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Impact of traditional processing on proximate composition, folate, mineral, phytate, and alpha-galacto-oligosaccharide contents of two West African cowpea (*Vigna unguiculata L. Walp*) based doughnuts



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ABSTRACT

Doughnuts made from cowpea, a highly nutritious pulse, are frequently consumed in West Africa. As processing may affect their nutritional composition, cowpea processing into two doughnut types (ata and ata-doco) was characterized, and samples collected from 12 producers in Cotonou, Benin. Proximate composition, folate, mineral, phytate, and alpha-galacto-oligosaccharide contents were determined in the raw material, intermediate products, and doughnuts. Mass balance was assessed during ata production to monitor folate and alpha-galacto-oligosaccharides distribution, and to determine what steps most influenced their concentration. Ata was prepared with dehulled-soaked seeds, and ata-doco with whole or partially dehulled, non-soaked and dry-milled seeds. After both types of doughnuts production, lipid content increased by 11–33 times compared with raw seeds, due to oil absorption during deep-frying. Milling led to an increase of iron content by 50–57% (ata) and 21–75% (ata-doco production). Alpha-galacto-oligosaccharide contents decreased by 22–57% after whipping during ata-doco, but not during ata production. The mass balance assessment showed significant reductions of folate (-50%) and alpha-galacto-oligosaccharides (-33%) after dehulled seed washing and soaking during ata production. This study showed that the impact of traditional processing on the nutritional value of cowpea-based doughnuts is strong, but highly variable depending on the doughnut type and producers' practices.

1. Introduction

Encouraging the production and consumption of pulses worldwide is important because of their numerous beneficial characteristics in terms of environmental impact and nutritional value. Indeed, pulses are an important source of plant proteins in low and middle income countries, where access to animal proteins is often limited (FAO, 2016). They also have high contents of fibre, and of some important minerals and vitamins. Cowpea (*Vigna unguiculata L. Walp*) also called black-eyed pea, black-eyed bean, or field peas (Unal et al., 2006), is the most produced and consumed pulse in West Africa. It is well adapted to high temperature and drought. Thus, this crop represents a good option to face climate changes (Carvalho et al., 2017) and to improve food security in low and middle income countries. In West African countries, cowpea is

consumed alone, or in combination with cereals (Akinyele and Akinlosotu, 1987). As pulses have an amino acid pattern complementary to that of cereal grains, such pulse-cereal association leads to a protein mix of good nutritional quality (Jayathilake et al., 2018). Cowpea seeds are also a good source of health-promoting components (Awika and Duodu, 2017), such as phenolic acids and flavonoids. Moreover, they contain soluble and insoluble fibre, and many other functional compounds, including B group vitamins, like folate (vitamin B9) and thiamine (vitamin B1) (Gonçalves et al., 2016). Cowpea seeds are also a good source of minerals (potassium, phosphorus, calcium, sulphur, magnesium, iron, zinc, manganese, and copper) (Harmankaya et al., 2016). However, they contain some chelating components (e.g. phytate or polyphenols) that reduce the mineral bioavailability (Rogério et al., 2014). Another major constraint is the presence of

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alpha-galacto-oligosaccharides in the seeds and in most cowpea-based dishes. These indigestible oligosaccharides cause abdominal discomfort and flatulence (Ndubuaku et al., 1989). Winham and Hutchins (2011) reported that many people avoid consuming some pulses due to the intestinal gas they generate.

Cowpea is widely consumed in Benin, a country located in West Africa, in various dishes prepared following combinations of unit operations that can affect their nutritional value. Madode et al. (2011) identified 18 different cowpea traditional dishes in Benin among which 10 (abla, abobo, adjagbé, adowè, ata, ataclè, atassi, ata-doco, magni-magni, djongoli) are popular in the southern cities of the country. Most dishes are prepared from seeds, but cowpea leaves are sometimes used for the preparation of sauces. Among the seed-based dishes, in Benin and in most other West-African countries, the cowpea-based doughnuts known as ata (with a light-brown colour crust) are widely consumed for breakfast or as a snack at any time of the day, in combination with cereal porridges, fried yam, sweet potato fries, akassa or lio (i.e. cooked fermented maize dough) (Madode et al., 2011). Ata (Benin), akara (Nigeria), koose (Ghana) (Madode et al., 2011) and even acarajé (Brazil) (Rogério et al., 2014) refer to the same product: a doughnut prepared by deep-frying in oil from a batter made from dehulled and soaked cowpea seeds. This dish is prepared daily at home and by street food vendors in urban and rural areas (Henshaw et al., 2000). In Benin, ata-doco is another type of cowpea doughnut that differs from ata by the dark-brown colour of the crust due to partial or no dehulling. The unit operations during the preparation of these doughnuts can reduce the micronutrient contents due to fractionation, diffusion, thermal degradation during frying, or oxidation reactions. This reduction could compromise the nutritional interest of cowpea seeds, when eaten under the form of doughnuts.

Previous studies have described the production of ata and ata-doco doughnuts-or similar products-and analysed their proximate composition, mineral content and phytate content (Madode et al., 2011; Rogério et al., 2014; Feitosa et al., 2015). However, they reported only the nutritional composition of the final products-as consumed-and did not focus on the effect of the intermediary processing steps. Moreover, to our knowledge, no study has monitored the changes in folate and alpha-galacto-oligosaccharide contents during ata and ata-doco production, although cowpea seeds are rich in this vitamin and this anti-nutritional factor (Gonçalves et al., 2016).

The first objective of the present work was to characterize the traditional processing of cowpea into ata and ata-doco, as carried out in small production units in Benin and its effects on the proximate composition, micronutrient (minerals, vitamin B9) and anti-nutritional factor contents (phytate and alpha-galacto-oligosaccharide) in the raw material, intermediate and final products, depending on the used processing variants. The second objective was to identify the processing steps that are responsible for the loss of folate and alpha-galacto-oligosaccharides during ata production. Therefore, a mass balance assessment was performed in small production units to monitor the folate and alpha-galacto-oligosaccharides distribution in the different fractions generated at each step.

2. Materials and methods

2.1. Materials

2.1.1. Collection and pre-treatment of cowpea seeds

The characterization of the traditional processing of cowpea (*Vigna unguiculata* L. Walp) into ata and ata-doco was performed, using a commonly consumed local cowpea cultivar named Atchawé-Tola (white seeds), grown in Azowlissé (a rural town in Ouémé department, Benin) in 2017, and provided by a farmer under the control of the International Institute of Tropical Agriculture (IITA, Benin). The assessment of the folate and alpha-galacto-oligosaccharide losses at each unit operation of cowpea processing for ata production (mass balance assessment) was

carried using the same cowpea cultivar grown in 2019 and purchased from a local farmer in the same department. Before use, cowpea seeds were cleaned by removal of damaged grains, insects and stones, placed at -20 °C for one week to destroy insect eggs, and then stored in a dry place at room temperature until processing.

2.1.2. Traditional processing into ata or ata-doco and sampling

All the producers used the same batch of raw material: cowpea seeds (1 kg) were provided to 12 cowpea-based doughnut's producers randomly selected in different areas of Cotonou (Benin) for processing into ata (n = 6) and ata-doco (n = 6), according to their own traditional procedures.

For laboratory analyses, 33 intermediates and 14 final products were sampled: *cleaned and dehulled seeds, soaked grits, whipped dough and ata doughnuts* (during ata production); *flour, whipped dough and ata-doco doughnuts* (during ata-doco production) (Fig. 1). Duration of soaking, whipping and frying as well as frying temperatures were recorded during the preparation of both doughnuts.

The collected samples were immediately placed in an icebox for transportation to the laboratory where they were stored at -20 °C.

2.1.3. Sample preparation for composition analysis

● Freeze-drying and milling

Fifty grams of each frozen sample were freeze-dried and milled using a laboratory mill IKA M20 (IKA Labortechnik, Staufen, Germany). All freeze-dried and milled samples were then stored at 4 °C until analysis (proximate composition, mineral, alpha-galacto-oligosaccharide and phytate contents).

● Cryo-milling

Twenty grams of each frozen sample were milled under liquid nitrogen using a laboratory mill IKA M20 (IKA Labortechnik, Staufen, Germany) to obtain an homogeneous powder immediately placed into an opaque bag to avoid humidification through ambient air, and stored at -20 °C. Cryo-milled samples were used for folate analysis.

2.1.4. Reagents, enzymes, reference materials and standards

The test kit used for the analysis of total dietary fibre (K-TDFR kits), as well as verbascose (purity > 95 %), were purchased from Megazyme (Bray Co. Wicklow, Ireland). The following chemicals were purchased from Carlo Erba (Val de Reuil, France): petroleum ether, ethanol, hydrogen peroxide, and nitric acid. Hydrochloric acid (37 %) was purchased from Fluka (Honeywell Fluka, Muskegon, USA). The following chemicals were purchased from Sigma-Aldrich (St Louis, USA): α-amylase from Aspergillus oryzae (A9857), protease from Streptomyces griseus (P5147), sodium ascorbate (A4034), galactose (G-0750), glucose (G-7528), fructose (F-2543), sucrose (S-7903), raffinose pentahydrate (206679), and Sodium hydroxide. The conjugase from chicken pancreas (P2002) was purchased from R-Biopharm (Berlin, Germany), and Folic Acid Casei Medium was purchase form Difco (Sparks, MD, USA). Reference materials were purchased from IRMM (Geel, Belgium): White cabbage (BCR-679) and Wholemeal flour (BCR-121).

