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Pauline Pinel, Cecile Barron, Denis Cassan, Mélina Robert, Claire Bourlieu-Lacanal, Valérie Micard

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Culinary properties, nutritional quality, and *in vitro* starch digestibility of innovative gluten-free and climate smart cowpea-based pasta

P. Pinel, C. Barron, D. Cassan, M. Robert, C. Bourlieu-Lacanal [1], V. Micard [*,1]

Univ Montpellier, INRAE, Institut Agro, JRU IATE, Montpellier, France

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ABSTRACT

This study investigates the processability, culinary and rheological properties, biochemical composition and *in-vitro* starch digestibility of new gluten-free pasta formulated with whole cowpea flour alone or combined with teff and/or amaranth leaf flour(s). These pasta are compared with three wheat-based pasta with fiber content increasing from 4% to 16% g/100g, 16% g/100g being the average fiber content of cowpea-based pasta. The pasta were processed using low temperature extrusion and drying. The antioxidant properties of amaranth leaf flour facilitated extrusion by limiting excessive aggregation of the dough during hydration/mixing that is attributed to lipoxygenase activity of cowpea flour. The optimal cooking time and cooking losses of cowpea-based pasta were similar to wheat-based pasta with comparable fiber content, highlighting fiber as a key factor influencing culinary properties. Adding teff to cowpea-based pasta reduced cooking losses and firmness. Although some micronutrients were lost during pasta processing and cooking, the consumption of a cooked portion of 100 g of dry cowpea-based pasta still covered FAO nutritional recommendations for protein, fiber, iron, zinc, and vitamin B9 targeting adult women. Adding amaranth leaves helped meet recommended beta-carotene levels. The *in vitro* slowly digestible starch content of cowpea-based pasta is similar to or higher than that of wheat-based pasta.

1. Introduction

Pasta traditionally made from durum wheat semolina is a non-perishable, affordable and widely consumed staple food. It is an important source of carbohydrates (75 g/100 g dried pasta) and enables consumers to keep their glycemic index low (Granfeldt &Bjorck, 1990), but is also a significant source of protein (13–15 g/100 g dried pasta) (Canadian Nutrition File). However, in the fight against malnutrition and food related diseases, its high gluten content, lack of certain essential amino acids, low fiber content (3 g/100 g) and limited micronutrient content (Canadian Nutrition File) call for optimization of the nutritional composition of pasta.

In recent years, new unconventional ingredients have been added to traditional pasta, including whole wheat, and various cereal or legume flours. Their incorporation increases the fiber content and/or the quantity and quality of protein and/or micronutrients (Hirawan & Beta, 2014; Laleg, Cassan, Barron, Prabhasankar, & Micard, 2016). Among other benefits, associating legumes and cereals in pasta allows a better balance between lysine and sulfured essential amino acids while simultaneously reducing the gluten content (Wu, 2010). However, incorporating whole cereal or legume flours that are richer in fibers also increases antinutritional factors such as phytate, oxalate, trypsin inhibiting factors and negatively impacts protein digestibility or iron and zinc bioavailability (Samtiya, Aluko & Dhewa, 2020). Concerning starch digestibility, partial or total replacement of durum wheat semolina by legume in pasta, keeps its interesting low glycemic index (predicted or *in vivo*) as reported by Greffeuille et al. (2015) or Osorio-Díaz, Agama-Acevedo, Mendoza-Vinalay, Tovar, and Bello-Pérez (2008) with respectively, 35% faba bean or 40% chickpea addition.

Despite the nutritional benefits, total or partial replacement of durum wheat semolina by legume flours, with their high lipoxygenase content, can lead to the production of large dough aggregates during mixing that, in turn, cause extrusion problems (Laleg, Cassan, Abecassis, & Micard, 2021). The use of technical aids such as antioxidants, counterbalances this difficulty and makes it possible to make pasta with up to 100% legume flours (Laleg et al., 2021) but the addition of whole cereals (with or without gluten) or whole legume flours affects the structure of the pasta and reduces its culinary quality, defined as limited cooking

* Corresponding author.
E-mail address: valerie.micard@institut-agro.fr (V. Micard).
[1] The two authors have equal participation to this work.

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loss, desirable firmness and a bright yellow color (Pagani, Lucisano & Mariotti, 2007). Specifically, it increases its cooking loss, increases or decreases its firmness and darkens its color, thereby reducing consumer acceptance (Carini, Curti, Minucciani, Antoniazzi, & Vittadini, 2014; Laleg et al., 2016, 2017). Additives as well as specific processing conditions (i.e. extrusion-cooking, high temperature drying) can be used to offset the reduction in quality. However, additives may be perceived as unnatural by consumers, and the necessary processing conditions may have a negative impact on nutritional qualities by reducing lysine and micronutrient contents, (Petitot, Abecassis, & Micard, 2009) and process sustainability.

In this context, using gluten-free flours originating from crops that are resilient to diseases and climate hazards to formulate pasta enables nutritional optimization to be combined with ecological concerns. Climate-smart gluten-free flours can be obtained from legumes, cereals and other raw materials (FAO, 2017; Rachid, Bin Mushtaq, Farooq & Zulqurnain, 2021). Among these materials, the cowpea legume is of particular interest as it is resistant to drought (Ngalamu, Odra & Tongun, 2015) and extensively grown in Africa (9.5 million tons in 2022; FAOSTAT). Both drought resistant teff, a gluten-free cereal, amaranth, a pseudo-cereal, can grow in poor soils (Emmambux & Taylor, 2013). Leaves of amaranth are a source of fiber, vitamin C, carotenoids and minerals (Shukla et al., 2006), which make them an interesting ingredient. Only a few studies have been conducted of the impact of incorporating cowpea, teff or amaranth leaves on the nutritional and/or culinary properties of pasta (Bergman, Gualberto, & Weber, 1994; Borneo & Aguirre, 2008; Hager, Lauck, Zannini, & Arendt, 2012; Kahlon & Chiu, 2015; Cárdenas-Hernández et al., 2016; Lawal et al., 2021). Moreover, all the studies concerned used additives and/or high temperature drying to make the pasta, so the question is whether the additives can be omitted to reinforce both the clean label approach and the nutritional quality of the pasta.

In this context, the aim of the present work objective was to investigate the combined impact of formulation and processing on the structure, culinary properties, biochemical composition and *in vitro* starch digestibility of new gluten-free pasta formulated by linear programming (Pinel et al., 2024) using whole cowpea flour alone or combined with teff and/or amaranth leaf flour(s). To preserve the nutritional and clean label potential of these new pasta, we included no additives and only used low temperatures for extrusion and drying.

2. Material and methods

2.1. Material

Refined and whole durum wheat semolina and wheat bran were provided by La Semoulerie de Bellevue (Marseille, France), Markal (Saint Marcel lès Valence, France) and Alpina Savoie (Chambéry, France), respectively. Whole climate-smart gluten-free African cereals, legumes and other flours were kindly provided by the H2020 Innofood Africa project partners: teff (white), cowpea (white; *Bechuana*) and amaranth leaf flours were provided by Birkuta (UK), the University of Pretoria (South Africa) and Makerere University (Uganda), respectively. The median diameter (D50) of particle size of refined and whole durum wheat semolina, wheat bran, teff flour, cowpea flour and amaranth leaf flour were 309 μm, 320 μm, 391 μm, 87.5 μm, 260 μm and 441 μm, respectively.

