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Nutritional optimization through linear programming of climate-smart and gluten free pasta

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ABSTRACT

Designing food formulations is an important approach to meet a set of nutritional needs and to address malnutrition issues. Linear programming is an appropriate tool for designing novel nutritionally optimized formulations based on a combination of gluten-free climate-smart crops. Four nutritionally optimized cowpea-based pasta formulations in association or not with teff and/or amaranth leaves (AL) were designed to meet a woman's protein requirements in terms of quantity and quality, fibers, ω6/ω3, iron, zinc and B9 vitamin, while limiting antinutritional factors such as phytates. To predict the processability of the flours into pasta and the visual acceptance of future pasta by consumers, the antioxidant and oxidant capacities of flours were measured and their color compared with the color of durum wheat semolina (DWS). The formulation combining cowpea and AL had the highest nutritional composition and lowest impact of phytates. The formulation with cowpea, teff and AL seems to be the easiest to process thanks to its lower lipoxygenase activity and the higher antioxidant capacity, followed by the teff-cowpea formulation. The color of the formulation that only contained cowpea was closest to DWS.

1. Introduction

The increase in malnutrition (undernutrition, overnutrition and micronutrient deficiency) requires appropriate solutions to fight this worldwide public health problem. Nutritional optimization of staple food makes it possible to supply essential nutrients to a large proportion of the population. Pasta is consumed worldwide and world production reached 16.9 million tons in 2021 (https://internationalpasta.org/annual-report/). Dry pasta is non-perishable, affordable, traditionally made from Durum Wheat Semolina (DWS) using a simple three step process (hydration-mixing, extrusion or lamination, drying) achievable at different scales (from homemade to industrial scale). However, despite some nutritional benefits such as high carbohydrate content with a low glycemic index (Atkinson, Brand-Miller, Foster-Powell, Buyken, & Goletzke, 2021) and non-negligible protein content (13 g/100g dried pasta), traditional pasta contains gluten, lacks some essential Amino Acids (AA) (notably lysine) and has only small quantities of fibers (3 g/100g dried pasta) and micronutrients (Canadian Nutrition File = https://food-nutrition.canada.ca/cnf-fce/?lang=eng).

Pasta formulations can be improved by adding natural food ingredients and/or fortifiers such as whole wheat semolina and/or legume flour to partially or totally replace the DWS. Such formulations increase the quantity and quality of protein, and/or fiber and/or micronutrients (Giménez et al., 2012; Laleg, Cassan, Barron, Prabhasankar, & Micard, 2016; Vignola, Bustos & Pérez, 2018). Adding legumes to pasta improves its essential AA balance and composition, as legumes are rich in lysine but deficient in sulfur-containing AA that are largely present in wheat (Wu, 2010). However, they contain antinutritional factors (ANF) such as phytate and trypsin inhibitors, that affect micronutrient (iron and zinc) bioavailability and protein digestibility. Even when reduced by processing such as germination, soaking or cooking, as reviewed by Samtiya, Aluko, and Dhewa (2020), the high concentrations of ANF in legumes must be taken into account. However, it has also been demonstrated that adding large quantities of legume flour, which is rich in lipoxygenase (LOX) to pasta, has a negative impact on the extrusion step (Laleg, Cassan, Abecassis, & Micard, 2021). Furthermore, total or partial substitutions of DWS by legume flours or even by whole wheat semolina can affect the organoleptic qualities of pasta such as its bright yellow color, and consequently affect consumer acceptance (Carini, Curti, Minucciani, Antoniazzi, & Vittadini, 2014).

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Consequently, to satisfy future consumers, these changes must be taken into account when developing new nutritionally optimized pasta.

The next step in optimizing the formulation of pasta could be the use of "climate-smart gluten free" (hereafter C-GF) raw materials of high nutritional value, i.e. crops that are resilient to diseases and climate hazards. In this case, nutritional optimization is combined with agroecological approaches and has already resulted in impressive progress in terms of food system sustainability (Roos et al., 2022). In Africa, a wide range of traditional crops have both agronomic and nutritional benefits and are socially and culturally accepted, but are currently not fully valued.

In recent years, linear programming, which is mainly used for diet optimization (for a review see Van Dooren, 2018), has also been increasingly used for food nutrition optimization. In the latter case, linear programming has mainly been used to formulate products that meet the needs of targeted populations, e.g. for therapeutic purposes, or for food for children (Bechman, Phillips & Chen, 2015; Wirawan, Fahmida, Purwestri, Timan, & Hegar, 2022; Ngume, Katalambula, Munyogwa, Mongi, & Lyeme, 2023) and to a lesser extent to optimize the nutritional composition of specific foods for the general population such as ice cream (Lopes, Martins Mesquita, Valença de Sousa & Ferreira-Dias, 2018) or yogurt (Dusabe, Chacha, Vianney, & Raymond, 2022).

The aim of the present study was to develop nutritionally optimized pasta formulations incorporating 100% C-GF raw materials such as whole cereal, pseudo-cereal, legume or others that are widely produced in Africa, by using linear programming, and to predict the processability and color of these future pasta products.

2. Material and methods

2.1. Material

Wholegrain African cereals, legumes and other climate-smart gluten free (C-GF) flours were provided by the H2020 Innofood Africa project partners: Cowpea white (*Bechuana*) (CW), Bambara groundnut (creamish white) (BG) and Orange Flesh Sweet Potato (OFSP) were provided by the University of Pretoria (South Africa); Amaranth grain (white) (AG), Amaranth leaves (AL) and Finger millet (FM) (Naromill 5-SEC915-light tan color) flours were provided by Makerere University (Uganda); Faba bean (Kontu) (FB) and Teff (white) (TEF) flours were provided by Vihreähärkä (Finland) and Birkuta (UK), respectively. In the remainder of this article, the term "flours" refers to wholegrain African C-GF flours (from cereals, legumes and other sources).

2.2. Characterization of flours

Particle size distribution of flours was measured in triplicate using a Beckman Coulter LS 13 320 XR laser diffraction analyzer (Beckman Coulter, Fullerton, USA).

The biochemical composition of all the flours was determined and predicted for all the formulations obtained by linear programming. Aminograms (NF EN ISO 13903) were produced by CAPINOV laboratory (Landerneau, France). Kjeldahl method (NF V 03-050, 1970) was used to determine total protein content using a conversion factor that accounts for the difference in AA profiles between flours (Sosulki & Imafidon, 1990). The Chemical Scores (CS) of the flours were calculated according to Block and Mitchell (1946) based on FAO (2011) recommendations for essential AA for people over 3 years old. Total lipid and fatty acid contents were obtained by Folch extraction and gas chromatography according to Cancalon et al. (2023). Total and damaged starch as well as sugar (glucose, fructose, sucrose) and phytic acid contents were analyzed using commercial kits (Megazyme, Co. Wicklow, Ireland); AACC method 76–13.01 and method 76–31.01 for total and damaged starch; K-SUFRG kit for sugars; K-PHYT kit for phytic acid). Trypsin Inhibitor Activity (TIA) was analyzed according to standard ISO 14902 (2009) and ash content according to ISO EN 2171:210. Total, soluble and insoluble fibers (MI MONU85 Enzymatic method and AOAC 991.43), iron, zinc (MI MONU05 ICP-OES), B9 vitamin (MP-01309-DE:2021-03) and beta carotene (NF EN 12823-2) were performed by CAPINOV laboratory (Landerneau, France). Characterizations were performed in triplicate with the exception of characterizations operated by CAPINOV laboratory, expressed in duplicate.