2.2. Methods

2.2.1. Composition analysis

● Proximate composition and energy determination

Total dry matter (DM), crude protein, lipid and ash contents were determined using standard methods (AOAC, 1995). Soluble and insoluble dietary fibre contents were determined with the Megazyme K-TDFR kit, as described by Njoumi et al. (2019). Before analysis, all frozen

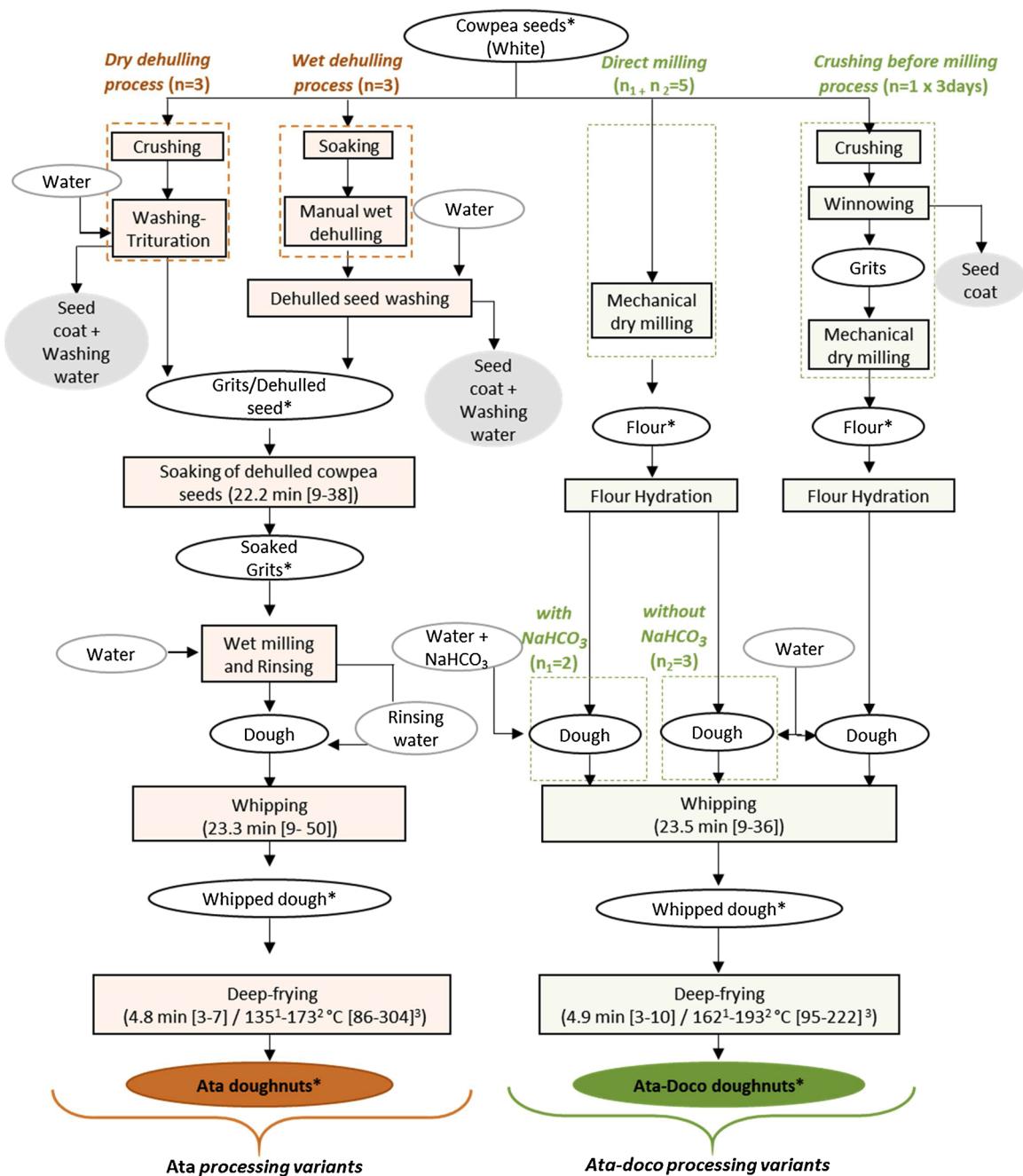


Fig. 1. Diagram showing the different steps of cowpea seed processing into Ata and Ata-doco. ¹: Mean initial temperature (T₀); ²: Mean final temperature (T_F); ³: [Min value T₀ - Max value T_F]; * indicates sampling points; n: number of producers; circle with grey outline: inputs; grey circle: by-products; circle with black outline: raw material and intermediate products; rectangular frame: different processing steps (light orange for Ata and light green for Ata-doco) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

doughnuts were lyophilized and cut into small pieces. Each sample was reduced into powder in petroleum ether using an ultra-turrax IKA T10 (IKA Labortechnik, Staufen, Germany), then filtered, and dried overnight at ambient temperature to obtain a defatted powder. Powdered samples of Ata and Ata-doco doughnuts were pooled based on the processing variant used by the producers (Fig. 1), yielding 5 pooled samples: 2 for Ata processing and 3 for Ata doco processing. After that, samples were successively digested with α -amylase, protease and amyloglucosidase, and then filtered. After filtration, extracts were precipitated with 95 % ethanol warmed at 70 °C (extract/ethanol; 1/4; v/v) for soluble fibre determination, and the filtration residue was used for insoluble dietary fibre quantification. Available carbohydrates (AC) were calculated by difference: AC (DM) = (100 g - (protein content +

lipid content + total dietary fibre + ash content)). Energy was determined using the Atwater coefficients: 4 kcal.g⁻¹ for proteins and available carbohydrates, 9 kcal.g⁻¹ for lipids, and 2 kcal.g⁻¹ for dietary fibres (FAO, 2003).

● Minerals

Total iron, zinc, calcium, and magnesium were determined in cowpea seeds, intermediate products and doughnuts, as described by Njoumi et al. (2018). Briefly, after wet mineralization of the freeze-dried samples with a mixture of hydrogen peroxide/nitric acid (1/7; v/v), using an Ethos 1 microwave digestion system (Milestone, Sorisole, Italy), minerals were determined by optical emission spectrometry using

an inductively coupled plasma - optical emission spectrometer ICP-OES 5100 apparatus (Agilent Technologies, Les Ulis, France). White cabbage (BCR-679) was used as reference material, with certified mineral contents of 5.5 ± 0.3 mg iron .100 g⁻¹ DM, 8.0 ± 0.3 mg zinc .100 g⁻¹ DM, 777 ± 65 mg calcium .100 g⁻¹ DM, and 136 ± 13 mg magnesium .100 g⁻¹ DM. The average mineral contents obtained during this study were 5.7 ± 0.4 mg iron .100 g⁻¹ DM, 7.0 ± 2.7 mg zinc .100 g⁻¹ DM, 798 ± 49 mg calcium .100 g⁻¹ DM, and 128 ± 24 mg magnesium .100 g⁻¹ DM (on n = 10 analyses).

● Folate (B9 vitamin) quantification

Total folate was determined by enzymatic extraction and microbiological assay, using the method described by [Kariluoto and Piironen \(2009\)](#) and adapted by [Bationo et al. \(2020\)](#). Briefly, 0.5 g of each sample was digested with α -amylase (20 mg.mL⁻¹ in 1% sodium ascorbate) and conjugase (100 mg.mL⁻¹ in ultrapure water) at 37 °C for 3 h. After this first enzymatic treatment, protease (3 mg.mL⁻¹ in 1% sodium ascorbate) was added and the mixture was incubated at 37 °C for 1 h. Sample extracts were diluted and pipetted into a 96-well microtiter plate (Greiner Bio-One, Frickenhausen, Germany). Folic Acid Casei Medium inoculated with *Lactobacillus rhamnosus* ATCC 7469 was added to all wells and incubated at 37 °C for 18 h. Wholemeal flour (BCR-121) was used as reference material to check the accuracy of the total folate quantification method using the same analytical procedure. After incubation, folate was quantified using a microplate reader (Tecan, Infinite M200 PRO, Lyon, France) set at 590 nm to measure the turbidity due to bacterial growth.

The certified total folate content for the reference material BCR-121 (whole wheat flour) was 50 ± 7 µg.100 g⁻¹ DM. The average total folate content obtained during this study was 47 ± 6 µg.100 g⁻¹ DM (average value for n = 49 analyses).

● Phytic acid

Phytic acid (IP6) content was determined by high-performance anion-exchange chromatography (Dionex, Sunnyvale, CA, USA) after sample extraction in 0.5 M HCl (0.1/5 ; w/v) at 100 °C for 6 min, according to the method described by [Lestienne et al. \(2005\)](#).

● Alpha-galacto-oligosaccharides

Alpha-galacto-oligosaccharides (raffinose, stachyose and verbascose) and their potential degradation products (saccharose, galactose, fructose, glucose) were extracted as described by [Njoumi et al. \(2019\)](#) with some modifications. For samples used to assess the effect of processing variants, 3 mL of ethanol (78%; v/v) was added to 80 mg of each lyophilized sample for extraction in two rounds at 80 °C for 20 min. Supernatants were dehydrated in a vacuum centrifuge concentrator RC 10 (Thermo Fisher Scientific, Asheville, NC, USA), and the dry extracts were dissolved in 2 mL of pure water, before injection.

The samples collected for the mass balance assessment were not lyophilized, and 80–700 mg were used, depending on the dry matter content of each sample (higher quantity was required for samples with low dry matter content). Alpha-galacto-oligosaccharides extraction followed the same procedure, but samples of soaking water were injected directly after filtration through cellulose acetate filters (0.2 µm).

Alpha-galacto-oligosaccharides separation and quantification were carried out by high performance anion exchange chromatography with pulsed amperometric detection using a Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System (Thermo Fisher Scientific, Sunnyvale, CA, USA) equipped with a Dionex™ CarboPac™ PA210-Fast-4 µm analytical column (150 × 4 mm) and a Dionex™ CarboPac™ PA210-Fast-4 µm guard column (30 × 4 mm). Elution was performed at 30 °C with isocratic flux at 0.8 mL.min⁻¹ of 12 mmol.L⁻¹ KOH. Injection volume ranged from 10 to 25 µL. Sugars were identified by comparison of the

retention time with that of appropriate standards (galactose, glucose, fructose, sucrose, raffinose pentahydrate, verbascose).

