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Optimization using response surface methodology of amylolytic capacity of maize *Atp-Y* and *coca-sr* varieties: *In vitro* digestibility capacity, physico-chemical and functional properties of optimal sample

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A R T I C L E I N F O

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A B S T R A C T

The use of sprouted cereals for the development of flavor, improvement of biochemical, physicochemical and techno-functional properties in beer production, improvement of nutritional quality and viscosity of supplementary foods is widespread in Africa. The objective of the current work was to evaluate the effect of malting conditions and variety on the amylolytic capacity, protein structures, physicochemical and functional properties of maize (*Atp-Y* and *Coca-sr* varieties). The Response Surface Methodology (RSM) using the central composite design (CCD) was applied to 5 independent factors, namely soaking time (18–42 h), vegetable salt concentration (0.5–1.2%), soaking temperature (25–41 °C), germination time (80–195 h) and maturation time (17.5–42 h). The optimal flours obtained were then subjected to physicochemical, digestibility, functional and antioxidant analysis. The analysis of the mathematical regression model showed that the linear and quadratic effects of all independent variables showed significant effects ($p < 0.05$) on the amylolytic activity of the *Atp-Y* variety. The optimal malting conditions were 25.12 h soaking in the presence of 0.52% vegetable salt at a temperature of 25.54 °C followed by 144.37 h germination and 37.65 h maturation for *Atp-Y*. *Coca-sr*, on the other hand, required 1.608 h soaking in the presence of 1.11% vegetable salt at a temperature of 36.63 °C followed by 144.37 h germination and 27.07 h maturation. The production of optimal samples using above optimal conditions reduced the anti-nutrients, pH, mass density, water holding capacity and swelling rate; improved the availability of minerals (Ca and Mg), bioactive compounds and the antioxidant properties. The FTIR spectra revealed similar peaks and different intensities for the beta-sheets as the major secondary structure of the proteins. Therefore, the application of these malting conditions would improve the enzymatic activity of these two maize varieties for their application in malting, beer production and digestibility of resistant starches.

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1. Introduction

Maize is the third most consumed cereal in the world after rice and wheat [1]. Between them, they account for more than 2.5 billion tons of cereals produced per year, making them the most available crops on the planet, ahead of tubers. Maize is the most widely grown and consumed cereal in Africa [2,3]. It is a vital source of nutrients for populations in developing countries, particularly for its starch content (nearly 80%) [4]. In sub-Saharan Africa and Cameroon in particular, maize is one of the main staple foods of the population and is used in the preparation of several traditional dishes and beverages such as maize cake, couscous, the traditional fermented drink called 'sha'a' and complementary gruels [5–8]. The use of this cereal involves some physical and chemical transformations such as boiling, roasting, soaking, fermentation and germination that have beneficial or undesirable effects on its nutritional and functional properties [9]. About germination, it is commonly applied in Africa by women for the production of alcoholic and non-alcoholic beverages [10].

Cereal malting which involved soaking for 12–48 h at room temperature, germination for 48–120 h with an optional maturation step and drying 45 °C for 48 h is a very important process in cereal processing [11,12]. During this process, the seeds are released from dormancy and lipolytic, proteolytic, fibrolytic and amylolytic enzymes, which can degrade the various macronutrients into simple molecules of interest are activated [13]. The intensity of these activities are influenced by several parameters mostly related to the malting conditions [14]. To this end, several studies have focused on the traditional malting of cereals and its effect on the physic-chemical of grains. It is the case of the work done by Dewar et al. [15], which revealed the influence of moisture content, germination temperature and malting time on the quality of malted cereals, particularly on amylolytic and proteolytic activities. Additionally, Kayode et al. [16] demonstrated the influence of variety and malting conditions on the enzymatic capacity of cereals. Similarly, Klang et al. [17–19] showed that variety, soaking time and germination time influenced the flowability of two varieties of maize and rice respectively. Taylor et al. [20], showed that grain variety, soaking time, soaking in dilute formaldehyde (0.05%) and NaOH (0.2%) improved the quality of malt produced. It is noted that to our knowledge none of these authors has evaluated the combined effect of soaking temperature, soaking time, germination, maturation and plant salt concentration on the activity of amylases produced mainly during germination of the two maize varieties as well as the evaluation of the physico-chemical characteristics of the malts produced.

Moreover, malting has also been reported to improve the nutritional value of proteins by about 30–50%, digestibility of macronutrients (carbohydrates, fats and proteins), food flavor, texture, antioxidant properties through the synthesis of new biologically active compounds such as terpenoids, the availability of phenols and free amino acids (FANs) which are great importance in beer production, as well as other physical properties of foods. It has also been observed to reduces the content of anti-nutrients such as phytates and oxalates [21–23].

In addition, the enzyme market is growing at an unprecedented rate of 6.75% per annum due to its numerous applications in the food, paper, biofuel, beverage and rubber industries … It is expected to grow from USD 8.18 billion in 2018 to USD 13.79 billion in 2026. Among these enzymes, hydrolases, particularly amylases, are of great interest given their applications in various industrial fields, mainly as food additives in bread-making, jams, etc. [24]. This shows the interest in improving the production conditions of plant amylases, as those of animal origin pose an extraction problem [25]. The quality of malted cereals is highly dependent on their diastatic power especially the activities of β- and α-amylolytic enzymes [24] as well as its protein content [26]. The interest in using cheap salt solutions in the germination process of cereals lies in the calcium richness of plant tegument which is additionally a cofactor of amylolytic activity [27].

This information is of great importance to the brewing and bread-making industries. This led us to optimize the amylase production capacity in the two maize varieties (*Atp* and Coca-sr). Optimization by response surface methodology (RSM) has long been used in this sense and has proven to be an effective tool, especially in highlighting the interactions between different factors that can influence a response [17–19]. The Central Composite Design (CCD) is one of the Response Surface Methodology that has the advantage to allowing a good estimation and efficiency of the optimal response [28]. The present work was therefore initiated to evaluate the influence of crop variety and malting conditions on the diastatic power, physicochemical properties and digestive capacity of amylases of two maize varieties.

2. Material and methods

2.1. Material

The plant material used for this work consisted of two maize varieties (Zea mays L): *Atp-Y* and *Coca-sr* obtained from the multipurpose station of the Institute of Agricultural Research for Development (IRAD) in Dschang in February 2019. Once purchased, these were transported to the laboratory for processing into germinated maize meal. The plant ash used as an input during soaking, was obtained from unripe banana peels collected in the city of Bafoussam in the ''Toungang village'' district.

2.2. Methods

2.2.1. Process for the production of vegetable ash

Unripe banana peels collected in the Toungang village in Bafoussam were sun-dried for 7 days and then incinerated in a muffle furnace calibrated at 300 °C for 5 h to eliminate all organic matter. The ash was ground in an ordinary mill and then stored in polyethylene bags to prevent air exchange and moisture pick-up.

2.2.2. Optimization of the production conditions for malted maize meal (Atp-Y and Coca-sr varieties)

2.2.2.1. Production process for malted maize meal. The methodology is the one described by Traoré et al. [29]. The maize grains of the *Atp-Y* and *Coca-sr* varieties were sorted, washed and soaked for 18–42 h in vegetable salt solutions, at concentrations varying from 0.5 to 1.2% (soaking ratio 1/1.4; w/v) and at a soaking temperature varying between 25 and 41 °C. The fifty four [54] were then drained, spread on a fine perforated cloth and cleaned by sprinkling water before germination in an incubator at 25 °C. The sample lots received 1 sprinkling/per 24 h of 15 mL/100 g of grain for a period ranging from 80 to 195 h. Following germination, they were matured by covering them with a dark wrapping between 17.5 and 42 h. The germination process was stopped by putting the grains at the end of the maturation in a "Venticell" oven at a temperature of 45 ± 0.5 °C for 45 h for drying. It should be noted that the concentration of input used during soaking, soaking temperature, soaking time, germination time and maturation time of each experiment were given by the experimental design. After drying, the roots and shoots were removed and grains were ground with a polymixer (Model POLYMIX, KINETICA) and then sieved (Ø = 300 μm). The sprouted flours obtained were packed in plastic bags and stored in a desiccator before further use for the evaluation of amylolytic capacity and physicochemical characterization. It should be noted that in each trial, the pH of the soaking water was measured before and after soaking, and the water absorption capacity and moisture content of the grains were also assessed.

2.2.2.2. Selection of the optimization design, factors, experimental domain and responses. The Response Surface Methodology (RSM) was used to optimize the 5 selected factors with the central composite design as the optimization scheme using Minitab 18.1. The choice of factors was

based on previous work and preliminary tests on other designs. The Doehlert design has the advantage of presenting a reduced number of experiments while limiting itself to the boundaries of the domain [17,18]. In addition, preliminary tests with the Box-Behnken design showed that this design, although it can be used for up to three levels of data, has the disadvantage of also having a narrower study area. The central composite design has the interesting property of presenting a uniform distribution of experimental points in the space of the factors, and also makes it possible to carry out the tests of a factorial design (−1/+1), supplemented by experiments in the centre of the study domain (0) and star tests (−2.366/+2.366); this gives each factor 5 levels. Regarding the factors, those selected were the concentration of plant salt during soaking (0.5–1.2%, X2), the soaking temperature (25–41 °C, X3), the soaking time (18–42h, X1), the germination time (80–195 h, X4) and the maturation time (17.5–42 h, X5). These factors were chosen based on previous work and factor screening [11,17,19].