2.2. Processing flour into pasta

In a previous study, four pasta formulations were theoretically nutritionally optimized by linear programming (Pinel et al., 2024). The different types of pasta were obtained from 8 gluten-free whole flours: teff, finger millet, faba bean, Bambara groundnut, cowpea, amaranth grain and leaves, orange fleshed sweet potato. Several criteria were respected during processing including FAO nutritional recommendations for adult women concerning the quality and quantity of protein, ω6/ω3 ratio, fiber, zinc, iron, beta-carotene and vitamin B9 for one meal i.e., based on 3 meals per day. The four optimized formulations, hereafter CW, CW-AL, CW-TEF and CW-TEF-AL, all based on cowpea, are listed in Table 1. In addition to the cowpea-based pasta, three wheat-based pasta controls were formulated: a 100% durum wheat semolina (S pasta; standard pasta with ~~4%~~ g/100g fiber content), a 100% whole wheat semolina (WS pasta; standard whole wheat pasta with ~~10%~~ g/100g fiber content) and a high fiber semolina (HFS) pasta made of 74.5% durum wheat semolina and 24.5% durum wheat bran with similar particle sizes (D50 = 309 ± 3 and 391 ± 4 μm, respectively) with ~~16%~~ g/100g fiber content, which is close to the fiber content of cowpea-based pasta. S, WS and HFS pasta were processed on the PLANET platform (JRU IATE, Montpellier, France) using a laboratory-scale single screw pasta extruder (Sercom, Montpellier, France). S, WS and HFS formulations (500 g) were hydrated at 47, 48 and ~~47%~~ g/100g (d.b.), respectively; their hydration percentage were calculated using a farinograph (Bradender, OGH, Duisburg, Germany) as described in Laleg et al. (2021). The ingredients were combined and mixed for 20 min at 120 rpm. CW, CW-AL, CW-TEF, CW-TEF-AL were processed as described for wheat-based pasta in Laleg et al. (2021) with minor modifications: for CW and CW-AL, the flours were hydrated to ~~42%~~ g/100g (d.b.) with cold tap water and, for CW-TEF and CW-TEF-AL, to ~~45%~~ g/100g (d.b.), and mixed for 15 min at 120 rpm. Ascorbic acid (Louis François, Marne la vallée, France, E300) was added in the hydration water (375 mg/500g flour d.b.) to delay oxidation and limit the formation of excessive dough aggregates during the processing of cowpea-based pasta. The agglomeration properties of the doughs were evaluated in triplicate by weighing the amount of all agglomerates bigger than 4 mm (Petitot, Boyer, Minier, & Micard, 2010). All the wheat and cowpea-based doughs were extruded at low temperature (40 °C, 31 rpm, 9×10^6 to 1.4×10^7 Pa, spaghetti die) and dried in a pilot-scale drier (CTS, Hechingen, Germany) at low temperature (55 °C, relative humidity = 80%) to reach ~~12%~~ g/100g w.b. final moisture content (Petitot et al., 2010). S, WS, HFS, CW, CW-AL, CW-TEF and CW-TEF-AL dry spaghetti had a diameter of 1.57, 1.55, 1.52, 1.46, 1.46, 1.46 and 1.45 μm respectively. All the pasta were produced in quadruplicate and pooled into a single batch for further analyses. For biochemical analyses and supramolecular structure of protein network, the dry or optimally cooked pasta were freeze-dried and ground before analyses.

2.3. Culinary properties of pasta

OCT was determined according to the AACC 66-51 method on 7 cm dry spaghetti cooked in Evian mineral water. A special attention had to be paid on OCT of gluten-free pasta, as the disappearance of the central core of gluten-free pasta is more difficult to determine than the one of gluten-based pasta. Further analyses were performed on pasta cooked at their own OCT. Water uptake was measured as described by Petitot et al. (2010). Cooking losses were obtained using the AACC method 66-50 and expressed as ~~%~~ g/100g of dry pasta (d.b.). A Minolta Chromameter (Model CR-400, Minolta Co., Osaka, Japan) was used to determine the color of dry and cooked pasta in terms of L* (lightness), a* (redness) and b* (yellowness). Each measurement of OCT, cooking loss and color was performed on three different cooking batches. Firmness of cooked pasta

Table 1
Formulations (% w/w d.b.) of the four cowpea-based pasta optimized by Pinel et al. (2024) using linear programming.

Flours	CW	CW-AL	CW-TEF	CW-TEF-AL
Cowpea	100	90	60	55
Teff	/	/	40	35
Amaranth leaves	/	10	/	10

AL = Amaranth leaves; CW =Cowpea; TEF = Teff.

after 5 min resting time was determined with a TA-XT plus (Stable Micro Systems, Scarsdale, USA) using the AACC method 66–52.01 as described in Petitot et al. (2010) (contact force 0.05 N). Five measurements were taken on three different cooking batches (5 × 3 cooking batch, resulting in n = 15 characterizations).

2.4. Particle size distribution of the raw materials and the pasta

Particle size distribution of refined and whole durum wheat semolina, durum wheat bran, and wheat-based and cowpea-based pasta (i.e. ground dry pasta, and ground OCT cooked freeze-dried pasta) were analyzed with a laser diffraction particle size analyzer Coulter LS 13320XR (Beckman Coulter Inc., Fullerton, USA) in dry mode with a laser diffraction refractive index of 1.54. Particle size is described using the median diameter (D_{50}) calculated from the particle size distribution expressed as volume.

2.5. Biochemical composition of dry and cooked pasta

Aminograms, protein, fiber, lipid, starch, sugar, vitamin, mineral, ash, phytic acid and trypsin inhibitor activity (TIA) of all ground dry and OCT cooked freeze-dried pasta were determined using the methods detailed in Pinel et al. (2024). Oxalates were determined using an enzymatic assay kit (OXALATE-100; Libios, Vindry-sur-Turdine, France). All analyses were performed in triplicate and are reported on a dry weight basis. The median diameter (D50) of particle size of ground dry and OCT cooked freeze-dried pasta were: 522 μm, 541 μm, 566 μm, 524 μm, 533 μm, 562 μm and 516 μm for dry, and 261 μm, 231 μm, 290 μm, 249 μm, 217 μm, 267 μm, 259 μm for OCT cooked freeze-dried S, WS, HFS, CW, CW-AL, CW-TEF and CW-TEF-AL respectively.

2.6. Pasta structure

2.6.1. Microstructure of cooked pasta

Pasta were cooked at their OCT and cross sections (8 μm thick) were cut at −16 °C using a cryomicrotome (Microm HM 520, Walldorf, Germany) with a cryoprotector (Cellpath, Newtown, UK) (Laleg et al., 2016). The sections were stained with acridine orange in acidified water (1 g/L) (Sigma Aldrich Co., USA) and then with Congo red 1 g/L (RAL Diagnostic, Sweden). Light microscopy was performed using a Ti2 microscope (Nikon, Japan) equipped with a color digital camera (Fi3, Nikon, Japan) and with a plan-APO 10× objective (Nikon, Japan).

2.6.2. Supramolecular structure of protein network of dry and cooked pasta

Proteins were extracted from ground dry and OCT cooked freeze-dried pasta in triplicate using the method of Petitot et al. (2009) with modifications. Two extractions were conducted in parallel: one at 60 °C for 80 min with sodium dodecyl sulfate (SDS) buffer (SDS, 0.1M) and a second with SDS-dithioerythritol (DTE) buffer (20 mM DTE) in the same conditions, followed by 5 min sonication. After centrifugation for 30 min at 18000 rpm, the supernatant protein content of each extract was estimated using the N amount measured using the Kjeldahl method (NF V 03–050, 1970) with a multiplicative factor of 5.88, 5.78, 5.73, 5.24, 5.25, 5.37 and 5.37 for S, WS, HFS, CW, CW-AL, CW-TEF and CW-TEF-AL, according to their amino acid composition (Mariotti, Tome & Mirand, 2008). The proteins in the first SDS-soluble extract were weakly bound. Disulfide-bonded proteins, DTE-soluble proteins, were obtained by subtracting the proteins in fraction 2 (SDS + DTE soluble) and in fraction 1 (SDS-soluble proteins). The SDS + DTE non-extractable protein, covalent bonded, were calculated as the difference between the total protein in the pasta and the protein in the SDS + DTE fraction. All protein fractions are expressed as a percentage of total protein.

2.7. Starch digestibility of cooked pasta

All pasta samples were cooked at their OCT, after which 10 ± 1 g were ground for 10 s in a grinder (IKA A10, Staufen, Germany) to obtain a particle size close to that of pasta masticated by humans (Englyst, Kingman, & Cummings, 1992). Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were determined using a K-DSTRS enzymatic assay kit (Megazyme, Co. Wicklow, Ireland). RDS and SDS correspond to, respectively, starch digested in 20 min or between 20 and 120 min. RS is the starch that is still not digested after 4 h (Englyst et al., 1992). Each measurement was performed in triplicate.

2.8. Statistics

Results are expressed as means ± standard deviation. Variance analysis (ANOVA), Fisher's least significant difference (LSD) test and Pearson's correlation matrix were used to compare means at the 5% significance level using XlStat (Addinsoft, v 2022, Paris, France). Different letters indicate means with significant difference at $p < 0.05$.

3. Results and discussion

Our results are presented and discussed paying particular attention to the impact of the addition of teff and/or amaranth leaves on the culinary properties, nutritional composition and starch *in vitro* digestibility of cowpea-based pasta. In addition, the innovative cowpea-based pasta is compared to the three wheat-based pasta to focus on the impact of the absence of gluten and of the high fiber content on the above-mentioned pasta properties.

3.1. Ability of flours to be processed into pasta

Formulations are processable into pasta if they agglomerate during the hydration-mixing step at a specific particle size without blocking the following extrusion step. Table 2 lists the percentage of dough particles more than 4 mm in size, a factor known to complicate extrusion or even make it impossible (Laleg et al., 2021; Petitot et al., 2010) for the four hydrated cowpea-based formulations. The hydration-mixing step of cowpea alone generated 8 to 24 times more particles >4 mm in size than when associated with 40% teff (CW-TEF) or 10% amaranth leaves (CW-AL). The combined addition of amaranth leaves and teff to cowpea (CW-TEF-AL) did not further reduce the proportion of dough particle >4 mm in size.