● Micronutrient quantification in defatted doughnuts

To assess the “passive dilution effect” due to oil uptake during frying, the micronutrient content of “defatted samples” was calculated on a dry matter basis with the following equation:

$$C_{df} = \frac{C_0}{100 - F_s} * 100$$

Where C_{df} is the micronutrient content in the defatted sample, C₀ the micronutrient concentration in the sample diluted with oil, and F_s the fat content (% DM) of the sample. This formula allows assessing the part of decrease caused by other phenomena than oil uptake, such as enzymatic degradation, diffusion, oxidation or thermal inactivation.

2.2.2. Mass balance assessment

To identify the unit operations that have the greatest effect on folate and alpha-galacto-oligosaccharide contents, a mass balance assessment was carried during ata production in small processing units. Ata doughnuts were produced by three producers using the dry dehulling processing in Cotonou (Benin). Batches of 2 kg of cowpea seeds were provided to each producer. The weight of all containers used for collecting the fractions obtained at each step was recorded, as well as the weight of the produced fractions (raw material, intermediate products, by-products, and doughnuts). The samples collected from the different fractions were also weighed. These weights were taken into account in the calculation to determine the exact mass at each processing step. The DM contents of all samples were measured, and combined with the folate and sugar contents to calculate the amounts of folate (in µg) and alpha-galacto-oligosaccharide (in mg) in the raw material, intermediate products, and final products. Then, the folate and alpha-galacto-oligosaccharide distribution in the produced fractions was expressed as the percentage of their initial quantity in the raw material.

2.3. Statistical analysis

Data were analysed with the Statgraphics Centurion 16.2 software (FranceStat, Neuilly, France). To describe the nutritional composition of intermediate and final products, means and standard deviations were calculated, and differences between samples were assessed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test, with a significance level set at p < 0.05.

To assess the effect of the different processing factors on the nutrient contents of ata and ata-doco doughnuts, two-level (ata) and three-level (ata-doco) nested ANOVAs were carried out. For example, the linear model for a two-way unbalanced nested arrangement, with two nested factors (A: fixed factor with i levels, and B: random factor with j levels) was:

$$y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{k(j)}$$

where: Y_{ijk} is the kth observation within the jth level of factor B within the ith level of factor A; μ is the overall mean; α_i is the fixed effect due to the ith level of factor A; $\beta_{j(i)}$ is the random effect due to the jth level of factor B nested within the ith level of A; and $\varepsilon_{k(j)}$ is the residual error of the observation Y_{ijk}.

For ata doughnuts, “dehulling type” was used as fixed factor (two levels: dry dehulling and wet dehulling) and nested within the factor “producer” (six levels: 1–6). For ata-doco, the first factor was “milling type” (two levels: direct milling and crushing before milling) nested within the factor “use of NaHCO₃” (two levels: with and without NaHCO₃) that in turn, was nested within the factor “producer” (six levels: 1–6). The variance associated with each factor was calculated and expressed as a percentage.

General linear models were used to evaluate the association between processing parameters and doughnut nutrient content (proximate composition, mineral, folate, phytate and alpha-galacto-oligosaccharide):

$$y = \beta_0 + \beta_1 * (\text{factor 1}) + \beta_2 * (\text{factor 2}) + \dots + \beta_k * (\text{factor } k)$$

where β_0 is the constant, and β_{1-k} the coefficients associated with each factor.

The factors considered were the processing parameters: number of washing of the grits after dehulling, soaking duration, soaking water volume/seed volume ratio, number of milling cycles, whipping duration, and frying time and temperatures (ata); type of milling, whipping duration, quantity of NaHCO₃ added to the dough, and frying time and temperatures (ata-doco). For both doughnut types, the effect of frying parameters on the mineral contents was not assessed. Models were adjusted, by removing the not significant factors (based on a statistical significance level set at $p < 0.05$).

3. Results and discussion

3.1. Characterization of ata and ata-doco processing

During the traditional ata processing (all variants), the main steps were dehulling, soaking, wet milling, whipping, and frying (Fig. 1). Ata doughnuts were prepared from dehulled seeds obtained by dry or wet dehulling. Wet dehulling was performed manually using seeds previously wetted with tap water for softening the seed coat. For dry dehulling, cowpea seeds were coarsely milled into grits using local mills equipped with two metallic grindstones. The crushing step was followed by washing and manual rubbing to remove the seed coats. Grits obtained after dehulling was soaked and milled into a dough using the same type of mills previously used for crushing. During ata production, the mean duration of cowpea grits soaking, dough whipping and frying were 22 ± 11 min, 23 ± 15 min, and 5 ± 2 min respectively, for 1 kg of cowpea processed. The mean frying temperature varied between 135 °C at the beginning (mean T₀) and 173 °C at the end of cooking (mean T_F). For ata-doco production, the main steps were dry milling, whipping and frying. Whole seeds were directly milled (direct milling variant) or crushed grossly and then winnowed before milling into flour (crushing

before milling variant) (Fig. 1). Then, water was added to the flour to obtain a dough. Among the five producers of ata-doco doughnuts who used the direct milling variant, two added NaHCO₃ to the dough as leavening agent during whipping. Whatever the variant, the mean whipping and frying times were 23 ± 9 min and 5 ± 2 min respectively, with frying temperatures ranging between 162 (mean T₀) and 193 °C (mean T_F). The variability observed for the frying temperature for both doughnuts could be due to the different energy sources used by the producers (firewood, charcoal and wood shavings).

3.2. Effect of traditional processing variants on ata and ata-doco nutritional composition

3.2.1. Proximate composition of ata and ata-doco doughnuts

The proximate composition of cowpea seeds, ata and ata-doco is presented in Table 1. The mean protein content of the raw material was 22 g.100 g⁻¹ DM, which is in the range reported by Madode et al. (2012) (i.e. 22–30 g.100 g⁻¹ DM) for thirty cowpea varieties grown in West Africa. Protein content in cowpea seeds can vary depending on the cultivar (Jayathilake et al., 2018), environmental conditions and agronomic practices (Dakora and Belane, 2019; Sebetha et al., 2014). The lipid content of the cowpea seeds in our study was 1.6 g.100 g⁻¹ DM, which is rather low, as usually observed in pulses (Dilis and Trichopoulou, 2009). The total dietary fibre content of cowpea seeds was high (15 g.100 g⁻¹ DM) and on average 83 % of fibres were insoluble. Other studies reported similar results for dietary fibre content in cowpea seeds (Eashwarage et al., 2017; Sreerama et al., 2012).

The protein, ash, and total (soluble and insoluble) dietary fibre contents were significantly lower in ata than ata-doco doughnuts ($p < 0.05$). As expected, lipid content increased sharply during the doughnut preparation. The mean lipid content of ata doughnuts was significantly higher than that of ata-doco (48 and 28 g.100 g⁻¹ DM for ata and ata-doco, respectively). McWatters (1983) obtained similar results for doughnuts prepared from dehulled-soaked cowpea and whole cowpea flour (that presented lipid contents of 32 g.100 g⁻¹ DM and 21 g.100 g⁻¹ DM, respectively). The higher oil absorption by doughnuts prepared with dehulled and soaked seeds compared with those prepared with whole flour could be due to the dough particle size that is influenced by the milling conditions. Indeed, wet milling generates finer particles than

Table 1
Proximate composition of the cowpea seeds and doughnuts (ata and ata-doco) according to different processes.

Products	Process	Dry matter (g/100g)	Protein (g/ 100g DM)	Fat (g/100g DM)	Available carbohydrate** (g/ 100g DM)	Ash (g/100g DM)	Dietary fibres (g/100g DM)			Energy (Kcal/100g)
							IDF	SDF	TDF	
Cowpea seeds		89 ± 0.3	22.0 ± 1.0	1.6 ± 0.0	58 ± 1.1	3.3 ± 0.0	12.4 ± 1.0	2.5 ± 0.4	± 1.2	309 ± 1
Ata	Wet dehulling (n=3)	58 ± 1.0 ^a	14.0 ± 1.4 ^a	42.7 ± 2.0 ^b	31 ± 3.4 ^{ab}	2.6 ± 0.9 ^a	8.4 ± 0.4 ^b	1.2 ± 0.3 ^a	9.6 ± 0.4 ^b	337 ± 12 ^b
	Mechanical dry dehulling (n=3)	61 ± 9.7 ^a	14.2 ± 3.2 ^a	52.9 ± 13 ^c	24 ± 16 ^a	2.9 ± 0.2 ^a	5.2 ± 0.6 ^a	1.3 ± 0.4 ^a	6.5 ± 0.3 ^a	388 ± 36 ^c
	Direct milling (+) NaHCO ₃ (n=2)	54 ± 3.6 ^a	17.1 ± 1.2 ^b	32.4 ± 2.4 ^a	36 ± 3.1 ^b	3.4 ± 0.3 ^a	8.6 ± 1.1 ^b	2.2 ± 0.3 ^b	10.8 ± 1.5 ^b	285 ± 17 ^a
Ata-doco	Direct milling (-) NaHCO ₃ (n=3)	53 ± 3.5 ^a	17.9 ± 1.0 ^b	25.6 ± 5.5 ^a	40 ± 6.6 ^b	5.4 ± 1.4 ^b	9.0 ± 0.9 ^b	2.3 ± 0.4 ^b	11.3 ± 0.8 ^b	254 ± 8 ^a
	Crushing before milling (n=1) *	58 ± 4.4 ^a	15.8 ± 0.8 ^{ab}	27.1 ± 2.5 ^a	41 ± 2.2 ^b	6.0 ± 1.0 ^b	8.3 ± 0.6 ^b	1.7 ± 0.6 ^{ab}	10.0 ± 1.2 ^b	283 ± 30 ^a
	P-value	ns	0.0005	<0.00001	0.002	<0.00001	0.02	0.0013	0.0028	<0.00001
Ata vs. Ata- doco	Ata	59 ± 6.8	14.1 ± 2.4	47.8 ± 10	27 ± 12	2.8 ± 0.6	7.1 ± 1.8	1.2 ± 0.3	8.3 ± 1.7	362 ± 37
	Ata-doco	56 ± 4.3	16.9 ± 1.3	27.8 ± 4.6	39 ± 4.7	5.1 ± 1.5	8.6 ± 0.8	2.1 ± 0.5	10.7 ± 1.2	273 ± 18

n: number of producers.

