% of dry weight), amino acids, fat, carbohydrates, various vitamins and others micro-nutrients (minerals. trace elements). Therefore insects offer an important nutritional resource for humans and are worthy of development [3,4,5]. Recent reports in Nigeria confirm that insects are indeed excellent sources of protein and other important nutrients [6]. Aside from high quality protein, the study also found important supplements like minerals and vitamins, even in dried form. Protein content can range as high as 29.8 percent for Analeptes trifasciata F., and good quality vitamins A, B2 and C are found in Apis mellifera L. In the Philippines, the nutritive value of *camaru* (mole cricket, Gryllotalpa sp.) was analysed and results showed that a 150g serving of the dish provided 28 and 74 percent of the daily protein and energy requirement, respectively, of average Filipinos between the ages of 19 and 49 [7].

In Cote d'Ivoire, caterpillars are also prized and consumed. Imbrasia oyemensis is one of the most widely eaten insects in the Western and Southern Côte d'Ivoire. This caterpillar, sold at high prices on Abidjan markets, is consumed by an important population fringe of the country in replacement of meat and fish [8]. The present paper focuses on the functional properties of fullfat and defatted flours of dried Imbrasia ovemensis larvae. Indeed, functional properties physicochemical foods are intrinsic of characteristics, which affect the behaviour of protein in food systems during processing, manufacturing, storage and preparation [9]. This project was undertaken to assess the functional properties of the flour made from Imbrasia ovemensis larvae. Such functional properties could be used to determine the suitability or otherwise of this larvae as food additive.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Samples

Dried caterpillars /. *oyemensis* were obtained from the "Gouro" market of Adjamé (Abidjan, Côte d'Ivoire). After collection, they were sorted and free from any kind of waste. Then, dried caterpillars (2kg) were ground using a porcelain motar for get the full-fat flour. For defatted flour, cold extraction method was used. Full-fat flour was placed in a conical flask and mixed with hexane; the mixture was stirred by mechanical shaker for 16 hours and then filtered. The residue was washed again with hexane and filtered. The resulting residue was dried in an open air at 45 °C for 24 hours. The dried flours were then stored at 0 °C for further analysis.

2.2 Protein Fractionation

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

2.3 Protein Content

Each solubility fraction was assayed for protein content according to the method of Lowry et al. [11] using bovine serum albumin (BSA) as the protein standard. In addition, nitrogen content of unsoluble fraction (residue) was determined by micro-Kjeldahl method [12].

2.4 Dispersibility

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

according to the method of Mora-Escobedo et al. [13]. One gram of the flour was dispersed in distilled water in a 100mL stoppered measuring cylinder. Then distilled water was added to reach a volume of 30mL, the mixture was stirred vigorously and allowed to settle for three hours, the volume of settled particles was subtracted from 30 and multiplied by 100 and reported as percentage dispersibility.

2.5 Volume and Bulk Density Measurement

The volume and bulk density were determined according to the modified method of Okezie and Bello [14], by pouring 2g of flour into a 10mL measuring cylinder, and then holding the cylinder on a vortex vibrator for 1 min to obtain a constant volume of the sample. The volume of the sample was recorded against the scale on the cylinder. The bulk density value was calculated as the ratio of mass of the powder and the volume occupied in the cylinder.

2.6 Determination of Wettability

The method described by Onwuka [15] was adopted. One gram (1g) of each flour sample was measured into a 10cm³ measuring cylinder. The cylinder was inverted at 10cm above the water contained in 600 ml beaker. The finger was used to close the cylinder disallowing the flour sample from falling. By removing the finger and giving the cylinder a gentle tap, the flour sample was discharged into the water surface. The time taken by the sample to get completely wet was recorded as the time of wettability.