Laleg et al. (2021) showed that the aggregation phenomenon is due to enzymatic oxidation, notably the action of lipoxygenases that catalyze PUFA oxidation. Table 2 also lists the theoretical agglomeration potential of cowpea-based formulations reported by Pinel et al. (2024) according to the lipoxygenase activity and antioxidant capacity (i.e. obtained by determining the ability of flour to reduce ABTS radicals) of the raw materials. Putting the ratio oxidant/antioxidant of formulations with their agglomeration behavior experimentally measured here into perspective, we confirm that the predictions made by Pinel et al. (2024) were correct for CW and CW-TEF-AL which had, respectively, the

Table 2
Agglomerates >4 mm, lipoxygenase activity and antioxidant capacity of doughs mixed for 15 min.

Composition of mixed doughs	Agglomerates >4 mm%	Lipoxygenase activity U/mg d.b	Antioxidant capacity mmol TEAC/kg d.b
CW	96.6 ± 13.4	3122 ± 20	15.6 ± 2.2
CW-AL	11.7 ± 1.7	2810 ± 18	31.0 ± 14.2
CW-TEF	4.1 ± 1.8	1873 ± 12	13.6 ± 9.4
CW-TEF-AL	3.7 ± 1.0	1717 ± 11	29.2 ± 8.9

Mean +standard deviation of three replications; Lipoxygenase activity and antioxidant capacity are taken from Pinel et al. (2024); TEAC = Trolox equivalent antioxidant capacity; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaves; CW-TEF = 60% cowpea +40% teff; CW-TEF-AL = 55% cowpea +35% teff + 10% amaranth leaves.

highest and lowest oxidant/antioxidant ratio and the highest and lowest agglomeration behaviors upon mixing. They are thus the respectively the most difficult and the easiest formulations to process.

According to Pinel et al. (2024), it should be more difficult to predict the agglomeration behavior of the CW-AL and CW-TEF formulations as they combine high lipoxygenase activity with high antioxidant capacity, and low lipoxygenase activity with quite low antioxidant activity, respectively. Their hydration-mixing showed that CW-AL was in fact three times more susceptible to agglomeration than CW-TEF (Table 2). Therefore, the 1.6-fold decrease in lipoxygenase activity (reported by Pinel et al. (2024) with the addition of teff in cowpea formulation) reduced the particle size of the dough compared to the two-fold increase in their antioxidant capacity (reported with the addition of amaranth leaves to cowpea formulations). Even if lipoxygenase activity therefore appears to be an interesting parameter to anticipate the potential future agglomeration during the hydration-mixing step, here we demonstrate that the relation between its activity and the amount of large agglomerates is not linear. Further studies including different raw materials are thus needed to establish a predictive model.

3.2. *Culinary properties of pasta*

Culinary properties such as optimal cooking time (OCT), water uptake, firmness and color of cowpea-based and wheat-based pasta are listed in Table 3. The OCT and water uptake of wheat-based pasta decreased around by 20% and cooking loss increased by 51% when its fiber content increased from 4~~%~~ to 16~~%~~ g/100g. The decrease in OCT was almost two times greater than the 10% decrease described by Aravind, Sissons, Egan, and Fellows (2012) for durum wheat semolina pasta whose fiber content increased from 5~~%~~ to 14~~%~~ g/100g. Firmness decreased drastically by 45% between S pasta and the two other wheat pasta richer in fibers (WS and HFS; with 10~~%~~ g/100g and 16~~%~~ g/100g of fiber respectively) with no difference between the two. Aravind et al. (2012) also reported a marked decrease in firmness in the case of 0~~%~~–10~~%~~ g/100g added bran, and stabilization with 10~~%~~–30~~%~~ g/100g bran added to wheat pasta (5~~%~~–14~~%~~ g/100g fiber). The OCT, water uptake and cooking losses of cowpea-based pasta containing 13.4~~%~~–19.7~~%~~ g/100g of fibers and no gluten, were closer to those of HFS pasta (16~~%~~ g/100g fiber). Cooking losses of cowpea-based pasta and HFS ranged between 9.5 and 11.3 g/100g dry weight of the pasta, almost twice higher than the value of S, was higher than the threshold of 7–8 g/100g reported for a pasta of a good quality (Sissons, Abecassis, Marchylo & Cubadda, 2012, pp. 231–234). Cooking losses higher than 7–8 g/100g Cooking losses of 10–11%, (i.e. almost twice higher than the value of S pasta), have been also described by Garcia-Valle, Bello-Pérez, Agama-Acevedo, and Alvarez-Ramirez (2021) and Rosa-Sibakov et al. (2016) for 100% chickpea and faba bean pasta. Their results are in agreement with those of In addition, Bergman, Gualberto & Weber (19946), who reported a cooking loss of from 8 to 9.2 g/100g for a soft wheat pasta enriched with 10–30% cowpea flour, far less than the 55%–100% cowpea flour used in our cowpea-based pasta. In cowpea-based pasta, the addition of teff did not impact OCT but decreased cooking losses, which may be linked to the lower fiber content of CW-TEF compared to CW (13.4 vs.17.7 g/100g). In association with amaranth leaves, the addition of teff led to a decrease in OCT and also decreased cooking losses compared to CW despite an increase in fiber content in the tripartite pasta. The simultaneous addition of three raw matters could have led to a higher disruption of the pasta structure facilitating starch gelatinization compared to CW but not leading to destructuration nor to higher cooking losses.

Table 4 shows the Pearson correlation matrix that highlights some linear relationships between biochemical and structural parameters and culinary properties of interest for all wheat-based and cowpea-based pasta. Despite the use of the same die, the diameter of cooked pasta (Fig. 1) ~~which~~ is larger in wheat-based than in cowpea-based pasta (1.57 vs. 1.45 mm). In addition to important differences in pasta protein network and structure between the two pasta, this could be also linked to the lower starch content of cowpea-based pasta compared to wheat-based pasta (41–56% vs. around 70%). Indeed, during cooking, the swelling of starch granules due to gelatinization, may have taken up less place compared with wheat-based pasta, thus reducing the diameter, which was also reported by Flores-Silva et al. (2014). Diameter of pasta is positively correlated with water uptake and OCT, and negatively correlated with cooking losses. A decrease in pasta diameter reduces the time water needs to reach the center of the pasta and reduces the pasta OCT, consequently a positive correlation was found between OCT and water uptake. The culinary parameters were also all governed by fiber content, i.e., induced by the presence of numerous fibers (seed coats of cowpea, envelopes of wheat grain, etc.) observed by microscopy in cooked HFS and cowpea-based pasta (Fig. 1). They alter the continuity of whole pasta structure and could disrupt the pasta protein network. This promotes nutrient losses during cooking and accelerates the penetration of water into pasta, which speeds up starch gelatinization and hence reduces the OCT. Indeed, a negative correlation was observed (−0.788) between OCT and cooking loss in our pasta, which are rich in fibers. In addition to the diameter and fiber content of pasta, the starch content also impacts culinary properties, as reported in Laleg et al. (2016). Cowpea-based pasta have a lower starch content, 41–56~~%~~ g/100g vs. around 70~~%~~ g/100g for S and WS pasta (Table 5) which can explain the lower OCT and water uptake. The agglomeration state of protein network also plays a positive role, but to a lesser extent, as observed by Laleg et al. (2016) in 100% legume pasta, especially in

Table 3
Culinary properties of wheat-based and cowpea-based pasta.