IDF: Insoluble dietary fibres; SDF: soluble dietary fibres; TDF: Total dietary fibres.

Results are the mean ± standard deviations (samples were collected from the indicated number of producers and analysed in duplicate). *: Samples were collected from this producer on three different days. **: Values were obtained by calculation.

Values in a same column with different superscript letters are significantly different ($p < 0.05$, Newman–Keuls test was used for post–hoc comparison).

dry milling, and products with a predominantly fine particle size distribution have a very high oil absorption capacity (Moreira et al., 1997; Singh et al., 2004). Another possible cause of the difference of oil absorption could be the higher soluble fibre content of the dough used for ata-doco preparation. By swelling in the presence of water, soluble fibre can increase the dough viscosity and this could influence oil absorption. Indeed, Lee and Inglett (2007) showed that the incorporation of oat β-glucans into a wheat flour batter reduces oil absorption during deep-frying, because of the high water retention capacity and viscosity generated by these polysaccharides. Moreover, a study on poori, a traditional wheat-based doughnut consumed in India, showed that the use of fibre-rich flour (4.2 % total dietary fibres, 1.3 % soluble dietary fibres) allowed reducing the oil content by 20 % compared with the control sample Yadav and Rajan (2012). In this study, the major consequences of oil absorption during frying were the passive reduction of the proportions of other nutrients in both doughnut types and the strong increase of the energy value (Table 1). Thus, the energy value of ata doughnuts was significantly higher than that of ata-doco ($p < 0.05$), due to the high lipid content of ata doughnuts, which was twice higher than the one of ata-doco doughnuts.

The producers' practices influenced the proximate composition of the doughnuts. The two-level nested ANOVA showed that 85–97 % of the variability observed in ata doughnut proximate composition (dry matter, protein, lipid and ash contents) was due to inter-producer variability (Table 1S, supplementary data). A specific analysis of the processing parameters that can lead to this producer-related variability highlighted a negative effect of dry dehulling, soaking duration, and initial frying temperature (T_0) on protein content ($p < 0.05$, Table 2S, supplementary data). Ash content was negatively correlated with soaking duration and positively correlated with the number of milling cycles ($p < 0.05$, Table 2S, supplementary data).

For ata-doco doughnuts, 55 % and 60 % of the observed variability of protein and lipid contents was explained by inter-producer variability, respectively (Table 3S, supplementary data). Protein content variability

was related also to the "milling type" factor (27 %), with higher mean protein content in doughnuts prepared using the "direct milling" than the "crushing before milling" variant (17 g.100 g⁻¹ DM vs 16 g.100 g⁻¹ DM, $p < 0.05$). Moreover, by studying the effect of processing parameters on protein content, a negative correlation was observed with the variant "crushing before milling" and the initial frying temperature ($p < 0.05$, Table 2S, supplementary data). The analysis of the impact of the processing parameters showed that frying duration and initial frying temperature (T_0) were positively correlated with the lipid content. The nested ANOVA showed that the ash content of ata-doco doughnuts was mainly influenced by the factors "use of NaHCO₃" and "producer" (that contributed to 44 % and 37 % of the variance, respectively).

3.2.2. Changes in micronutrients and anti-nutritional factors during ata and ata-doco production

● Minerals

Cowpea seeds contained appreciable amounts of minerals with nutritional interest, particularly iron and magnesium (Table 2). Iron content of doughnuts increased during processing for both types. This increase in iron content of both doughnut types could be due to contamination from various possible sources throughout processing. Some authors reported that exogenous iron from pots and mills can be added to the food during processing (Adish et al., 1999; Hama-Ba et al., 2019). Greffeuil et al. (2011) observed this contamination after maize milling into flour. The processing steps before frying did not lead to a significant reduction of zinc, calcium and magnesium content in both doughnut types. However, a passive decrease in mineral contents was observed after frying due to oil absorption by the dough. Indeed, the calculation of the mineral content in defatted ata doughnuts showed zinc, calcium, and magnesium contents (5.2 mg.100 g⁻¹, 83 mg.100 g⁻¹ and 188 mg.100 g⁻¹ DM, respectively), similar to that in the whipped dough. The mean iron, zinc, calcium and magnesium contents calculated

Table 2
Changes in mineral, phytate and folate contents (on a dry matter basis) during ata and ata-doco processing.

Dish	Processing	Products	Iron (mg/100g DM)	Zinc (mg/100g DM)	Calcium (mg/100g DM)	Magnesium (mg/100g DM)	Phytate (mg/100g DM)	Folate (μg/100g DM)
Ata	Wet dehulling (n=3)	Raw cowpea seeds	5.8 ± 0.0 ^a	3.9 ± 0.0 ^c	78 ± 1 ^d	163 ± 1 ^c	979 ± 81 ^{cd}	362 ± 33 ^e
		Dehulled seeds	4.5 ± 0.1 ^a	3.9 ± 0.1 ^c	61 ± 12 ^c	144 ± 2 ^b	1049 ± 82 ^d	318 ± 43 ^d
		Soaked grits	4.5 ± 0.2 ^a	4.0 ± 0.1 ^c	65 ± 9 ^c	143 ± 2 ^b	1044 ± 72 ^d	261 ± 23 ^c
		Whipped dough	11.5 ± 6.8 ^{bc}	3.6 ± 0.3 ^c	86 ± 23 ^d	135 ± 8 ^b	871 ± 99 ^b	195 ± 21 ^b
	Mechanical dehulling (n=3)	Ata	8.9 ± 3.0 ^{ab}	2.3 ± 0.4 ^a	44 ± 9 ^{ab}	91 ± 17 ^a	562 ± 101 ^a	98 ± 29 ^a
		Dehulled seeds	4.6 ± 0.2 ^a	3.5 ± 0.3 ^c	53 ± 3 ^{bc}	140 ± 5 ^b	988 ± 69 ^{cd}	305 ± 22 ^d
Ata-doco	Direct milling with NaHCO ₃ (n=2)	Soaked grits	4.7 ± 0.4 ^a	3.9 ± 0.1 ^c	55 ± 5 ^{bc}	139 ± 6 ^b	982 ± 38 ^{cd}	293 ± 38 ^d
		Whipped dough	13.4 ± 6.7 ^c	4.0 ± 0.1 ^c	60 ± 2 ^c	137 ± 7 ^b	919 ± 63 ^{bc}	204 ± 30 ^b
		Ata	7.1 ± 2.5 ^a	2.7 ± 0.5 ^b	37 ± 1 ^a	92 ± 22 ^a	519 ± 105 ^a	113 ± 27 ^a
		Raw cowpea seeds	5.8 ± 0.0 ^A	3.9 ± 0.0 ^{BC}	78 ± 1 ^{DE}	163 ± 1 ^F	979 ± 81 ^E	362 ± 33 ^F
		Flour	7.5 ± 1.4 ^A	3.9 ± 0.2 ^{BC}	77 ± 3 ^{DE}	153 ± 5 ^{DE}	940 ± 76 ^E	273 ± 41 ^D
	Direct milling without NaHCO ₃ (n=3)	Whipped dough	8.4 ± 2.3 ^A	4.2 ± 0.0 ^C	86 ± 0 ^F	153 ± 7 ^{DE}	814 ± 14 ^C	180 ± 44 ^{AB}
		Ata-doco	5.5 ± 0.6 ^A	3.0 ± 0.0 ^A	58 ± 1 ^A	113 ± 2 ^A	601 ± 79 ^A	207 ± 46 ^{BC}
		Flour	7.2 ± 0.3 ^A	4.1 ± 0.1 ^{BC}	78 ± 5 ^{DE}	160 ± 4 ^{EF}	929 ± 85 ^E	302 ± 41 ^{DE}
	Crushing + Milling (n=1)*	Whipped dough	7.6 ± 1.0 ^A	3.9 ± 0.2 ^{BC}	86 ± 5 ^F	156 ± 5 ^{DE}	800 ± 42 ^C	306 ± 38 ^E
		Ata-doco	5.5 ± 0.9 ^A	3.0 ± 0.2 ^A	69 ± 7 ^{BC}	127 ± 8 ^C	641 ± 79 ^{AB}	176 ± 36 ^{AB}
		Flour	37.9 ± 10 ^C	3.9 ± 0.0 ^{BC}	75 ± 8 ^{CD}	153 ± 3 ^{DE}	980 ± 47 ^E	287 ± 36 ^{DE}
		Whipped dough	37.9 ± 13 ^C	3.8 ± 0.2 ^B	84 ± 6 ^{EF}	149 ± 5 ^D	876 ± 41 ^D	232 ± 26 ^C
		Ata-doco	28.3 ± 12 ^B	2.9 ± 0.2 ^A	66 ± 6 ^B	121 ± 5 ^B	686 ± 45 ^B	157 ± 10 ^A

n: number of producers.

In each sub-column, values with different superscript letters are significantly different ($p < 0.05$, Newman–Keuls test was used for post–hoc comparison).

Results are the mean ± standard deviation (samples were collected from the indicated number of producers). *: Samples were collected from this producer on three different days.

in defatted ata-doco were $4.1 \text{ mg.}100 \text{ g}^{-1}$, $90 \text{ mg.}100 \text{ g}^{-1}$ and $168 \text{ mg.}100 \text{ g}^{-1}$ DM, respectively. These results show the oil dilution effect on mineral contents.