2.7 Water Absorption Capacity and Water Solubility Index

The water absorption capacity and solubility index of flours from *Imbrasia oyemensis* larvae were evaluated according to Phillips et al. [16] and Anderson et al. [17] methods, respectively. The full-fat and defatted flours from *Imbrasia oyemensis* larvae (2g) were each weighed into a centrifuge tube and 50mL distilled water added. The content of the centrifuge tube was shaken for 30 min in a KS 10 agitator. The mixture was kept in a water-bath (37 °C) for 30 min and centrifuged (Ditton LAB centrifuge, UK) at 5000 rpm for 15 min. The resulting sediment (M2) was weighed and then dried at 105 °C to constant weight (M1). The WAC was then calculated as follows:

WAC (%) =
$$\frac{M2 - M1}{M2}$$
 X 100

While the WSI was calculated using the following equation:

WSI (%) =
$$\frac{M0 - M1}{M0}$$
 X 100

M0 is the original weight of sample.

2.8 Determination of Oil Absorption Capacity

For the oil absorption capacity, the method of Beuchat [18] was used. One gram of each flour sample was mixed with 10mL of oil for 30 min in a mixer (Vari-whirl-mixing control set at fast speed). The sample was then allowed to stand at room temperature for 30 min. It was then centrifuged at 5000 rpm for 30 min, using a spinner (Ditton LAB centrifuge, UK) and the volume of the supernatant noted in a 10mL graduated cylinder. The density of the oil was determined too. The volume of oil absorbed was multiplied by the density of the oil to determine the weight of oil so absorbed.

OAC (%) =
$$\frac{(V1 - V2) \times P}{W} \times 100$$

Where:

V = Initial volume of oil used 1

V = Volume remaining (not absorbed) 2

P = density of the oil used

W = Weight of sample

2.9 Foam Capacity and Foam Stability

The foam capacity (FC) and stability (FS) of flours from *Imbrasia oyemensis* larvae were studied according the method of Coffman and Garcia [19]. Three (3) g of each flour were transferred into clean, dry and graduated (50mL) cylinders. The flour samples were gently levelled and the volumes noted. Distilled water (30 mL) was added to each sample; the cylinder was swirled and allowed to stand for 120 min while the change in volume was recorded every 15 min.

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

$$FS(\%) = \frac{FC}{FC_0} \times 100$$

Where V_0 is the original volume of sample (mL), Vt is the total volume after different times (mL) and FC₀ is the foam capacity (FC) at 0 min.

2.10 Determination of Emulsion Capacity and Stability

The method of Beuchat [18] was used. 2g of each flour sample and 50 ml distilled water were blended at room temperature for 30 sec in Philips blender at 1600 rpm. After complete dispersion, vegetable oil (Gino) was added continuously in 10mL portions from a burette. Blending continued until the emulsion breakpoint (where a separation into two layers/phases) was observed. The emulsion capacity was expressed asmL of oil emulsified per gram of sample and was expressed as %:

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

Where:

 $W = weight of sample \\ V_E = Volume of emulsion layer E \\ V = Total volume of mixture$

For the emulsion stability, the emulsion so prepared was then allowed to stand in a 250mL graduated cylinder over time and the volume of the emulsion layer read. The stability was measured in terms of the amount of oil that was retained in the emulsion layer and given by:

$$\mathrm{ES}(\%) = \frac{\mathrm{V}_{\mathrm{ET}} \ge 100}{\mathrm{V}}$$

 V_{ET} = Emulsion volume at Time (T) V = total volume of the mixture

2.11 Determination of Swelling Index

The swelling capacity of the samples was determined using the method of Lin et al. [20], with slight modification. One gram (1g) of the

flour sample was dispersed in 10mL of cold distilled water in a graduated centrifuge tube. The suspension was left at room temperature for 5 min to absorb water but not to swell. After 5 min the mixture was centrifuged at 2000 rpm for 30 min and the volume of the sediment recorded as initial volume. Another 1g of the sample was dispersed in a centrifuge tube of known weight and the suspensions heated in boiling water for 30 min. The suspension was cooled to room temperature under the tap water and then centrifuged at 2000 rpm for 30 min. using a magnetic stirrer. The volume of the heated sediment was recorded as final volume:

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

2.12 Statistical Analysis

All experiments in this study are reported as means of three replicate analyses. One-way analysis of variance (ANOVA) was carried out to compare the mean values of the two flours. Differences in the mean values were determined using Duncan's multiple range tests (SAS, 1990).