The choice of study domains for each factor was first based on previous work [11,19,30] and then preliminary tests using a definitive design within the domain of each factor were conducted to determine whether there was a significant difference ($P<0.05$) between them in terms of the response being assessed. If this was not observed, an adjustment of the study domain was made followed by a test to confirm the difference between the bounds. As regards the choice of response, the one chosen was amylolytic activity. Following the choice of the different factors and study domains, operations were performed on these factors to work with dimensionless variables (coded variables). These

Table 1
Experimental matrix, experimental and predicted values of amylolytic activity according to the different tests.

N°	Real and coded (values in brackets) variables X_1 (h) X_2 (%) X_3 (°C) X_4 (h) X_5 (h)					Experimental values *Atp-Y Coca-sr*		Predicted values *Atp-Y Coca-sr*	
1	30.00(0)	0.85(0)	33.00(0)	137.50(0)	0.77(-α)	4.92 ± 1.56^{aB}	8.46 ± 0.06^{bA}	4.47^{b}	9.36^{a}
2	30.00(0)	0.85(0)	33.00(0)	137.50(0)	58.75(+α)	10.37 ± 0.82^{aA}	10.47 ± 0.02^{aA}	10.01^{b}	8.88^{b}
3	58.39(+α)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	8.07 ± 0.66^{aB}	16.95 ± 0.32^{bA}	6.95^{b}	22.57^{a}
4	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	16.31 ± 0.51^{aB}	27.71 ± 2.63^{aA}	13.51^{b}	23.45^{b}
5	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	12.68 ± 0.93^{aB}	18.85 ± 0.25^{bA}	13.51^{a}	23.45^{a}
6	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	12.83 ± 0.71^{aB}	21.82 ± 1.40^{bA}	13.51^{a}	23.45^{a}
7	30.00(0)	0.85(0)	14.07(-α)	137.50(0)	29.75(0)	12.42 ± 1.67^{aA}	9.49 ± 1.30^{aB}	11.34^{b}	9.41^{a}
8	30.00(0)	0.85(0)	51.93(+α)	137.50(0)	29.75(0)	1.66 ± 0.53^{aB}	$4.45 \pm 0.05a^{A}$	1.94^{a}	3.94^{b}
9	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	13.88 ± 0.74^{aB}	23.49 ± 0.42^{aA}	13.51^{b}	23.45^{a}
10	30.00(0)	0.85(0)	33.00(0)	273.55(+α)	29.75(0)	8.97 ± 0.56^{aB}	9.75 ± 0.87^{aA}	8.26^{b}	10.31^{a}
11	30.00(0)	0.85(0)	33.00(0)	1.46(-α)	29.75(0)	0.87 ± 0.03^{aA}	0.33 ± 0.00^{aB}	0.78^{a}	-0.82^{b}
12	1.61(-α)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	10.28 ± 0.65^{aB}	31.19 ± 1.03^{aA}	10.59^{a}	24.98^{b}
13	30.00(0)	1.678(+α)	33.00(0)	137.50(0)	29.75(0)	7.52 ± 0.74^{aB}	17.32 ± 2.10^{aA}	8.25^{a}	16.67^{b}
14	30.00(0)	0.022(-α)	33.00(0)	137.50(0)	29.75(0)	12.00 ± 1.59^{aA}	11.40 ± 0.00^{aB}	10.47^{b}	11.47^{a}
15	42.00(+1)	1.20(+1)	25.00(-1)	80.00(-1)	17.50(-1)	4.98 ± 1.02^{aB}	12.55 ± 0.17^{aA}	5.77^{a}	11.71^{b}
16	18.00(-1)	1.20(+1)	41.00(+1)	80.00(-1)	17.50(-1)	2.45 ± 0.37^{aB}	13.74 ± 1.94^{aA}	3.55^{a}	14.85^{a}
17	42.00(+1)	1.20(+1)	41.00(+1)	195.00(+1)	42.00(+1)	7.59 ± 1.52^{aB}	19.95 ± 0.25^{aA}	8.43^{a}	17.06^{b}
18	18.00(-1)	1.20(+1)	25.00(-1)	195.00(+1)	42.00(+1)	9.60 ± 0.82^{aB}	14.57 ± 2.26^{aA}	10.65^{a}	15.94^{a}
19	42.00(+1)	0.50(-1)	41.00(+1)	80.00(-1)	17.50(-1)	0.32 ± 0.12^{aB}	7.48 ± 0.74^{aA}	0.58^{a}	5.91^{b}
20	18.00(-1)	0.50(-1)	41.00(+1)	80.00(-1)	17.50(-1)	5.42 ± 0.42^{aB}	9.51 ± 1.01^{aA}	5.15^{a}	10.96^{b}
21	18.00(-1)	1.20(+1)	41.00(+1)	80.00(-1)	42.00(+1)	6.96 ± 0.48^{aB}	12.49 ± 0.00^{aA}	6.27^{b}	11.86^{b}
22	18.00(-1)	1.20(+1)	25.00(-1)	80.00(-1)	17.50(-1)	6.47 ± 025^{aB}	14.13 ± 0.49^{aA}	5.35^{b}	13.46^{b}
23	18.00(-1)	0.50(-1)	25.00(-1)	80.00(-1)	42.00(+1)	12.16 ± 0.73^{aA}	11.44 ± 0.76^{aA}	13.44^{a}	11.30^{a}
24	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	13.28 ± 1.25^{aB}	25.66 ± 0.42^{aA}	13.51^{a}	23.45^{b}
25	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	13.51 ± 0.65^{aB}	23.16 ± 0.37^{aA}	13.51^{a}	23.45^{a}
26	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	15.66 ± 0.82^{aB}	22.99 ± 0.82^{aA}	13.51^{b}	23.45^{a}
27	42.00(+1)	1.20(+1)	25.00(-1)	195.00(+1)	17.50(-1)	11.18 ± 0.46^{aB}	16.16 ± 0.70^{aA}	11.53^{a}	15.55^{b}
28	42.00(+1)	0.50(-1)	25.00(-1)	80.00(-1)	42.00(+1)	9.45 ± 0.42^{bB}	13.02 ± 0.68^{aA}	11.10^{a}	12.65^{a}
29	42.00(+1)	0.50(-1)	41.00(+1)	80.00(-1)	42.00(+1)	5.82 ± 0.12^{aA}	4.39 ± 0.23^{aB}	4.15^{b}	3.65^{b}
30	18.00(-1)	0.50(-1)	25.00(-1)	195.00(+1)	17.50(-1)	10.97 ± 0.45^{aB}	12.62 ± 0.28^{aA}	10.73^{a}	12.85^{a}
31	42.00(-1)	0.50(-1)	25.00(-1)	195.00(+1)	17.50(-1)	10.01 ± 0.45^{aB}	16.07 ± 0.66^{aA}	10.46^{a}	15.87^{a}
32	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	12.88 ± 1.02^{aB}	23.16 ± 1.21^{aA}	13.51^{a}	23.45^{a}
33	18.00(-1)	1.20(+1)	25.00(-1)	195.00(+1)	17.50(-1)	8.55 ± 0.71^{aB}	13.93 ± 0.71^{aA}	9.54^{a}	14.09^{a}
34	18.00(-1)	0.50(-1)	41.00(+1)	195.00(+1)	42.00(+1)	8.76 ± 0.42^{bB}	11.42 ± 1.20^{aA}	9.78^{a}	12.72^{a}
35	18.00(-1)	1.20(+1)	41.00(+1)	195.00(+1)	17.50(-1)	8.81 ± 0.59^{aB}	16.62 ± 1.20^{bA}	8.54^{b}	18.46^{a}
36	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	13.61 ± 0.57^{aB}	22.99 ± 0.42^{aA}	13.51^{a}	23.45^{a}
37	18.00(-1)	0.50(-1)	25.00(-1)	80.00(-1)	17.50(-1)	9.59 ± 0.28^{aA}	10.43 ± 0.64^{bA}	9.38^{a}	13.69^{a}
38	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	10.48 ± 0.79^{bB}	24.06 ± 0.61^{aA}	13.51^{a}	23.45^{a}
39	42.00(+1)	1.20(+1)	41.00(+1)	80.00(-1)	42.00(+1)	1.61 ± 0.26^{bB}	5.89 ± 1.53^{aA}	3.46^{a}	6.81^{a}
40	18.00(-1)	1.20(+1)	25.00(-1)	80.00(-1)	42.00(+1)	8.31 ± 0.53^{aA}	9.14 ± 0.45^{bA}	8.06^{a}	11.90^{a}
41	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	13.25 ± 0.56^{aB}	20.46 ± 1.93^{bA}	13.51^{a}	23.45^{a}
42	42.00(+1)	0.50(-1)	25.00(-1)	195.00(+1)	42.00(+1)	12.66 ± 0.05^{aB}	19.51 ± 1.27^{aA}	12.43^{a}	18.45^{b}
43	18.00(-1)	1.20(+1)	41.00(+1)	195.00(+1)	42.00(+1)	11.53 ± 1.16^{aB}	16.58 ± 1.03^{bA}	9.67^{b}	18.89^{a}
44	18.00(-1)	0.50(-1)	41.00(+1)	80.00(-1)	42.00(+1)	9.12 ± 0.56^{aA}	5.47 ± 0.64^{bB}	9.21^{a}	7.15^{a}
45	30.00(0)	0.85(0)	33.00(0)	137.50(0)	29.75(0)	14.28 ± 1.99^{aB}	27.43 ± 1.96^{aA}	13.51^{b}	23.45^{b}
46	18.00(-1)	0.50(-1)	25.00(-1)	195.00(+1)	42.00(+1)	13.60 ± 1.34^{aA}	13.29 ± 1.40^{aA}	13.19^{b}	13.88a
47	42.00(+1)	0.50(-1)	41.00(+1)	195.00(+1)	17.50(-1)	3.11 ± 0.56^{bB}	12.31 ± 2.16^{aA}	4.30^{a}	11.28^{b}
48	42.00(+1)	1.20(+1)	41.00(+1)	80.00(-1)	17.50(-1)	1.95 ± 0.25^{aB}	8.39 ± 1.87^{aA}	1.24^{b}	8.25^{a}
49	42.00(+1)	1.20(+1)	25.00(-1)	80.00(-1)	42.00(+1)	9.53 ± 0.70^{aB}	11.95 ± 0.02^{aA}	7.98^{b}	11.70^{a}
50	42.00(+1)	0.50(-1)	41.00(+1)	195.00(+1)	42.00(+1)	5.59 ± 0.14^{aA}	11.90 ± 1.16^{aA}	6.28^{a}	12.44^{a}
51	42.00(+1)	0.50(-1)	25.00(-1)	80.00(+1)	17.50(-1)	6.56 ± 0.43^{bB}	15.66 ± 0.33^{aA}	7.54^{a}	13.48^{b}
52	18.00(-1)	0.50(-1)	41.00(+1)	195.00(+1)	17.50(-1)	6.93 ± 0.46^{bB}	14.10 ± 0.32^{aA}	7.30^{a}	13.12^{b}
53	42.00(+1)	1.20(+1)	41.00(+1)	195.00(+1)	17.50(-1)	9.09 ± 0.64^{aB}	16.09 ± 1.75^{aA}	7.80^{b}	15.08^{a}
54	42.00(+1)	1.20(+1)	25.00(-1)	195.00(+1)	42.00(+1)	11.81 ± 0.25^{aB}	19.88 ± 2.11^{aA}	12.15^{a}	18.96^{b}