The Oxidant and Antioxidant Capacities (AOC) of all the flours were measured and predicted by calculation for formulations obtained by linear programming. Free phenolic compounds were analyzed using the modified Folin-Ciocalteu method corrected by polyvinylpolypyrrolidone (PVPP) (Bridi et al., 2014; Hainal, Ignat, Volf, & Popa, 2011). Phenolic extraction was carried out in MilliQ water/-Ethanol (40:60 v/v) solution for 1 h at 50 °C. The proportion of Folin reagent (Sigma-Aldrich, St Louis, 0.2N) to the sample was 2.5:1. Absorbance was measured at 730 nm with a microplate reader (TECAN, Spark, Männedorf, Switzerland). Gallic acid (Sigma-Aldrich, St Louis, US) was used as a standard, and the results are expressed in mg of gallic acid equivalent (GAE)/g sample. Values obtained with PVPP were subtracted from the total phenolic compounds to obtain the final concentrations of phenolic compounds in the flours. The AOC of all the flours were determined in triplicate as Trolox Equivalent Antioxidant Capacity (TEAC) according to Rosa, Barron, Gaiani, Dufour, and Micard (2013) (the method adapted from Serpen, Gokmen, Pellegrini, and Fogliano (2008)). The LOX activity of the flours was evaluated as detailed in Szymanowska, Jakubczyk, Baraniak, and Kur (2009). Briefly, after LOX extraction, the volume of reactive mixture was 249.7 μL with 34 μL of substrate (linoleic acid 2.5 mM), 10 μL of sample (supernatant) and 205.7 μL of phosphate buffer (pH 5.5). One LOX activity unit corresponds to an increase of 0.001 absorbance in 1 min at 234 nm (25 °C). Results are expressed in U/mg flour. All characterization analyses were performed in triplicate and on dry weight basis.

A Minolta Chromameter (Model CR-400, Minolta Co., Osaka, Japan) was used to determine the color of all flours and formulations (flour mixture) in terms of L* (lightness), a* (redness) and b* (yellowness). Each measurement was performed in triplicate.

2.3. Linear programming for nutritional optimization

Linear programming solves optimization problems i.e. finds the minimum or the maximum of a linear function (the objective function) under a set of linear constraints (equalities or inequalities figuring the limits of the problem) as reviewed in Vishwakarma, Genu Dalbhagat, Mandliya & Niwas Mishra (2022). The simulations were run several times with the General Algebraic Modeling System (GAMS) software version 23.8 using the CONOPT solver by varying the number of flours involved in formulation (one to three). The equation of our models can be written as:

$$Min \sum_j Phytates_j * X_j$$

$$\sum_i a_{ij} * X_j \geq b_i$$

$$X_j \geq 0$$

where $Phytates_j$ represents the quantity of phytates in 1 g of flour j and X_j is the quantity of flour j used to make 100 g of dry pasta. a_{ij} is the quantity of nutrient i in 1 g of flour j and the b_i are the criteria used for the resulting formulation.

2.4. Statistical analysis

Results are expressed as means ± SD. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test were used to compare means at the 5% significance level using Microsoft XLSTAT software 2022 (Addinsoft, Paris, France). Different letters indicate statistically significant differences ($P \leq 0.05$). Externalized biochemical analyses on the composition of flours (fibers, amino acid details, zinc, iron, beta-carotene and B9 vitamin) are expressed as means ± uncertainty.

3. Results and discussion

3.1. Biochemical composition of flours

Tables 1 and 2 list the composition of the flours used for nutritional optimization by linear programming. The FM and TEF cereal flours have 1.2 times higher starch content than pseudo cereals (i.e. AG) and up to 2.5 times more than legume flours (i.e. BG, CW and FB). Legume flours contain up to 4 times more protein than cereal flours and up to 1.8 times more than the pseudo-cereal flour. As can be seen in Table 2, which details AA composition, all legume and pseudo-cereal flours have a CS above 100, whereas all cereals, TEF and FM have limited lysine contents that reduce their CS to 86 and 65, respectively. These values are higher than the CS found by Taylor and Taylor (2017) and Audu et al. (2018) for TEF (65) and FM (39), respectively. Lipids are present in small amounts (<4%) in flours except in BG and AG (9 and 11% d.b., respectively) making them interesting sources of this nutrient with a higher caloric density. The ω6/ω3 ratio is highly variable among flours, varying from 0.2 to 81, with no trend in cereals versus legumes or pseudo-cereals. A nutritionally interesting ω6/ω3 ratio ≤5 was only found in AL and CW and to a lesser extent in TEF. Conversely, AG have a very high ω6/ω3 ratio (>80) in accordance with Opute (1979) who found a lipid content between 10 and 17% d.b. with an ω6/ω3 ratio between 55 and 102 depending on the variety of amaranth concerned. Sugars (i.e. glucose, fructose and sucrose), are barely present (<3%) in flours except in OFSP flour that has 10 times higher sugar content than the other sources. Wholegrain flours have an interestingly high fiber content that range from 9 to 46% d.b., with, in decreasing order: AL > all legumes > cereals. The fiber content of cereal flours (9–13% d.b.) is in the same range as that of whole wheat flours (10–16%) (Rainakari, Rita, Putkonen, & Pastell, 2016) i.e. three time higher than the fiber content reported in refined wheat flour by Bader Ul Ain et al. (2019).

Zinc and iron, two micronutrients that are largely limited in optimized plant-based diets (Dussiot et al., 2021), are respectively, 2 to 8 times and 1.5 to 7 times higher in AL than in other flours (Table 1). The concentration of zinc in AL is in accordance with Canadian Nutrition File values (https://food-nutrition.canada.ca/cnf-fce/) but iron content is 2 times higher. According to Wei Wong et al., 2019, leafy vegetables like AL are indeed a good source of zinc which comes from the soil. Since the flour is prepared using traditional African methods, the higher iron content of AL could also be at least partially explained by soil contamination, as reported by Baye, Guyot, Icard-Verniere, and Mouquet-Rivier (2013). Beta-carotene is only found in OFSP and AL flours with 5% and 17 % d.b., respectively. The main source of vitamin B9 is CW but results concerning vitamin B9 should be interpreted with caution due to their high standard deviations from the liquid chromatography method (coupled with mass spectroscopy).