For ata doughnuts, the nested ANOVA (Table 1S, supplementary data) showed that more than 60 % of the variability of iron, calcium, and magnesium content was due to the inter-producer variability. Conversely, the variability of zinc content was very low and not related to any factor. The analysis of the different processing parameters that could cause this producer-related variability of iron, calcium, and magnesium contents in ata doughnuts showed a negative effect of the number of dehulled-seed washing steps on iron content ($p < 0.05$, Table 2S, supplementary data), and a negative effect of the number of dehulled-seed washing steps and of soaking duration on magnesium and calcium content ($p < 0.05$, Table 2S, supplementary data). These decrease in iron, calcium and magnesium contents of ata doughnuts could be due to leaching from the grits during washing.

For ata-doco, the iron content variability was related to the factor "milling type" (84 % of variance, Table 3S, supplementary data), with higher iron content in the "crushing before milling" than in the "direct milling" variant ($28 \pm 10 \text{ mg.}100 \text{ g}^{-1}$ DM vs. $5.5 \pm 1 \text{ mg.}100 \text{ g}^{-1}$), which shows the influence of processing types on iron contamination. Zinc content variability was also low and more related to the factor "producer" (50 %). This producer-related variability was not caused by the processing parameters. The calcium and magnesium content variability was mainly due to the factor "use of NaHCO_3 " (52 % of the variance for calcium, and 56 % for magnesium). Indeed, the calcium and magnesium contents of ata-doco doughnuts prepared with NaHCO_3 were lower than those of doughnuts prepared without NaHCO_3 ($58 \pm 1 \text{ mg.}100 \text{ g}^{-1}$ DM vs. $68 \pm 6 \text{ mg.}100 \text{ g}^{-1}$ DM for calcium and $113 \pm 3 \text{ mg.}100 \text{ g}^{-1}$ DM vs. $124 \pm 7 \text{ mg.}100 \text{ g}^{-1}$ DM for magnesium).

● Folate

The total folate content of the initial raw material was $362 \pm 33 \mu\text{g.}100 \text{ g}^{-1}$ DM (Table 2). Hoppner and Lampi (1993) found a similar folate content of $367 \pm 28 \mu\text{g.}100 \text{ g}^{-1}$ DM in cowpea seeds purchased from a local market in Canada. During the production of both doughnut types, a reduction in folate content was observed after each step. The mean total folate content of ata-doco doughnuts was significantly higher than that of ata doughnuts ($177 \pm 36 \mu\text{g.}100 \text{ g}^{-1}$ DM vs. $111 \pm 29 \mu\text{g.}100 \text{ g}^{-1}$ DM). This difference in the mean folate content of ata and ata-doco doughnuts could be due to the absence of soaking during ata-doco production. Indeed, a negative effect of soaking duration on folate content was observed during ata production (Table 2S, supplementary data for details). Different studies showed the effect of the soaking on folate content in legumes. Arcot et al. (2002) observed a reduction of 31 % of total folate after soaking of soybeans overnight at room temperature. In their study, the recovery of folate from soaked soybeans and the soaking water was almost equal to 100 % of the initial amount in the soybeans showing that the loss was due to leaching into the soaking water. During soaking at 30°C of whole cowpea seeds, Coffigniez et al. (2019) also observed folate diffusion, but at a much lower rate than at 60°C and 95°C . In our study, the negative effect of soaking duration observed could be due to an increased diffusion due to the high surface area generated by the small size of the grits.

The nested ANOVA showed that the total folate content variability for ata was mainly related to the factor "producer" (91 % of variance, Table 1S, supplementary data). Indeed, the total folate content was negatively correlated with the dry dehulling variant, the number of dehulled seed washing steps, and the soaking duration ($p < 0.05$, Table 2S, supplementary data). For ata-doco, 87 % of the observed variability was due to inter-producer variability (Table 3S, supplementary data), possibly due to the NaHCO_3 amount added to the dough (positive correlation, $p < 0.05$, Table 2S supplementary data).

● Phytate

Phytate content of both doughnut types was only slightly affected by processing (Table 2), although it decreased at some production steps. During ata production, dehulling did not have any effect on phytate contents. This could be due to phytic acid localization in the seed. In pulses, phytic acid is stored in globoids, located in protein bodies that are in the seed cotyledons (Reddy, 2002). Thus, the dehulling is not an efficient way to reduce phytate in cowpea seeds. Similarly, soaking did not significantly reduce the phytate content of dehulled seeds. Lestienne et al. (2005) found that soaking at 30°C for 24 h significantly reduced phytate content in millet, maize, rice and soybean, but not in cowpea. Ologhobo and Fetuga (1984) observed a reduction of 20–28 % in phytate contents in three cowpea cultivars after soaking at 27°C for 3 days. Thus, the lack of effect observed in our study may be due to the short soaking duration (between 9 and 38 min). In our study, phytate content in the dough used for ata preparation decreased by 11 % and 6% during the whipping step compared with soaked seeds, for the wet and dry dehulling variants, respectively. This small effect of whipping on phytate content was also observed during ata-doco preparation. The use of NaHCO_3 during ata-doco processing did not affect phytate contents. The mean final phytate content was significantly higher in ata-doco than in ata doughnuts ($656 \text{ mg.}100 \text{ g}^{-1}$ DM for ata-doco vs. $526 \text{ mg.}100 \text{ g}^{-1}$ DM for ata). The variability in the doughnut phytate content was mostly related to the factor "producer" (63 % and 75 % of the variance for ata and ata-doco, respectively). For ata, this producer-related variability might be due to the number of grits washing steps and the duration of soaking that were negatively correlated with phytate content ($p < 0.05$, Table 2S, supplementary data). For ata-doco, the inter-producer variability was not explained by the processing parameters.

● Alpha-galacto-oligosaccharides

The total alpha-galacto-oligosaccharide content in cowpea seeds reached $3554 \text{ mg.}100 \text{ g}^{-1}$ DM, and stachyose content was 8–10 times higher than that of verbascose and raffinose, as already reported for other legume seeds (Njoumi et al., 2019). The changes in alpha-galacto-oligosaccharide contents during ata and ata-doco processing are shown in Fig. 2. During ata production, raffinose (27 %) and verbascose (28 %) content increased after wet dehulling. Sreerama et al. (2010) studied alpha-galacto-oligosaccharides distribution in cotyledon, embryonic axis and seed coat of two pulse species: chickpea (*Cicer arietinum L.*) and horse gram (*Macrotyloma uniflorum L. Verdc.*). They found higher concentrations of these oligosaccharides in the cotyledon, but stachyose was present in higher amounts also in the embryonic axis. This could explain the increase observed after wet dehulling because the removal of the seed coat, which does not contain raffinose and verbascose, could lead to a passive increase of these compounds in dehulled seeds. After dehulling during ata preparation, a decrease of 15–20 % in the content of the three alpha-galacto-oligosaccharide forms was observed up to the "whipped dough" step, whatever the variant. The reduction after whipping was more significant (total alpha-galacto-oligosaccharide content was halved) during ata-doco processing. This reduction most probably results from the alpha-galactosidase activity in cowpea seeds (Coffigniez et al., 2018) or provided by exogenous factors, such as *Aspergillus niger* (Somari and Balogh, 1992). Bulgarelli et al. (1988) reported that *A. niger* is among the predominant microbiological populations in the dough used for akara (i.e. ata) preparation. After frying, the difference in alpha-galacto-oligosaccharide content of both doughnuts was compensated by the higher oil absorption in ata, and the mean total alpha-galacto-oligosaccharide contents of ata and ata-doco doughnuts were $2695 \pm 792 \text{ mg.}100 \text{ g}^{-1}$ DM and $2680 \pm 255 \text{ mg.}100 \text{ g}^{-1}$ DM, respectively. When calculated in defatted doughnuts, the total alpha-galacto-oligosaccharide content was significantly higher in defatted ata than in ata-doco ($4623 \text{ mg.}100 \text{ g}^{-1}$ DM vs. $3713 \text{ mg.}100 \text{ g}^{-1}$ DM, respectively).