X_1: soaking time (h); X_2: salt concentration (%); X_3: soaking temperature (°C); X_4: germination time (h); X_5: maturation time (h).
Means with the same letter along the line are not significantly different at 5%.

operations made it possible to make the effects of natural variables, which are not necessarily expressed in the same units, comparable and to generate a matrix of 54 trials (divided into 32 factorial trials, 10 in the stars and 12 in the centre of the domain) as shown in Table 1 below.

2.2.2.3. Mathematical model proposal. The proposed model simply had the property of representing the experimental response studied well in the experimental area of interest and thus obtained an estimate of the value of the studied responses of acceptable quality. The polynomial models, because of their simplicity, are the ones that have been chosen. On the performance of the experiments, the amylolytic activity is taken as the answer (Y); I the constant; a, b, c, d and e the linear coefficients; f, g, h, i, j, k, l, m, n and o the interaction coefficient; p, q, r, s and t the square coefficients. We therefore assumed a second degree model, for five variables. The model is presented by Equation (1) below:

$$Y = I + ax_1 + bx_2 + cx_2 + dx_2 + ex_2 + fx_1x_2 + gx_1x_3 + hx_1x_4 + ix_1x_5 + jx_2x_3 + kx_2x_4 + lx_2x_5 + mx_3x_4 + nx_3x_5 + ox_4x_5 + px_1^2 + qx_2^2 + rx_3^2 + sx_4^2 + tx_5^2 + \varepsilon \text{ (error)} \quad (1)$$

2.2.2.4. Validation of models. In order to predict responses in the area defined for the study, it was important to validate the empirical models obtained. To do so, the determination coefficient (R^2), the Absolute Mean Deviation Analysis (AMDA) which provides information on the average handling error, the desirability and the Bias factor (Bf) were determined [31]. For the model to be validated, the value of the determination coefficient (R^2) must be greater than 75%; Absolute Mean Deviation Analysis must be equal to 0, desirability greater than 0.5 and Bias factor (Bf) must be between 0.75 and 1.25.

2.2.2.5. Evaluation of the response

2.2.2.5.1. Production of crude enzyme extracts. This was carried out following the method described by Tambo et al. [32] with slight modification. To 1 g of malt flour, 4 mL of 100 mM phosphate buffer (pH 6) was added, then the mixture was vortexed for 5 min and left to stand for 1 h. The mixture was then centrifuged at 4500 rpm for 15 min using a "Heraeus" oblique centrifuge. The supernatant collected as crude extracts was bottled and stored in a freezer at −10 °C for 2 h before further centrifugation under the same conditions. The supernatant collected from this second centrifugation was stored under the same conditions before use.

2.2.2.5.2. Determination of amylolytic activity (diastatic power). This was carried out according to the saccharogenic method described by Bernfeld [33]. One [1] mL of soluble starch (1%) previously prepared in 100 mM phosphate buffer (pH 6) was pre-incubated at 45 °C for 20 min and 100 µl of enzyme extract diluted at 1/25 was added to it. The mixture was incubated for 10 min at the same temperature. The reaction was stopped by adding 0.5 mL of DNS reagent (3,5-dinitrosalicylic acid). The mixture was heated in a "Heraeus" boiling water bath for 5 min and then cooled to room temperature. After the addition of 5 mL of distilled water, the absorbance was read at 540 nm against a blank. The enzyme activity is defined as the amount of enzyme that allows the formation of 1 µmol of maltose per minute.

2.2.2.6. Determination of soluble protein content. This was carried out using the Biuret method slightly modified by Tambo et al. ([32]. To this end, 10 mL of distilled water was added to 0.5 g of sprouted flour. The mixture was macerated for 1 h and then centrifuged (Heraus centrifuge) at room temperature for 30 min at 3500 rpm. The resulting suspension was filtered and the extract was used to quantify soluble proteins with bovine serum albumin (BSA) as standard. To 1 mL of extract, 8 mL of freshly prepared biuret solution was added and the mixture was allowed to stand for 30 min before reading the absorbance at 540 nm against the blank. The soluble protein content is expressed in mg.

2.2.2.7. Determination of free amino nitrogen (FAN) content. This was carried out according to the method described by the European Brewery Convention [34] using glycine as a reference amino acid. To do this, the FANs were extracted in 5% trichloroacetic acid previously heated to 30 °C at a ratio of 1/100 w/v for 1 h. The mixture was then centrifuged at 4500 rpm for 10 min. After centrifugation, 1 mL of clear supernatant is diluted to 25 mL in a volumetric flask with distilled water. Two [2] mL of the diluted sample was pipetted into a test tube and 1 mL ninhydrin color reagent was added, then mixed well. After this, the test tubes were covered with glass marbles, heated for exactly 25 min in a constantly boiling water bath, cooled for 20 min in a water bath at room temperature, then add 5 mL of the diluent to each tube and mix well. Within 1 h read the samples at 570 nm. The results are calculated as mg FAN/100 g dry mass of malt, which can be obtained from the following Equation (2) below:

$$\textit{Free Amino Nitrogen}\ (\frac{mg}{100}g) = \frac{\text{Abs of the sample}*400*100}{\text{Abs of standard}*\text{dry matter of the sample}} \quad (2)$$

2.2.2.8. Proteolytic activity. To detect the presence of protease activity in the crude extract sample and in the amylase enriched fraction, a modified method using azocasein presented by Garcia de Fernando and Fox [35], was used. Two hundred (200) µl of azocasein solution 2% (w/v) and 300 µl of a buffer solution of sodium bicarbonate 0.5% (w/v), pH 8.0 were added to 100 µl of samples. The mixture was incubated at room temperature for 24 h, and then the reaction was stopped by adding 500 µl of 20% (w/v) trichloroacetic acid. The mixture was well mixed, incubated at room temperature for 5 min and then centrifuged for 5 min at 4500 rpm. Five hundred (500) µl of supernatant were taken and added to 1000 µl of 1 M NaOH, and absorbance was read at 440 nm. One unit of activity was defined as the amount of activity that gives a change of one unit of absorbance at 440 nm/min/mL of enzyme preparation.

2.2.2.9. α-Amylase activity. The α-amylase activity was determined according to the Bernfeld [33] method modified by Giamarchi and Trèche [36]. The α-amylase activity was determined according to the same principle as the diastatic power after extraction of the enzymes with $CaCl_2$ solution (3.3 g/l) and inactivation of β-amylase at 68 °C for 15 min.

2.2.2.10. Malt yield, malt loss and kolbach index. The method described Embashu et al. [37] has permit to determine the malt yield, malt loss and kolbach index folloxing Equations (3)–(5) below:

$$\textit{Malt loss}\ (\%) = \frac{\text{corn weight (g)} - \text{malt weight (g)}}{\text{corn weight (g)}} * 100 \quad (3)$$

$$\textit{Malt yield}\ (\%) = 100 - \text{Malt loss (\%)} \quad (4)$$

$$\textit{Kolbach Index}\ (\%) = \frac{\text{soluble protein content}}{\text{crude protein content of the sample}} * 100 \quad (5)$$

2.2.2.11. Determination of germinative capacity. Germinative capacity is, the ability of the grain to germinate fully and with vigor, which is essential to normal malting was determined following method used by Ebbah et al. [38]. This was repeated five independent times (n = 6) per variety. To a 90 mm petri dishes, placed 100 kernels of two maize varieties with two layers of filter paper (Whatman N° 1) wetted with 4 mL of water. The petri dishes with corn kernels were placed in an incubator at 25 °C. The germinated kernels were counted and removed at 24, 48 and 72 h. The germination energy was calculated using the following Equation (6).

$$\textit{Germinative capacity}\ (\%) = \frac{(n24 + n48 + n72)}{3} \quad (6)$$

where: n24, n48, n72 – numbers of germinated kernels at 24, 48 and 72 h.