3. RESULTS AND DISCUSSION

3.1 Soluble and Insoluble Fractions

Table 1 presents the Protein content of the soluble and insoluble fractions of the caterpillar flours. As regards these results, glutelin was found to be the major component in both full-fat (31.20%) and defatted (32.21%) fours. It is well known that glutelin is generally associated to elasticity and toughness properties in flours [21]. Albumin was the second component with content of 11.96% in crude flour and 18.51% in defatted flour. These results suggest that defatting of Imbrasia oyemensis flour significantly increase its glutelin and albumin contents. The statistical analysis showed no significant difference in globulin (4.18% in full-fat flour and 4.19% in defatted flour) and prolamin (4.44% in full-fat fullfat flour and 4.16% in defatted flour) contents.

3.2 Functional Properties

The functional properties of flours play important role in the manufacturing of products. In present study the functional properties of full-fat and defatted *Imbrasia oyemensis* flours were evaluated.

3.2.1 Nitrogen solubility profile

The solubility of a protein is the thermodynamic manifestation of the equilibrium between proteinprotein and protein solvent interactions [22]. Solubility of a protein is one of the critical functional attributes required for use as food ingredient, because it greatly influences other properties such as emulsification, gelation and foaming [23]. Thus, it is an important property governing the functional behavior of proteins and their potential application to food processing. The pH effect on protein solubility is shown in Fig. 1. It reveals lower solubility in alkaline media. Higher solubility values were obtained in acid media and the isoelectric points (IEP) values are 4, and 7.6. At the isoelectric point, there is no net charge on the protein; resulting in no repulsive interactions and the protein-protein interactions disfavoring solubility [24]. The minimum solubility was found to be 0.11 and 0.13mg/mL (at pH 4) and 0.0 and 0.03mg/mL (at pH 7.6) for full-fat and defatted flours, respectively. On either sides of this pH, the solubility was increased up to maximum values at the extreme levels of pH. Generally, the dependency of the solubility on pH has been attributed to the change in the net charges carried by the flour protein as the pH changes [25]. The high net charge acquired at both acid and alkaline pH's caused arise in solubility due to unfolding of the flour protein with the degree of unfolding being greater at acidic than the alkaline pH [22]. The possession of 2 isoelectric points (IEP 4 and 7.6) suggests that Imbrasia oyemensis has more than one major protein constituent.

3.2.2 Dispersibility and wettability

The dispersibility of a mixture in water indicates its reconstitutability [26]. The better temperature, ionic composition, pH and agitation degree of the solvent are major factors affecting dispersibility [23]. This property is a means of comparing the solubility of a protein in water, and this property is widely used in the flour and powder studies. The dispersibility of full-fat and defatted I. ovemensis flours is shown in Table 2. The results indicated that defatted flour had higher dispersibility than full-fat flour. Also, for both, it was higher at low concentration (2%) than high. As shown in Table 2 the dispersibility of full-fat flour decreased significantly (P≤0.05) with increasing the level of I. oyemensis protein concentration (2%, 4%, 6%, 8% and 10%). As regards defatted flour, it also decreased

significantly (P≤0.05) but until concentration of 6%.

observed during making of bread, macaroni and cookies [27].

These results indicated that increasing of protein concentration wasn't favourable for a better dispersibility of *I. oyemensis* flours. On the over side, defatting increased significantly this dispersibility suggesting that fats might play a negative role on this functional property. The dispersibility of a mix in water indicates its reconstitutionability. The higher the dispersibility, the better the reconstitution property [26]. Higher dispersibility enhances the emulsifying and foaming properties of proteins, which was

As regards wettability, it is an important property. when protein powders are dispersed to produce aqueous beverages and batters [28]. Wettability of proteins is affected by surface polarity, topography, texture, area and by the size and microstructure of the protein particles [29]. The results presented in Table 2 shows that full-fat flour wet slowly (50.33min) while defatted sample wet very quickly (9.67min). This might be due to presence or absence of fat. So, defatting greatly influences the wettability.