Variability in the total alpha-galacto-oligosaccharide content was

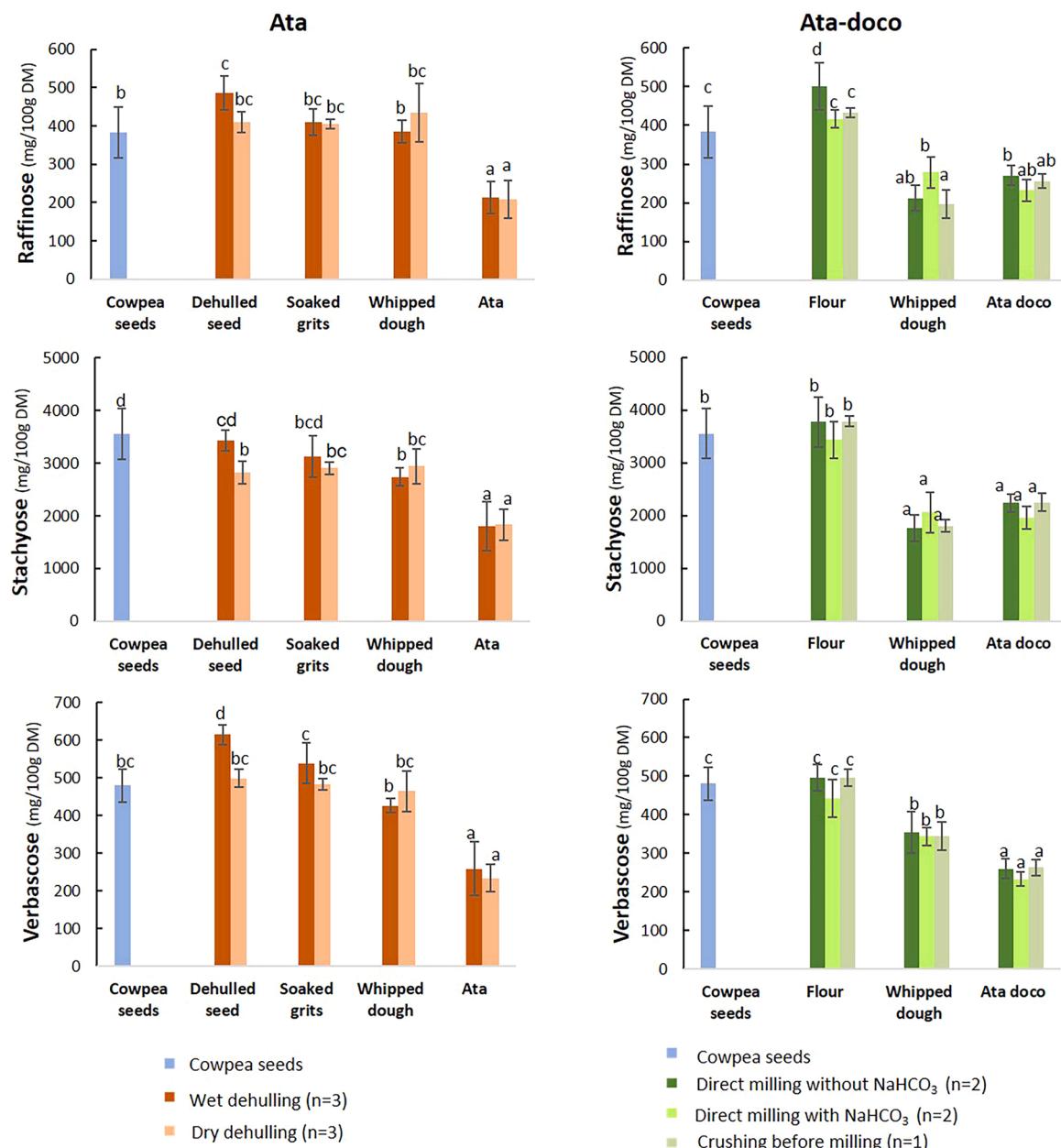


Fig. 2. Raffinose, stachyose and verbascose content (on a dry matter basis) during ata and ata-doco) processing. n: number of producers, Different superscript letters correspond to significant difference between samples ($p<0.05$, Newman-Keuls test was used for post-hoc comparison).

mostly caused by the factor "producer" (>97 %) in ata doughnuts and by the factors "producer" (37 %) and "use of NaHCO_3 " (33 %) in ata-doco. Specifically, the total mean alpha-galacto-oligosaccharide content was higher in ata-doco doughnuts prepared without than with NaHCO_3 (2764 mg.100 g⁻¹ DM vs. 2427 mg.100 g⁻¹ DM).

3.3. Mass balance of dry matter, folate and alpha-galacto-oligosaccharide during ata production

3.3.1. Dry matter

Dry matter losses and gains were observed during ata processing based on the dry dehulling process (variant presented on the left in Fig. 1) (Fig. 3). The dehulling step (i.e. coarse milling followed by washing and manual rubbing) resulted in an average loss of 16 % of the dry matter (8.6 % due to diffusion into the washing water and 7.1 % from the seed coats). Only one among the three producers performed two cycles of crushed-seed washing, but this did not result in a

significant difference compared with the other producers because the second soaking water contained only 2% of the initial dry matter. After soaking, the amount of dry matter in the grits was about 78 % of the initial dry matter of cowpea seeds. After milling, on average, 60 % of the initial dry matter was recovered as dough, and 12 % was found in the water used for mill rinsing. The addition of the mill rinsing water to the dough, to adjust the dough consistency during whipping, allowed recovering, on average, 3% of dry matter. Oil absorption by the whipped dough during frying was highly variable, resulting in a mean dry matter gain of about 27 ± 16 %.

3.3.2. Folates

The initial total folate content of the cowpea seeds used for the mass balance assessment was 274 $\mu\text{g}.100 \text{ g}^{-1}$ DM (Table 3), which was lower than that of the cowpea seeds used for the study of the processing variants (Table 2). Analysis of the distribution of the initial amount of folate in the different fractions generated during ata production (Fig. 3)

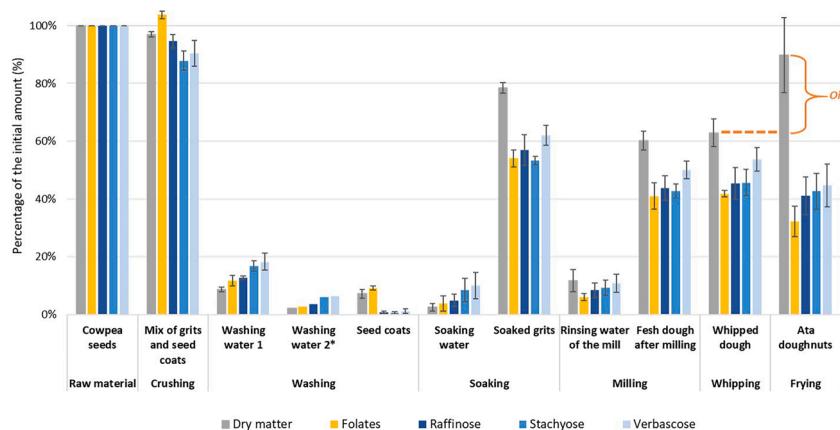


Fig. 3. Dry matter, folate and alpha-galactoside distribution in the different production fractions during the traditional processing of ata (calculated using the dry weight of each fraction and component content; data are the mean values for the samples collected from three producers). Oil: proportion of dry matter gained due to oil absorption during frying.

Table 3

Dry matter, total folate and alpha-galactoside content in the different fractions during ata processing.

Fractions	Dry matter (g/100 g)	Folate (µg/100 g DM)	Raffinose (mg/100 g DM)	Stachyose (mg/100 g DM)	Verbascose(mg/100 g DM)
Cowpea seeds	89.5 ± 1.5 ^h	274 ± 9 ^c	469 ± 7 ^d	4150 ± 137 ^e	658 ± 19 ^c
Mix of grits and seed coats	90.7 ± 1.1 ^h	293 ± 15 ^{cd}	456 ± 37 ^d	3761 ± 195 ^{de}	614 ± 42 ^c
Washing water 1	2.3 ± 0.7 ^a	378 ± 28 ^{ef}	727 ± 132 ^e	7814 ± 749 ^f	1304 ± 190 ^d
Washing water 2*	0.7 ^a	348 ^{de}	797 ^f	11391 ^g	1931 ^e
Seed coats	20.2 ± 2.6 ^c	366 ± 49 ^{ef}	49 ± 11 ^a	330 ± 153 ^a	99 ± 46 ^a
Soaking water	1.0 ± 0.2 ^a	429 ± 147 ^f	963 ± 124 ^g	14840 ± 2527 ^h	2794 ± 559 ^f
soaked grits	43.0 ± 1.8 ^f	189 ± 15 ^b	342 ± 40 ^c	2825 ± 157 ^c	521 ± 45 ^c
Mill rinsing water	14.5 ± 3.1 ^b	152 ± 52 ^{ab}	336 ± 30 ^c	3290 ± 174 ^{cd}	608 ± 35 ^c
Freshly milled dough	38.8 ± 1.3 ^g	187 ± 21 ^b	340 ± 27 ^c	2952 ± 20 ^c	548 ± 22 ^c
Whipped dough	32.5 ± 1.8 ^d	183 ± 17 ^b	338 ± 41 ^c	3012 ± 174 ^c	562 ± 23 ^c
Ata doughnuts	54.4 ± 2.8 ^g	98 ± 8 ^a	214 ± 23 ^b	1965 ± 176 ^b	327 ± 42 ^b

Values in a same column with different superscript letters are significantly different ($p < 0.05$, Newman–Keuls test was used for post-hoc comparison).

Results are the mean ± standard deviation (samples were collected from 3 producers and analysed in triplicate for folate and in duplicate for alpha-galactoside and dry matter quantification).

* : Only producer P3 used two rounds of washing.

Showed that approximately 24 % of the folate present in the cowpea seeds was found in the washing water and seed coat during the washing step. Indeed, it has been shown that folate vitamers in cowpea seeds are mainly concentrated in the embryonic axis (Coffigniez et al., 2019). As after dehulling and washing, the embryonic axis is discarded, this could explain part of the folate loss. Moreover, dehulling leads to the loss of fine particles from cotyledon, which are also washed out. After the soaking step, a loss of 26 % of the initial folate was recorded and only 4% was in the soaking water. The other 22 % might be due to folate degradation. Indeed, some of the folate vitamers are very sensitive to pH and oxygen (Strandler et al., 2015; Xue et al., 2011). Low pH and presence of oxygen can lead to oxidative degradation or cause conversion to other forms, sometimes without vitaminic activity (Coffigniez et al., 2019). In this study, milling resulted in an additional average loss of 13 % of the initial folate. The addition of the mill rinsing water, which contained about 6% of the initial folate, to the dough during whipping did not result in a significant difference between freshly milled dough (41 % of the initial folate) and whipped dough (42 %). Frying also strongly affected folate contents. However, the main part of the decrease observed was related to oil absorption by the dough during frying, and only approximately 10 % of the decrease could be explained by thermal degradation.