2.2.2.12. Determination of starch hydrolysis kinetics. The starch hydrolysis capacity of the two optimal samples and porcine pancreatic alpha-amylase (No. 7545, Sigma-Aldrich, St. Louis, MO) was conducted following the method by Goni et al. [39] with slight modifications. In fact, a stock solution of α-amylase was prepared by mixing 40 mg of Porcine pancreatic alpha-amylase (No. 7545, Sigma-Aldrich, St. Louis, MO) in 100 mL of 0.2 M phosphate buffer (pH 6.9) and centrifuged for 10 min at 2500 g. Crude amylase extracts were prepared as previously described. To assess the digestive capacity of these samples, one [1] mL of freshly prepared 1% (w/v) starch was introduced into test tubes and pre-incubated in a thermostatically controlled water bath at 37 °C for 20 min. Subsequently, 100 μl of each enzyme solution was added and hydrolysis was monitored for 0, 10, 25, 40, 65, 90 and 120 min. After incubation, 500 μl of 3–5 dinitrosalicyclic acid was immediately added and the mixture was heated for 5 min to inactivate the enzymes. The solutions after cooling were used to determine a glucose concentration in the supernatant using a spectrophotometer (Thermo scientific, GENESYS 20) at a wavelength of 540 nm.

2.2.3. Chemical composition, color, physical and functional analyses of the various samples

2.2.3.1. Chemical composition. The two optimal samples produced as described above were subjected to different analyses. The proximate chemical composition (moisture, crude protein, crude fat, crude fiber, total digestible carbohydrate and energy calorie) analyses of different samples were done in triplicates according to standard methods described by AOAC [40]. Total lipids were extracted in Soxhlet and protein determination was done by the Kjeldahl method which is based on the transformation of organic nitrogen into ammoniacal nitrogen by mineralization with concentrated sulphuric acid. Reducing sugar was also evaluated through Fischer and Stein [41] method using 3,5-dinitrosalicylic acid reagent (DNSA). The standard method described by AOAC [40] also made it possible to assess the carbohydrate content using Equation (7):

$$\begin{aligned}\text{Digestibles carbohydrates content } (\%) = 100 - (&\text{moisture } (\%) + \text{ash}(\%) \\ &+ \text{proteins } (\%) + \text{lipids } (\%) \\ &+ \text{Fibers } (\%))\end{aligned} \tag{7}$$

The starch content was evaluated by the method described by Jarvis and Walker [42]. The amylose content of the different samples was quantified by the rapid colorimetric method as described by Chrastyl [43]. The amylopectin content will be deducted from the amylose content, with the difference in relation to the starch content of the samples being determined by the following Equation (8) below:

$$\textit{Amylopectin content } (\%) = 100 - \textit{amylose content } (\%) \tag{8}$$

The determination of ash content was assayed by the standard method described by AOAC [40] after incinerating the sample at 560 °C in an oxidizing atmosphere. Mineral contents (Ca, Mg, Na, K and Cu) were determined after the initial digestion of ash in HNO_3/HCl, then analyzed using atomic absorption spectrophotometry (AAS).

2.2.3.2. Color analysis. The tristimulus l*, a*, b* parameters of corn samples were determined after standardization with a white tile using a Colorflex-EZ benchtop color difference meter (A60-1014-593, Hunter Associates, Reston, VA, USA). Samples were placed in the plastic sample holder to cover the base of the plastic and digital color photos were taken in duplicate with the Colorflex. The total browning index (BI) was calculated according to Equation (9) given below [44].

$$\textit{Browning Index} = \frac{100*(x - 0.31)}{0.172} \tag{9}$$

with: $x = \frac{(a+1.75l)}{5.645l+(a-3.012b)}$

2.2.3.3. Determination of antinutrients, secondary metabolite content and antioxidants activities of different samples. The phytate content of our different samples was determined using the iron III chloride colorimetric titration method described by AOAC [40]. Two [2] g of sample were introduced in a 250 mL Erlen, then 100 mL of 2% HCL were introduced and the whole was left to stand for 3 h. The solution was then filtered with whatman paper N°3. Fifty [50] mL of filtrate were introduced in a 250 mL Erlen with 107 mL of distilled water. Ten [10] mL of 0.3% ammonium thiocyanate has been added as an indicator. The whole was titrated with a standard solution of $fecl_3$ which contains 0.00195 g of iron per mL.

The potassium permanganate colorimetric titration of Day et al. [45] was applied to quantify the oxalates. Two [2] g of sample were introduced in a 250 mL Erlen, then 150 mL of 1.5 M H_2SO_4 were introduced and the whole was left to stand for 5 h. The solution was then filtered with whatman paper N°3. Seventy five [75] mL of filtrate were introduced in a 250 mL Erlen. The whole was titrated with 0.1 M potassium permanganate and the content determined from the following Equation (10):

$$0.1\ ml\ KMNO4\ = 0.00450g\ \ \text{D'oxalate} \tag{10}$$

The trypsin inhibitor content of the samples was determined by the standard AOAC [40] method using albumin as a control. In a 100 mL beaker containing 0.5 g of sample, 50 ml of NaOH (10 mM) were introduced and the pH was adjusted 9.6 with NaOH or HCl (1 M). The mixture was stirred at room temperature for 3 h and stored at 4 °C overnight. This mixture was centrifuged the next day and the supernatant obtained was used to estimate the trypsin inhibitor content of the samples. The trypsin inhibitory capacity was calculated from Equation (11) below:

$$\textit{Trypsin}\ \frac{mg}{100}g = \frac{(\text{Abs Standard} - \text{Abs sample}) \times \text{dilution factor}}{19 \times \text{sample (g)}} \times 100 \tag{11}$$

The picrate paper methodology of Makkar et al. [46] was used to quantify the hydrocyanic acid released from the sample. For phenols and flavonoids, protocols using the Folin-Ciocalteu reagent of Gao et al. [47] allowed their quantification. The results were expressed as mg GAE/g and mg EC/g respectively. Condensed and hydrolyzed tannins were respectively determined using vanillin-HCl and ferric chloride method as described by Gaytán-Martínez et al. [48]. The results were expressed as milligrams of Catechin equivalent per gram of sample on dry basis. Saponins were quantified using the method described by Koziol [49]. Antioxidant properties were also assessed including the ABTS free radical scavenging assay using method described by Siwela et al. [50], FRAP and the DPPH radical scavenging assay using the method described by Moodley et al. [51].

2.2.3.4. Percentage contribution, physical and functional properties of the different flours. The percentage contribution to the recommended nutrient intake was expressed as a % of the RDA (Recommended Daily Allowances) using Equation (12) below:

$$\text{RDA } (\%) = \frac{X}{Y} x100 \tag{12}$$

with: X as value of the nutrient in sampleY: standard value of the nutrient.

Bulk density was determined according to Okaka et al. [52]. A fifty [50] g flour sample was taken into a 100 mL measuring cylinder and the

volume occupied by the sample was noted. Further, the sample with a measuring cylinder was tapped 100 times, and volume was observed. The bulk and tap densities, Hausner ratio and porosity were calculated using Equations (13)–(15) below.

$$\text{Bulk and tapped density} = \frac{\text{weight of sample (g)}}{\text{volume occupied by the sample}} \quad (13)$$

$$\textit{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad (14)$$

$$\textit{Porosity}\ (\%) = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} * 100 \quad (15)$$

pH was determined in 10% aqueous solution at room temperature using a pH meter (GlowGeek Advanced Portable pH meter) according to the standard method described by AOAC [40]. The standard method described by AFNOR [53], was used to evaluate titrable acidity. For functional properties, swelling rate and water holding capacity were determined following the methods described by Okezie and Bello [54] modified by Tambo et al. [2,3] and Lin et al. [55] respectively.

2.2.3.5. Fourier transmission-infrared (FTIR) spectroscopy of optimal maize samples. The FTIR spectra of the samples were obtained using a FTIR spectrophotometer (Jasco FT/IR-4100, ATR PRO ONE). Background spectra of the instrument were collected before samples (0.1 g of each optimal corn flour) were mounted on the instrument and the spectra were recorded with characteristic peaks in wavenumbers from 400 to 4000 cm^{-1} at 4 runs per scan.

2.2.4. Statistical analysis

The estimated data in triplicates were expressed as mean ± standard deviations using the analysis of variance (ANOVA) to determine the degree of significance ($p < 0.05$) of each of these effects. The significance of each factor was determined by the Fisher test. The regression equations were also subjected to the Fisher test to determine the coefficient of determination R^2. The calculations were carried out using MINITAB 18.1 software (IBM STATISTICS, USA). Graphical representations of the contour and surface response curves of the postulated models were made using SIGMA PLOT 12.0 software. IBM SPSS™ software version 25.0 and the Duncan test allowed us to verify the existence of significant differences between the predicted and experimental response within the two maize varieties.