	Wheat-based pasta			Cowpea-based pasta			
	S	WS	HFS	CW	CW-AL	CW-TEF	CW-TEF-AL
Optimal cooking time (min)	11.2 ± 0.3^{a}	10.3 ± 0.3^{b}	9.0 ± 0.0^{c}	8.8 ± 0.3^{cd}	8.6 ± 0.1^{d}	8.6 ± 0.1^{d}	7.4 ± 0.1^{e}
Water uptake (% d.b)	167.9 ± 2.1^{a}	158.6 ± 3.5^{a}	133.1 ± 0.7^{bc}	126.0 ± 11.8^{cd}	129.8 ± 6.5$_{bcd}$	118.3 ± 3.0^{d}	137.6 ± 9.9^{b}
Cooking loss (% d.b)	6.3 ± 0.1^{e}	8.9 ± 0.4^{d}	9.5 ± 0.1^{cd}	11.3 ± 0.4^{a}	10.7 ± 0.1^{ab}	10.1 ± 0.2^{bc}	9.9 ± 0.8^{c}
Firmness (N)	8.7 ± 0.7^{ab}	5.1 ± 0.3^{d}	4.8 ± 0.3^{d}	9.1 ± 0.3^{a}	8.6 ± 0.2^{b}	6.1 ± 0.5^{c}	5.9 ± 0.2^{c}
Color of pasta							
Dry pasta							
L*	45.0 ± 0.5^{a}	38.8 ± 0.3^{b}	36.8 ± 0.1^{c}	22.8 ± 0.1^{c}	21.5 ± 0.2^{d}	21.6 ± 0.0^{d}	21.0 ± 0.1^{d}
a*	1.5 ± 0.1^{f}	7.7 ± 0.3^{b}	8.1 ± 0.1^{a}	5.9 ± 0.1^{c}	3.3 ± 0.0^{e}	5.3 ± 0.0^{d}	3.4 ± 0.1^{e}
b*	19.8 ± 0.4^{a}	17.1 ± 0.4^{b}	14.1 ± 0.1^{c}	1.3 ± 0.0^{c}	−1.0 ± 0.1^{d}	−0.9 ± 0.0^{d}	−1.4 ± 0.0^{e}
Cooked pasta							
L*	58.7 ± 1.2^{a}	54.4 ± 1.2^{b}	50.0 ± 1.4^{c}	43.3 ± 0.6^{d}	37.4 ± 1.4^{f}	41.8 ± 0.4^{d}	39.3 ± 0.8^{e}
a*	−0.7 ± 0.0^{f}	4.2 ± 0.2^{c}	6.1 ± 0.1^{b}	7.0 ± 0.4^{a}	2.7 ± 0.2^{d}	6.8 ± 0.1^{a}	2.9 ± 0.2^{e}
b*	16.3 ± 0.4^{a}	14.6 ± 0.8^{b}	15.3 ± 0.7^{b}	10.4 ± 0.7^{c}	8.0 ± 0.5^{d}	7.7 ± 0.2^{d}	9.1 ± 0.1^{d}

Means with the same letter within a row are not significantly different ($p < 0.05$); Mean ± standard deviation of three repetitions (except for firmness n = 15); S = 100% durum wheat semolina pasta; WS = whole durum wheat semolina pasta; HFS = 100% durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaf pasta; CW-TEF = 60% cowpea +40% teff pasta; CW-TEF-AL = 55% cowpea +35% teff + 10% amaranth leaf pasta.

Table 4
Pearson's correlation matrix between parameters of wheat-based and cowpea-based pasta.

Variables	OCT (min)	Cooking loss (%)	Water uptake (%)	Firmness (g)	Fibers (%)	Proteins (%)	Starch (%)	Covalently bonded proteins (%)	Diameter of pasta (mm)
OCT (min)	**1**	**−0.788**	**0.789**	0.181	**−0.850**	−0.546	**0.796**	0.742	**0.913**
Cooking loss (%)		**1**	**−0.867**	0.019	**0.926**	**0.766**	**−0.900**	**−0.879**	**−0.839**
Water uptake (%)			**1**	0.018	**−0.821**	0.642	**0.801**	**0.780**	**0.869**
Firmness (g)				**1**	−0.010	0.642	−0.319	−0.400	−0.168
Fibers (%)					**1**	0.713	**−0.935**	**−0.812**	**0.913**
Proteins (%)						**1**	**−0.909**	**−0.950**	**−0.800**
Starch (%)							**1**	**0.945**	**0.899**
Covalently bonded proteins (%)								**1**	**0.922**
Diameter (mm)									**1**

Fiber, protein and starch content of dry pasta; OCT = Optimal cooking time; Values in bold differ from 0 with a significant level of $p < 0.05$.

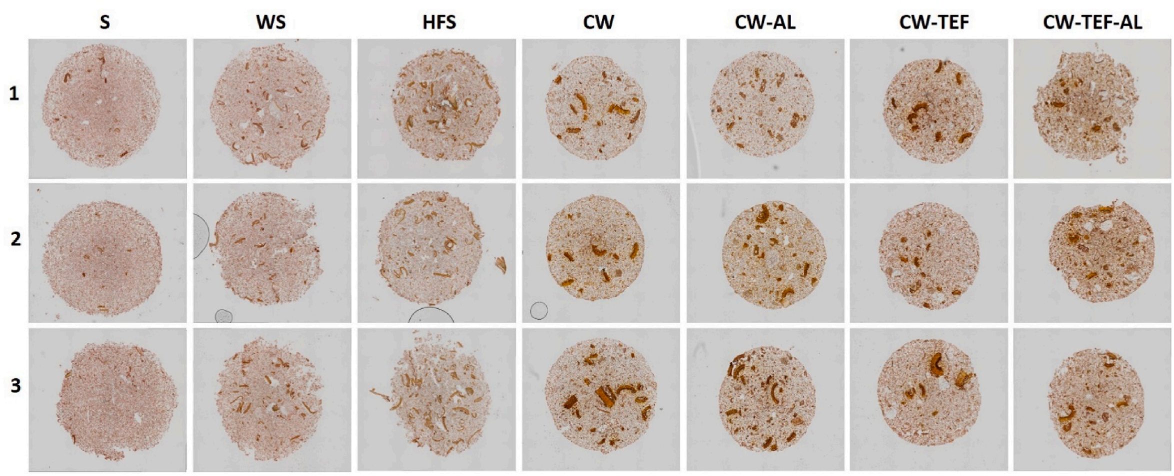

Fig. 1. Light microscopy images of wheat-based and cowpea-based cooked pasta. Cross sections stained with acridine orange.
Field of view for all the images = 3.5 mm*3 mm; S = durum wheat semolina pasta; WS = whole durum wheat semolina pasta; HFS = durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaves; CW-TEF = 60% cowpea +40% teff; CW-TEF-AL = 55% cowpea +35% teff +10% amaranth leaves; Three replicates (1,2,3) corresponding to 3 different strands of pasta. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cooking losses, as shown in Fig. 2. The agglomeration of protein network is related to the ratio of covalent *versus* weak protein bonds (Laleg et al., 2017). Despite similar covalent-bonded protein contents in dry wheat-based and cowpea-based pasta (34–39% of DTE-soluble ~~plus~~ and non-extractable proteins) except for CW (22%), the cooking step causes significantly different changes in protein aggregation between wheat-based and cowpea-based pasta. Cooking increased the protein agglomeration particularly in wheat-based pasta. In contrast, cooked CW and CW-AL pasta had a lower protein agglomeration state, with only 38% of total protein bonded by covalent bonds (Fig. 2), followed by CW-TEF and CW-TEF-AL (52%), while wheat-based pasta had the highest protein agglomeration state (76–87%).

In contrast to all the other culinary parameters, in all our pasta, there was almost no correlation between firmness and the other culinary properties and only a limited correlation was found with either diameter, fiber and starch contents, or with the covalently-bounded protein content. Only the percentage of proteins seemed to be slightly positively correlated with firmness in all the pasta as reported by Del Nobile, Baiano, Conte, and Mocci (2005). The firmness of CW and CW-AL was similar to that of S pasta, whereas the addition of 40% teff alone or combined with amaranth leaf flour in cowpea-based pasta led to a 33–35% decrease in firmness. Among cowpea-based pasta, protein content was shown to be able to play a role, as, interestingly, a linear increase ($R^2 = 0.99$) in firmness was observed as a function of protein content. This was also observed by Rosa-Sibakov et al. (2016) in pasta made of 100% faba bean flour or starch.

The color of the pasta has a significant influence on consumer preferences. Among wheat-based pasta, a decrease in L* and b*, and an increase in a* was observed between S and WS/HFS for dry and cooked pasta. In comparison to wheat-based pasta, all dry cowpea-based pasta were darker (lower L*) with lower yellowness (b*) (Table 3). The decrease in L* was also reported by Petitot et al. (2010) and Bergman et al. (1994) for pasta made with 35% faba bean and 30% cowpea flour compared to dry pasta made from durum wheat. Concerning redness (a*), cowpea-based pasta containing amaranth leaves (alone or combined with teff) were closer to WS pasta, and between S pasta and WS. When cooked, the same trend was observed but the difference in b* between wheat-based and cowpea-based pasta was less marked, and a* which shifts towards HFS in those with no amaranth leaf. The color of the raw material is one of the parameters that has the most influence on the final color of the pasta due to the presence of anthocyanin and carotenoid pigments in cowpea and amaranth leaf flours, as reported by Laleg et al. (2016) for pasta made with 100% legume flour. Fiber and ash can also influence pasta color as reported for wheat-based pasta. According to Aravind et al. (2012), the addition of 10–30% bran to wheat pasta leads to a lower L*, higher a* and lower b* in dry pasta. Finally, the enzymatic oxidative reactions that occur during pasta processing may cause browning and hence to a decrease in L* and to an increase in the a* value of dry pasta in cowpea-based pasta, as reported by Laleg et al. (2016).

Table 5
Biochemical composition of dry and cooked wheat-based and cowpea-based pasta.

