Heliyon 10 (2024) e38171

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Formulation of nutritious and functional meal-based biscuits from mixture of soybean, papaya fruit pulp, and baobab fruit pulp flours

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ARTICLE INFO

Keywords:
Glycine max L.
Carica papaya L.
Adansonia digitata L.
Mixture design
Biscuits
Safety
Antioxidant activity
Optimization

ABSTRACT

Background and objective: The current trend in the formulation of convenience foods like biscuits is directed towards using local ingredients endowed with health benefits effects. The present study aimed at valorizing local crops and fruits as substitutes for wheat flour (WF) and sugar in the formulation of healthy, nutritious and functional meal-based biscuits.
Methods: Soybean (*Glycine max* L.), papaya fruit pulp (*Carica papaya* L.) and baobab fruit pulp (*Adansonia digitata* L.) flours were produced, characterized, and used to formulate biscuits following a simplex centroid mixing design. The physicochemical, microbiological, sensory and antioxidant properties of the biscuits were assessed.
Results: The results showed that protein and lipid contents of the biscuits increased significantly ($p < 0.05$) with the proportion of soybean flour (SF) in the formulation. The significant increase ($p < 0.05$) in the mineral content of the biscuits was proportional to the incorporation of papaya fruit pulp (PFPF) and baobab fruit pulp (BFPF) flours in the formulation. The energetic value of the formulated biscuits was higher than those made with 100 % WF. The incorporation of SF and BFPF contributed to a significant increase ($p < 0.05$) in the crude fibres' content of biscuits. Biscuits made with SF, PFPF and BFPF were safe for human consumption. They were accepted by the panelists; rich in bioactive compounds (total phenolic, flavonoids), and displayed high antioxidant activities. The optimization procedure revealed that the optimum formulation (with the highest desirability of 0.81) was WF 25 g, SF 51.86 g, PFPF 8.06 g and BFPF 15.06 g
Conclusion: This study indicates that baobab fruit pulp, papaya fruit pulp and soybean flours can be used as a substitute for WF in the formulation of functional biscuits.

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https://doi.org/10.1016/j.heliyon.2024.e38171
Received 12 July 2024; Received in revised form 27 August 2024; Accepted 18 September 2024
Available online 19 September 2024

Significance of the research: This study suggests that the formulated meal-based biscuits might have the potential to be used to fight/prevent malnutrition and cardiometabolic diseases, and to boost the immune system while reducing the dependence on wheat.

1. Introduction

The trend for convenience foods has significantly increased this decade thanks to consumers' eating habits that have changed. Indeed, the current social pressure makes people have busier lifestyles and adopt the snacking culture to manage their food intake. They spend less time cooking homemade foods, particularly women who actively participate in business life to improve their level of income. Among the convenience foods, biscuits figure out as the most common ready-to-eat snack foods that are consumed and prized by the whole population independent of their age [1,2]. Biscuits can be defined as sweet or savory flour-based baked delights, typically hard, unleavened and flat, with different sizes, shapes, flavors and textures. The global market of biscuits proliferates throughout the world and is estimated to reach $110 billion by 2025. However, the major ingredient used in biscuit formulation is WF for which health issues such as gluten intolerance are associated [3,4]. Hence, consumers' interest has shifted towards gluten-free, sugar-free, low calories, nutritional, and functional biscuits, because of their positive effect on gut microbiota, blood sugar level, satiety, and cardiometabolic diseases. Considering the cultural conditions of wheat which is limited to a few countries and the current international conflict context that has led to an increase in the importation costs of wheat, there is a need for reducing the use of WF in biscuits formulation. In this light, substituting WF with flour from other locally available food matrices rich in compounds (fibres, proteins, minerals, vitamins and phenolic compounds) endowed with biological activities appears as a suitable and sustainable alternative [5].

Among these food ingredients, soybean due to its richness in proteins containing essential amino acids, poly-unsaturated fatty acids, crude fibres and phenolic compounds attracts attention and is positioned as one of the ideal substitutes for wheat [6]. Ghoshal and Kaushik [7] showed that replacing WF with soy flour resulted in fortified biscuits having higher phenolic compounds and antioxidant capacities, with improved organoleptic and nutritional characteristics, as well as shelf life.

Other important nutrients sought by consumers in biscuits are fibres, minerals and phenolic compounds due to their health benefits effects. Fruits are generally the main source of these functional nutrients [8,9]. This is the case for baobab fruit pulp [10,11] and papaya fruit pulp [12]. These two fruits consumed by the whole class of the population are less expensive and sustainably produced in the country. They also contain high proportions of sugars which suggests their possible use as a substitute for sugars in biscuit formulation. To our knowledge, there is a gap in the literature regarding the optimal use of these fruits associated with soybean in the formulation of meal-based biscuits. The novelty of this work lies in the application of response surface methodology to formulate biscuits with local crops' flours along with reduced amount of WF combined with known functionality, baking and nutritional value which has not been analyzed before. The present study has as objective to valorize some local resources including soybean, papaya and baobab a substitute for WF in the production of nutritious biscuits endowed with functional properties.

2. Materials and methods

2.1. Vegetal materials

The vegetal materials used in this study were papaya fruits (*Carica papaya* L.), soybean (*Glycine max* L.) and baobab fruits (*Adansonia digitata* L.). Twenty kilograms of mature fruits of papaya (red Solo cultivar) of about 850 g each were sampled at the Mfoundi market in the city of Yaoundé, Centre Region, Cameroon. Five kilograms of soybean (variety TGX 1835) and 7 kg of dried baobab fruits were respectively collected from selected sellers at the Mfoundi and Mokolo markets, in the city of Yaoundé. All samples were channelled to the laboratory for analysis.

Other materials including WF (Grand Moulin brand, Douala, Cameroon), eggs, sugar (Sosucam brand, Mbandjock, Cameroon), salt (Sasel brand, Douala, Cameroon), baking powder (Alsa brand, France) and margarine (Jadida brand, Douala, Cameroon) were procured from a supermarket in the city of Yaoundé, Cameroon.

2.2. Production of flours

2.2.1. Papaya fruit pulp flour

PFPF was produced according to the method described by Pathak et al. [13]. The mature fresh fruits showing no deterioration were washed, peeled, de-seeded and then cut into thin 5 mm strips. The strips were dried at 50 °C for 72 h in a ventilated oven (Heraeus, Germany), ground (Moulinex, France) and sieved (Ø = 450 μm) to obtain a fine powder. The lumps collected were again crushed and sieved. The resulting flour was weighed, sealed in polyethylene plastic bags and stored at room temperature (25 ± 2 °C).

2.2.2. Soybean flour

The samples of soybeans were sorted, roasted for 10 min, and dehulled. Non-dehulled seeds were manually removed by hand-picking. The dehulled seeds were crushed and sieved (Ø = 450 μm). The resulting flour was weighed, sealed in polyethylene plastic bags and stored at room temperature (25 ± 2 °C).

2.2.3. Baobab fruit pulp flour

The samples of dried baobab fruits were sorted and pulped to remove seeds, fibres and other debris. The pulp obtained was sieved and then ground to obtain a fine powder. The powder thus obtained was again sieved using a sieve with a particle size of 450 μm. The obtained flour was weighed, sealed and stored at room temperature (25 ± 2 °C).

2.3. Analyses of biscuit ingredients

2.3.1. Physicochemical analyses

The moisture, crude fibres and ash contents of the flours were assessed using the AOAC 925.10, AOAC 978.10 and AOAC 942.05 methods, respectively [14]. Total sugars were determined using the method described by Dubois et al. [15]. The lipids were evaluated according to the method of Bourely [16] while the proteins were estimated using the Kjeldahl method [17]. The total polyphenols and flavonoid contents were determined following the methods of Fapetu et al. [18] and Faiqoh et al. [19], respectively.

2.3.2. Functional analyses

The functional properties of biscuit ingredients assessed in this study were the water absorption capacity (WAC) and the water solubility index (WSI) using the method of Heywood et al. [20] and the oil absorption capacity (OAC) following the method of Malomo et al. [21].

2.4. Baking and biscuits processing

2.4.1. Biscuit production process

The biscuits were produced using the experimental process described by Mouafo et al. [4]. The following ingredients were used: PFPF, BFPF, SF, WF, eggs, sugar, salt, baking powder and margarine. Margarine and sugar were creamed thoroughly until obtaining a homogeneous, snowy and smooth mixture. To the cream obtained, eggs were added and the mixture was stirred. Then, the mixture of flours (PFPF, BFPF, SF, WF), salt, and baking powder were sieved (Ø = 450 μm) and added to the creamy dough. After homogenization, the dough was rolled out using a wooden rolling pin on a plate and shaped using a biscuit mold (0.5 cm thick discs of 6 cm diameter). The biscuits were placed on baking trays greased with a thin layer of margarine and introduced in an oven (Memmert IN110, Germany) and baked for 25 min at 180 °C. The biscuits were removed from the oven, cooled at room temperature (25 ± 2 °C), and conditioned into polyethylene bags.