Concerning ANF, the phytic acid content in flours ranges from 0.08 to 1.6 g/100 g d.b. It is known that, due to its negative charge, phytic acid reduces the bioavailability of divalent mineral cations by forming electrostatic complexes (Sandberg, 2002). The bioavailability of iron is affected if the molar ratio of phytate/iron is above 1 (FAO/IZiNCG, 2018), which is the case of all flours (Table 1) except AL and OFSP. Molar ratios of phytate/zinc below 5 indicate high bioavailability of zinc, which is the case of AL and is almost reached by OFSP. A ratio higher than 15, which is the case of the other flours (17-36) corresponds to low zinc bioavailability (15%) (FAO, 2004). The highest phytate/zinc ratio was found in cereals and FB flours. Only AL flour combines a phytate/iron below 1 with a phytate/zinc ratio below 5, meaning this flour is highly nutritious. TIA which negatively affects protein digestibility by blocking the enzyme receptor sites (Samtiya et al., 2020), is undetectable in cereals and in OFSP whereas in legume flours, it ranges from 650 to 1000 mg/100 g d.b. This is in accordance with the range found by Laleg et al. (2016): 750–1100 mg TIA/100 g d.b. for faba bean, lentils and black gram flours. With their impact on macro and micronutrient bioavailability, ANF should be reduced in optimized product formulations to conserve high nutritional values.

3.2. Linear programming

Linear programming was used to select the mix of flours for pasta formulation that minimizes the concentration of phytic acid among all possible combinations of cereal, legume and other African raw material flours in order to meet FAO nutritional recommendations for important nutrients for adults (especially women). Calculations were made on the basis of a 100 g portion of dry pasta (i.e. the amount usually recommended by pasta manufacturers) for an adult at one meal, and on the hypothesis of three meals a day (common throughout the industrialized world). One hundred grams of dry pasta should therefore provide one third of the FAO daily nutritional recommendations. Nutritional

Table 1
Biochemical composition and antinutritional factors of gluten free climate smart wholegrain flours.

	AG	AL	BG	CW	FB	FM	OFSP	TEF
Protein (*g*/100 g d.b*)* (conversion Factor)	13.9 ± 0.2^{d} (5.27)	12.2 ± 0.1^{e} (5.34)	18.0 ± 0.2^{c} (5.25)	21.2 ± 0.2^{b} (5.24)	25.0 ± 0.0^{a} (5.00)	6.3 ± 0.1^{g} (5.52)	6.0 ± 0.0^{h} (5.02)	8.6 ± 0.0^{f} (5.57)
Starch (*g*/100 g d.b*)* (% damaged starch)	58.2 ± 0.4^{b} (8.3 %)	2.8 ± 0.2^{f} (42.0 %)	38.9 ± 1.0^{c} (0.4 %)	33.5 ± 2.1^{d} (4.8 %)	27.0 ± 0.2^{e} (1.7 %)	68.5 ± 1.0^{a} (1.4 %)	39.5 ± 0.9^{c} (34.2 %)	66.9 ± 0.8^{a} (9.2 %)
Fiber (*g*/100 g d.b.*)* (% insoluble fiber)	11.0 ± 2.0 (72 %)	45.9 ± 2.0 (95 %)	22.3 ± 2.0 (82 %)	22.8 ± 2.0 (92 %)	25.6 ± 2.0 (88 %)	12.7 ± 2.0 (99 %)	13.8 ± 2.0 (76 %)	8.8 ± 2.0 (68 %)
Lipids (*g*/100 g d.b.*)*	11.4 ± 1.2^{a}	1.2 ± 0.4^{de}	9.2 ± 0.3^{b}	1.9 ± 0.5^{d}	1.7 ± 0.2^{d}	1.7 ± 0.1^{d}	0.4 ± 0.1^{e}	4.1 ± 0.5^{c}
ω6/ω3 ratio	81.2	0.2	14.9	1.8	14.6	6.4	8.8	5.4
Sugars[a] *(g/*100 g d.b.*)*	1.78 ± 0.02^{c}	2.08 ± 0.10^{c}	2.94 ± 0.17^{b}	2.10 ± 0.12^{c}	1.57 ± 0.03^{c}	0.71 ± 0.04^{d}	29.85 ± 1.11^{a}	1.64 ± 0.06^{c}
Ash *(g/*100 g d.b.*)*	2.26 ± 0.13^{de}	12.53 ± 0.93^{a}	2.61 ± 0.20^{cde}	3.36 ± 0.11^{b}	2.90 ± 0.21^{bcd}	2.67 ± 0.53^{bcde}	3.27 ± 0.20^{bc}	1.98 ± 0.20^{e}
Zinc *(mg/*100 g d.b.*)*	5.49 ± 0.51	12.45 ± 1.10	3.61 ± 0.32	4.06 ± 0.37	5.67 ± 0.50	2.19 ± 0.20	1.43 ± 0.20	4.00 ± 0.36
Iron *(mg/*100 g d.b.*)*	14.31 ± 2.00	44.00 ± 5.90	2.70 ± 0.36	24.81 ± 3.40	6.35 ± 0.84	11.40 ± 1.49	6.37 ± 0.87	6.11 ± 0.83
Beta-carotene *(mg/*100 g d.b.*)*	<0.01 ± 0.00	16.70 ± 5.01	<0.01 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	5.30 ± 1.59	<0.01 ± 0.00
Vitamin B9 *(μg/*100 g d.b.*)*	85.9 ± 25.8	419.4 ± 125.8	77.5 ± 23.3	678.5 ± 203.6	107.4 ± 32.2	21.9 ± 6.6	107.1 ± 32.1	106.6 ± 32.0
Phytic acid *(g/*100 g d.b.*)*	1.03 ± 0.05^{c}	0.17 ± 0.01^{f}	0.61 ± 0.02^{e}	0.79 ± 0.04^{d}	1.63 ± 0.04^{a}	0.80 ± 0.01^{d}	0.08 ± 0.00^{g}	1.16 ± 0.01^{b}
TIA *(mg/*100 g d.b.*)*	200 ± 7^{d}	110 ± 7^{e}	994 ± 21^{a}	821 ± 33^{b}	650 ± 14^{c}	$<DL^{f}$	$<DL^{f}$	$<DL^{f}$
Phytate/Iron *(molar ratio)*	6.1	0.3	19.1	2.7	21.7	5.9	1.1	16.1
Phytate/Zinc *(molar ratio)*	18.6	1.4	16.7	19.3	28.5	36.2	5.5	28.7

[a] Glucose + Fructose + Sucrose; AG = Amaranth grains; AL = Amaranth leaf; BG = Bambara groundnut; CW = Cowpea white; FB = Faba bean; FM = Finger millet; OFSP = Orange fleshed sweet potato; TEF = Teff; TIA = Trypsin Inhibitor Activity; <DL = below detection limit; Means of triplicate.

Table 2
Essential amino acids (g/100g protein), chemical score.