3. Results and discussion

3.1. Modelling of amylolytic activity

Table 1 shows the influence of different variables (steeping time, germination and maturation, steeping temperature and salt concentration) and variety on the amylolytic activity of maize malts. Similarly, the influence of steeping conditions on the water content, water absorption capacity and pH variation of the steeping solution was also determined. This resulted in a variation in amylolytic activities from 0.32 UI (trial 19) to 16.31 UI (trial 4) in the *Atp-Y* while it has been found from 0.33 UI (trial 11) to 31.19 (trial 12) in the *Coca-sr* variety. With the exception of trials 14, 29 and 44, the *Coca-sr* variety showed significantly ($p < 0.05$) higher amylolytic activities. Klang et al. [17], also reported a higher liquefying power of *Kassaï* maize malt compared to *Atp-Y*. Furthermore, there was a variation in amylolytic activities with malting conditions. Gujjaiah and Kumari [56] demonstrated that proteolytic and amylolytic activities were influenced by steeping and germination conditions. Ali et al. [57] further demonstrated that soaking beyond 36 h would cause a decrease in enzyme activity as it would lead to seed asphyxiation, loss of soluble proteins and delay in metabolic processes. Furthermore, according to Yaredi et al. [58], the low amylase activity observed under certain conditions is also the effect of a partial lifting of dormancy inhibition due to reduced germination time or inactivation beyond the required maturation time. The predicted and experimental responses of the two varieties were significantly ($p < 0.05$) affected in some trials. The variety-independent values are higher than those of Irakoze et al. [59], indicating that a combination of these factors has beneficial effects.

3.2. Influence of soaking conditions on water holding capacity, water content and pH

From the analysis of the influence of the soaking conditions on the pH and the variation of this parameter (see Table 2), it appears that it was mainly influenced by the plant salt concentration of the different trials. Nevertheless, a lowering of the pH after soaking was noted. This would be linked to a diffusion of the ions contained in the salts towards the seed. This variation was more important in the trials where a high soaking temperature was required. Rahman et al. [60] revealed in their study that an increase in soaking time and temperature led to an embrittlement of the grain wall facilitating the entry of soaking solutions by osmosis. Several authors [61,62] noted a significant improvement in the quality of sorghum malts when saline solutions were used during steeping. They attributed this to the cofactors (Mg^{2+}, Ca^{2+}) of the enzymatic activities present, the lowering of the steeping time due to these ions, the removal of phenols and tannins that can complex soluble proteins. A decrease of 0.98–4 pH units was noted between the beginning and the end of soaking in both varieties without a real significant effect. The effect of pH would therefore be associated with the different nature of the amylases in the two varieties. Kolawole and Kolawole [12], noted that soaking sorghum grains in a basic solution improved α-amylase activity in contrast to the slightly acidic solution and had no effect on β-amylase activity. These results suggest a strong preponderance of α-amylase activity in the variety *Atp-Y*. Tambo et al. (2018) noted the same observations with the same maize malt variety. The results obtained are in agreement with those of Perveen et al. [63] who demonstrated that pH and concentration played a great importance in the quality of sorghum malts.

The absorption capacity and moisture content of the grains are influenced by parameters such as soaking temperature, soaking time, presence or absence of saline solutions, hardness and seed size [19,59,62]. These are very important parameters in the germination process and the activation of enzymes. From Table 2 below, it appears that these two parameters are significantly ($p < 0.05$) correlated with temperature and soaking time. The peak of amylolytic activity was observed at 41.09% water holding capacity and 36.28% moisture content for the variety *Atp-Y*. It was observed at 23.67% and 17.26% moisture content and holding capacity respectively for the variety *Coca-sr*. This variation could be explained by the starting moisture content of the two grains, the location of the amylases, the nature of the amylases and the grain size [64]. The lowest activities were noted at water holding capacities of 44.32 and 39.23% for moisture contents of 39.68 and 36.55% for *Atp-Y* and *Coca-sr*, respectively. This can be explained by the loss of soluble proteins through osmosis, dilution of reagents, asphyxiation of grains leading to a limitation of metabolic phenomena, destruction and loss of enzymes located at the cell wall [65]. The optimum moisture contents reported in this study are far lower than those of Klang et al. [17,18]; Bwanganga et al. [62]; which ranged from 39 to 42% moisture content in maize and sorghum seeds. This would be related to the fact that this study combined time, temperature and saline solutions during soaking. These observations demonstrate the importance of the combination of the above factors in improving germination and reducing steeping time.

3.3. Validation of mathematical models, contribution of variables, coefficient, p-value and sums of squares of variables

Table 3 below presents the validation of the mathematical models, variable contribution, coefficient, *p-value* and sums of squares of the

Table 2
Experimental matrix of the influence of soaking conditions on water holding capacity, water content and pH.