2.4.2. Optimization of flour formulations

A simplex centroid mixture design was used to formulate flours for biscuits' production. The independent variables were WF (X_1), PFPF (X_2), SF (X_3) and BFPF (X_4). Preliminary experiments considering the targeted specifications for the biscuits which were a maximum reduction of WF and the satisfaction of the required daily intakes for nutrients and energy, were used to define the domain of variation of the selected independent variables which were: 25–65 g for WF, 25–65 g for SF, 5–10 g for PFPF, and 5–20 g for BFPF. The experimental matrix showing the 16 trials is presented in Table 1. The responses assessed on prepared biscuits in triplicate for each flour formulation were pH, ash, crude fibres, total proteins, total aerobic mesophilic flora, yeasts and moulds count, general

Table 1
Effects of the different factors on the physicochemical, microbiological, antioxidant and sensorial attributes of biscuits.

Biscuits	Factors				Responses							
	WF (g)	SF (g)	PFPF (g)	BFPF (g)	pH	Ash (g/100gMS)	Proteins (g/100gMS)	Crude fibres (g/100gMS)	TAMF (Log UFC/g)	YM (Log UFC/g)	General Acceptability	DPPH IC_{50} (μg/mL)
BS1	65	25	5	5	7.53	2.97	12.73	2.93	2.75	0.00	6.40	5.75
BS2	25	65	5	5	7.03	4.07	16.41	4.90	2.64	1.48	5.20	1.60
BS3	60	25	10	5	6.21	3.29	10.39	3.44	2.33	0.00	6.25	6.10
BS4	25	60	10	5	6.83	3.52	18.73	3.87	2.90	0.00	5.65	1.54
BS5	50	25	5	20	6.33	4.15	11.32	6.28	3.76	0.00	6.00	5.10
BS6	25	50	5	20	6.63	4.26	14.90	6.53	2.14	0.00	5.65	1.20
BS7	45	25	10	20	6.13	4.70	9.06	6.80	3.00	0.00	6.65	4.90
BS8	25	45	10	20	6.22	5.36	13.37	7.26	2.18	0.00	6.45	0.90
BS9	65	25	5	5	7.53	2.99	12.73	2.90	2.67	0.00	6.40	5.75
BS10	25	65	5	5	7.13	4.00	16.58	5.13	2.67	1.40	5.20	1.64
BS11	60	25	10	5	6.16	3.34	10.74	3.43	2.34	0.00	6.30	6.00
BS12	25	60	10	5	7.03	3.54	18.42	3.90	2.80	0.00	5.65	1.35
BS13	50	25	5	20	6.34	4.18	11.45	6.10	3.75	0.00	6.05	5.11
BS14	100	0	0	0	8.83	2.07	7.15	2.09	2.75	1.03	6.80	7.60
BS15	90	0	10	0	7.76	2.25	6.54	2.28	3.18	1.13	6.25	5.35
BS16	80	0	0	20	5.50	3.63	6.50	4.50	2.18	1.21	6.10	4.85

TAMF = total aerobic mesophilic flora; YM = yeasts and moulds, WF = wheat flour, PFPF = papaya fruit pulp flour, BFPF = baobab fruit pulp flour, SF = soybean flour.

acceptability and antioxidant activity. The means of each response were used to calculate coefficients which were related to coded variables by a first-order model as shown by the equation below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 \quad (1)$$

Where Y are the response, X_1, represents the WF, X_2 PFPF, X_3 SF, and X_4 BFPF, and b_1, b_2, b_3 and b_4 their respective linear coefficients.

The model adequacies were checked by the coefficient of determination R^2, adjusted R^2 (adj-R^2), the lack-of-fit test and the F and P-values. The model was considered valid when R^2 and adj-R^2 were greater than 80 %, F and P significant at the statistical significance level of 5 % [22]. To determine the optimum formulation leading to maximizing the different responses, the multi-response optimization based on the desirability method was adopted [23]. To confirm the validity of the models for predicting the optimum responses, experiments were conducted in triplicate under the optimum conditions, and both predicted and experimental values of the responses were compared using a Student *t*-test.

2.4.3. Physicochemical characterization of processed biscuits

For the proximal composition of processed biscuits, humidity, ash, total sugar, total protein, lipid and crude fibres were determined using the above-described methods. The total proteins, total sugars and lipids contents of each sample of biscuits were used to calculate their energetic value following the method proposed by AOAC [14]. The pH and titratable acidity of biscuits were measured using the method described by Ferreira et al. [24].

2.4.4. Microbiological quality of processed biscuits

To control the microbiological quality of processed biscuits, enumeration of the total aerobic mesophilic flora, total and fecal coliforms, *E. coli*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., yeasts and moulds, were performed. Under aseptic conditions, 25 g of biscuit samples were ground, mixed with 225 mL of sterile saline (0.9 %, w/v), left on the bench for 30 min and serially ten-folds diluted (10^{-1} to 10^{-6}) [25]. The total aerobic mesophilic flora was enumerated by bottom inoculation on Plate Count Agar medium [26], while total coliforms, fecal coliforms, *E. coli*, *Staphylococcus* spp., yeasts and moulds were enumerated by surface inoculation on McConkey agar [27], Eosin Methylene Blue agar [27], Mannitol Salt agar [28], and Sabouraud agar supplemented with chloramphenicol [29], respectively. *Salmonella* spp. and *Shigella* spp. were assessed in three steps (pre-enrichment in sterile peptone water, enrichment in sterile Selenite cysteine broth, and streaking on Salmonella and Shigella agar following the method ISO 6579-1 [30]. All the culture media were from LiofilChem (Italy) and microbial loads were expressed as Log CFU/g of biscuits.

2.4.5. Sensory evaluation of processed biscuits

The sensory parameters of biscuits produced from the 16 formulations were assessed by a trained panel of 25 participants according to the method described by Eyenga et al. [31]. The panelists who regularly consumed biscuits were of both sexes (10 men and 15 women), available, aged of 18 years and more, non-smokers, and in good health with no allergy to any of the ingredients used in the preparation of biscuits. They were all volunteers who signed an informed consent sheet after being aware of the objectives of the study. Panelists were instructed not to eat or drink (except water) 1 h before the session. The sensory test was carried out in a large, purpose-built facility and the panelists assessed the perceived sensory characteristics (color, tenderness, surface roughness, hardness, friability, crispness, taste, odor and general acceptability) of the biscuits on a 9-point scale ranging from 0 (extremely dislikes) to 9 (extremely like). The different biscuits were introduced in a white dish, coded and randomly submitted to the panelists. They were accompanied by a glass of water to rinse their mouth between two samples.

2.4.6. Antioxidant activity of processed biscuits

The ability to scavenge free radicals was exploited in this study to assess the antioxidant activity of biscuit samples. The method described by Uddin et al. [32] based on the ability of biscuits to scavenge the DPPH (2.2-diphenyl-1-picrylhydrazyl) free radical was used. Briefly, biscuits were prepared in three solvents (ethanol, methanol, distilled water) at concentrations of 0.0, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 μg/mL. Then, 1 mL of each solution was pipetted and introduced into a tube containing 4 mL of DPPH solution (0.2 mM in methanol). After homogenization, the mixture was kept in darkness for 30 min at room temperature (25 ± 2 °C). An UVmini-1240 spectrophotometer (Shimadzu, Japan) was used to read the optical density at 536 nm against the blank (solvent). Vitamin C (Ascorbic acid) was used as standard. The scavenging activity of the DPPH radical was calculated as follows:

$$DPPH\ scavenging\ activity\ (\%) = \frac{(OD_{control} - OD_{sample})}{OD_{control}} \times 100 \quad (2)$$

With ODcontrol = optical density of the solution free of extract, and ODsample = optical density of the solution containing biscuit extract at a given concentration. The DPPH inhibition percentage was plotted as a function of the biscuit concentrations and the plot was used to calculate IC50 which refers to the concentration at which 50 % of DPPH radical is inhibited.

2.5. Statistical analysis

All experiments performed in this study were replicated at least three times. Statgraphic Centurion XVI version 16.1.18 (StatPoint Technologies, Inc., Virginia, USA) was used to calculate means, standard deviation, and standard errors, perform analysis of variance, and Duncan multiple range test. The significance level was set at 5 %. The mixture design, regression analysis, and principal component analysis were performed using Minitab 18 version 18.1 (Minitab, Inc., USA) while graphs were plotted using Sigma Plot 12.5 version 12.5.0.38 (Systat Software, Inc., Chicago IL, USA).