Table 1. Protein content of the soluble and insoluble fractions Imbrasia oyemensis flours

Flour		Insoluble fraction (%)						
samples	Albumin	Globulin	Prolamin	Glutelin				
Full-fat	11.96±0.09 ^d	4.18±0.44 ^a	4.44±0.70 ^a	31.20±1.47 [†]	7.85±0.30 [°]			
Defatted	18.51±0.24 ^e	4.19±0.08 ^ª	4.16±0.14ª	32.21±0.59 ^g	6.77±0.30 ^b			
	Values given are the averages of at least three experiments \pm SE							



Fig. 1	. Effect of	pH on	protein	solubility of	of Imbr	asia	oyemensis	flours
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Table 2. Dispersibility and wettability of Imbrasia oyemensis flours

Jr			Dispersib	ility (%)		Wettabilit
nples	2%	4%	6%	8%	10%	(min)

Flour		Wettability				
samples	2%	4%	6%	8%	10%	(min)
Full-fat	88.73±2.63 [°]	80.90±2.32 ^ª	74.64±0.63 [†]	71.61±0.67 ^e	59.71±1.65 ^d	50.33±0.58 ^b
Defatted	94.61±2.26 ⁹	91.12±2.31 [°]	84.89±1.86 ^b	83.51±0.31 ^{ab}	81.63±0.33 ^a	9.67 ±0.58 ^a
	Value	an airran are the	average of at l	a a at three average	manta ICE	

Values given are the averages of at least three experiments ±SE

3.2.3 Bulk density

Bulk density is a measure of heaviness of a flour sample. It is important for determining packaging requirements, material handling and application in wet processing in the food industry [28]. The bulk density of full-fat and defatted I. ovemensis flour was found to be 1.00 and 1.04g/mL (Table 3). It worth to note that the bulk density reported in the present study for the two samples is higher than the previously reported values for I. belina (0.67-0.71g/mL) [30]. Higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness which is an important factor in convalescent and child feeding [31]. Thus, the full-fat and defatted I. oyemensis flour could possibly be used in food formulation as supplement for child food. The high bulk density of *I. ovemensis* flours indicates that they would serve as good thickeners in food products.

Table 3. Bulk density of Imbrasia oyemensis flours

Flour samples	Bulk density (g/mL)				
Full-fat	1.00±0.0005ª				
Defatted	1.04±0.02 ^b				
Values given are the averages of at least three					
experiments ±SE					

3.2.4 Water and oil absorption capacity

Hydration or rehydration is the first and perhaps most critical step in imparting desirable functional properties to proteins in a food system. Interactions of water and oil with flours are very important in food transformation because of their effects on the flavour and texture of foods. Intrinsic factors affecting water binding properties of food flours with relatively high protein contents include amino acid composition. protein conformation and surface polarity/hydrophobicity [32]. Water absorption capacity describes flour water association ability under limited water supply. The results of water and oil absorption capacity of the two samples are shown in Table 4. The defatted I. ovemensis flour (65.44 to 86.89%) had higher water absorption capacity (WAC) than the full-fat flour (56.90 to 66.07%). These values are higher than those reported for the Jackfruit seed flour (25%) [33] and for African yam bean (17%) [34]. The results obtained are however lower than those of Cirina forda (300.00%) reported by Omotoso [35] and than those of mushroom flours reported by Aremu et al. [36]. This shows that I. ovemensis is slowly hydrophilic. The difference in protein structure and the presence of different hydrophilic carbohydrates might be responsible for variation in the WAC of the flours. Indeed, flours with high WAC have more hydrophilic constituents such as polysaccharides [36].

The highest oil absorption capacity (OAC) was found in defatted flour at 2% of sample (84.08%) and the lowest in full-fat flour at 10% of sample (56.71%). Oil absorption capacity is important since oil acts as flavour retainer and increases the palatability of foods [23]. The result obtained shows that *I. oyemensis* flours are high flavour retainer and may therefore find useful application in food systems such as ground meat formulations.