Wheat-based pasta				Cowpea-based pasta			
Dry pasta	**S**	**WS**	**HFS**	**CW**	**CW-AL**	**CW-TEF**	**CW-TEF-AL**
Protein (*g*/100 g d.b*)* (conversion Factor)	13.4 ± 0.1^{h} (5.88)	12.9 ± 0.1^{i} (5.78)	13.5 ± 0.1^{h} (5.73)	21.0 ± 0.4^{c} (5.24)	20.5 ± 0.2^{b} (5.25)	16.6 ± 0.1^{f} (5.37)	16.4 ± 0.1^{f} (5.37)
Starch (*g*/100 g d.b*)*	72.9 ± 1.8^{b}	68.2 ± 1.8^{c}	57.7 ± 0.8^{e}	43.2 ± 1.5^{i}	41.1 ± 1.1^{j}	55.9 ± 0.3^{ef}	50.3 ± 0.7^{g}
Fiber (*g*/100 g d.b.*)* (% insoluble fiber)	3.5 ± 0.8 (86%)	9.6 ± 2.0 (94%)	16.3 ± 2.0 (95%)	17.7 ± 2.0 (89%)	19.7 ± 2.0 (93%)	13.4 ± 2.0 (92%)	16.6 ± 2.0 (94%)
Lipids (*g*/100 g d.b.*)*	1.4 ± 0.0^{f}	1.4 ± 0.0^{fg}	1.4 ± 0.1^{f}	1.6 ± 0.1^{e}	1.6 ± 0.0^{e}	2.3 ± 0.0^{c}	2.5 ± 0.0^{a}
Sugars[a] (*g*/100 g d.b.*)*	0.9 ± 0.0^{de}	1.2 ± 0.1^{c}	1.7 ± 0.1^{a}	0.5 ± 0.0^{h}	0.8 ± 0.0^{f}	1.7 ± 0.1^{a}	1.5 ± 0.1^{b}
Ashes (*g*/100 g d.b.*)*	1.0 ± 0.1^{k}	1.6 ± 0.0^{i}	2.1 ± 0.1^{h}	3.6 ± 0.1^{c}	4.6 ± 0.1^{a}	3.0 ± 0.1^{e}	4.2 ± 0.1^{b}
Zinc (*mg*/100 g d.b.*)*	1.9 ± 0.2	4.3 ± 0.4	4.9 ± 0.4	4.1 ± 0.4	5.4 ± 0.5	4.4 ± 0.4	5.1 ± 0.5
Iron (*mg*/100 g d.b.*)*	4.4 ± 0.6	4.5 ± 0.6	4.7 ± 0.6	40.0 ± 5.3	52.6 ± 6.9	27.4 ± 3.6	32.8 ± 4.3
Beta-carotene (*mg*/100 g d.b.*)*	<0.01	<0.01	<0.01	<0.01	1.40 ± 0.42	<0.01	1.17 ± 0.35
Vitamin B9 (*μg*/100 g d.b.*)*	19 ± 6	30 ± 9	34 ± 10	504 ± 151	493 ± 148	320 ± 96	337 ± 101
Cooked pasta	**S**	**WS**	**HFS**	**CW**	**CW-AL**	**CW-TEF**	**CW-TEF-AL**
Protein (*g*/100 g d.b*)*(conversion Factor)	13.0 ± 0.0^{g} (5.88)	12.5 ± 0.1^{h} (5.78)	13.1 ± 0.1^{g} (5.73)	22.1 ± 0.0^{a} (5.24)	21.4 ± 0.1^{b} (5.25)	17.0 ± 0.2^{e} (5.37)	16.9 ± 0.3^{e} (5.37)
Starch (*g*/100 g d.b*)*	76.3 ± 0.9^{a}	69.8 ± 1.6^{c}	59.9 ± 1.2^{d}	46.8 ± 0.5^{h}	41.4 ± 0.6^{ij}	55.3 ± 0.2^{f}	50.0 ± 0.8^{g}
Fiber (*g*/100 g d.b.*)*(% insoluble fiber)	4.5 ± 1.2 (84%)	11.2 ± 2.0 (88%)	18.9 ± 2.0 (94%)	20.9 ± 2.0 (92%)	23.6 ± 2.0 (97%)	16.3 ± 2.0 (90%)	19.7 ± 2.0 (90%)
Lipids (*g*/100 g d.b.*)*	1.4 ± 0.0^{gh}	1.3 ± 0.0^{h}	1.3 ± 0.1^{h}	1.7 ± 0.1^{d}	1.7 ± 0.0^{d}	2.5 ± 0.0^{a}	2.4 ± 0.1^{a}
Sugars[a] (*g*/100 g d.b.*)*	0.5 ± 0.1^{gh}	0.8 ± 0.1^{ef}	0.9 ± 0.1^{d}	0.3 ± 0.0^{h}	0.3 ± 0.0^{f}	0.6 ± 0.1^{g}	0.6 ± 0.0^{gh}
Ashes (*g*/100 g d.b.*)*	0.7 ± 0.0^{l}	1.2 ± 0.0^{j}	1.6 ± 0.0^{i}	2.7 ± 0.0^{f}	3.6 ± 0.0^{c}	2.4 ± 0.0^{g}	3.2 ± 0.0^{d}
Zinc (*mg*/100 g d.b.*)*	1.9 ± 0.2	4.5 ± 0.5	5.4 ± 0.5	4.7 ± 0.5	5.6 ± 0.6	4.4 ± 0.4	5.4 ± 0.5
Iron (*mg*/100 g d.b.*)*	6.2 ± 0.9	5.5 ± 0.8	4.8 ± 0.7	53.2 ± 7.8	48.4 ± 7.1	33.1 ± 4.9	43.8 ± 6.4
Beta-carotene (*mg*/100 g d.b.*)*	<0.01	<0.01	<0.01	0.02 ± 0.01	1.04 ± 0.31	0.01 ± 0.00	0.90 ± 0.27
Vitamin B9 (*μg*/100 g d.b.*)*	<10	14 ± 4	18 ± 6	323 ± 97	302 ± 91	196 ± 59	211 ± 63

Means with the same letter within a row are not significantly different ($p < 0.05$); Mean ± standard deviation of three repetitions; S = durum wheat semolina pasta; WS = whole durum wheat semolina pasta; HFS = durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaf pasta; CW-TEF = 60% cowpea +40% teff pasta; CW-TEF-AL = 55% cowpea +35% teff + 10% amaranth leaf pasta.

[a] Sugar = glucose + fructose + sucrose.

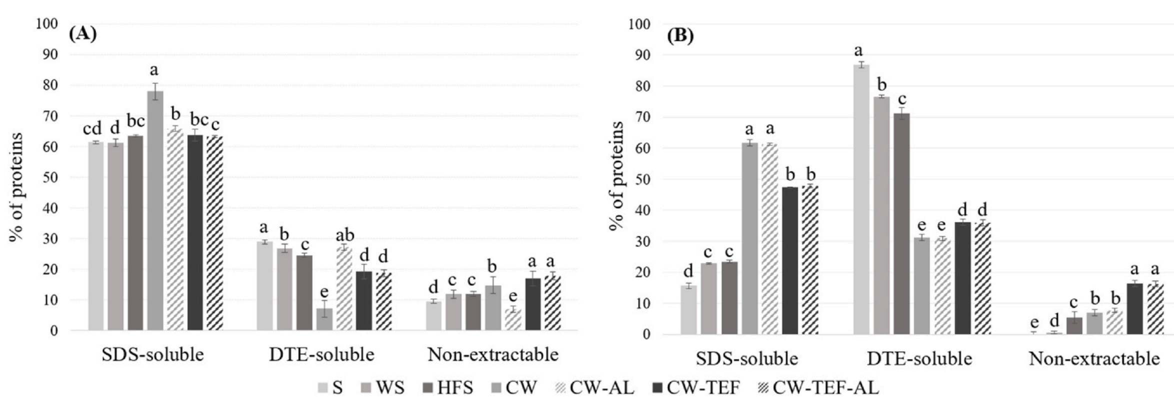

Fig. 2. Protein solubility in sodium dodecyl sulfate (SDS) and dithioerythritol (DTE) of dry (A) and cooked (B) wheat-based and cowpea-based pasta
Bars with the same letter within the same fraction are not significantly different ($p < 0.05$); Mean ± standard deviation of three repetitions; S = durum wheat semolina pasta; WS = whole durum wheat semolina pasta; HFS = durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaves; CW-TEF = 60% cowpea +40% teff; CW-TEF-AL = 55% cowpea +35% teff + 10% amaranth leaves.

3.3. Nutritional quality of dry and cooked pasta

3.3.1. Biochemical composition of dry pasta

Table 5 presents the biochemical composition of dry cowpea-based pasta and wheat-based pasta. The starch, fiber, ash and zinc contents (only formulations containing AL) of all dry cowpea-based pasta are closer to HFS. However, they are 1.2–1.6 times richer in protein, 6 to 12 times richer in iron, 9 to 27 times richer in B9 vitamin and have a twice higher chemical score than all the wheat-based pasta (Table 6). Among all dry cowpea-based pasta, CW-AL pasta were shown to have the highest nutritional potential, especially that concerning fibers and micronutrients.