3. Results and discussion

3.1. Proximate composition of the flours

The proximate composition of the WF, BFPF, PFPF and SF is depicted in Table 2. The highest water contents (13.34 ± 0.23 and 19.37 ± 0.15 %) were recorded with BFPF and PFPF, probably due to the hygroscopic properties of fruit flours as they contain more soluble sugars which easily bind water molecules [33]. BFPF scored the highest ash content (6.55 ± 0.15 g/100 g DM) followed by PFPF (5.31 ± 0.25 g/100 g DM) and SF (4.29 ± 0.41 g/100 g DM). Crude fibres were mostly found in BFPF (8.57 ± 0.76 g/100 g DM) and PFPF (5.74 ± 0.86 g/100 g DM). This can be ascribed to the fact that fruits contain generally high crude fibres compared to cereals and leguminous [8,9]. The highest total carbohydrates were obtained with WF (69.96 ± 2.56 g/100 g DM), BFPF (67.21 ± 1.48 g/100 g DM) and PFPF (61.77 ± 0.57 g/100 g DM). SF was characterized by the high contents in proteins (36.00 ± 2.49 g/100 g DM) and lipids (23.95 ± 1.57 g/100 g DM). This characteristic is specific to leguminous crops [34]. Bioactive compounds including polyphenols and flavonoids were present in all flours at contents which vary significantly ($p<0.05$) from one ingredient to another. The highest total polyphenols content was obtained with BFPF (1227.21 ± 5.03 mg GAE/100 g DM) while PFPF scored the highest flavonoids (710.03 ± 0.83 mg QE/100 g DM).

3.2. Techno-functional properties of the flours

Some techno-functional properties of the flours were assessed. The results presented in Table 3 showed that PFPF has the greatest WAC (1121.40 ± 23.95 %), followed by BPFP (823.14 ± 33.75 %). The high WAC values obtained with PFPF and BFPF could be attributed to their high contents of hydrophilic compounds opposite to starch which constitute the main carbohydrates of WF and SF. This result is consistent with the findings of Adeyanju and Bamidele [12]. In addition, the highest crude fibres' contents of PFPF and BFPF observed in this study can also justify their high WAC. Indeed, fibres are known for their great capacity to absorb water [35].

An indication of the quantity of soluble solids that are present in flour is its WSI [36]. The highest WSI was recorded with BFPF (62.57 ± 0.84 %). This can be ascribed to the nature of constituents present in BFPF. Indeed, WF is mainly constituted of starch, which is generally less soluble in water [4]. SF besides starch, also contains insoluble constituents such as lipids and non-polar proteins, which contribute to reducing its solubility in water.

An interesting property of flour used for baking industries is its OAC [4]. The use of flours with high OAC leads to products with improved palatability, mouth feel, flavor retention and shelf life [37]. In this study, SF showed the highest OAC (266.80 ± 4.49 %). This can be justified by the huge amount of proteins in SF. Indeed, proteins are amphiphilic compounds containing both polar and non-polar side chains. Their non-polar side chain can bind the oil hydrocarbon chain side leading to increased OAC. A similar observation was noticed by Maboh et al. [37].

3.3. Formulation of biscuits from the different flours

Table 1 presents the experimental matrix obtained under experimental conditions described by the mixture design. Globally, it was noticed stochastic variations in the physicochemical, microbiological, antioxidant and sensorial qualities of biscuits according to the proportions of the different flours in the mixture.

3.3.1. Modeling

The multilinear regression analysis of each response obtained the estimations of the model coefficients and the respective equations were as follows:

Table 2
Proximal composition of the flours from the different ingredients.

Parameters	Flours			
	WF	PFPF	BFPF	SF
Water content (g/100 g)	11.81 ± 0.53^b	19.37 ± 0.15^d	13.34 ± 0.23^c	8.34 ± 0.34^a
Ash (g/100 g DM)	0.34 ± 0.05^a	5.31 ± 0.25^d	6.55 ± 0.15^b	4.29 ± 0.41^c
Lipids (g/100 g DM)	4.14 ± 0.12^b	6.19 ± 0.36^c	1.89 ± 0.08^a	23.95 ± 1.57^d
Crude fibres (g/100 g DM)	1.19 ± 0.09^a	5.74 ± 0.86^c	8.57 ± 0.76^d	2.96 ± 0.62^b
Total carbohydrates (g/100 g DM)	69.96 ± 2.56^c	61.77 ± 0.57^b	67.21 ± 1.48^c	24.46 ± 1.85^a
Proteins (g/100 g DM)	12.56 ± 1.37^c	1.62 ± 0.07^a	5.44 ± 0.33^b	36.00 ± 2.49^d
Total polyphenols (mg GAE/100 g DM)	248.50 ± 0.93^b	656.15 ± 2.10^b	1227.21 ± 5.03^b	424.76 ± 0.44^b
Flavonoids (mg QE/100 g DM)	79.50 ± 0.11^b	710.03 ± 0.83^b	566.57 ± 6.72^b	231.42 ± 0.76^b

DM = dry matter, GAE = gallic acid equivalent, QE = quinin equivalent. WF = wheat flour, PFPF = papaya fruit pulp flour, BFPF = baobab fruit pulp flour, SF = soybean flour. Values bearing the different superscript letters in the same raw are significantly different at $p<0.05$.

Table 3
Techno-functional properties of the flours from the different ingredients.

Flours	WAC (%)	WSI (%)	OAC (%)
BFPF	823.14 ± 33.75^c	62.57 ± 0.84^c	107.02 ± 7.09^a
WF	116.41 ± 6.05^a	26.55 ± 1.40^a	143.00 ± 9.42^b
PFPF	1121.40 ± 23.95^d	30.12 ± 1.14^b	104.52 ± 4.05^a
SF	331.91 ± 4.27^b	30.77 ± 0.98^b	266.80 ± 4.49^c

WAC = water absorption capacity, WSI = water solubility index, OAC = oil absorption capacity. WF = wheat flour, PFPF = papaya fruit pulp flour, BFPF = baobab fruit pulp flour, SF = soybean flour. Values bearing the same superscript letters in the same raw are not significantly different at $p<0.05$.

$$pH = 7.14\, X_1 + 4.31\, X_2 + 7.20\, X_3 + 5.42\, X_4 \tag{3}$$

$$Ash = 2.92\, X_1 + 6.15\, X_2 + 3.42\, X_3 + 6.48\, X_4 \tag{4}$$

$$Proteins = 12.27\, X_1 + 3.00\, X_2 + 18.73\, X_3 + 8.97\, X_4 \tag{5}$$

$$Crude\ fibres = 2.90\, X_1 + 6.46\, X_2 + 3.78\, X_3 + 11.75\, X_4 \tag{6}$$

$$TAMF = 3.19\, X_1 + 0.86\, X_2 + 3.35\, X_3 + 4.05\, X_4 \tag{7}$$

$$Yeasts\ and\ moulds = 1.65\, X_1 + 7.34\, X_2 + 2.24\, X_3 - 0.16\, X_4 \tag{8}$$

$$General\ acceptability = 6.36\, X_1 + 0.99\, X_2 + 6.00\, X_3 + 3.81\, X_4 \tag{9}$$

$$Antioxidant\ activity = 1.25\, X_1 + 4.77\, X_2 + 11.21\, X_3 + 15.81\, X_4 \tag{10}$$

Where X_1 refers to WF, X_2 to PFPF, X_3 to SF and X_4 to BFPF.

3.3.2. Validation of models' parameters

As observed in Table 4, the R^2 and adj-R^2 values of the different models were all higher than 80 %, indicating that these models explained more than 80 % of the variability in the respective responses. These values were close to standard values for validating models [22]. The F and P-values of the models demonstrated that they were significant. The lack-of-fit test of the different models were non-significant ($p>0.05$), indicating the strength of the models for the experimental data. All these results confirm the satisfactory fit of the regression models to the experimental data.

3.3.3. Effect of the different flours' formulations on the nutritional quality of the biscuits

Fig. 1 presents the biscuit-based meals prepared from the 16 formulations of flours from different matrices using a mixture design.

These biscuits were analyzed and the effect of flour's formulation on their nutritional quality is presented in Table 5. Water contents ranging from 5.55 (BS14) to 8.77 % (BS7) were obtained. The water contents of biscuits were all higher than the threshold value of 5 % recommended by the FAO for biscuits which will be stored for a long period [38]. This could be attributed to the WAC of the ingredients used in biscuits' formulation and suggests their handling with respect of good hygiene practices to avoid contamination as they might offer suitable conditions for microbial proliferation. Odeseye et al. [39] also highlighted the reduced shelf life of biscuits with high water content. Ash that reflects the mineral content of biscuits was also assessed. The ash content of biscuits significantly increases with the proportion of BFPF and PFPF in the formulation (Table 5). This can be explained by the high ash content of these fruit flours. Varastegani et al. [40] also reported that the ash content of biscuits increases with the addition PFPF in the formulation. Yusufu and Akhigbe [41] highlighted that the substitution of WF with PFPF contributes to an increase in the ash content of biscuits. The results of this study suggest the potential of biscuits BS5, BS6, BS7, BS8 and BS13 to boost the immune system and to prevent/manage cardiometabolic diseases.

Table 4
Values of the parameters used for statistical validation of models.

Parameters	pH	Ash	Proteins	Fibers	TAMF	Yeasts and moulds	General Acceptability	Antioxidant activity
R^2	88.24	85.13	90.63	94.03	87.38	84.67	88.41	86.01
Adjusted R^2	87.66	80.18	87.51	92.05	80.17	80.00	84.55	81.78
F	6.45	17.18	29.03	47.33	1.13	0.98	4.21	6.11
P-value	0.01	0.0005	0.0001	0.00	0.03	0.04	0.04	0.02
Absolute Error	0.17	0.19	0.72	0.25	0.32	0.99	0.37	0.21
Type Error	0.28	0.31	1.16	0.46	0.43	1.47	0.53	0.96
Lack of fit	0.89	0.33	0.61	0.88	0.56	0.86	0.36	0.42

TAMF = total aerobic mesophilic flora.