3.3.3. Alpha-galacto-oligosaccharides

The initial amounts of raffinose, stachyose and verbascose were reduced by 5%, 12 % and 10 %, respectively, after dehulling (Fig. 3). Moreover, 22 %, 32 % and 36 % of the initial amounts of raffinose,

stachyose and verbascose, respectively, were found in the seed coats and washing water. After grits soaking, the amount of initial alpha-galacto-oligosaccharides found in the soaking water and soaked grits (the two fractions generated after this step) allowed deducing that 18–33% of the initial alpha-galacto-oligosaccharides were lost due to degradation. Moreover, an important increase of the initial amounts of galactose, glucose and fructose, which are alpha-galacto-oligosaccharide degradation products, was simultaneously observed (Fig. 1S, supplementary data). The initial amount of these sugars increased by 23-fold for galactose, 33-fold for glucose, and 14-fold for fructose. The higher decrease in alpha-galacto-oligosaccharide content of the whipped doughs used for ata-doco (half the one of ata) observed during the study of the traditional processing variants could be explained by this higher production of galactose observed after grits soaking during ata production. Indeed, alpha-galactosidase activity can be reduced by the presence in the medium of some inhibitors, particularly galactose (Coffigniez et al., 2018). According to Dey and Pridham (1969), 6.2 mmol.L⁻¹ and 25 mmol.L⁻¹ of galactose inhibit alpha-galactosidase activity by 70 % and 90 % respectively. Indeed, in our study, the galactose content of the raw material was 0.3 mmol.L⁻¹, and increased to 8.4 ± 1 mmol.L⁻¹ in the soaked grits, which were milled into dough (Fig. 1S, supplementary data). After soaking, residual losses of alpha-galacto-saccharide were observed up to the frying step.

3.4. Potential contribution of ata and ata-doco to the recommended nutrient intakes

In West Africa, cowpea-based doughnuts are consumed for breakfast and as a snack food. The average adult serving is 3–4 doughnuts, which corresponds to approximately 100 g. The consumption of 100 g of ata and ata-doco provides 8.2 ± 0.9 g and 9.3 ± 0.8 g of protein (Table 4S, supplementary data). For an adult weighing 65 kg, such serving of ata and ata-doco would contribute to 15 and 17 % respectively of the recommended nutrient intake (RNI) for protein, which is estimated at 54 g of protein/day (FAO and OMS, 2007). The dietary fibre intake associated to the consumption of 100 g of both doughnuts would be around 4.9 ± 0.9 g and 5.9 ± 0.6 g for ata and ata-doco respectively. These values represent 16–20% of the daily fibre recommended intake which is estimated at 30 g per day by (ANSES, 2016). This serving could also provide 8% and 13 % of the iron RNI, 11 % and 11 % of the zinc RNI, and 20 % and 26 % of the magnesium RNI for ata and ata-doco, respectively. Folate intake from 100 g of ata and ata-doco would represent 16 % and 24 % of the folate RNI that is estimated at 400 µg/day for adults (FAO-WHO, 2004). Based on these results both doughnuts can be regarded as a source of protein, dietary fibre, magnesium and folate (Codex, 1997). Calcium intake from both doughnut types is low (less than 4% of the RNI) (Table 5S, supplementary data). However, this serving also provides high amounts of lipids, much more elevated for ata (28 ± 5 g) than for ata-doco (15 ± 3 g).

4. Conclusion

This study highlighted the complex effects of traditional processing on the proximate composition, mineral, folate, phytate, and alpha-galacto-oligosaccharide contents of two cowpea-based doughnuts, and their variability in function of the doughnut type and processing variant. Part of these effects could be attributed to some processing parameters (e.g. soaking duration, milling type), but the role of the producers' practices remained significant, hindering the overall understanding of the whole processing effect. Mineral content was only slightly affected by the processing. Although folate losses were significant, the folate content in the final products was enough to qualify these doughnuts as a source of folate. Globally, ata-doco showed a better nutritional value than ata, with higher protein, fibre and folate and lower lipid contents. This work allowed identifying the processing steps that influence the nutritional quality of cowpea-based doughnuts, with the perspective of optimizing their preparation in the future. A study in controlled conditions could help to better understand and master the factors leading to folate and alpha-galacto-oligosaccharide losses due to degradation (around 20 %) during soaking. The important oil absorption observed during frying led to a passive reduction of the other nutrient contents. This high lipid intake counterbalances the nutritional interest of cowpea-based doughnuts. Therefore, more studies are also needed to determine the processing conditions to limit oil absorption.

CRediT authorship contribution statement

L. Akissoé: Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Y.E. Madodé:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Y.M. Hemery:** Conceptualization, Methodology, Formal analysis, Visualization, Writing - review & editing, Supervision. **B.V. Donadje:** Investigation, Formal analysis. **C. Icard-Vernière:** Conceptualization, Methodology, Writing - review & editing, Supervision. **D.J. Houhouigan:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition. **C. Mouquet-Rivier:** Conceptualization, Methodology, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

- Adish, A.A., Esrey, S.A., Gyorkos, T.W., Jean-Baptiste, J., Rojhani, A., 1999. Effect of consumption of food cooked in iron pots on iron status and growth of young children: a randomised trial. Lancet 353, 712–716. [https://doi.org/10.1016/S0140-6736\(98\)04450-X](https://doi.org/10.1016/S0140-6736(98)04450-X).
- Akinyele, I.O., Akinlosotu, A., 1987. Contribution of cowpea (*Vigna unguiculata*) in a mixed diet to the nutrient intake of rural children in Ibadan. Br. J. Nutr. 58, 31–39. <https://doi.org/10.1079/BJN19870066>.
- ANSES, 2016. Actualisation des repères du PNNS : élaboration des références nutritionnelles. Rapport d'expertise collective. Available at: <https://www.anses.fr/r/system/files/NUT2012SA0103Ra1-1.pdf>.
- AOAC, 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists International, Washington DC.
- Arcot, J., Wong, S., Shrestha, A.K., 2002. Comparison of folate losses in soybean during the preparation of tempeh and soymilk. J. Sci. Food Agric. 82, 1365–1368. <https://doi.org/10.1002/jsfa.1197>.
- Awika, J.M., Duodu, K.G., 2017. Bioactive polyphenols and peptides in cowpea (*Vigna unguiculata*) and their health promoting properties: a review. Journal of Functional Foods, Special issue on pulses 38, 686–697. <https://doi.org/10.1016/j.jff.2016.12.002>.
- Bationo, F., Humblot, C., Songré-Ouattara, L.T., Hama-Ba, F., Le Merre, M., Chapron, M., Kariluoto, S., Hemery, Y.M., 2020. Total folate in West African cereal-based fermented foods: bioaccessibility and influence of processing. J. Food Compos. Anal. 85, 103309 <https://doi.org/10.1016/j.jfca.2019.103309>.
- Bulgarelli, M.A., Beuchat, L.R., McWATTERS, K.H., 1988. Microbiological quality of cowpea paste used to prepare Nigerian Akara. J. Food Sci. 53, 442. <https://doi.org/10.1111/j.1365-2621.1988.tb07726.x>.
- Carvalho, M., Lino-Neto, T., Rosa, E., Carnide, V., 2017. Cowpea: a legume crop for a challenging environment: cowpea for a challenging environment. J. Sci. Food Agric. 97, 4273–4284. <https://doi.org/10.1002/jsfa.8250>.
- Codex, 1997. Guidelines for Use of Nutrition and Health Claims. Revised in 2004. Amended in 2001, 2008, 2009, 2010, 2011, 2012 and 2013. (No. CAC/GL 23-1997).
- Coffigniez, F., Briffaz, A., Mestres, C., Ricci, J., Alter, P., Noel, D., Bohuon, P., 2018. Kinetic study of enzymatic α -galactosidase hydrolysis in cowpea seeds. Food Res. Int. 113 <https://doi.org/10.1016/j.foodres.2018.07.030>.
- Coffigniez, F., Rychlik, M., Sanier, C., Mestres, C., Striegel, L., Bohuon, P., Briffaz, A., 2019. Localization and modeling of reaction and diffusion to explain folate behavior during soaking of cowpea. J. Food Eng. 253, 49–58. <https://doi.org/10.1016/j.jfoodeng.2019.02.012>.
- Dakora, F.D., Belane, A.K., 2019. Evaluation of protein and micronutrient levels in edible cowpea (*Vigna Unguiculata L. Walp.*) leaves and seeds. Front. Sustain. Food Syst. 3 <https://doi.org/10.3389/fsufs.2019.00070>.
- Dey, P.M., Pridham, J.B., 1969. Substrate specificity and kinetic properties of α -galactosidases from *Vicia faba*. Biochem. J. 115, 47–54.
- Dilis, V., Trichopoulou, A., 2009. Nutritional and health properties of pulses. Med. J. Nutrition Metab. 1, 149–157. <https://doi.org/10.1007/s12349-008-0023-2>.
- Eashwarage, I.S., Herath, H.M.T., Gunathilake, K.G.T., 2017. Dietary Fibre, Resistant Starch and in-Vitro Starch Digestibility of Selected Commonly Consumed Legumes (Mung Bean, Cowpea, Soybean and Horse Gram) in Sri Lanka, 7, p. 7.
- FAO (Ed.), 2003. Food Energy: Methods of Analysis and Conversion Factors: Report of a Technical Workshop, Rome, 3–6 December 2002. FAO food and nutrition paper. Food and Agriculture Organization of the United Nations, Rome. Available at: http://www.fao.org/uploads/media/FAO_2003_Food_Energy_02.pdf.
- FAO, 2016. Pulses Are Praised for Their Health, Environmental and Economic Benefits. How Can Their Full Potential Be Tapped? Retrieved from <http://www.fao.org/fsnorum/activities/discussions/pulses> [Google Scholar].