N°	Real and coded (values in brackets) variables X_1 (h) X_2 (%) X_3 (°C) X_4 (h) X_5 (h)					Soaking pH	*Atp-Y* pH after soaking	*Coca-sr* pH after soaking	*Atp-Y* moisture content (%)	*Coca-sr* moisture content (%)	*Atp-Y* water retention capacity (%)	*Coca-sr* water retention capacity (%)
1	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	0.77 (-α)	11.03 ± 0.06	7.16 ± 0.05^a	7.94 ± 0.26^a	37.79 ± 0.66^a	36.19 ± 0.59^a	40.17 ± 0.43^a	36.07 ± 0.19^b
2	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	58.75 (+α)	11.03 ± 0.04	7.22 ± 0.04^a	7.21 ± 0.04^a	37.07 ± 0.69^a	36.93 ± 0.11^a	40.80 ± 0.49^a	39.22 ± 0.55^a
3	58.39 (+α)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	12.88 ± 0.06	6.07 ± 0.03^a	6.21 ± 0.02^a	40.68 ± 2.76^a	37.82 ± 1.53^b	43.79 ± 0.90^a	41.66 ± 0.55^a
4	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.01 ± 0.01	7.14 ± 0.10^b	7.98 ± 0.12^a	36.28 ± 0.05^a	35.31 ± 0.09^b	41.09 ± 1.17^a	35.14 ± 0.07^b
5	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.02 ± 0.02	7.11 ± 0.09^b	7.50 ± 0.04^a	36.81 ± 0.28^a	35.59 ± 0.18^b	40.60 ± 2.24^a	35.42 ± 0.07^b
6	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.08 ± 0.01	7.18 ± 0.13^b	7.91 ± 0.38^a	35.84 ± 0.54^a	35.67 ± 0.47^a	40.50 ± 0.61^a	36.38 ± 0.44^b
7	30.00 (0)	0.85 (0)	14.07 (-α)	137.50 (0)	29.75 (0)	11.07 ± 0.01	6.07 ± 0.03^b	7.97 ± 0.03^a	33.96 ± 0.23^a	31.49 ± 0.19^b	40.15 ± 3.34^a	34.13 ± 1.00^b
8	30.00 (0)	0.85 (0)	51.93 (+α)	137.50 (0)	29.75 (0)	11.55 ± 0.05	6.27 ± 0.07^a	6.10 ± 0.35^a	42.91 ± 0.20^a	43.58 ± 0.18^a	49.20 ± 0.47^b	54.63 ± 0.15^a
9	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.22 ± 0.03	7.39 ± 0.09^b	7.79 ± 0.06^a	37.62 ± 0.37^a	35.52 ± 0.30^b	41.34 ± 1.16^a	35.66 ± 0.54^b
10	30.00 (0)	0.85 (0)	33.00 (0)	273.55 (+α)	29.75 (0)	11.21 ± 0.01	7.27 ± 0.04^a	7.01 ± 0.23^a	36.67 ± 1.88^a	35.97 ± 0.27^a	41.65 ± 0.19^a	39.11 ± 1.52^a
11	30.00 (0)	0.85 (0)	33.00 (0)	1.46(-α)	29.75 (0)	11.22 ± 0.03	7.24 ± 0.06^a	7.01 ± 0.11^a	37.70 ± 0.21^a	36.55 ± 0.23^b	41.51 ± 0.06^a	39.23 ± 0.27^b
12	1.61 (-α)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.04 ± 0.01	10.08 ± 0.00^a	10.17 ± 0.06^a	24.68 ± 1.00^a	23.67 ± 0.21^a	20.37 ± 0.05^a	17.26 ± 0.80^b
13	30.00 (0)	1.678 (+α)	33.00 (0)	137.50 (0)	29.75 (0)	12.46 ± 0.01	7.66 ± 0.01^a	7.78 ± 0.01^a	35.75 ± 0.55^a	37.24 ± 0.33^a	41.26 ± 1.19^a	34.97 ± 0.92^b
14	30.00 (0)	0.022 (-α)	33.00 (0)	137.50 (0)	29.75 (0)	9.99 ± 0.06	5.87± 0.43^a	5.34 ± 0.23^a	38.31 ± 0.71^a	38.04 ± 2.30^a	42.51 ± 0.35^a	41.85 ± 1.42^a
15	42.00 (+1)	1.20 (+1)	25.00 (-1)	80.00 (-1)	17.50 (-1)	11.19 ± 0.02	7.40 ± 0.05^a	7.30 ± 0.01^a	38.68 ± 0.26^a	35.25 ± 0.39^b	41.40 ± 1.52^a	35.02 ± 0.22^b
16	18.00 (-1)	1.20 (+1)	41.00 (+1)	80.00 (-1)	17.50 (-1)	11.42 ± 0.06	7.71 ± 0.02^a	7.81 ± 0.28^a	36.06 ± 0.07^a	35.92 ± 0.30^a	40.94 ± 0.48^a	36.71 ± 1.07^b
17	42.00 (+1)	1.20 (+1)	41.00 (+1)	195.00 (+1)	42.00 (+1)	11.42 ± 0.06	7.37 ± 0.07^a	7.99 ± 0.21^a	38.42 ± 0.25^a	37.82 ± 1.57^a	43.52 ± 0.76^a	36.97 ± 0.49^b
18	18.00 (-1)	1.20 (+1)	25.00 (-1)	195.00 (+1)	42.00 (+1)	11.27 ± 0.04	8.13 ± 0.13^a	8.47 ± 0.14^a	34.77 ± 1.93^a	32.58 ± 0.23^a	38.47 ± 0.47^a	31.09 ± 0.29^b
19	42.00 (+1)	0.50 (-1)	41.00 (+1)	80.00 (-1)	17.50 (-1)	11.14 ± 0.03	6.19 ± 0.00^a	6.99 ± 0.22^a	39.68 ± 1.74^a	39.29 ± 1.39^a	44.32 ± 1.13^a	44.94 ± 1.01^a
20	18.00 (-1)	0.50 (-1)	41.00 (+1)	80.00 (-1)	17.50 (-1)	11.14 ± 0.03	7.09 ± 0.17^a	7.06 ± 0.25^a	34.09 ± 1.53^a	36.83 ± 0.00^a	41.11 ± 0.34^a	38.20 ± 0.55^b
21	18.00 (-1)	1.20 (+1)	41.00 (+1)	80.00 (-1)	42.00 (+1)	11.36 ± 0.06	7.68 ± 0.01^a	7.81 ± 0.15^a	36.10 ± 0.75^a	35.90 ± 1.01^a	40.84 ± 0.31^a	35.65 ± 0.11^b
22	18.00 (-1)	1.20 (+1)	25.00 (-1)	80.00 (-1)	17.50 (-1)	11.31 ± 0.01	8.19 ± 0.06^a	8.21 ± 0.04^a	33.00 ± 0.66^a	32.30 ± 0.25^a	38.63 ± 0.78^a	32.39 ± 0.70^b
23	18.00 (-1)	0.50 (-1)	25.00 (-1)	80.00 (-1)	42.00 (+1)	11.04 ± 0.01	7.13 ± 0.04^a	7.05 ± 0.20^a	34.28 ± 2.08^a	32.75 ± 0.37^a	38.58 ± 0.12^a	32.76 ± 1.44^b
24	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.25 ± 0.01	7.39 ± 0.02^a	7.45 ± 0.08^a	36.75 ± 0.29^a	35.12 ± 0.93^a	41.24 ± 0.34^a	35.71 ± 0.32^b
25	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.36 ± 0.06	7.12 ± 0.14^a	7.16 ± 0.49^a	37.16 ± 0.23^a	35.38 ± 0.84^a	41.56 ± 1.53^a	36.54 ± 0.28^b
26	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.21 ± 0.01	7.13 ± 0.07^a	6.63 ± 0.38^a	37.61 ± 2.15^a	36.97 ± 0.33^a	41.62 ± 1.01^a	41.44 ± 1.96^a
27	42.00 (+1)	1.20 (+1)	25.00 (-1)	195.00 (+1)	17.50 (-1)	11.51 ± 0.01	7.39 ± 0.02^a	6.90 ± 0.05^b	36.20 ± 0.57^a	35.33 ± 2.44^a	42.34 ± 0.50^a	36.09 ± 0.45^b
28	42.00 (+1)	0.50 (-1)	25.00 (-1)	80.00 (-1)	42.00 (+1)	10.99 ± 0.01	7.03 ± 0.04^a	6.59 ± 0.04^b	38.18 ± 0.47^a	36.10 ± 0.14^b	42.67 ± 0.96^a	37.48 ± 0.21^b
29	42.00 (+1)	0.50 (-1)	41.00 (+1)	80.00 (-1)	42.00 (+1)	10.99 ± 0.01	6.25 ± 0.01^b	6.92 ± 0.23^a	39.31 ± 1.15^a	40.24 ± 2.72^a	44.19 ± 0.68^a	44.68 ± 1.46^a
30	18.00 (-1)	0.50 (-1)	25.00 (-1)	195.00 (+1)	17.50 (-1)	10.95 ± 0.20	6.60 ± 0.14^b	7.78 ± 0.18^a	40.88 ± 1.02^a	31.97 ± 0.33^b	37.25 ± 0.09^a	30.55 ± 0.40^b
31	42.00 (-1)	0.50 (-1)	25.00 (-1)	195.00 (+1)	17.50 (-1)	10.98 ± 0.01	7.01 ± 0.01^a	6.57 ± 0.01^b	37.04 ± 0.13^a	35.26 ± 0.20^b	42.60 ± 0.81^a	35.53 ± 0.11^b
32	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.32 ± 0.01	7.31 ± 0.08^a	6.81 ± 0.00^b	36.77 ± 1.18^a	34.39 ± 0.38^b	41.46 ± 0.60^a	35.90 ± 0.33^b
33	18.00 (-1)	1.20 (+1)	25.00 (-1)	195.00 (+1)	17.50 (-1)	11.31 ± 0.02	8.21 ± 0.02^a	8.22 ± 0.10^a	33.63 ± 0.14^a	32.87 ± 0.93^a	41.47 ± 0.28^a	33.65 ± 1.26^b
34	18.00 (-1)	0.50 (-1)	41.00 (+1)	195.00 (+1)	42.00 (+1)	11.14 ± 0.03	7.03 ± 0.07^a	6.92 ± 0.08^a	35.72 ± 0.21^b	36.91 ± 0.08^a	42.89 ± 2.40^a	37.84 ± 0.96^b
35	18.00 (-1)	1.20 (+1)	41.00 (+1)	195.00 (+1)	17.50 (-1)	11.41 ± 0.00	7.67 ± 0.07^a	7.47 ± 0.15^a	34.74 ± 0.61^a	35.48 ± 0.32^b	41.28 ± 0.74^a	35.84 ± 0.35^b
36	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.35 ± 0.04	7.13 ± 0.10^a	6.93 ± 0.18^a	37.02 ± 0.43^a	35.96 ± 0.01^b	42.48 ± 1.76^a	36.39 ± 0.54^b

(*continued on next page*)

Table 2 (*continued*)

N°	Real and coded (values in brackets) variables X_1 (h) X_2 (%) X_3 (°C) X_4 (h) X_5 (h)					Soaking pH	*Atp-Y* pH after soaking	*Coca-sr* pH after soaking	*Atp-Y* moisture content (%)	*Coca-sr* moisture content (%)	*Atp-Y* water retention capacity (%)	*Coca-sr* water retention capacity (%)
37	18.00 (-1)	0.50 (-1)	25.00 (-1)	80.00 (-1)	17.50 (-1)	11.06 ± 0.00	7.05 ± 0.01^a	6.93 ± 0.13^a	35.13 ± 1.13^a	32.47 ± 0.38^b	40.42 ± 3.30^a	32.64 ± 0.72^b
38	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.31 ± 0.04	7.26 ± 0.03^b	7.80 ± 0.01^a	36.30 ± 0.90^a	36.26 ± 0.64^a	41.74 ± 0.06^a	35.71 ± 0.87^b
39	42.00 (+1)	1.20 (+1)	41.00 (+1)	80.00 (-1)	42.00 (+1)	11.74 ± 0.03	7.27 ± 0.14^b	7.82 ± 0.00^a	39.63 ± 0.25^a	37.80 ± 0.94^a	48.04 ± 3.31^a	38.75 ± 0.81^b
40	18.00 (-1)	1.20 (+1)	25.00 (-1)	80.00 (-1)	42.00 (+1)	11.54 ± 0.02	8.06 ± 0.13^a	8.05 ± 0.12^a	34.68 ± 0.42^a	33.01 ± 2.79^a	38.34 ± 0.97^a	32.58 ± 0.65^b
41	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.29 ± 0.10	7.27 ± 0.21^a	7.78 ± 0.11^a	38.78 ± 4.45^a	36.99 ± 0.16^b	41.58 ± 0.46^a	36.51 ± 0.62^b
42	42.00 (+1)	0.50 (-1)	25.00 (-1)	195.00 (+1)	42.00 (+1)	11.00 ± 0.00	7.01 ± 0.05^a	6.80 ± 0.08^a	38.41 ± 0.23^a	34.87 ± 0.19^b	42.41 ± 0.05^a	36.68 ±1.54^b
43	18.00 (-1)	1.20 (+1)	41.00 (+1)	195.00 (+1)	42.00 (+1)	11.37 ± 0.04	7.75 ± 0.04^a	7.57 ± 0.22^a	35.66 ± 0.41^a	35.05 ± 0.38^a	38.96 ± 0.17^a	35.65 ± 1.17^a
44	18.00 (-1)	0.50 (-1)	41.00 (+1)	80.00 (-1)	42.00 (+1)	11.08 ± 0.01	7.16 ± 0.04^a	6.85 ± 0.15^a	35.82 ± 0.88^a	36.10 ± 0.91^a	41.63 ± 0.20^a	38.59 ± 1.13^a
45	30.00 (0)	0.85 (0)	33.00 (0)	137.50 (0)	29.75 (0)	11.29 ± 0.10	6.95 ± 0.16^b	7.64 ± 0.09^a	38.98 ± 0.14^a	36.40 ± 0.00^b	41.39 ± 1.12^a	37.47 ± 1.14^b
46	18.00 (-1)	0.50 (-1)	25.00 (-1)	195.00 (+1)	42.00 (+1)	11.04 ± 0.04	6.61 ± 0.07^b	8.12 ± 0.16^a	41.69 ± 1.44^a	31.89 ± 1.21^b	38.02 ± 0.25^a	30.00 ± 0.23^b
47	42.00 (+1)	0.50 (-1)	41.00 (+1)	195.00 (+1)	17.50 (-1)	11.23 ± 0.24	6.40 ± 0.13^a	6.44 ± 0.14^a	40.02 ± 0.53^a	39.99 ± 1.91^a	45.19 ± 0.30^a	46.45 ± 1.22^a
48	42.00 (+1)	1.20 (+1)	41.00 (+1)	80.00 (-1)	17.50 (-1)	11.79 ± 0.02	7.34 ± 0.01^b	7.70 ± 0.05^a	38.96 ± 0.79^a	37.93 ± 01.70^a	42.85 ± 0.64^a	40.85 ± 1.29^a
49	42.00 (+1)	1.20 (+1)	25.00 (-1)	80.00 (-1)	42.00 (+1)	11.79 ± 0.02	7.37 ± 0.04^a	6.98 ± 0.06^a	37.26 ± 0.73^a	36.33 ± 0.69^a	41.41 ± 0.66^a	35.32 ± 0.18^b
50	42.00 (+1)	0.50 (-1)	41.00 (+1)	195.00 (+1)	42.00 (+1)	11.06 ± 0.05	6.37 ± 0.14^a	6.64 ± 0.01^a	41.09 ± 0.25^b	42.63 ± 0.00^a	44.87 ± 0.81^a	46.23 ± 0.05^a
51	42.00 (+1)	0.50 (-1)	25.00 (-1)	80.00 (+1)	17.50 (-1)	11.11 ± 0.01	6.69 ± 0.11^a	6.77 ± 0.05^a	46.22 ± 1.70^a	35.48 ± 0.98^b	46.72 ± 6.64^a	35.85 ± 0.41^b
52	18.00 (-1)	0.50 (-1)	41.00 (+1)	195.00 (+1)	17.50 (-1)	11.11 ± 0.05	7.00 ± 0.07^a	6.83 ± 0.04^a	36.59 ± 0.49^a	35.58 ± 0.03^a	42.06 ± 0.49^a	38.66 ± 1.29^a
53	42.00 (+1)	1.20 (+1)	41.00 (+1)	195.00 (+1)	17.50 (-1)	11.88 ± 0.02	6.78 ± 0.06^b	7.92 ± 0.02^a	39.23 ± 0.52^a	38.80 ± 0.85^a	43.11 ± 0.06^a	40.50 ± 0.99^a
54	42.00 (+1)	1.20 (+1)	25.00 (-1)	195.00 (+1)	42.00 (+1)	11.68 ± 0.23	7.39 ± 0.23^b	8.36 ± 0.20^a	41.77 ± 1.78^a	36.38 ± 2.07^b	41.81 ± 1.57^a	34.99 ± 0.41^b