Despite the nutritional interest of cowpea-based pasta, some anti-nutritional factors limit their benefit. The phytic acid content of dry cowpea-based pasta is more than twice higher than that of S and closer to WS in CW-AL and closer to HFS in the three others cowpea-based pasta (Table 7). The use of whole cowpea flours to make cowpea-based pasta and use of the outer layer of the wheat kernel (bran) to make WS and HFS pasta explains why. Indeed, Chouchene, Micard & Lullien-Pellerin (2018) reported a high concentration of phytic acid in the outer layer of the wheat kernel and in the germ of pea seeds whereas the concentration of phytic acid in cowpea-based pasta remained twice lower than in pasta made with 100% faba bean and lentil flours (1.5~~%~~ g/100g and 1.8~~%~~ g/100 g d.b. respectively; Laleg et al., 2016). Trypsin inhibitors (whose activity was evaluated by measuring TIA) were detected in all the dry cowpea-based pasta but were undetectable in all the wheat-based pasta (Table 7). These results are in accordance with those reported by Otegbayo, Adebiyi, Bolaji, and Olunlade (2018) and

Table 6
Essential amino acids (g/100 g protein) and chemical score of dry and cooked wheat-based and cowpea-based pasta.

	Wheat-based pasta			Cowpea-based pasta			
Dry pasta	**S**	**WS**	**HFS**	**CW**	**CW-AL**	**CW-TEF**	**CW-TEF-AL**
His	2.3 ± 0.3	2.5 ± 0.3	2.83 ± 0.4	3.9 ± 0.5	3.6 ± 0.5	3.3 ± 0.4	3.4 ± 0.4
Ile	3.6 ± 0.5	3.6 ± 0.5	3.58 ± 0.5	5.0 ± 0.6	4.9 ± 0.6	4.6 ± 0.6	4.8 ± 0.6
Leu	7.3 ± 0.9	7.5 ± 1.0	7.25 ± 0.9	9.2 ± 1.2	8.9 ± 1.2	8.5 ± 1.1	9.0 ± 1.2
Lys	2.3 ± 0.3	2.6 ± 0.3	2.75 ± 0.4	6.7 ± 0.9	6.5 ± 0.8	5.1 ± 0.7	5.5 ± 0.7
Met	1.7 ± 0.1	1.7 ± 0.1	1.75 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	2.2 ± 0.1	2.3 ± 0.1
Phe	4.9 ± 0.6	4.9 ± 0.6	4.75 ± 0.6	6.8 ± 0.9	6.5 ± 0.8	6.2 ± 0.8	6.4 ± 0.8
Thr	2.8 ± 0.4	3.0 ± 0.4	3.08 ± 0.4	4.5 ± 0.6	4.4 ± 0.6	4.0 ± 0.5	4.2 ± 0.5
Trp	1.1 ± 0.2	1.1 ± 0.2	1.25 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.2	1.4 ± 0.3
Val	4.2 ± 0.5	4.3 ± 0.6	4.42 ± 0.6	6.0 ± 0.8	5.8 ± 0.8	5.6 ± 0.7	5.9 ± 0.8
Chemical score	49	54	57	116	122	105	114
Cooked pasta	**S**	**WS**	**HFS**	**CW**	**CW-AL**	**CW-TEF**	**CW-TEF-AL**
His	2.6 ± 0.3	2.7 ± 0.3	3.1 ± 0.4	3.9 ± 0.5	3.8 ± 0.5	3.4 ± 0.4	3.4 ± 0.4
Ile	3.9 ± 0.5	4.0 ± 0.5	3.9 ± 0.5	5.3 ± 0.7	5.2 ± 0.7	4.8 ± 0.6	4.8 ± 0.6
Leu	7.9 ± 1.0	8.0 ± 1.0	7.9 ± 1.0	9.7 ± 1.3	9.5 ± 1.2	9.1 ± 1.2	9.1 ± 1.2
Lys	2.6 ± 0.3	2.7 ± 0.4	3.0 ± 0.4	7.3 ± 0.9	7.0 ± 0.9	5.4 ± 0.7	5.7 ± 0.7
Met	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	2.6 ± 0.2	2.3 ± 0.1
Phe	5.6 ± 0.7	5.2 ± 0.7	5.0 ± 0.7	6.9 ± 0.9	6.9 ± 0.9	6.5 ± 0.8	6.6 ± 0.9
Thr	2.9 ± 0.4	3.3 ± 0.4	3.5 ± 0.5	4.5 ± 0.6	4.4 ± 0.6	4.3 ± 0.6	4.4 ± 0.6
Trp	1.1 ± 0.2	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.3
Val	4.6 ± 0.6	4.8 ± 0.6	4.9 ± 0.6	6.3 ± 0.8	6.1 ± 0.8	5.9 ± 0.8	5.8 ± 0.8
Chemical score	53	57	63	117	123	112	118

Mean ± standard deviation; S = durum wheat semolina pasta; WS = whole durum wheat semolina pasta; HFS = durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaf pasta; CW-TEF = 60% cowpea +40% teff pasta; CW-TEF-AL = 55% cowpea +35% teff + 10% amaranth leaves; Cys = Cystein; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Trp = Tryptophan; Tyr = Tyrosine; Val = Valine.

Table 7
Antinutritional factors of dry and cooked wheat-based and cowpea-based pasta.

	Wheat-based pasta			Cowpea-based pasta			
Dry pasta	**S**	**WS**	**HFS**	**CW**	**CW-AL**	**CW-TEF**	**CW-TEF-AL**
Phytic acid *(g/100 g d.b.)*	0.35 ± 0.02^{g}	0.67 ± 0.02^{f}	0.87 ± 0.01^{c}	0.81 ± 0.03^{d}	0.72 ± 0.02^{e}	0.82 ± 0.03^{d}	0.82 ± 0.02^{d}
TIA *(mg/100 g d.b.)*	$< DL^{f}$	$< DL^{f}$	$< DL^{f}$	624 ± 31^{a}	630 ± 38^{a}	397 ± 20^{b}	413 ± 1^{b}
Oxalates *(mg/100 g d.b.)*	45 ± 3^{g}	56 ± 6^{fg}	69 ± 6^{ef}	24 ± 3^{h}	307 ± 32^{c}	77 ± 8^{e}	418 ± 21^{a}
Phytate/Iron *(molar ratio)*	6.7	12.6	15.7	1.7	1.2	2.5	2.1
Phytate/Zinc *(molar ratio)*	18.2	15.5	17.6	19.4	13.3	18.5	15.9
Cooked pasta	**S**	**WS**	**HFS**	**CW**	**CW-AL**	**CW-TEF**	**CW-TEF-AL**
Phytic acid *(g/100 g d.b.)*	0.36 ± 0.01^{g}	0.72 ± 0.01^{e}	0.98 ± 0.01^{a}	0.87 ± 0.02^{c}	0.83 ± 0.01^{d}	0.92 ± 0.02^{b}	0.90 ± 0.02^{bc}
TIA *(mg/100 g d.b.)*	$< DL^{f}$	$< DL^{f}$	$< DL^{f}$	196 ± 5^{c}	176 ± 7^{d}	156 ± 1^{e}	156 ± 4^{e}
Oxalates *(mg/100 g d.b.)*	50 ± 3^{g}	50 ± 3^{g}	67 ± 5^{ef}	10 ± 2^{i}	197 ± 17^{d}	80 ± 7^{e}	328 ± 12^{b}
Phytate/Iron *(molar ratio)*	4.9	11.0	17.3	1.4	1.5	2.4	1.7
Phytate/Zinc *(molar ratio)*	18.6	15.7	18.0	18.3	14.6	20.7	18.0

Means with the same letter within a row are not significantly different ($p < 0.05$); Mean ± standard deviation of three repetitions; S = durum wheat semolina pasta; WS = whole durum wheat semolina pasta; HFS = durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaves; CW-TEF = 60% cowpea +40% teff; CW-TEF-AL = 55% cowpea +35% teff + 10% amaranth leaves; < DL = Below detection level.

Kostekli and Karakaya (2017), who found no TIA in either refined wheat or whole wheat flours. Among the four dry cowpea-based pasta, CW-TEF and CW-TEF-AL had 1.5 lower TIA due to the low TIA of teff flour (Pinel et al., 2024). Finally, the concentration of soluble oxalates ranged from 24 to 418 mg/100g in dry cowpea-based pasta. The highest values (13–17 times higher than the lowest value) were measured in CW-AL and CW-TEF-AL, due to the high concentration of soluble oxalate (446 mg/100g) in amaranth leaves reported by Pingle and Ramasastri (1978). Nevertheless, CW-AL still have the highest nutritional potential.