- FAO, OMS (Eds.), 2007. Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint FAO/WHO/UNU Expert Consultation (WHO Technical Report Series 935). WHO, Geneva. Available at : https://www.who.int/nutrition/publications/nutrientrequirements/WHO_TRS_935/en/, WHO technical report series.
- FAO-WHO (Ed.), 2004. Vitamin and Mineral Requirements in Human Nutrition, 2. ed. Geneva. Available at: <https://apps.who.int/iris/bitstream/handle/10665/42716/9241546123.pdf>.
- Feitosa, S., Korn, M. das, Pinelli, M., Oliveira, T., Bozzo, E., Greiner, R., Almeida, D., 2015. Content of minerals and antinutritional factors in akara (Fried cowpea food). *Int. J. Food Process. Technol.* 2, 42–50. <https://doi.org/10.15379/2408-9826.2015.02.02.06>.
- Gonçalves, A., Goufo, P., Barros, A., Domínguez-Perles, R., Trindade, H., Rosa, E.A.S., Ferreira, L., Rodrigues, M., 2016. Cowpea (*Vigna unguiculata* L. Walp), a renewed multipurpose crop for a more sustainable agri-food system: nutritional advantages and constraints. *J. Sci. Food Agric.* 96, 2941–2951. <https://doi.org/10.1002/jsfa.7644>.
- Greffeuille, V., Polycarpe Kayodé, A.P., Icard-Vernière, C., Gnimadi, M., Rochette, I., Mouquet-Rivier, C., 2011. Changes in iron, zinc and chelating agents during traditional African processing of maize: effect of iron contamination on bioaccessibility. *Food Chem.* 126, 1800–1807. <https://doi.org/10.1016/j.foodchem.2010.12.087>.
- Hama-Ba, F., Mouquet-Rivier, C., Diawara, B., Weltzien, E., Icard-Vernière, C., 2019. Traditional African dishes prepared from local biofortified varieties of pearl millet: acceptability and potential contribution to iron and zinc intakes of burkinabé young children. *Front. Nutr.* 6 <https://doi.org/10.3389/fnut.2019.00115>.
- Harmankaya, M., Ceyhan, E., Çelik, A.S., Sert, H., Kahraman, A., Özcan, M.M., 2016. Some chemical properties, mineral content and amino acid composition of cowpeas (*Vigna sinensis* (L.) Savi). *Qual. Assur. Saf. Crop. Foods* 8, 111–116. <https://doi.org/10.3920/QAS2014.0487>.
- Henshaw, F.O., Uzochukwu, S.V.A., Bello, I.Y., 2000. Sensory properties of akara (fried cowpea paste) prepared from paste stored at low storage temperatures. *Int. J. Food Prop.* 3, 295–304. <https://doi.org/10.1080/10942910009524635>.
- Hoppner, K., Lampi, B., 1993. Folate retention in dried legumes after different methods of meal preparation. *Food Res. Int.* 26, 45–48. [https://doi.org/10.1016/0963-9969\(93\)90104-Q](https://doi.org/10.1016/0963-9969(93)90104-Q).
- Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B.C., Nammi, S., Liyanage, R., 2018. Cowpea: an overview on its nutritional facts and health benefits: nutritional and health properties of cowpea. *J. Sci. Food Agric.* 98, 4793–4806. <https://doi.org/10.1002/jsfa.9074>.
- Kariluoto, S., Pironen, V., 2009. Total folate. In: Shewry, P.R., Ward, J.L. (Eds.), *HEALTHGRAIN Methods, Analysis of Bioactive Components in Small Grain Cereals*. AACC International. St Paul, Minnesota, USA, pp. 59–68.
- Lee, S., Inglett, G.E., 2007. Effect of an oat β-glucan-rich hydrocolloid (C-trim30) on the rheology and oil uptake of frying batters. *J. Food Sci.* 72, E222–E226. <https://doi.org/10.1111/j.1750-3841.2007.00326.x>.
- Lestienne, I., Icard-Vernière, C., Mouquet, C., Picq, C., Trèche, S., 2005. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* 89, 421–425. <https://doi.org/10.1016/j.foodchem.2004.03.040>.
- Madode, Y.E.E., Houssou, A.P., Linnemann, A., Hounhouigan, D., Nout, M.J., Boekel, M., 2011. Preparation, consumption, and nutritional composition of west African cowpea dishes. *Ecol. Food Nutr.* 50, 115–136. <https://doi.org/10.1080/03670244.2011.552371>.
- Madode, Y.E.E., Linnemann, A.R., Nout, M.J.R., Vosman, B., Hounhouigan, D.J., van Boekel, M.A.J.S., 2012. Nutrients, technological properties and genetic relationships among twenty cowpea landraces cultivated in West Africa. *Int. J. Food Sci. Technol.* 47, 2636–2647. <https://doi.org/10.1111/j.1365-2621.2012.03146.x>.
- McWatters, K.H., 1983. Compositional, physical, and sensory characteristics of akara processed from Cowpea Paste and Nigerian cowpea flour. *Cereal Chem.* 60, 333–336.
- Moreira, R.G., Sun, X., Chen, Y., 1997. Factors affecting oil uptake in tortilla chips in deep-fat frying. *J. Food Eng.* 31, 485–498. [https://doi.org/10.1016/S0260-8774\(96\)00088-X](https://doi.org/10.1016/S0260-8774(96)00088-X).
- Ndubuaku, V.O., Uwaegbute, A.C., Nnanyelugo, D.O., 1989. Flatulence and other discomforts associated with consumption of cowpea (*Vigna unguiculata*). *Appetite* 13, 171–181. [https://doi.org/10.1016/0195-6663\(89\)90010-X](https://doi.org/10.1016/0195-6663(89)90010-X).
- Njoumi, S., Bellagha, S., Icard-Vernière, C., Picq, C., Amiot, M.J., Mouquet-Rivier, C., 2018. Effects of cooking and food matrix on estimated mineral bioavailability in Mloukhiya, a Mediterranean dish based on jute leaves and meat. *Food Res. Int.* 105, 233–240. <https://doi.org/10.1016/j.foodres.2017.11.020>.
- Njoumi, S., Amiot, M.J., Rochette, I., Bellagha, S., Mouquet-Rivier, C., 2019. Soaking and cooking modify the alpha-galacto-oligosaccharide and dietary fibre content in five Mediterranean legumes. *Int. J. Food Sci. Nutr.* 0, 1–11. <https://doi.org/10.1080/09637486.2018.1544229>.
- Ologhobo, A.D., Fetuga, B.L., 1984. Distribution of phosphorus and Phytate in some Nigerian varieties of legumes and some effects of processing. *J. Food Sci.* 49, 199–201. <https://doi.org/10.1111/j.1365-2621.1984.tb13706.x>.
- Reddy, N.R., 2002. Occurrence, distribution, content, and dietary intake of phytate. In: Reddy, N.R., Sathe, S.K. (Eds.), *Food Phytates*. CRC Press, Boca Raton, FL, pp. 25–52.
- Rogério, W.F., Greiner, R., Nunes, I.L., Feitosa, S., Furtunato, D. M. da N., Almeida, D.T. de, 2014. Effect of preparation practices and the cowpea cultivar *Vigna unguiculata* L. Walp on the quality and content of myo-inositol phosphate in akara (fried bean paste). *Food Sci. Technol.* 34, 243–248. <https://doi.org/10.1590/fst.2014.0040>.
- Sebetha, E.T., Modis, A.T., Owoeye, L.G., 2014. Cowpea crude protein as affected by cropping system, site and nitrogen fertilization. *J. Agric. Sci.* 7 <https://doi.org/10.5539/jas.v7n1p224>.
- Singh, A., Hung, Y.-C., Phillips, R.D., Chinnan, M.S., McWatters, K.H., 2004. Particle-size distribution of cowpea flours affects quality of Akara (fried cowpea paste). *J. Food Sci.* 69, 243–249. <https://doi.org/10.1111/j.1365-2621.2004.tb13623.x>.
- Somari, R.I., Balogh, E., 1992. Hydrolysis of raffinose and stachyose in cowpea (*Vigna unguiculata*) flour, using α-galactosidase from *Aspergillus niger*. *World J. Microbiol. Biotechnol.* 8, 564–566. <https://doi.org/10.1007/BF01238789>.
- Seerama, Y.N., Neelam, D.A., Sashikala, V.B., Pratape, V.M., 2010. Distribution of nutrients and Antinutrients in milled fractions of chickpea and horse gram: seed coat phenolics and their distinct modes of enzyme inhibition. *J. Agric. Food Chem.* 58, 4322–4330. <https://doi.org/10.1021/jf903101k>.
- Seerama, Y.N., Sashikala, V.B., Pratape, V.M., Singh, V., 2012. Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: evaluation of their flour functionality. *Food Chem.* 131, 462–468. <https://doi.org/10.1016/j.foodchem.2011.09.008>.
- Strandler, H.S., Patring, J., Jägerstad, M., Jastrebova, J., 2015. Challenges in the determination of unsubstituted food folates: impact of stabilities and conversions on analytical results. *J. Agric. Food Chem.* 63, 2367–2377. <https://doi.org/10.1021/jf504987n>.
- Unal, H., Isik, E., Can Alpsoy, H., 2006. Some physical and mechanical properties of black-eyed pea (*Vigna unguiculata* L.) Grains. *Pak. J. Biol. Sci.* 9, 1799–1806. <https://doi.org/10.3923/pjbs.2006.1799.1806>.
- Winham, D.M., Hutchins, A.M., 2011. Perceptions of flatulence from bean consumption among adults in 3 feeding studies. *Nutr. J.* 10, 128. <https://doi.org/10.1186/1475-2891-10-128>.
- Xue, S., Ye, X., Shi, J., Jiang, Y., Liu, D., Chen, J., Shi, A., Kakuda, Y., 2011. Degradation kinetics of folate (5-methyltetrahydrofolate) in navy beans under various processing conditions. *LWT - Food Sci. Technol.* 44, 231–238. <https://doi.org/10.1016/j.lwt.2010.05.002>.
- Yadav, D.N., Rajan, A., 2012. Fibres as an additive for oil reduction in deep fat fried poori. *J. Food Sci. Technol.* 49, 767–773. <https://doi.org/10.1007/s13197-010-0218-7>.