X_1: soaking time (h); X_2: salt concentration (%); X_3: soaking temperature (°C); X_4: germination time (h); X_5: maturation time (h).
Means with the same letter along the line are not significantly different at 5%.

variables. They give an idea of the degree of influence of the different variables on the evaluated responses and the direction of variation of these effects. The proposed models show that the different factors increase or decrease the different assessed responses in different proportions. These observations show that the choice and the domains of the factors have been well done. It also shows that these mathematical models could be applied in the production of sprouted flours with high amylolytic activity and reducing sugar content, which is of great importance in the fermentation process in the brewing industry and the improvement of the energy density of complementary foods in infant nutrition. It follows from Table 3 below that the amylolytic activity of the *Atp-Y* variety was significantly ($p < 0.05$) influenced by all linear and quadratic effects of the variables as well as the interactions of soaking time-salt concentration, salt concentration-soaking temperature, time-soaking temperature and salt concentration-sprouting time. The *Coca-sr* variety was not influenced by the linear effect of soaking and ripening time and the quadratic effect of soaking time. However, it is significantly ($p < 0.05$) influenced by the soaking time-temperature and salt concentration-soaking temperature interactions. Klang et al. [17–19]; Irakoze et al. [59] also demonstrated the influence of steeping and germination temperature, steeping and germination time on the amylolytic activity, fluidity, FAN content and extraction rate of sorghum and maize malts. The analysis of the coefficients of the different variables shows that all quadratic effects negatively affected the activity in both varieties. Indeed, an exponential increase in soaking time results in water uptake beyond the limit (23 and 36% for *Coca-sr* and *Atp-Y* respectively) which would lead to a decrease in metabolic phenomena, exponential growth of fermentative microorganisms and loss of enzymes through leaching. Gujjaiah and Kumari [56] reported an almost six-fold decrease in proteolytic activity in some cereals when soaking and germination doubled. The same is true of Nadeem et al. [64] who noted a decrease in germination capacity (from 99% to 87%) of yellow maize beyond 24 h soaking. Saline solutions influence the pH of the soaking solution and water uptake. They are sources of cofactors for the enzymatic activity. A doubling of this factor during soaking would lead to a high alkanisation of the solution resulting in increased embrittlement of the cereal cell wall followed by a high uptake of water, ions and a change in the general acid-base catalysis mechanism of amylases to base due to a change in the ionization state of the amino acids (Glutamic and Aspartic acids) of the active site of amylases [66]. Most of the development of enzyme activity in seeds takes place during germination and continues during maturation. It allows the activation of numerous enzymes (phytases, amylases, proteases, lipases, glucanases …) following the lifting of the dormant state. These parameters are strongly influenced by the duration. Indeed, Ratnavathi and Ravi [67] showed that amylolytic activity dropped after 4 days of germination. Hassani et al. [68], showed that the optimum of alpha and beta-amylase activities was 6 and 5 days respectively. Linear effects of soaking time and input concentration also showed a negative effect on the activity of the *Atp-Y* variety while soaking time as well as interactions with soaking temperature and input concentration lowered the amylolytic activity in the *Coca-sr* variety. The latter (soaking time and interactions with input concentration and soaking temperature) rather showed the opposite effect in the *Atp-Y* variety. This also reveals different application possibilities. In addition, the linear effects of germination and ripening time as well as the interactions of soaking time-input concentration, soaking

Table 3
Validation of mathematical models, contribution of variables, coefficient, *p-value* and sums of squares of variables.

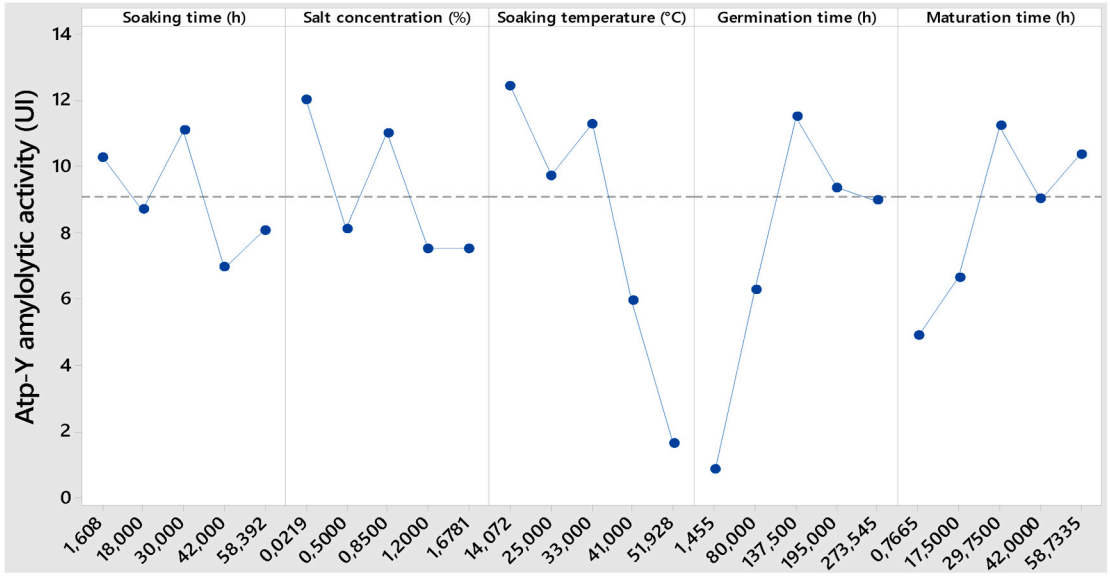
Coca-sr Atp-Y								
Source	Coeff	RC (%)	*P*	SS	*P*	RC (%)	SS	Coeff
Model		90.88	**0.000**	2089.74	**0.000**	92.79	777.177	
X_i (linear)	23.45	15.68	**0.000**	360.52	**0.000**	44.49	372.646	13.51
X_1	−1.240	0.49	0.194	11.18	**0.001**	3.05	25.537	−1.82
X_2	2.60	2.27	**0.007**	52.13	**0.029**	1.13	9.497	−1.11
X_3	−2.74	2.51	**0.005**	57.74	**0.000**	20.36	170.527	4.70
X_4	5.57	10.40	**0.000**	239.03	**0.000**	12.87	107.795	3.74
X_5	−0.24	0.02	0.793	0.45	**0.000**	7.08	59.289	2.77
X_iX_i (square)		68.16	**0.000**	1567.21	**0.000**	40.12	336.028	
X_1X_1	0.33	0.67	0.860	0.20	**0.000**	3.01	42.773	−4.74
X_2X_2	−9.38	4.29	**0.000**	167.58	**0.000**	2.31	32.885	−4.16
X_3X_3	−16.77	19.31	**0.000**	535.40	**0.000**	8.68	89.935	−6.88
X_4X_4	−18.70	26.89	**0.000**	665.69	**0.000**	17.19	153.934	−9.00
X_5X_5	−14.33	16.99	**0.000**	390.70	**0.000**	8.93	74.832	−6.27
X_iX_j (interactions)		7.05	**0.021**	162.01	**0.002**	8.18	68.504	
X_1X_2	−2.17	0.21	0.391	4.80	**0.024**	1.22	10.214	3.16
X_1X_3	−6.78	2.04	**0.010**	46.98	**0.007**	1.78	14.895	3.82
X_1X_4	4.51	0.90	0.080	20.75	0.110	0.59	4.926	2.20
X_1X_5	2.18	0.21	0.389	4.85	0.605	0.06	0.499	−0.70
X_2X_3	5.75	1.47	**0.027**	33.82	**0.016**	1.42	11.863	3.41
X_2X_4	2.04	0.19	0.419	4.26	**0.006**	1.92	16.119	3.97
X_2X_5	1.15	0.06	0.647	1.36	0.168	0.43	3.639	−1.89
X_3X_4	4.18	0.78	0.103	17.84	0.407	0.15	1.290	1.12
X_3X_5	−1.99	0.18	0.430	4.06	0.990	0.00	0.000	0.02
X_4X_5	4.78	1.01	0.064	23.30	0.106	0.60	5.059	−2.23
Error		9.12		209.73		7.21	60.369	
Inadequation		5.96	0.566	137.13	0.723	4.34	36.342	
Pure error		3.16		72.60		2.87	24.027	
		100.00				100.00		
Validation of the model								
R^2		90.88				92.79		
Adjust R^2		85.35				88.42		
Bias factor		1.002				1.00		
AADM		0.02				0.01		