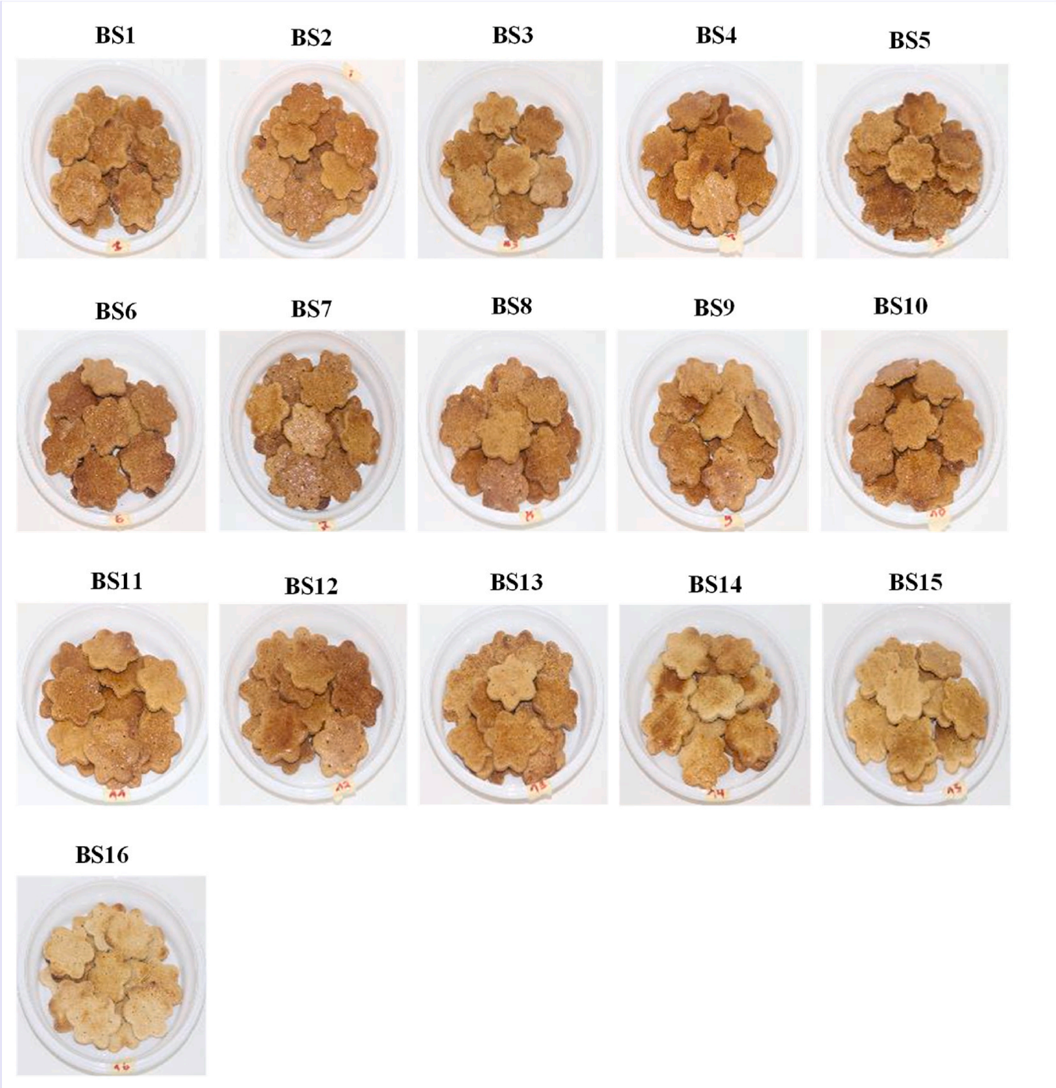

Fig. 1. Biscuits formulated from the different flours using a mixture design.

As shown in Table 5, the crude fibres' content of biscuits ranges significantly ($p < 0.05$) from 2.09 (BS14) to 7.26 g/100 g DM (BS8). Globally, the crude fibres' content increases with the proportions of BFPF in the mixture, as well as the proportion of SF and PFPF. This can be ascribed to the high crude fibres' content of these flours compared to WF. Mounjouenpou et al. [42] also noticed that cookies made with 20 % BFPF contained more crude fibres than the ones made with 100 % WF. The results obtained in this study suggest that biscuits BS5, BS6, BS7, BS8 and BS13 with high crude fibres' contents might be useful as a functional food to prevent/manage diseases including digestive disorders, constipation, diverticular disease, hemorrhoids, heart diseases, colon cancer and diabetes. However, further studies on dietary fibres should bring more insights into these health benefit potentials as with crude fibres, it might be an underestimation of fibres due to their limited relevance in human nutrition.

Proteins represent nutrients required for growth and several physiologic functions. Table 5 shows the influence of flour formulation on the protein content of biscuits. The protein content of biscuits varies significantly ($p < 0.05$) from 6.50 (BS16) to 18.74 g/100 g DM (BS2). It can be observed that the protein content of biscuits increases with the proportion of SF in the formulation. This is due to the high protein content of SF. An increase in the protein content of biscuits following the incorporation of SF as a substitute for WF was reported by Ghoshal and Kaushik [7]. Given that soybeans contain essential amino acids, vitamins, polyphenolic compounds and minerals [34], BS2, BS4, BS10 and B12 might be useful in the management of malnutrition and cardiometabolic diseases.

Fats represent the principal elements that contribute to the sensory attributes of biscuits including softness, mouth feeling and retention of flavors [31,37,43]. As for the proteins content of biscuits where an increase in the proportion of SF in the formulation was noticed, a similar behavior was noticed with the lipids content of biscuits (Table 5). This is probably due to the high lipids content of SF and also to the high oil absorption capacity of SF. Indeed, during the baking process, lipids are lost when they are not held in the dough,

Table 5
Nutritional values of the biscuits prepared from different formulations of flours.

Biscuits	Lipids (g/100gDM)	Ash (g/100gDM)	Crude fibres (g/100gDM)	Water content (g/100gDM)	Proteins (g/100gDM)	Total carbohydrates (g/100gDM)	Energetic value (Kcal/100gDM)
BS1	17.39 ± 0.55^{b}	2.97 ± 0.38^{b}	2.93 ± 0.01^{b}	5.72 ± 0.31^{a}	12.73 ± 0.02^{f}	58.74 ± 0.21^{e}	442.45 ± 2.01^{e}
BS2	22.05 ± 0.81^{e}	4.07 ± 0.01^{f}	4.90 ± 0.07^{f}	7.00 ± 0.05^{c}	18.73 ± 0.09^{j}	47.90 ± 0.56^{b}	485.71 ± 4.15^{g}
BS3	17.35 ± 0.49^{b}	3.29 ± 0.05^{c}	3.44 ± 0.21^{c}	8.16 ± 0.02^{f}	10.39 ± 0.23^{d}	61.34 ± 0.07^{e}	443.13 ± 3.18^{e}
BS4	24.17 ± 0.87^{f}	3.52 ± 0.01^{d}	3.87 ± 0.14^{d}	7.53 ± 0.02^{d}	16.41 ± 0.22^{i}	42.14 ± 0.31^{a}	461.13 ± 2.01^{f}
BS5	17.38 ± 0.49^{b}	4.15 ± 0.03^{g}	6.28 ± 0.14^{g}	7.97 ± 0.11^{e}	11.32 ± 0.05^{e}	52.86 ± 0.04^{c}	413.21 ± 2.29^{b}
BS6	20.34 ± 0.32^{d}	4.26 ± 0.12^{g}	6.53 ± 0.14^{h}	5.46 ± 0.80^{a}	14.90 ± 0.02^{h}	48.48 ± 0.56^{b}	436.66 ± 0.72^{d}
BS7	17.53 ± 0.25^{b}	4.70 ± 0.07^{g}	6.80 ± 0.21^{h}	8.77 ± 0.11^{h}	9.06 ± 0.39^{c}	53.11 ± 0.02^{d}	406.55 ± 1.20^{a}
BS8	18.90 ± 0.42^{c}	5.36 ± 0.03^{h}	7.26 ± 0.06^{i}	7.89 ± 0.10^{h}	13.37 ± 0.22^{g}	47.20 ± 0.56^{b}	412.38 ± 2.44^{b}
BS9	17.82 ± 0.87^{bc}	2.99 ± 0.13^{b}	2.90 ± 0.04^{b}	5.74 ± 0.20^{a}	12.73 ± 0.31^{f}	58.00 ± 0.48^{e}	443.33 ± 3.18^{e}
BS10	22.07 ± 0.42^{e}	4.00 ± 0.07^{f}	5.13 ± 0.17^{f}	7.09 ± 0.32^{c}	18.42 ± 0.49^{j}	46.94 ± 0.91^{b}	481.77 ± 0.52^{g}
BS11	17.47 ± 0.47^{b}	3.34 ± 0.08^{c}	3.43 ± 0.27^{c}	8.46 ± 0.06^{g}	10.74 ± 0.54^{d}	60.00 ± 0.61^{e}	446.39 ± 1.58^{e}
BS12	23.33 ± 0.08^{f}	3.54 ± 0.10^{d}	3.90 ± 0.20^{d}	7.45 ± 0.11^{d}	16.58 ± 0.09^{i}	43.33 ± 0.54^{a}	460.07 ± 0.06^{f}
BS13	17.91 ± 0.85^{bc}	4.18 ± 0.02^{g}	6.10 ± 0.19^{g}	8.07 ± 0.01^{e}	11.45 ± 0.15^{e}	51.55 ± 0.88^{c}	413.29 ± 3.58^{b}
BS14	17.36 ± 0.45^{b}	2.07 ± 0.13^{a}	2.09 ± 0.07^{a}	5.55 ± 0.12^{a}	7.15 ± 0.05^{b}	65.75 ± 1.32^{f}	447.93 ± 6.21^{e}
BS15	16.77 ± 0.28^{ab}	2.25 ± 0.03^{a}	2.28 ± 0.20^{a}	7.95 ± 0.14^{e}	6.54 ± 0.30^{a}	64.17 ± 0.20^{f}	433.86 ± 2.22^{d}
BS16	15.79 ± 1.00^{a}	3.63 ± 0.03^{e}	4.50 ± 0.21^{e}	6.60 ± 0.01^{b}	6.50 ± 0.06^{a}	62.94 ± 1.07^{f}	419.98 ± 4.52^{c}