	AG	AL	BG	CW	FB	FM	OFSP	TEF	FAO (2011)
His	3.1 ± 0.4	2.2 ± 0.3	3.8 ± 0.5	3.7 ± 0.5	3.1 ± 0.4	3.0 ± 0.4	3.4 ± 0.4	2.5 ± 0.3	1.6
Ile	4.1 ± 0.5	4.4 ± 0.6	5.0 ± 0.7	4.9 ± 0.6	4.8 ± 0.6	4.6 ± 0.6	6.8 ± 0.9	4.4 ± 0.6	3.0
Leu	6.2 ± 0.8	7.7 ± 1.0	9.2 ± 1.2	8.8 ± 1.1	8.4 ± 1.1	10.9 ± 1.4	10.6 ± 1.4	8.4 ± 1.1	6.1
Lys	6.3 ± 0.8	5.7 ± 0.7	8.5 ± 1.1	8.8 ± 1.1	7.5 ± 1.0	3.1 ± 0.4	4.8 ± 0.6	4.1 ± 0.5	4.8
Met + Cys	5.0 ± 0.7	3.6 ± 0.5	3.2 ± 0.4	2.5 ± 0.3	2.4 ± 0.4	6.8 ± 0.8	4.4 ± 0.5	7.4 ± 0.8	2.3
Phe + Tyr	7.8 ± 1.0	8.5 ± 1.1	10.0 ± 1.3	9.7 ± 1.3	8.7 ± 1.1	9.4 ± 1.2	16.0 ± 2.1	10.5 ± 1.4	4.1
Thr	4.0 ± 0.5	4.3 ± 0.6	4.1 ± 0.5	4.2 ± 0.5	4.2 ± 0.5	4.6 ± 0.6	8.2 ± 1.1	4.4 ± 0.6	2.5
Trp	1.6 ± 0.3	1.7 ± 0.3	1.1 ± 0.2	1.4 ± 0.3	1.1 ± 0.2	1.5 ± 0.3	2.4 ± 0.5	1.5 ± 0.3	0.7
Val	4.8 ± 0.6	6.0 ± 0.8	6.1 ± 0.8	5.9 ± 0.8	5.5 ± 0.7	7.4 ± 1.0	11.3 ± 1.5	5.8 ± 0.8	4.0
Chemical score	102	119	137	106	104	65	100	86	

AG = Amaranth Grains; AL = Amaranth leaf; BG = Bambara groundnut; CW = Cowpea white; FB = Faba bean; FM = Finger millet; OFSP = Orange fleshed sweet potato; TEF = Teff; Cys = Cystein; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Trp = Tryptophan; Tyr = Tyrosine; Val = Valine; means ± uncertainty.

constraints to the resulting formulations were as follow: (i) CS > 100, (ii) ω6/ω3 ratio ≤5 (FAO, 2008), (iii) protein >14g (WHO/FAO, 2007), 8 <fibers< 25 g (WHO/FAO, 2003), iron >9.8 mg, zinc>1.6 mg, beta carotene >130 μg and B9 vitamin >1 mg (FAO, 2004) in 100 g of dry pasta. The quantity and quality of protein in pasta are among the main criteria used in simulations because protein content is often lower in plant-based protein products than in most animal-based protein sources such as milk, eggs, and meat (as reviewed in Hertzler, Lieblein-Boff, Weiler, and Allgeier (2020)). If minimal attention is paid to the sources and proportions of plant protein, it is relatively easy to achieve plant protein intakes that surpass both the quality and quantity of those from animal sources. For example, Dimina, Remond, Huneau, and Mariotti (2021) and Rojas Conzuelo, Robyr, and Kopf-Bolanz (2022) used linear programming for plant-based protein products (mixture of flours, yogurt, drink) to obtain a balanced blend of AA. Although pasta are not a source of lipids, some of the flours we studied (i.e. AG and BG) contain around 10% of lipids, which justifies a constraint on lipid quality as the ω6/ω3 criterion. The maximum constraint on fibers was set at 25 g/100g dry pasta, defined by EFSA, the European Food Safety Authority, (2010) as “adequate for normal laxation in adults”. As micronutrient deficiencies are common and negatively affect a wide range of health functions, it is worth including the micronutrients with the most common deficiencies i.e. iron, zinc, B9 and A vitamins (with beta-carotene as precursor of A vitamin) in new product formulations (Bailey, West & Black, 2015). FAO recommendations (daily recommendation divided by three) were those for male and female adults (70 kg - 2700 kcal for male and 60 kg - 2200 kcal for female). Different iron or zinc recommendations depending on their bioavailability do exist (FAO, 2004). We chose values corresponding to recommendations for women considering 10% and 30% iron and zinc bioavailability, respectively. An iron bioavailability of 10% makes it possible to cover populations from both emerging and Western countries for which iron bioavailability was considered as 5–10% and 12–15%, respectively (FAO, 2004). Moderate zinc bioavailability (30%) is advised by FAO (2004) if the diet contains animal and plant-based protein, which is the case of the majority of the world population.

In addition to nutritional criteria, a formulation criterion was imposed in linear programming as follows: AL and OFSP flours must represent less than or equal to 10% of the final formulation. This level of fortification is often used to improve nutritional quality without negatively impacting the processing and properties of pasta. Lawal et al. (2021) and Cárdenas-Hernández et al. (2016) reported an AL fortification from 2% to 6% in pasta.

As unlike TIA, phytic acid is less affected by the pasta processing (Laleg et al., 2016; Laleg et al., 2017), the nutritional optimized formulations selected are those that minimize phytic acid content (the objective) while meeting all the above criteria (the constraints).

The simulations were run several times. We investigated three types of formulations among all the flours. The first type (Type 1) is a single 100% legume formulation. The second type (Type 2) associates each legume with each cereal or pseudo-cereal. The third type (Type 3) of formulations associates legume and/or cereal/pseudo-cereal and other raw materials (AL or OFSP). In order to obtain results for Types 1 and 2, the beta carotene criterion was removed because this micronutrient is only provided by AL and OFSP. The four optimized nutritional formulations of types 1, 2 and 3 resulting from linear programming are presented in Table 3. They contain cowpea as the main ingredient and in some, TEF with or without AL. Formulations containing AL meet all the constraints (CW-AL and CW-TEF-AL) whereas CW and CW-TEF do not meet the beta-carotene criterion.

Although linear programming has often been used in diet

Table 3
Theoretical biochemical composition and antinutritional factors of the four nutritionally optimized formulations of pasta (obtained by calculation).