3.2.5 Emulsifying properties

The emulsifying properties are usually attributed to the flexibility of solutes and exposure of hydrophobic domains. Food emulsions are thermodynamically unstable mixtures of immiscible liquids. The formation and stability of emulsion is very important in food systems such as salad dressing [37,38]. As shown in Table 5 the emulsion capacity was higher for defatted flour than full-fat flour at 2% proteins concentration and, also for both, it was higher at low concentration (2%) than high. The emulsion capacity of full-fat as defatted flour decreased significantly (P≤0.05) with increasing the level of I. oyemensis proteins concentration. These values were higher than those reported for defatted groundnut cake flour (28.10%) [37] and for roasted peanut [38]. The emulsifying activity results were 80.85 and 86.46% respectively for full-fat and defatted flour, whereas the emulsion stability was 84.76 and 87.21% for the two samples, respectively. The results obtained were much higher than those reported by Fekria et al. [37] for defatted groundnut cake flour and Omotoso [35] for Cirina forda larva protein. The capacity of proteins to enhance the formation and stabilization of emulsion is important for many applications in cakes, coffee whiteners, and frozen desserts. In these products varying emulsifying and stabilizing capacities are required because of different compositions and stresses to which these products are subjected [39].

3.2.6 Foaming capacity and foam stability

The foaming capacity (FC) of a protein refers to the amount of interfacial area that can be created by the protein and foam stability (FS) refers to the ability of protein to stabilize against gravitational and mechanical stresses [40].

Table 4. Water and oil absorption capacity of Imbrasia oyemensis flours

samples	8% 10%
Full-fat	60.23±1.47 ^d 56.71± 0.74 ^c
Defatted	70.26±0.44 ^a 62.68±0.57 ^e
Defatted	60.23± 70.26±

Values given are the averages of at least three experiments ±SE

Table 5. Emulsifying properties of Imbrasia oyemensis flours

Flour	Emulsion capacity (EC) (%)					Emulsion stability(ES) (%)				
samples	2%	4%	6%	8%	10%	2%	4%	6%	8%	10%
Full-fat	80,85±0,98 ^g	46,66±1,20 [†]	25,90±0,60 ^b	21,65±0,58 ^ª	16,84±0,23 ^d	84,76±0,34 ^a	91,22±0,73 ^h	79,72±0,25 [†]	88,14±0,35 [°]	84,58±0,61 ^ª
Defatted	86,46±1,38 ^h	43,80±1,63 ^e	24,43±0,72 ^b	21,61±0,64 ^a	14,93±0,11 [°]	87,21±0,31 ^{bc}	86,40±1,02 ^b	71,70±1,42 ^d	82,78±0,93 ^g	74,22±0,63 ^e

Values given are the averages of at least three experiments ±SE

Foam formation and foam stability are a function of the type of protein, pH, processing methods, viscosity and surface tension. The full-fat *I. oyemensis* flour showed no foam whereas the defatted flour had foaming capacity of 18.006% and foaming stability of 27.19% (Table 6). These values are greater than some insect flours as reported by some workers: *I. belina*, 11.8% [30] and *Rhyncophorus phoenicis*, 10% [41]. Akubor and Chukwu [42] reported that foams are used to improve the texture, consistency and appearance of foods. Graham and Phillips [43] linked good foamability with flexible protein molecules, which reduces surface tension.

Low foamability on the other hand can be related to highly ordered globular proteins, which resists surface denaturation. The basic requirements of proteins as good foaming agents are the ability to (i) Adsorb rapidly at air water interface during bubbling, (ii) Undergo rapid conformational change and rearrangement at the interface, and (iii) Form a cohesive viscoelastic film via intermolecular interactions. The first two factors are essential for better foamability whereas the third is important for the stability of the foam [40].

Table 6. Foaming properties of *Imbrasia* ovemensis flours

Flour samples	Foaming capacity (FC) (%)	Foaming stability (FS) (%)					
Full-fat	0.00 ^a	0.00 ^a					
Defatted	18.006±2.37 ^b	27.19±0.98 [▶]					
Values given are the averages of at least three							
	experiments ±SE						

4. CONCLUSION

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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