3.3.2. *Impact of pasta processing on their biochemical composition*

The biochemical composition of each dry cowpea-based pasta was compared with the composition of the corresponding flour formulations (from Pinel et al. (2024)) and with the composition of cooked cowpea-based pasta, in order to identify macro- or micronutrient losses throughout the course of pasta processing (hydration/mixing, extrusion and drying steps) (Fig. 3A) and during cooking (Fig. 3B). Depending on the formulation of the cowpea-based pasta, their processing resulted in 6–15% lipid losses, 18–22% fiber losses, 10–76% losses of sugars (excluding starch), 17–100% losses of beta-carotene and 25–29% losses of vitamin B9. The high losses of sugar observed in CW-AL and CW-TEF-AL, and the high losses of beta-carotene observed in CW-TEF and CW-TEF-AL could be due to the low initial contents of these nutrients (0.5–0.8 g sugar/100g and 0.01 mg beta-carotene/100g) in corresponding formulations, which in the event of even the slightest loss, would result in a high percentage of losses. Furthermore, despite having no significant impact on protein content, processing the pasta results in a 10–17% decrease in lysine in cowpea-based pasta, together with a 14% decrease in methionine observed in CW-TEF (results not shown). The observed decrease in lysine is in line with that reported by Dias Paes & Maga (2004) who found a 11% decrease in lysine content when extrusion was used. Starch, iron, zinc and ash are concentrated in pasta. The decrease in vitamin B9 and beta-carotene could be induced by oxidative reactions and light, as reported by Riaz, Asif, and Ali (2009). Indeed, Paiva & Russel (1999) reported the antioxidant capacity of

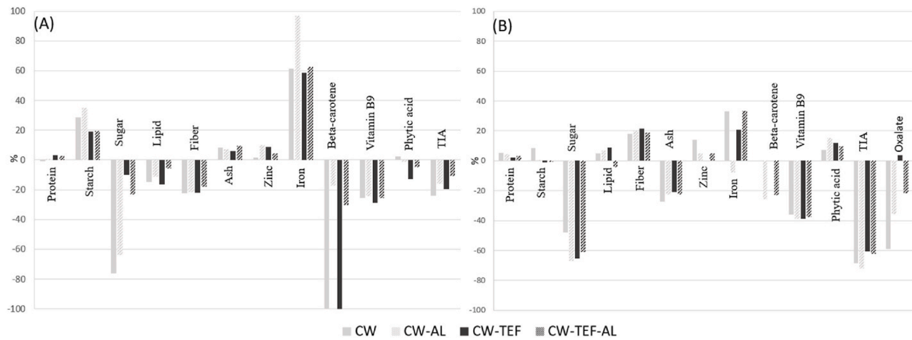

Fig. 3. Losses or concentration (in %) of macro- and micronutrients of interest between: A) Theoretical expectations (Pinel et al., 2024) and dry pasta B) Dry and cooked pasta
CW = 100% cowpea; CW-AL = 90% cowpea +10% amaranth leaves; CW-TEF = 60% cowpea +40% teff; CW-AL-TEF = 55% cowpea +35% teff +10% amaranth leaves; sugar = glucose + fructose + sucrose; positive values correspond to nutrient concentrations and negative values correspond to nutrient losses.

beta-carotene. As mentioned in section 3.1, the antioxidant capacity of pasta containing amaranth leaf flour helps reduce the oxidative reaction that occurs during hydration/mixing, and thus reduces the risk of over agglomeration of the dough. Cooking resulted in further losses of sugars (excluding starch) (48–67%), vitamin B9 (36–39%) and beta-carotene (23–26%) (Fig. 3B). Losses of beta-carotene were only observed in CW-AL and CW-TEF-AL as the beta-carotene content of CW-TEF and CW is already almost zero in dry pasta (<0.01 mg/100g) with no change during cooking. These results are similar to or lower than the results reported by Agte, Tarwadi, Mengale, Hinge, and Chiplonkar (2002), who found 32% and 46% losses of B9 and beta-carotene vitamin, respectively, during cooking of raw vegetables. Ash losses (21–27%) only occur during cooking. Protein content is not affected by cooking and essential amino acids may even be slightly concentrated (Table 6). Laleg et al. (2016) also reported no significant impact of cooking on the quantity or quality of protein in 100% lentil or faba bean pasta.

Concerning antinutritional factors, processing pasta only led to a 5–13% decrease in phytic acid in both cowpea-based pasta containing teff but to a larger (11–24%) decrease in TIA (Fig. 3A) in all cowpea-based pasta. Cooking reduced TIA 2.5–3 fold, and reduced the concentration of oxalate in cowpea-based pasta by 22–58% (with the exception of in CW-TEF) and the concentration of phytates (7–15%) in all cowpea-based pasta (Fig. 3B). TIA losses are in accordance with those reported by Zhao, Manthey, Chang, Hou, and Yuan (2005) who observed a 5 fold decrease in TIA during cooking of 80:20 w/w wheat: chickpea pasta. Indeed, the native structure of trypsin inhibitors is maintained by disulfide which, altered during thermal treatment, modifies the structure of the active site, consequently reducing TIA. The TIA of cooked cowpea-based pasta ranged from 156 to 196 mg, in line with the results obtained by Laleg et al. (2016), who found 248 and 152 mg TIA/100 g d. b. for 100% faba bean and lentil cooked pasta, respectively. Pingle and Ramasastri (1978) also reported that cooking had an impact on oxalates with a 90% decrease in soluble oxalates between raw and cooked amaranth leaves.

3.3.3. *Theoretical bioavailability of iron and zinc*

Iron and zinc bioavailability can be influenced by the presence of phytic acid which links with divalent cation thanks to its negative charge (Sandberg, 2002). Furthermore, soluble oxalates can form insoluble calcium oxalate, or other insoluble mineral salts (magnesium and iron), which decrease their bioavailability (Hodgkinson, 1981). Although indicators can be calculated to predict the impact of phytic acid on zinc and iron bioavailability, no indicators exist to approximate the impact of oxalates. The bioavailability of iron is affected if the molar ratio of phytate/iron is above 1 (FAO/IZiNCG, 2018). This ratio varied between 1.4 and 2.4 in cooked cowpea-based pasta (CW < CW-AL < CW-TEF-AL < CW-TEF), meaning that iron bioavailability is very likely impacted (Table 7). Even when iron bioavailability was affected, it was still much better than that reported by Wiesinger, Cichy, Hooper, Hart, and Glahn (2020) for cooked bean-based pasta (phytate/iron ratio between 10.0 and 13.6) and that of wheat-based pasta (4.9–17.3) (Table 7). Phytate/zinc ratios of less than 5, between 5 and 15, and more than 15, have been associated with high, moderate and low zinc bioavailability (FAO, 2004). Cowpea-based pasta had a molar ratio of 14.6 for CW-AL and ranged from 18.0 to 20.7 for CW-TEF-AL, CW-TEF and CW (CW-TEF-AL < CW < CW-TEF), making CW-AL the pasta with the highest iron and zinc bioavailability, better than all three wheat-based pasta (Table 7).

3.3.4. *Nutritional coverage of a portion of pasta*

FAO nutritional recommendations for adult women in one meal (i.e. 1/3 of daily requirements) covered by the consumption of a serving size of each cooked pasta (corresponding to 100 g of dry pasta w.b. which have been cooked) are presented in Fig. 4. The recommendation for coverage targets women as this population is at greater risk than men, particularly in terms of iron. The iron recommendation selected corresponds to 10% iron bioavailability as the phytate/iron ratio of cooked cowpea-based pasta was calculated as being above 1 and the general iron bioavailability in the diet in emerging and Western countries is considered to be, respectively, 5–10% and 12–15% (FAO, 2004). Concerning zinc, 30% zinc bioavailability was chosen for the FAO recommendation, in line with the theoretically moderate (30%) zinc bioavailability estimated from the phytate/zinc ratio of our cooked cowpea-based pasta.

Despite some nutrient losses due to pasta processing and cooking, and taking the negative impact of phytate on iron and zinc bioavailability into account, the consumption of a serving size of each cooked cowpea-based pasta more than matches FAO nutritional recommendations for protein, fiber and iron, zinc and vitamin B9 for adult women in one meal compared to S (Fig. 4). The consumption of a serving size of the two cooked cowpea-based pasta containing only 10% of amaranth leaf flour almost covers the beta-carotene recommendation for one meal (29–31% of the daily recommended amount). Among the wheat-based pasta, HFS is the closest to cowpea-based pasta in terms of fiber and zinc coverage in one meal, followed by WS pasta. However, none of the wheat-based pasta are able to cover the required quantity of iron, B9 and beta-carotene in one meal. A serving size of each of the four cooked cowpea-based pasta even reached 108–161% of daily iron recommendations, and CW-AL and CW-TEF-AL also reached, respectively, 103% and 106% of daily recommendation for zinc.