DM = dry matter. Values bearing different superscript letters on the same column are significantly different ($p < 0.05$).

leading to biscuits with low lipids content [4]. The high lipids content of biscuits BS2, BS4, BS6, BS10 and BS12 might suggest a reduced shelf life through the lipid oxidation process. However, the presence of phenolic compounds in SF might protect these biscuits from oxidation. Besides, the presence of phenolic compounds, ascorbic acid in BFPF [10] and β-carotene in PFPF might also contribute through their antioxidant capacity, in increasing the shelf life of these biscuits.

The total carbohydrate content of biscuits ranges from 46.94 (BS10) to 65.75 g/100 g DM (BS14). Globally, the total carbohydrate content of biscuits decreases as the proportion of SF in the formulation increases, probably because SF contains more proteins and lipids than carbohydrates. Hence, substituting WF with SF in the formulation leads to a decrease in the carbohydrate content of biscuits. Considering biscuits BS15 (90 % WF and 10 % PFPF) and BS16 (80 % WF and 20 % BFPF), the total carbohydrates content was not significantly ($p>0.05$) different to that of the control biscuit BS14 made with 100 % WF. This observation implies that the substitution of WF with BFPF or PFPF does not affect the carbohydrate content of biscuits, but increases the sweetening taste of these latters. The consequence is the reduction of the quantity of sugar used in biscuit preparation thus conferring health benefits effects to these products. Kulla et al. [44] also reported that papaya fruit pulp can be used as a substitute for sugar in biscuits' preparation.

The development of new products that target children and adults has energy as the main requirement [45]. Indeed, their energy requirements are high. The biscuits developed in this study showed energetic values that range from 406.55 (BS7) to 485.71 kcal/100 g DM (BS2). Globally, biscuits with a high proportion of SF in the formulation showed the highest energetic values (Table 5). This can be ascribed to the fact that lipids contribute more to energetic value compared to carbohydrates and proteins [46]. The richness of SF in lipids associated with their high OAC could explain the increase in energetic values biscuits as the proportion of SF increases in the

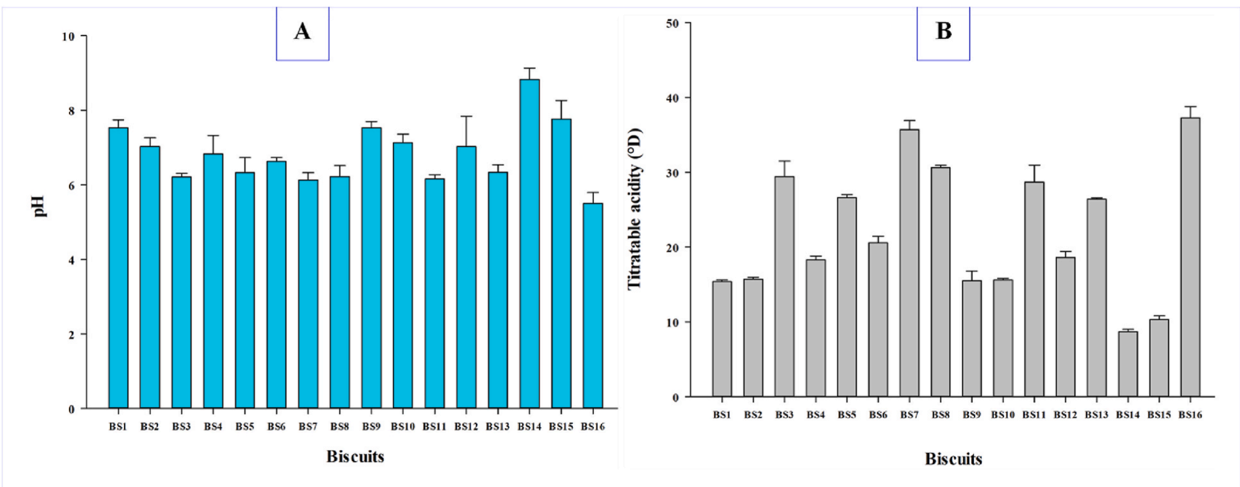

Fig. 2. Effect of the flour formulations on the pH (A) and titratable acidity (B) of the different biscuits.

formulation. Mouafo et al. [4] also noticed that biscuits with high energetic values are those for which the flour used for dough preparation contained high OAC. The results obtained in this study suggest that biscuits with energetic values higher than 400 kcal/100 g DM can be used as a food complement to fight against protein-energetic malnutrition.

3.3.4. Effects of the different flours' formulations on the pH and titratable acidity of the biscuits

pH is an important parameter of foods as it influences their shelf life. Fig. 2 shows that the pH and titratable acidity of biscuits are inversely proportional. They vary respectively from 5.50 (BS16) to 8.83 (BS14) and from 8.70 (BS14) to 37.28°D (BS16). Globally, an increase of BFPF in the formulation leads to a significant ($p<0.05$) decrease in the pH values of biscuits while increasing their titratable acidity. This could be attributed to the acidity of BPFP. Indeed, baobab fruit pulp contains many organic acids which are responsible for its acidic nature [10]. The results obtained in this study suggest that biscuits with high BFPF proportion might be of good microbiological quality and thus possess a good ability for preservation.

3.3.5. Effect of the different flours' formulations on the microbiological quality of the biscuits

The mean microbial loads (Log CFU/g) of biscuits are presented in Table 6. The TAMF provides an idea of the level of contamination and the variability of microorganisms that can be present in a product. In this study, the TAMF were present in all samples at loads ranging significantly ($p<0.05$) from 2.14 Log CFU/g (BS6) to 3.76 Log CFU/g (BS5). Globally, the TAMF was not associated with the proportion of a particular flour in the formulation. Considering the microbiological criteria for biscuits intended for human consumption, biscuits BS5, BS13 and BS15 showed TAMF loads higher than the threshold value which is 3 Log CFU/g [47]. They might be considered as unsuitable for human consumption. However, the safety of a product is not necessarily determined by its TAMF loads, but by the different groups of microorganisms present in that product [48].

Total and fecal coliforms were present in only a few samples of biscuits at loads ranging significantly ($p<0.05$) from 0.5 Log CFU/g (BS4) to 2.44 Log CFU/g (BS6) and from 1.07 Log CFU/g (BS14) to 2.11 Log CFU/g (BS6), respectively. Pathogens associated with good hygiene and manufacturing practices during biscuit production and handling including *E. coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus* spp. were not present in all biscuit samples independent of the formulation. This result is consistent with the findings of Mouafo et al. [4] and makes no doubt on the safety status of the biscuits and suggests their suitability for human consumption.

Yeasts were present in biscuits BS2, BS4, BS10, BS12, BS14 and BS15 while moulds were present in BS2, BS10, BS13, BS14 and BS15. The presence of these microorganisms could be associated with their spore-forming ability. A similar observation was reported by Mouafo et al. [4] with biscuits made with fermented sweet potato flour supplemented with mackerel. Compared to the norms for which a load of yeasts and moulds loads should not exceed 2 Log CFU/g, all the biscuits produced independent of the flour formulation might be considered safe for human consumption.

3.3.6. Effect of the different flours' formulations on the sensorial quality of the biscuits

New products were developed in this study using different flour formulations and submitted to descriptive sensory analysis to predict their acceptance by consumers. Color is a key factor in food quality and represents the product characteristic that significantly influences the customer's purchasing decision [4]. In this study, the color of the developed meal-based biscuits using flours from different crops was measured by a panel. As shown in Table 7, all biscuits received scores greater than 5.2. The highest score (6.95) was recorded for BS14 and the lowest score (5.2) with biscuits BS2 and BS10. Globally, although non-significant ($p > 0.05$), the color scores increase as the concentration of SF decreases in the formulation. This could be due to the brown color of the biscuits that became darker as the concentration of SF increased. That dark brown color seems not to be appreciated by the panelists. Besides, the richness in PFPF in reducing sugars that could be involved in the Maillard reaction with SF proteins during baking could also explain the dark

Table 6
Microbial loads (Log UFC/g) of biscuits prepared from the different formulations of flours.