Type of simulation	CW	CW-AL	CW-TEF	CW-TEF-AL
	1	3	2	3
Formulations	100 % CW	90 % CW + 10 % AL	60 % CW + 40 % TEF	55 % CW + 35 % TEF + 10 % AL
Protein *(g/100 g d. b)*	21.2 ± 0.2	20.3 ± 0.2	16.1 ± 0.1	15.9 ± 0.1
Starch (g/100 g d. b) **(% damaged starch)**	33.5 ± 1.8 (1.6 %)	30.4 ± 1.9 (5.1 %)	46.9 ± 1.3 (7.3 %)	42.1 ± 1.2 (7.5 %)
Fiber *(g/100 g d.b.)* (%insoluble fiber)	22.8 ± 1.8 (92 %)	25.1 ± 1.8 (93 %)	17.2 ± 1.4 (87 %)	20.2 ± 1.3 (89 %)
Lipids *(g/100 g d. b.)*	1.9 ± 0.4	1.8 ± 0.5	2.7 ± 0.4	2.6 ± 0.3
ω6/ω3 ratio	1.8	1.6	3.2	2.9
Sugars[a]*(g/100 g d. b.)*	2.1 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
Ash *(g/100 g d.b.)*	3.4 ± 0.1	4.3 ± 0.1	2.8 ± 0.1	3.8 ± 0.1
Zinc *(mg/100 g d. b.)*	4.06 ± 0.33	4.90 ± 0.35	4.04 ± 0.26	4.88 ± 0.26
Iron *(mg/100 g d. b.)*	24.8 ± 3.0	26.7 ± 3.1	17.3 ± 2.1	20.2 ± 2.0
Beta-carotene *(mg/100 g d.b.)*	0.02 ± 0.01	1.69 ± 0.50	0.01 ± 0.01	1.68 ± 0.50
B9 vitamin *(μg/100 g d.b.)*	678 ± 179	653 ± 184	450 ± 123	452 ± 113
Phytic acid *(g/100 g d.b.)*	0.79 ± 0.04	0.73 ± 0.04	0.94 ± 0.02	0.86 ± 0.02
TIA *(mg/100 g d.b.)*	821 ± 29	750 ± 30	493 ± 20	463 ± 18
Phytate/Iron *(molar ratio)*	2.7	2.3	4.6	3.6
Phytate/Zinc *(molar ratio)*	19.3	14.7	23.0	17.4

[a] = Glucose + Fructose + Sucrose; CW = 100% cowpea; CW-TEF = 60% Cowpea +40% Teff; CW-AL = 90% Cowpea +10% Amaranth leaf; CW-TEF-AL = 55% Cowpea +35% Teff + 10% Amaranth leaf.

optimization, and is now increasingly used to optimize food products, it has not previously been used to formulate pasta. A few authors used linear programming to improve the nutritional properties of other cereal based products, often with only a few criteria (Agrahar-Murugkar & Dixit-Bajpai, 2019; Agrahar-Murugkar, Dwivedi, Dixit & Kumar, 2018; Jiménez, Giménez, Farfán, & Sammán, 2019). Agrahar-Murugkar, Dwivedi, Dixit-Bajpai, and Kumar (2018) and Agrahar-Murugkar and Dixit-Bajpai (2019) studied nutritional optimized cookies and bread using linear programming in order to reach 10% of protein and 0.2% of calcium in both products. Jiménez et al. (2019) used linear programming with bread to obtain formulations that meet the dietary guidelines for Americans in terms of daily energy and macronutrient intakes while minimizing the cost. Our nutritional optimization of pasta accounts for both its macro and micronutrient contents. Moreover, emphasis was also placed on the AA balance and future nutrient bioavailability by minimizing the amount of ANF such as phytic acid in selected formulations never proposed in other studies.

3.3. *Theoretical nutritional quality of the four nutritionally optimized types of pasta*

The theoretical biochemical composition of CW, CW-AL, CW-TEF and CW-TEF-AL pasta were predicted by calculations based on the results obtained for flour listed in Table 3. The theoretical protein content ranges from 16 to 21% d.b. which is 1.2–1.6 times higher than that in standard DWS pasta (Canadian Nutrition File = https://food-nutrition.canada.ca/cnf-fce/index-eng.jsp). Thanks to linear programming constraints all cowpea-based pasta types have a CS above 100 (Table 4) reflecting a balanced essential AA profile, compared to a CS of only 44 reported by Messia et al. (2021) for DWS pasta due to its lysine deficiency. Theoretical fiber content ranges from 17 to 25% d.b. (25% corresponds to the maximum value of the fiber criterion) which is 4–5.5 times higher than DWS pasta, and even 2 to 3 times higher than in whole DWS pasta (Canadian Nutrition File). Compared to other legume-based pasta in the literature, CW and CW-AL pasta have similar protein content (around 23.5% d.b. for 100% chickpea or faba bean) but 2.5 to 3.5 times more fiber than 100% chickpea, faba bean or lentil pasta (Laleg et al., 2016; Suo et al., 2022) due to the use of de-husked faba beans and lentils in their studies, unlike CW, TEF and AL flours in our work. Compared to other gluten free legume-cereal based pasta, CW-TEF and CW-TEF-AL pasta have similar protein contents but around 2.5 times more fiber and zinc, and more than 2 times as much iron as hulled corn:broad bean (70:30) pasta (Gimenez, Drago, Bassett, Lobo, & Samman, 2016). With the highest level of fiber, zinc, iron and beta-carotene, a large quantity of protein and a CS above 100, CW-AL pasta has the highest nutritional potential among the four selected cowpea-based types of pasta.

Table 4
Essential amino acids (g/100g protein) and chemical score of the four nutritionally optimized pasta (obtained by calculation).

	CW	CW-A	CW-T	CW-TA
His	3.7 ± 0.4	3.6 ± 0.4	3.2 ± 0.3	3.1 ± 0.3
Ile	4.9 ± 0.6	4.9 ± 0.6	4.7 ± 0.4	4.7 ± 0.4
Leu	8.8 ± 1.0	8.7 ± 1.0	8.6 ± 0.8	8.6 ± 0.7
Lys	8.8 ± 1.0	8.5 ± 1.0	6.9 ± 0.7	6.9 ± 0.7
Met + Cys	2.5 ± 0.2	2.6 ± 0.3	4.4 ± 0.4	4.3 ± 0.3
Phe + Tyr	9.7 ± 1.1	9.6 ± 1.1	10.0 ± 0.9	9.9 ± 0.8
Thr	4.2 ± 0.5	4.2 ± 0.5	4.3 ± 0.4	4.3 ± 0.4
Trp	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.2
Val	5.9 ± 0.7	5.9 ± 0.7	5.8 ± 0.5	5.9 ± 0.5
Chemical score	107	112	142	140

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; Cys = Cystein; His = Histidine; Ile = Isoleucine; Leu = Leucine; Lys = Lysine; Met = Methionine; Phe = Phenylalanine; Thr = Threonine; Trp = Tryptophan; Tyr = Tyrosine; Val = Valine; means ± theoretical uncertainty.