3.4. *In vitro starch digestibility of cooked pasta*

Fig. 5 shows starch digestibility classified as rapidly digestible (RDS), slowly digestible (SDS), and resistant (RS) starch, according to Englyst et al. (1992). This classification makes it possible to predict the glycemic index of pasta (Englyst & Hudson, 1996). Cowpea-based pasta starch is

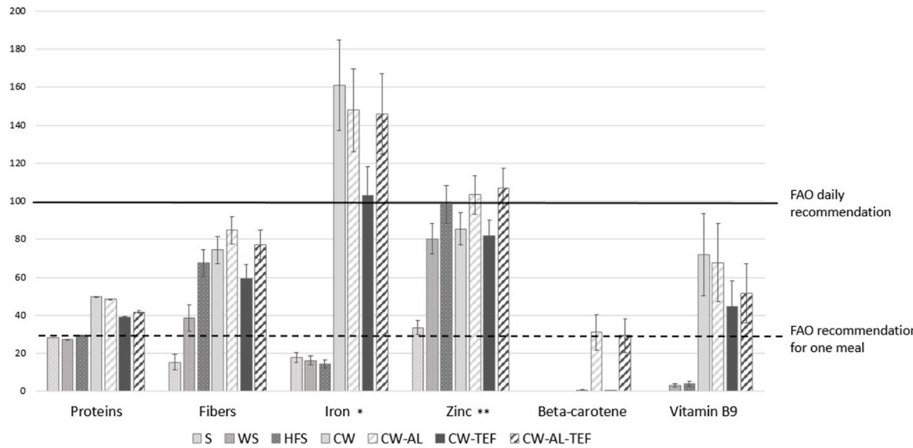

Fig. 4. Nutritional coverage (% coverage of FAO recommendation for women[1]) of a serving size of the cooked[#] cowpea-based pasta in comparison with wheat-based pasta

*# 100g of dry (w.b.) pasta that have been cooked and correspond to 268, 259, 233, 226, 230, 218 and 238 g of cooked S, WS, HFS, CW, CW-AL, CW-TEF and CW-TEF-AL pasta respectively; S = durum wheat semolina; WS = whole durum wheat semolina pasta; HFS = durum wheat semolina pasta enriched with bran; CW = 100% cowpea; CW-AL = 90% cowpea + 10% amaranth leaves; CW-TEF = 60% cowpea + 40% teff; CW-AL-TEF = 55% cowpea + 35% teff + 10% amaranth leaves; * Based on the FAO, 2004 recommendation for 10% iron bioavailability; ** Based on the FAO, 2004 recommendation for zinc bioavailability as moderate; [1]FAO Carbohydrates and Fibers= 2003 recommendation, Vitamin and Minerals = 2004 recommendation, Proteins = 2007 recommendation.*

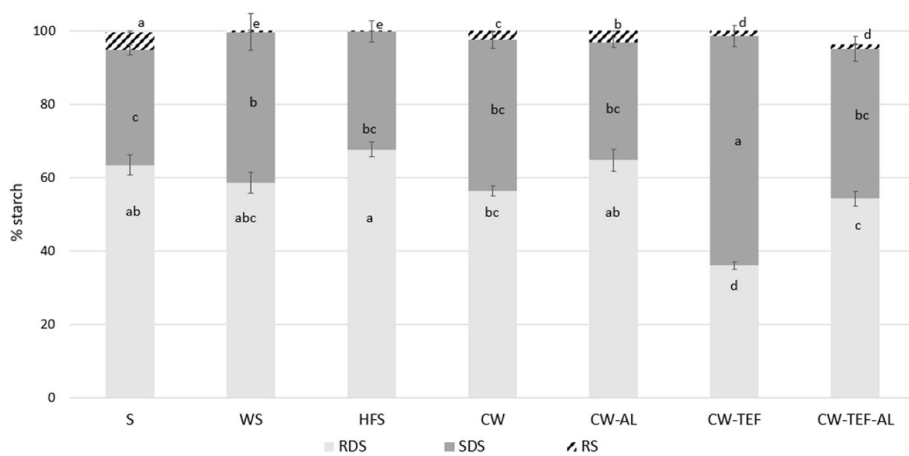

Fig. 5. Fractions of rapidly digestible (RDS), slowly digestible (SDS) and resistant (RS) starch (% total starch) of cooked cowpea-based pasta and wheat-based pasta Means with the same letter within the same starch fraction are not significantly different ($p < 0.05$); Means ± SD of three repetitions; S = 100% Durum wheat semolina pasta; WS = Whole durum wheat semolina pasta; HFS = 100% Durum wheat semolina pasta enriched with bran; CW = 100% Cowpea; CW-AL = 90% Cowpea +10% amaranth leaves; CW-TEF = 60% Cowpea +40% teff; CW-TEF-AL = 55% Cowpea +35% teff + 10% amaranth leaves; The “Remaining starch”, starch digested between 2h and 4h is not shown in this figure.

composed of 36–65~~%~~ g RDS/100g starch ~~RDS~~, 32–62~~%~~ g SDS/100g starch ~~of SDS~~ and 1–3~~%~~ g RS/100g starch ~~of RS~~. Their RDS contents are similar or lower than the RDS of wheat-based pasta, suggesting cowpea-based pasta has a low glycemic index. These results are in accordance with those of Pinel, Emmambux, Bourlieu, and Micard (2023) who reported that the addition of climate-smart gluten-free raw materials (legumes or cereals) to pasta leads to a slight to significant decrease in the RDS fraction or glycemic index compared to pasta made from durum wheat semolina. Among cowpea-based pasta, the addition of 10% amaranth leaves had no significant effect on the RDS fraction (Fig. 5) in contrast to the addition of 40% teff alone (i.e. CW-TEF) that decreased the RDS fraction by 35% (Fig. 5), making it a cowpea-based pasta that could have a lower GI than wheat-based pasta.

Although gluten-free cowpea-based pasta with a weaker protein network (i.e. lower disulfide bond proteins, Fig. 2) and more fibrous structures, disrupt the pasta structure (Fig. 1), have a similar or an even lower RDS fraction than wheat-based pasta can be explained by several parameters. Cowpea-based pasta have 1.2 to 1.6 times higher protein content (Table 5), which can act as a physical barrier to starch digestive enzymes (Rosa-Sibakov et al., 2016). In addition, by reducing digestive protease attacks, the presence of TIA in cooked cowpea-based pasta (Table 7) can enhance the protection of starch embedded in the protein network, which hinders its hydrolysis by amylase (Rooney & Pflugfelder, 1986). The decrease in the RDS fraction in CW-TEF among cowpea-based pasta could be explained by its higher state of protein agglomerate compared to CW and CW-AL (52% vs. 38% of protein linked by covalent bonds Fig. 2) combined with the fact it has the lowest fiber content of all the cowpea-based pasta (13~~%~~ g/100 g d.b. compared to 17–20~~%~~ g/100 g d.b., Table 5) maintains higher pasta structure integrity. This result is in line with that obtained by Giuberti, Gallo, Fiorentini, Fortunati, and Masoero (2015) who found a low predicted glycemic index of 46 with a combination of 60% teff and 40% common bean in pasta.

4. Conclusion

Far better than standard wheat pasta, cowpea-based pasta, nutritionally optimized through linear programming meets the nutritional recommendation of FAO for adult women in terms of proteins (quantity and quality), fiber, zinc, iron, vitamin B9 and beta-carotene (when amaranth leaves are added) despite the negative impact of pasta processing and cooking on vitamins. Even though some antinutritional factors persist after processing and cooking, the predicted bioavailability of iron and zinc are higher than those of all wheat-based pasta and exhibit a low predicted GI with culinary quality surpassing other legume pasta. The culinary qualities are mainly influenced by fiber content, which has a major impact on pasta structure. Other parameters can play a role, including protein content, and the absence of gluten which influence the state of protein agglomerate. Thanks to the antioxidant capacity of amaranth leaves, their addition to pasta formulation facilitates mixing and subsequently the extrusion process and significantly increases the micronutrient contents of pasta and their iron and zinc bioavailability. Adding teff flour to pasta formulation helps reduce cooking loss and also reduces firmness of cowpea-based pasta. Among the four cowpea-based pasta, CW-AL stands out as the best compromise in terms of technological feasibility, nutrient content, iron and zinc bioavailability, satisfactory predicted GI, and culinary qualities. Further evaluation of protein digestibility and sensory properties of cowpea-based pasta is needed and a life cycle assessment of cowpea production, is an interesting future perspective.

CRediT authorship contribution statement

P. Pinel: Writing – original draft, Investigation, Formal analysis, Data curation. **C. Barron:** Writing – review & editing, Data curation. **D. Cassan:** Investigation. **M. Robert:** Investigation. **C. Bourlieu-Lacanal:** Writing – review & editing, Validation, Supervision, Resources. **V. Micard:** Writing – review & editing, Validation, Supervision, Resources.

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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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Data availability

Data will be made available on request.

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