Biscuits	TAMF	Total coliforms	Fecal coliforms	Yeasts	Moulds
BS1	2.75 ± 0.16^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS2	2.64 ± 0.06^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.38 ± 0.12^{c}	1.48 ± 0.07^{d}
BS3	2.33 ± 0.02^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS4	2.90 ± 0.13^{cd}	0.50 ± 0.07^{b}	0.00 ± 0.00^{a}	0.10 ± 0.00^{b}	0.00 ± 0.00^{a}
BS5	3.76 ± 0.02^{f}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS6	2.14 ± 0.04^{a}	2.44 ± 0.02^{f}	2.11 ± 0.21^{d}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS7	3.00 ± 0.05^{d}	0.73 ± 0.04^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS8	2.18 ± 0.05^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS9	2.67 ± 0.03^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS10	2.67 ± 0.10^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.40 ± 0.00^{d}	1.40 ± 0.06^{d}
BS11	2.34 ± 0.07^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS12	2.80 ± 0.16^{cd}	0.60 ± 0.23^{de}	0.00 ± 0.00^{a}	0.13 ± 0.02^{b}	0.00 ± 0.00^{a}
BS13	3.75 ± 0.01^{f}	0.00 ± 0.07^{de}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
BS14	2.75 ± 0.04^{c}	1.76 ± 0.05^{d}	1.07 ± 0.17^{b}	1.12 ± 0.01^{f}	1.03 ± 0.11^{b}
BS15	3.18 ± 0.02^{e}	2.06 ± 0.02^{e}	1.70 ± 0.13^{c}	1.00 ± 0.05^{e}	1.13 ± 0.02^{b}
BS16	2.18 ± 0.04^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.21 ± 0.01^{c}
Norms	3	1	1	2	2

TAMF = total aerobic mesophilic flora, Norms = Microbiological criteria for foodstuffs of the European Commission Regulation. Values bearing different superscript letters on the same column are significantly different ($p < 0.05$).

Table 7
Sensory characteristics of the different biscuits.

Biscuits	Color	Tenderness	Surface roughness	Hardness	Friability	Crispness	Taste	Odor	General acceptability
BS1	6.40 ± 0.22^{d}	6.21 ± 0.14^{c}	6.10 ± 0.12^{b}	6.21 ± 0.50^{ab}	6.00 ± 0.13^{c}	6.15 ± 0.17^{b}	6.60 ± 0.26^{c}	6.00 ± 0.50^{c}	6.40 ± 0.45^{c}
BS2	5.20 ± 0.14^{a}	5.05 ± 0.67^{ab}	6.05 ± 0.13^{b}	6.05 ± 0.39^{ab}	6.10 ± 0.37^{c}	6.25 ± 0.14^{b}	6.55 ± 0.15^{c}	4.45 ± 0.28^{a}	5.20 ± 0.18^{a}
BS3	6.20 ± 0.31^{d}	5.84 ± 0.17^{ab}	5.84 ± 0.39^{ab}	5.89 ± 0.63^{a}	6.00 ± 0.16^{c}	6.45 ± 0.10^{bc}	5.60 ± 0.44^{b}	6.12 ± 0.25^{c}	6.25 ± 0.23^{c}
BS4	5.40 ± 0.12^{b}	5.35 ± 0.38^{a}	5.35 ± 0.39^{a}	6.12 ± 0.36^{ab}	5.45 ± 0.13^{a}	5.97 ± 0.50^{ab}	4.60 ± 0.43^{a}	5.00 ± 0.26^{b}	5.65 ± 0.38^{b}
BS5	6.00 ± 0.13^{d}	5.60 ± 0.15^{a}	5.00 ± 0.43^{a}	6.30 ± 0.17^{a}	5.80 ± 0.08^{b}	5.65 ± 0.37^{ab}	7.00 ± 0.13^{d}	6.20 ± 0.38^{c}	6.00 ± 0.17^{c}
BS6	5.63 ± 0.21^{bc}	5.15 ± 0.51^{a}	5.15 ± 0.46^{a}	6.30 ± 0.38^{ab}	5.95 ± 0.16^{bc}	5.65 ± 0.48^{ab}	6.25 ± 0.42^{c}	5.40 ± 0.26^{b}	5.65 ± 0.16^{b}
BS7	6.35 ± 0.30^{bc}	5.30 ± 0.38^{a}	6.30 ± 0.42^{b}	6.00 ± 0.48^{ab}	6.85 ± 0.16^{d}	6.35 ± 0.30^{bc}	6.90 ± 0.14^{cd}	6.65 ± 0.48^{c}	6.65 ± 0.29^{cd}
BS8	5.90 ± 0.19^{cd}	5.00 ± 0.94^{ab}	6.00 ± 0.51^{b}	6.50 ± 0.43^{ab}	6.25 ± 0.28^{c}	5.80 ± 0.13^{a}	6.60 ± 0.13^{c}	5.90 ± 0.23^{c}	6.45 ± 0.17^{c}
BS9	6.45 ± 0.52^{de}	6.20 ± 0.55^{bc}	6.10 ± 0.27^{b}	6.20 ± 0.38^{ab}	6.00 ± 0.17^{c}	6.10 ± 0.30^{a}	6.60 ± 0.16^{c}	6.00 ± 0.14^{c}	6.40 ± 0.50^{c}
BS10	5.20 ± 0.05^{a}	5.00 ± 0.81^{a}	6.00 ± 0.13^{b}	5.90 ± 0.33^{a}	6.00 ± 0.13^{de}	6.20 ± 0.19^{b}	6.50 ± 0.32^{c}	4.40 ± 0.16^{a}	5.20 ± 0.26^{a}
BS11	6.21 ± 0.27^{d}	5.86 ± 0.15^{ab}	5.86 ± 0.22^{ab}	5.95 ± 0.16^{a}	6.15 ± 0.31^{c}	6.47 ± 0.20^{bc}	5.75 ± 0.33^{b}	6.25 ± 0.21^{c}	6.30 ± 0.18^{c}
BS12	5.38 ± 0.29^{ab}	5.45 ± 0.31^{a}	5.45 ± 0.19^{a}	6.00 ± 0.45^{a}	5.40 ± 0.16^{a}	6.00 ± 0.14^{a}	4.55 ± 0.13^{a}	5.00 ± 0.18^{b}	5.65 ± 0.13^{b}
BS13	6.15 ± 0.19^{d}	5.60 ± 0.18^{a}	5.10 ± 0.16^{a}	6.20 ± 0.39^{ab}	5.80 ± 0.15^{b}	5.66 ± 0.45^{a}	7.10 ± 0.77^{d}	6.10 ± 0.29^{c}	6.05 ± 0.10^{c}
BS14	6.95 ± 0.15^{e}	6.40 ± 0.14^{c}	6.60 ± 0.15^{c}	6.75 ± 0.69^{ab}	6.00 ± 0.31^{c}	6.30 ± 0.29^{bc}	7.60 ± 0.26^{e}	7.30 ± 0.05^{d}	6.80 ± 0.19^{d}
BS15	6.60 ± 0.42^{e}	6.10 ± 0.16^{b}	5.55 ± 0.29^{a}	6.40 ± 0.47^{ab}	6.10 ± 0.06^{c}	6.60 ± 0.37^{c}	6.00 ± 0.22^{b}	6.65 ± 0.25^{c}	6.25 ± 0.34^{c}
BS16	6.85 ± 0.16^{e}	6.00 ± 0.21^{b}	5.40 ± 0.19^{a}	6.65 ± 0.49^{b}	6.10 ± 0.20^{c}	6.65 ± 0.11^{c}	7.25 ± 0.16^{d}	6.95 ± 0.64^{c}	6.10 ± 0.44^{c}

Values bearing different superscript letters in the same column are significantly different at $p<0.05$.

brown coloration observed in this study. Color modification following the incorporation of fruit flour in biscuits formulation was also reported by Lara et al. [49].

Table 7 shows that the tenderness of biscuits BS1, BS9, BS14, BS15 and BS16, with scores greater than 6, were the most appreciated by the panelists. A similar observation was noticed with the scores for biscuits' hardness. This can be explained by the WAC of the flours used in the formulation. Indeed, biscuits for which the highest scores of tenderness and hardness were obtained in this study, were characterized by their high proportion in WF. The highest WAC of PFPF, BPFP and SF which led to dough that was tough to knead could be the reason for which the derived biscuits were hard and thus less appreciated by the panelists. This result is consistent with the findings of Eyenga et al. [31].