Since several constraints were imposed by linear programming, the consumption of 100 g d.b. of all the cowpea-based pasta theoretically covers the FAO recommendations for protein, fiber, iron, zinc and B9 vitamin for adults in one meal (Fig. 1A, women; men not shown). The consumption of 100 g d.b. of the two types of pasta containing AL flour additionally covers the beta-carotene recommendation for one meal and approximately half the daily recommendation; the daily recommendation for vitamin B9 is largely covered by the consumption of 100 g d.b. of all cowpea-based types of pasta; daily recommendation for zinc (for 30% zinc bioavailability) is also reached with 100 g d.b. of CW-AL and CW-TEF-AL. These results show that the theoretical nutritional coverage is considerably improved by the consumption of cowpea-based pasta compared to DWS pasta especially for fibers and micronutrients. These results vary when the theoretical impact of pasta processing (i.e. extrusion, drying and cooking) is taken into account (Fig. 1B). To mimic it, a 20% reduction in insoluble fiber content (Laleg et al., 2016), 27%, 29%, 32% and 51% losses in iron, zinc, beta-carotene and B9 vitamin, respectively (Yaseen, 1993), were applied to previous theoretical nutrient contents of the pasta. Even when processing was taken into account, 100 g d.b. of all four cowpea-based types of pasta covered the FAO recommendations for protein, fiber, iron, zinc and B9 vitamin in one meal (Fig. 1B). Moreover, CW-AL and CW-TEF-AL still covered beta-carotene recommendations for one meal. However, with all cowpea-based pasta, daily B9 vitamin coverage would drop from more than 100% to 55–83%. In addition, CW-AL and CW-TEF-AL which cover daily zinc requirements, would only cover 70% of the requirements after processing.

Some ANF such as TIA or phytic acid can limit the nutritional benefit of the final food product. TIA is theoretically present in all the dry cowpea-based pasta varying from 460 to 820 mg/100g (Table 3), whereas undetectable TIA content was reported in wheat flour by Otegbayo, Adebiyi, Bolaji, and Olunlade (2018). Adding TEF leads to a 1.5 to 1.8 times reduction in theoretical TIA in dry pasta. TIA values of theoretical cowpea-based pasta are 2–5 times higher than TIA reported for 100% cooked legume-based pasta (152–248 mg/100g d.b.) by Laleg et al. (2016). As a 70% decrease was reported for TIA between flour vs cooked 100% faba bean and 80:20 w/w semolina:chickpea pasta by Laleg et al. (2017) and by Zhao, Manthey, Chang, Hou, and Yuan (2005) respectively, the theoretical TIA content of cowpea-based pasta is consequently certainly overestimated in comparison to the real content in the food product consumed. Considering a 70% decrease in TIA during pasta processing, the theoretical TIA in cooked cowpea-based pasta would be between 140 and 250 mg/100g d.b. which is in accordance with TIA content reported by Laleg et al. (2016) for cooked pasta. As pasta processing has a bigger impact on TIA than on phytic acid, the objective of linear programming was to obtain formulations that minimize phytic acid. Its theoretical content in cowpea-based pasta varies from 0.7 to 0.9% d.b. (Table 3) which is more than 2.5 times compared to results reported for DWS (Demir & Bilgicli, 2020; Wiesinger, Cichy, Hooper, Hart, & Glahn, 2020). Chouchene, Micard, and Lullien-Pellerin (2018) observed that the concentration of phytic acid was higher in the outer layer of wheat kernel, and in the germ of pea seeds. These fractions of seed are retained in whole flours such as those used to make cowpea-based pasta, which may explain the higher phytic acid content than in pasta made from DWS pasta. Compared to other legume-based pasta, Wiesinger et al. (2020) reported a slightly higher range of phytic acid content (0.8–1.0% d.b.) than for 100% bean-based pasta. The theoretical molar ratio of phytate/zinc varied from 14.7 to 23.0 in cowpea-based pasta, which correspond to low bioavailability of zinc (15%) (FAO, 2004). Lawal et al. (2022) reported moderate iron bioavailability (20–37%) in pasta made from 90 to 95% cassava and 5–10% amaranth leaves. The theoretical molar ratio phytate/iron of cowpea-based pasta is above 1 (from 2.3 to 4.6) which means the bioavailability of iron could be impacted. Wiesinger et al. (2020) reported a higher phytate/iron ratio, of between 10.0 and 13.6 in 100% bean-based pasta. If we apply a 3–20% loss of phytic acid (as reported by