The surface roughness and friability of biscuits were assessed by the panelists and the results are depicted in Table 7. Although there was no significant difference ($p>0.05$) between biscuits from the different flours' formulation, BS7 and BS14 showed the highest scores (6.85 and 6.7, respectively) regarding the biscuits' friability. While considering the surface roughness of biscuits, the highest scores were also recorded with BS7 (6.3) and BS14 (6.6). The differences in the surface roughness and friability scores of biscuits were not associated with the proportion of a particular flour in the formulation. This suggests that other parameters linked to the interaction between the different flour constituents should be investigated to bring more insights into the results obtained in this study.

As shown in Table 7, a significant ($p<0.05$) decrease in the crispness scores was recorded as the proportion of SF increases in the formulation. This could be due to the proximate composition of SF. Indeed, SF contains more lipids which contribute to reducing the crispness of biscuits. Chakrabarti et al. [43] and Eyenga et al. [31] also mentioned that the incorporation of flour rich in lipids in the formulation of biscuits contributes to increasing its softness and thus reduces its crispness. However, biscuits made of the mixture of either WF and PFPF or WF and BFPF showed the highest scores of crispness compared to other biscuits including the control ones BS14 made of 100 % WF. This can be explained by the proportions of soluble sugars brought by fruit flours in the formulation. These carbohydrates acted as hardening agents as they crystallize during biscuit cooling leading to a crisp product [50].

Taste is an important parameter that influences the acceptability of a product. In this study, panelists were asked to assess the taste of biscuits. The lowest scores were recorded with BS4 (4.5) and BS12 (4.6) while BS14 showed the highest score (7.6). BS7 with a score of 7.25 showed that BFPF might influence the biscuit taste. Important scores were also observed for biscuits BS5, BS6, BS7, BS8 and BS13, where the proportion of BFPF in the formulation was 20 %. This can be ascribed to the presence of flavoring compounds and the acidic nature of BFPF [51] that might have attracted panelists' interest.

Biscuits odor was assessed by the panelists and the results are presented in Table 6. The scores recorded vary from 4.4 (BS10) to 7.3

(BS14). Although non-significant ($p > 0.05$), odor scores decrease as the proportion of SF in the formulation increases. This can be explained by the fact that the biscuit odor which developed during baking through the Maillard reaction could be masked by SF constituents. Besides, the influence of SF, BFPF and PFPF constituents (including proteins, lipids, sugars, and phenolic compounds) through enzymatic and non-enzymatic reactions, leading to the distinct aroma when sugars are heated could explain the difference in odor obtained with the control biscuit BS14 for which the panelists were more familiar. A similar observation was reported by Karaoğlu and Kotancilar [52]. The authors highlighted that the interaction between flour constituents during baking can result in the development of an aroma that might be appreciated or not by the panelists.

Regarding the general acceptability, BS1, BS7, BS8, BS9, and BS14 showed the highest scores. The less appreciated ones were BS2 and BS10. High preferences for wheat-based biscuits were also reported by Srivastava et al. [53] and Mouafo et al. [4].

Globally, the most appreciated biscuits were BS1, BS3, BS7, BS8, BS9, BS11 and BS14. However, to select the best ones, other parameters such as nutrient contents, bioactive compounds contents, microbiological quality, antioxidant activity, and water content of biscuits should be considered.

3.3.7. *Effect of the different flours' formulations on the antioxidant activity of biscuits*

The antioxidant activity of meal-based biscuits formulated using different flours was assessed through their ability to scavenge free radicals because, in the human body, free radicals represent one of the main leading causes of oxidative stress. DPPH free radical scavenging activities were observed in all extracts from the different biscuits independent of the solvents used. That scavenging activity evolved in a concentration-dependent manner as a significant increase was noticed when the concentration of the extracts increased. A similar observation was reported by Mouafo et al. [4] while accessing the antioxidant activity of meal-based biscuits. One of the great ways to compare the scavenging activity of compounds is through their IC50 which represents the concentration of the compounds required to scavenge 50 % of DPPH free radicals [54]. In this study, the IC50 of the different extracts of biscuits were calculated and the results are depicted in Fig. 3. Although the extracts showed interesting scavenging activities with IC50 ranging from 0.90 ± 0.10 to 13.40 ± 0.21 μg/mL, they were all less active than ascorbic acid (0.020 ± 0.001 μg/mL) used as control.

Regarding the flour formulations, globally, biscuits BS2, BS4, BS6, BS8, BS10 and BS12 showed the highest DPPH scavenging activity as they scored the lowest IC50 values independent of the extraction solvents. This can be ascribed to the phytochemical substances and some proteins present in high quantities in these biscuits. Indeed, these biscuits were characterized by their low content in WF (25 %) for which the main constituent is starch. Hence, as WF increases in the formulation, the antioxidant activity decreases significantly. Amongst these biscuits, the most active one was BS8 (IC50 of 0.90 ± 0.10 μg/mL) characterized by a high content of BFPF and PFPF (25 % WF, 45 % SF, 10 % PFPF and 20 % BFPF). This can be explained by the polyphenolic compounds present in these fruit flours. Indeed, fruit flour contains not only free phenolic compounds but also a high-level of phenolics bound to fibres [8]. The stability of bound phenolics to heat treatment applied during the biscuit preparation process could justify the highest antioxidant activity of BS8 obtained in this study when BFPF and PFPF increase in the formulation opposite to SF. In addition, the high vitamin C content in BFPF as reported in the literature [10] can also explain the high antioxidant activity observed in this study. Besides, the ability of fibres to transport phenolic compounds through the upper gastrointestinal tract of humans to the colon where the gut microbiota metabolizes fibres and releases these polyphenol compounds [8], suggests several health benefits associated with the consumption of these biscuits. These health benefits might be the reduction of the risk of cardiometabolic diseases and the improvement of the gut microbiota.

Considering the extraction solvents, the results showed that ethanol concentrated more bioactive compounds present in the

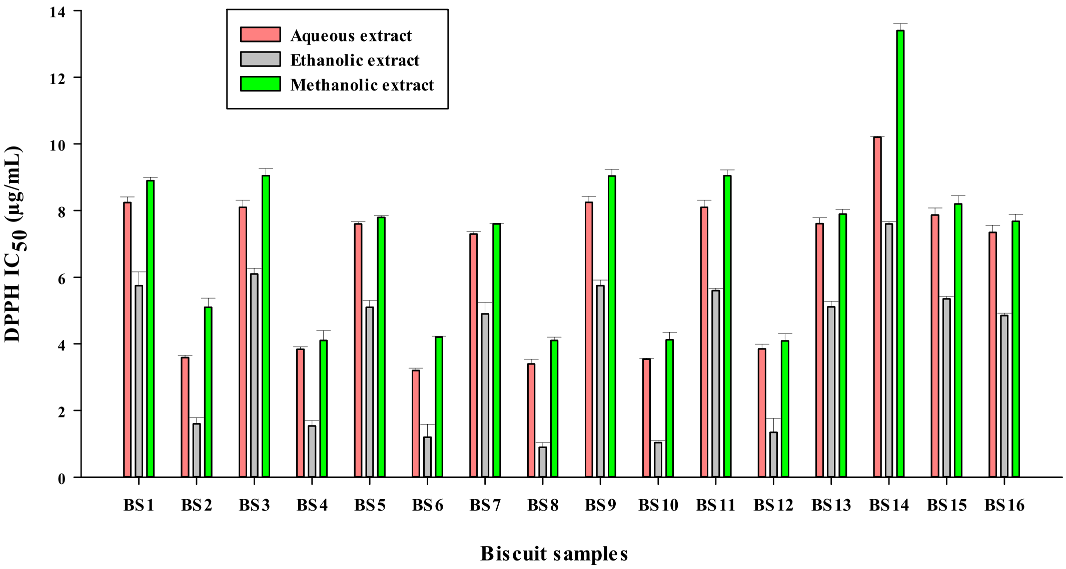

Fig. 3. Histograms showing the DPPH IC50 values of ethanolic, methanolic and aqueous extracts of the different biscuits.

different extracts, compared to water and methanol (Fig. 4). Indeed, the lowest IC50 values corresponding to the highest DPPH free radicals scavenging activity were recorded with ethanol as solvent. This observation can be ascribed to the ability of ethanol to extract more bioactive compounds, particularly phenolic compounds and flavonoids endowed with antioxidant activity [55,56]. As observed in Fig. 4, aqueous extracts were more active than the methanolic ones. This suggests that besides polyphenolic compounds and flavonoids, soluble compounds including some proteins and polysaccharides might be involved in the antioxidant activity. Indeed, they can donate active hydrogen to DPPH free radicals through their hydroxyl groups, leading to the scavenging of these radicals [62]. This observation is consistent with the findings of Mouafo et al. [4].

3.3.8. *Principal component analysis*

To visualize the relationship between the different variables and the individual biscuits obtained from different flour formulations, principal component analysis was performed. As shown in Fig. 4, the axes F1 and F2 expressed 89.89 % variation amongst the different samples. Four separated groups are denoted on the F1 × F2 plot. The first group includes biscuits BS2, BS10, BS4, BS12 and BS6, which are associated with proteins, lipids and energetic value. These biscuits are characterized by a high SF proportion in the formulation. This shows that to have biscuits with high proteins, lipids and energetic value, WF should be substituted by SF. It also suggests that these biscuits are suitable for the management of malnutrition. In addition, that group is opposite to a group which includes biscuits BS3, BS11 and BS5 correlated to TAMF, friability, general acceptability, taste, odor, and the DPPH IC_{50} of ethanolic, methanolic and aqueous extracts. Although these biscuits showed some interesting sensory attributes, they have a weak antioxidant activity. Indeed, as the IC_{50} is high, the DPPH scavenging activity is considered as weak. The biscuits of the first group (BS2, BS10, BS4, BS12 and BS6) that are opposite to this second group possessed the high antioxidant activity as they scored the lowest IC_{50} values. This observation shows that proteins significantly contributed to the antioxidant activity. Hence, these biscuits might also be suitable for the management of cardiometabolic diseases associated to oxidative stress. The third group is constituted of biscuits BS7, BS8 and BS16 which are correlated to crude fibres, ash, water content and titratable acidity. These biscuits are characterized by their high proportion of BFPF. This shows that the use of BFPF as a substitute for WF leads to biscuits with high contents of crude fibres and minerals. These biscuits should be chosen if the consumers' aim is the prevention/management of digestive disorders, heart diseases, colon cancer, diabetes, and cardiometabolic diseases or to boost the immune system. The fourth group is made of biscuits BS1, BS9, BS15 and BS14 which showed a good relationship with total carbohydrates, pH, tenderness, color, crispness, hardness, surface roughness, total and fecal coliforms, and yeasts and moulds. These biscuits are characterized by a high proportion of WF. This result shows that biscuits made with WF, although they possessed interesting sensory attributes and a high total carbohydrate content, their microbiological quality is poor and they do not possess potential health benefits for consumers.

3.3.9. *Optimum conditions for the different responses assessed on biscuits*

Following statistical analyses, the optimum conditions predicted by the models for each response were determined and the results are given in Table 8. Globally, the values of predicted optimum for all responses assessed were not significantly (p>0.05) different to experimental values, thus highlighting the validity of the models.

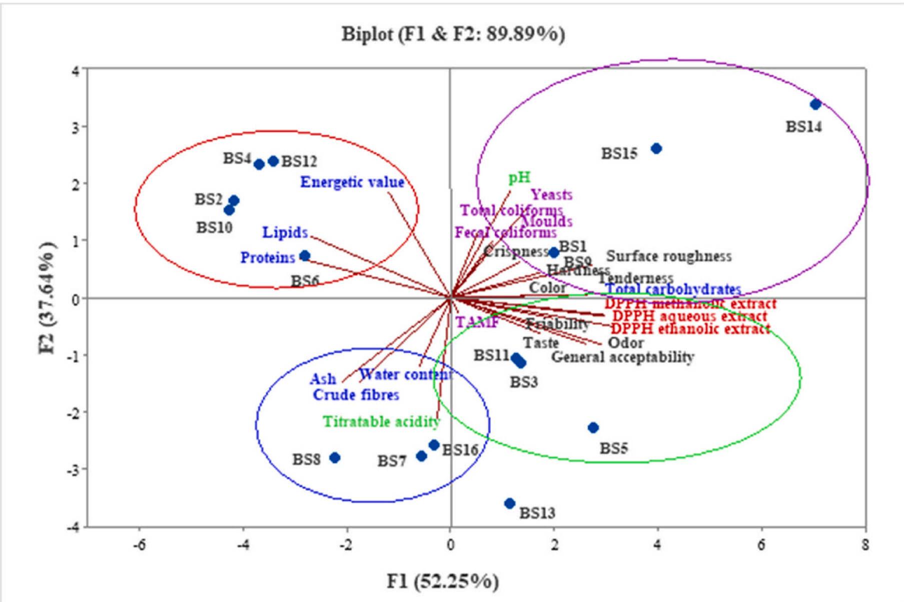

Fig. 4. Principal component analysis showing the distribution of different biscuits, their nutritional composition, their physico-chemical, microbiological, antioxidant and sensory parameters on the F1 & F2 axis system.

3.3.10. Multiple responses optimization

To determine the optimum condition that satisfies all the responses assessed, the desirability function was used. The desirability function enables the transformation of the different responses measured into values comprised between 0 (non-desirable) and 1 (most desirable).

When the response approaches the quality criteria that were set for biscuits, the desirability value becomes closer to 1 [23]. The total desirability was determined by combining the desirabilities of the different responses for simultaneous optimization. Hence, the simultaneous optimization of all responses was reduced to the optimization of one response: the total desirability. Using a computational approach through the software Minitab 19, the optimum point with the maximum desirability total value of 0.81 was selected. That optimum point corresponds to the following flour formulation: WF 25 g, SF 51.86 g, PFPF 8.06 g and BFPF 15.06 g. The formulation enables obtention of a biscuit having a pH of 6.53, ash of 4.04 g/100 g DM, proteins of 15.07 g/100 g DM, fibers of 6.00 g/100 g DM, TAMF of 2.99 Log CFU/g, yeasts and moulds count of 0.90 Log CFU/g, DPPH IC_{50} of 1.28 μg/mL and general acceptability of 7.07. While comparing the experimental and predicted values at the optimum point, no significant difference ($p>0.05$) was observed, thus confirming the adequacy of the model equations.

4. Conclusion

This study demonstrated that baobab fruit pulp, papaya fruit pulp and soybean flours can be used as a substitute for WF in the formulation of functional biscuits. Biscuits made from formulations with high proportions of WF do not have health benefits for consumers opposite to those with high SF that are rich in proteins and display high antioxidant activity. Biscuits with high proportions of BFPF are rich in crude fibres and minerals. The optimization procedure enables the determination of the proportions of ingredients in the formulations which leads to biscuits with optimum physicochemical, microbiological, antioxidant and sensory properties. Further experiments to characterize the bioactive compounds of these biscuits and assess their *in vivo* effects in mice models should be performed.

Funding

This research did not receive any specific grant from funding agencies in the commercial, public, or not-for-profit sectors. The study was self-funded.

Ethics and consent

The study was reviewed and approved by Institutional Ethic Committee of the Institute of Medical Research and Medicinal Plant Studies, under reference number 2017/0588/CEIRSH/ESS/MSP issued on December 7, 2017. Administrative Ethics approval was not required for this study because the food formulated contained approved ingredients that are normally and commonly consumed in the food habits of the participants. Biscuits were prepared under standard and approved conditions, and which are unlikely to need any specific requirements. Good hygiene practices were respected during the preparation and sensory evaluation following ISO/TC34/SC12 standards. The participants were informed on the objectives of the study, and the work was described in details. Any information that might be relevant to possible unidentified hazards were explained. They were also informed that what they do was ethical and their rights, dignity, safety, health and wellbeing will be respected. Participants from vulnerable groups, with any disease or allergy to the food ingredients used, or under medication were not included in the study. After being aware of this, voluntary participants that fit the recruitment criteria, have signed informed consent that they understand the purpose of the study, the confidentiality requirements, the anonymous publication of their biscuits' scores, and what is expected from them. They were allowed to withdraw from the panel at any time, without penalty or having to give a reason. The data were collected and recorded in accordance with the provisions of relevant data protection legislation of the country.

Data availability statement

The data associated with this study have not been deposited into a publicly available repository. Data will be made available on request.

CRediT authorship contribution statement

Hippolyte Tene Mouafo: Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Auréol Djommum Matuekam:** Writing – review & editing, Methodology, Investigation. **Igor Landry Petagou:** Writing – review & editing, Resources, Conceptualization. **Maxwell Wandji Ngeudjo:** Writing – review & editing, Software, Methodology. **Annick Manuela Bengue Baomog:** Writing – review & editing, Methodology, Investigation. **Patricia Marianne Ntsama:** Writing – review & editing, Supervision, Software. **Gabriel Nama Medoua:** Writing – review & editing, Supervision.

Table 8
Optimum conditions of flours' formulation for the different responses assessed on biscuits.

Responses	Formulation				Predicted value	Experimental value	*p*-value (*t*-test)
	X1 (g)	X2 (g)	X3 (g)	X4 (g)			
pH	45	25	10	20	6.14	6.11 ± 0.10	0.12
Ash	25	45	10	20	4.91	4.92 ± 0.21	0.85
Proteins	25	65	5	5	18.73	18.71 ± 0.30	0.46
Fibers	25	45	10	20	7.10	7.07 ± 0.45	0.19
TAMF	60	25	10	5	2.90	2.81 ± 0.24	0.47
YM	45	25	10	20	0.00	0.00 ± 0.00	0.89
GA	65	25	5	5	6.36	6.41 ± 0.28	1.07
DPPH IC_{50}	25	45	10	20	0.90	1.01 ± 0.10	1.42

X1 = Wheat flour, X2 = Soybean flour, X3 = Papaya fruit pulp flour, X4 = Baobab fruit pulp flour, TAMF = total aerobic mesophilic flora, YM = yeasts and moulds, GA = general acceptability.

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.

Acknowledgements

The authors acknowledge the panelists who accepted to evaluate the sensory attributes of biscuits. They also thank General Director of the Institute of Medical Research and Medicinal Plant Studies for providing facilities for the completion of this work.

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