Figure 1A

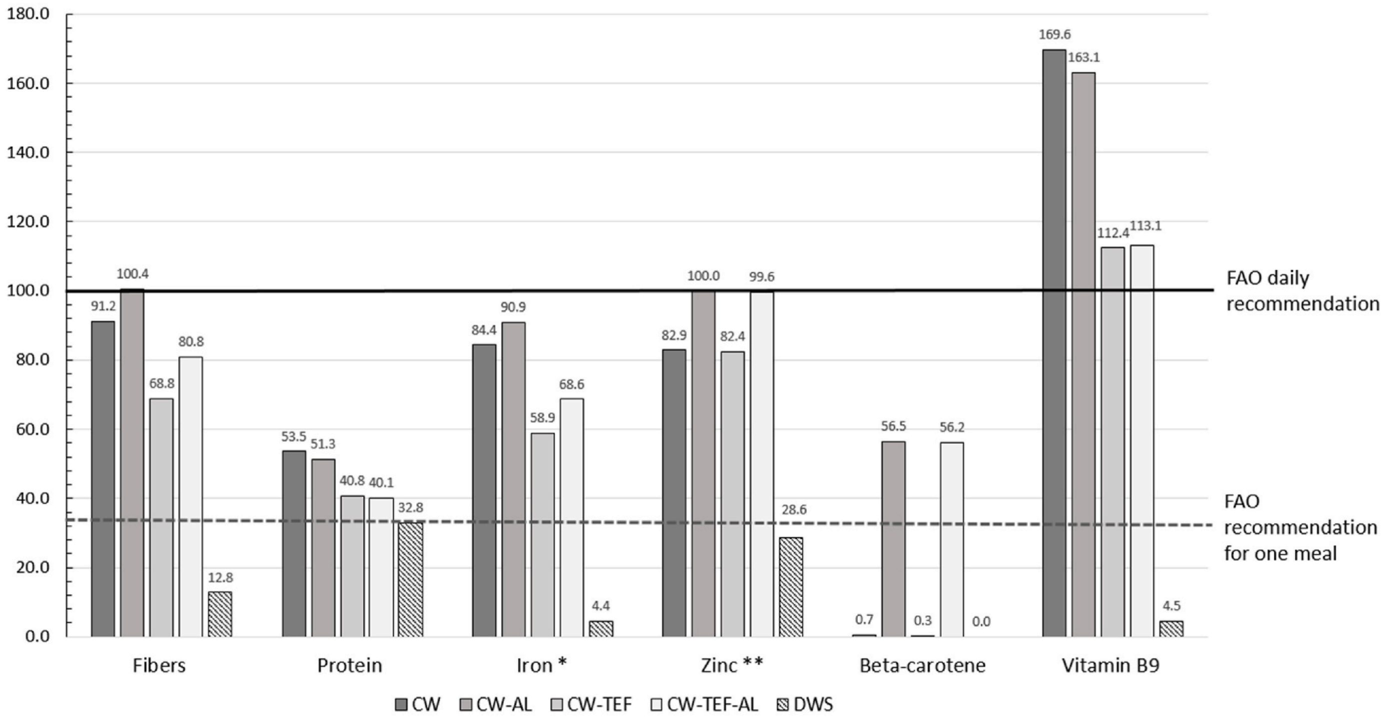

Figure 1B

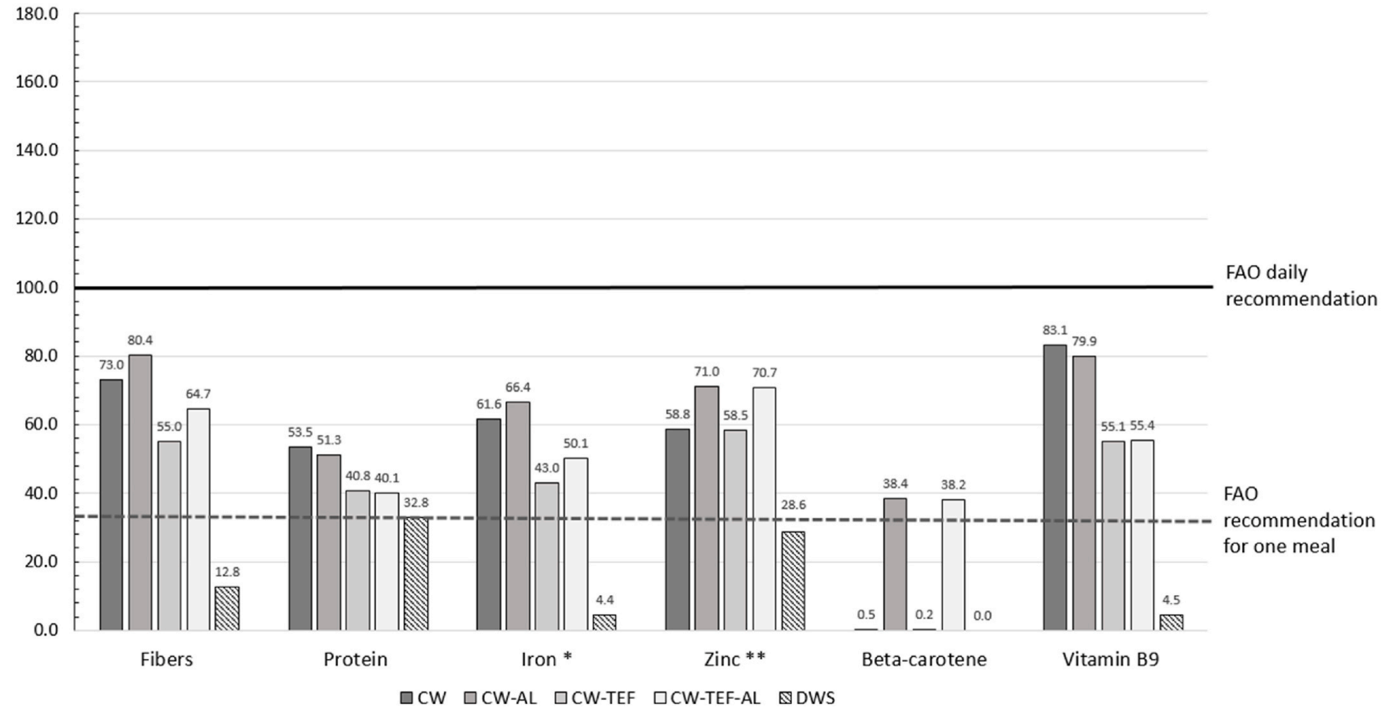

Fig. 1. A and B Theoretical nutritional coverage (% FAO recommendation for women (1)) of a serving size (100 g d.b.) of the dried (1A) or cooked (1B) cowpea-based pasta in comparison with durum wheat semolina pasta

Theoretical impact of the process for cooked pasta has been applied to nutrients according to Laleg et al. (2016) and Yaseen (1993) results. CW = 100% cowpea; CW-AL = 90%cowpea +10%amaranth leaf; CW-TEF = 60% cowpea +40% teff; CW-AL-TEF = 55% cowpea +35% teff +10% amaranth leaf; DWS = data from Canadian Nutrition File; * Based on FAO 2004 recommendation 10% iron bioavailability; ** Based on FAO 2004 recommendation 30% zinc bioavailability; (1) FAO Carbohydrates and fibers = 2003, Vitamin and minerals = 2004, Proteins = 2007

Laleg et al. (2016) for 100% legume-based pasta), the phytate/iron ratio would be 1.8 to 4.4, and the phytate/zinc ratio would be 11.8 to 22.3 depending on the cowpea-based pasta formulation. However, for all pasta except CW-TEF, iron bioavailability would still be negatively impacted, zinc bioavailability could increase from low to moderate after pasta processing. Among the four cowpea-based pasta we selected, CW-AL remains the most interesting formulation thanks to its lower phytate/zinc and phytate/iron ratio.

3.4. Pasta processing ability of nutritionally optimized flour formulation

The pasta processability of a flour mix containing cereal and legumes and especially its first hydration-mixing step can be drastically impacted and even rendered impossible by excessive granulation due to the action of oxidative enzymes like lipoxygenase (LOX) (Laleg et al., 2021). To counteract this phenomenon, an antioxidant compound can be added to the formulation (Laleg et al., 2021). Thus, to predict future difficulties during pasta processing, we calculated the concentration of AOC, free phenolic antioxidant compounds and oxidative capacity (i.e. LOX activity) of the four optimized formulations (CW, CW-AL, CW-TEF, CW-TEF-AL) based on flour measurements (Table 5).

LOX is expressed at particularly high levels in legumes, and is responsible for the catalysis of C18 PUFA oxidation (linoleic and linolenic acids being the main substrates) (Shi, Mandal, Singh, & Pratap Singh, 2020). Predicted LOX activity of the four cowpea-based pasta formulations ranges from 1700 to 3100 U/mg d.b. The high LOX content is due to the CW flour which has an even higher LOX level than that reported by Chang and McCurdy (1985) (2560 U/mg). Even if the addition of TEF reduced LOX activity in CW-TEF and CW-TEF-AL 1.5 to 1.8 times, it was still 4 times higher than the LOX activity reported by Laleg et al. (2021) for 100% faba bean or lentil pasta formulations. Predicted AOC and free phenolic content of the four cowpea-based pasta formulations range from 13 to 31 mmol TEAC/kg d.b and 2.4–4.0 μg GAE/mg d.b., respectively. This is 1.3–3 times better AOC and 1.3 to 2 times more free phenolic compounds than reported by Turco et al. (2016) for 100% faba bean pasta. This may be due to the use of whole flours in our study instead of refined faba bean flour, and by the impact of pasta processing on these compounds that was not taken into account in our predictions. Indeed, antioxidant phenolic compounds are present in greater amounts in the peripheral tissues of the grain, as in whole flours (Adom, Sporrells & Liu, 2005) and Turco, Bacchetti, Morresi, Padalino, and Ferretti (2019) reported a 40–62% decrease in polyphenol content after the pasta cooking step. In addition, adding only 10% of AL to cowpea-based pasta formulations doubled the AOC of the formulations. The AOC of AL is 11–17 times higher than that of CW and TEF flours, and concentrations of phenolic compounds are high, i.e. 1.5 to 15 times higher than in CW and TEF flours, respectively. The high AOC of AL was obtained even though it has the highest D50 particle size (i.e. the lowest specific surface area) compared to the other flours (1.7–5 times higher). The addition of AL also slightly increases the free phenolic antioxidant compounds but alone, they do not seem to explain the AOC value of flours. This could also be due to the co-occurrence of water- and fat-soluble antioxidant micronutrients (vitamins C and E and beta-carotene) in the flours (Sarker et al., 2022).

Table 5
Experimental and theoretical lipoxygenase and phenolic contents, antioxidant capacity of flours and nutritionally optimized formulations

	AL	TEF	CW	CW-AL	CW-TEF	CW-TEF-AL
AOC *(mmol TEAC/kg d.b)*	169.2 ± 21.8^{a}	10.6 ± 0 .7^{b}	15.6 ± 2.2^{b}	31.0 ± 14.2	13.6 ± 9.4	29.2 ± 8.9
Free phenolic content *(μg GAE/mg d.b)*	5.7 ± 0.5^{a}	0.4 ± 0.0^{c}	3.8 ± 0.1^{b}	4.0 ± 0.1	2.4 ± 0.1	2.8 ± 0.1
Lipoxygenase Activity *(U/mg d.b)*	< DL^{b}	< DL^{b}	3122 ± 20^{a}	2810 ± 18	1873 ± 12	1717 ± 11
Particle size D50 *(μm)*	440.9 ± 22.4^{a}	87.5 ± 0.4^{c}	259.7 ± 8.1^{b}			

Results of CW-AL, CW-TEF and CW-TEF-AL are predicted by calculation; AOC = Antioxidant capacity; GAE = Gallic acid equivalent; TEAC = Trolox equivalent antioxidant capacity; AL = Amaranth leaf; CW = 100% Cowpea; TEF; TEF = Teff; CW-TEF = 60% Cowpea +40% Teff; CW-AL = 90% Cowpea +10% Amaranth leaf; CW-TEF-AL = 55% Cowpea +35% Teff + 10% Amaranth leaf; < DL = below detection limit; Means of triplicates.

Considering both oxidative and AOC of the four cowpea-based pasta formulations, CW probably contains the highest proportion of dough aggregates during the hydration-mixing step, consequently resulting in the most difficult extrusion. The high AOC combined with lower LOX activity in the CW-TEF-AL suggest that this formulation will be the easiest to produce. The processability of a formulation with a high antioxidant content combined with high LOX activity as in CW-AL or with a lower AOC combined with lower LOX activity (CW-TEF) seems to be more difficult to predict.

3.5. Color of nutritionally optimized flour formulations

Color is one of the main criteria of pasta quality that can affect consumer acceptance, and today's consumers are used to bright yellow durum wheat semolina pasta. The color of dry pasta is impacted by several criteria including pigments, enzymatic activities, Maillard reaction or the color of the raw materials themselves (Anese, Nicoli, Massini, & Lerici, 1999; Borrelli, Troccoli, Di Fonzo, & Fares, 1999). The colors of nutritionally optimized cowpea-based formulations before pasta processing are listed in Table 6. The addition of AL in CW or CW-TEF decreases their lightness and redness due to the dark green color of leaf flour. The addition of TEF in formulations increases their redness. All cowpea-based formulations have 5–20% lower lightness, higher redness and 55–60% lower yellowness than DWS partly due to the use of legume flours (CW), the use of whole flour (CW, TEF and AL) and to the presence of specific pigments (carotenoids). This is in accordance with Fenn, Lukow, Humphreys, Fields, and Boye (2010), who found a decrease in lightness and an increase in redness with the proportional addition of legume flours (yellow pea or chickpea) to wheat flour. Moreover, Isik, Ozgoren and Sola (2022) found a lower lightness and yellowness, and a higher redness for whole wheat flour compared to refined wheat flour. These differences in the color of formulations before processing may be reflected in dry pasta and hence influence consumer acceptance. The formulation whose color is closest to DWS before pasta processing is CW. However, enzymatic reactions that may occur during processing (see section 3) seem to be stronger in the CW formulation than in the other cowpea-based formulations, and can lead to a change in color (enzymatic browning) after processing.

Table 6
Color of nutritionally optimized formulations before pasta processing.

	CW	CW-AL	CW-TEF	CW-TEF-AL	DWS
L*	65.71 ± 1.58^{b}	57.24 ± 2.18^{d}	62.07 ± 0.86^{c}	56.85 ± 1.88^{d}	69.48 ± 1.01^{a}
a*	0.56 ± 0.01^{c}	−0.01 ± 0.06^{d}	2.26 ± 0.03^{a}	1.36 ± 0.02^{b}	−1.92 ± 0.03^{e}
b*	10.53 ± 0.30^{b}	9.29 ± 0.51^{c}	9.06 ± 0.16^{c}	9.42 ± 0.44^{c}	23.26 ± 0.49^{a}

L* = Lightness; a* = redness; b* = yellowness; CW = 100% Cowpea; CW-TEF = 60% Cowpea +40% Teff; CW-AL = 90% Cowpea +10% Amaranth leaf; CW-TEF-AL = 55% Cowpea +35% Teff + 10% Amaranth leaf; DWS = Durum wheat semolina; Means of triplicates; Means with the same latter in a row are not significantly different ($p > 0.05$).

4. Conclusion

Based on our extensive characterization of 8 flours, applying linear programming made it possible to optimize pasta formulations that meet FAO recommendations for adults in terms of AA, proteins, lipid quality, fibers and several key micronutrients while simultaneously decreasing ANF. Linear programming constraints integrated nutritional recommendations of health interest and the maximum presence of AL and OFSP in the final mix. LOX and AOC capacity could be added in future simulations as new constraints to eliminate at least the most potentially difficult-to-produce formulations. CW-AL seems to be the formulation with the highest nutritional interest and processability potential, followed by CW-TEF-AL, CW and CW-TEF. However, one of limitation of our study at this stage is that the real processability of most interesting prototypes of pasta, *i.e.* cowpea-based, have only be determined by linear programming and now needs to be tested from theoretical to effective nutritional characterizations. The impact of the entire process, *i.e.* extrusion, drying and cooking as to be determined to highlight impact of these operation units on the nutritional potential and color of pasta.The study of optimized pasta will also require sensory analysis, and to have an impact on the health of low-income populations, further study is needed to integrate price into linear programming.

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CRediT authorship contribution statement

P. Pinel: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **S. Drogue:** Formal analysis, Software. **M.J. Amiot-Carlin:** Methodology. **C. Vannier:** Data curation. **C. Bourlieu-Lacanal:** Methodology, Supervision, Writing – original draft, Writing – review & editing. **V. Micard:** Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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