X_1: Soaking time; X_2: Plant salt concentration; X_3: Soaking temperature; X_4: Germination time; X_5: Ripening time; X_1X_1: Quadratic effect soaking time; X_2X_2: Quadratic effect plant salt concentration; X_3X_3: Quadratic effect soaking temperature; X_4X_4: Quadratic effect germination time; X_5X_5: Quadratic effect ripening time; X_1X_2: Interaction soaking time-plant salt concentration; X_1X_3: Interaction soaking time-soaking temperature; X_1X_4: Soaking time-sprouting time interaction; X_1X_5: Soaking time-maturing time interaction; X_2X_3: Plant salt concentration-soaking temperature interaction; X_2X_4: Plant salt concentration-sprouting time interaction; X_2X_5: Plant salt concentration-maturing time interaction; X_3X_4: Soaking temperature-sprouting time interaction; X_3X_5: Soaking temperature-maturing time interaction; X_4X_5: Sprouting time-maturing time interaction. Coeff: Coefficient Value; RC: Real Contribution; SS: Sum of Square; p: P-value; AMDA: Absolute Mean Deviation Analysis; R^2: determination coefficient.

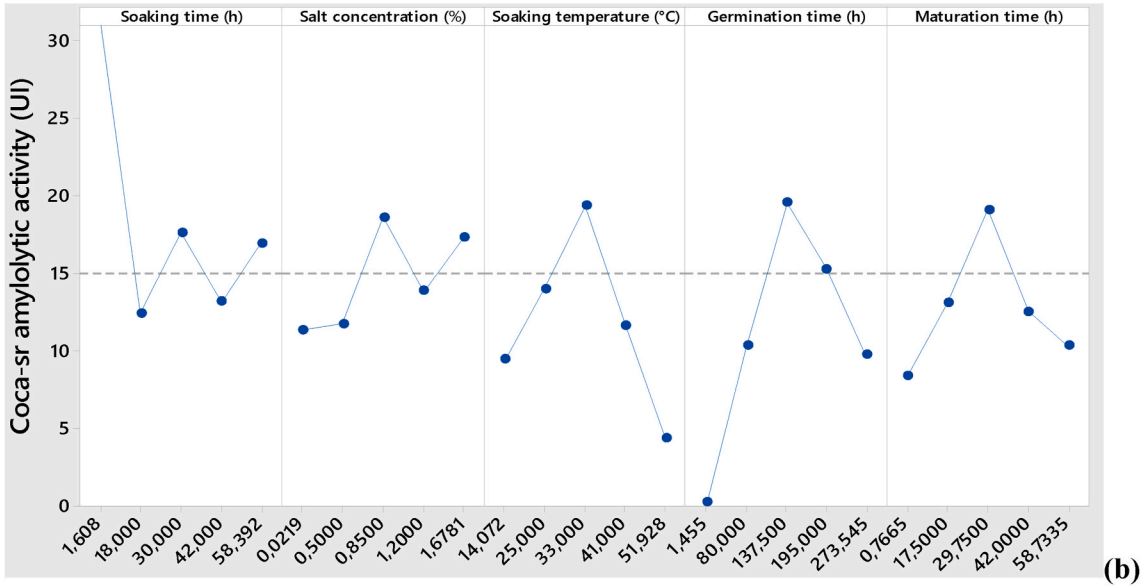
time-soaking temperature, input concentration-soaking temperature and finally input concentration-soaking time improved the amylolytic activity of the *Atp-Y* variety, whereas the linear effect of germination and the input concentration-soaking temperature interaction showed the same variations in *Coca-sr*. Progressive activation of hydrolases at the aleurone layer by gibberellins would be at the origin of these observations [69]. Similarly, the destruction of protein-tannin, protein--phenol and protein-phytate complexes followed by the leaching of these molecules under the combined action of temperature, soaking and salts would make it possible to improve the content of soluble proteins essential for enzymatic activity [70]. Activation of phytases and improvement of their activity under the effect of temperature and salts would allow the hydrolysis of phytates, thus releasing the metal cofactors essential for the activity of amylases. The table also shows that linear effects, mainly germination time, contributed most to the amylolytic activity of the *Atp-Y* variety, while quadratic effects, particularly germination time, had the greatest influence on the activity of the *Coca-sr* variety. These observations were confirmed with the sum of squares being higher for these variables. Several parameters such as Coefficient of Determination (R^2), Adjusted Coefficient of Determination (R^2 adj), Absolute Average Deviation Analysis (AADM) and Bias Factor (bf) are used to check the robustness of the model, the fit between the predicted and experimental models, the degree of agreement between the selected variables and the evaluated responses [19,71]. The R^2 and R^2 adj are 90.88 and 92.79% respectively for *Coca-sr* and *Atp-Y*. These two parameters are positively correlated and have all presented values above the standard values (R^2 and R^2 adj ≥75%) for validation of a mathematical model [72,73]. It can also be seen from Table 3 below that the Absolute Average Deviation Analysis (AADM) and the Bias Factor (bf) have values within the range of standard values (0–0.30 for AADM and 0.75–1.25 for bf) for all the responses evaluated regardless of the variety [74,75]. These results show that the five [5] selected factors can be applied in the production of high amylase malts.

3.4. Individual effect of different factors on amylolytic activity

From the analysis of the influence of the different variables on the amylolytic activity within the two varieties, it follows that the effects observed are different in the two varieties (Fig. 1). Klang et al. [19] also reported a different effect of soaking and germination time on the flowability of two rice malt varieties. The amylolytic activity of the *Atp-Y* variety increases with soaking time until a maximum at 30 h and decreases beyond that. On the other hand, it decreases with soaking time in the *Coca-sr* variety until it stabilizes at 30 h soaking. The increase in amylolytic activity with soaking is explained by the activation of the metabolic processes of the enzymes of this variety. It would also be linked to the denaturation of the enzymes during drying due to the high water content of the seeds [76]. The lowering of amylolytic activity would be the effect of a reduction of the oxygen content necessary for metabolic reactions and a dilution of the reagents necessary for germination. Indeed, Kanensi et al. [77], showed that the diastatic power and reducing sugar content of amaranth seeds increased up to 15 h of



(a)



(b)

Fig. 1. Effect of individual variables on amylolytic activity (a and b for *Atp-Y* and *Coca-sr* respectively).

soaking and dropped beyond that. The amylolytic activity is constant up to 0.5% concentration and decreases beyond the *Atp-Y* variety. The increase in activity with salt concentration is linked to the absorption of activity-activating ions, the destruction of activity-inhibiting anti-nutrients, the modification of pH and the weakening of cell walls facilitating water absorption. The drop-in activity is due to a high concentration of ion that creates steric hindrance at the active site, thereby reducing the enzymatic activity. Enzymes are metalloproteins whose activity is conditioned by the presence of ions acting as cofactors. Indeed, Tambo et al. [32] demonstrated that the amylolytic activity of crude extracts of potato (*1112* and *Local*) and maize (*Kassaï* and *Atp*) flour decreased with the concentration of Na^+ ion. In addition, this pH variation leads to a modification of the ionization state of the amino acids of the active site responsible for catalysis, which results in a modification of the amylase activity. Perveen et al. [63], also demonstrated that soaking in the presence of $NaHCO_3$ and $Mg(OH)_2$ improved the digestive and reducing sugar producing capacity of sprouted barley amylases, which was not the case for NaOH and KOH. Concerning the soaking temperature, there is an embrittlement of the epidermis membrane of the seeds facilitating the absorption of water, the absorption of the ions contained in the soaking water necessary to lift the dormancy state and the activation of soluble proteins. The activity increases up to 33 °C and drops beyond that for the *Coca-sr* variety. However, a drastic decrease was observed with the *Atp-Y* variety. Indeed, Helland et al. [78] showed a causal relationship between soaking temperature and amylase activity. Similarly, germination leads to an activation of metabolic and respiratory reactions. Indeed, it is during this phenomenon that most of the development of the diastatic power of cereals takes place. The peak of activity was observed at 137.5 h in both varieties and a decrease beyond this time. Indeed, Ratnavathi and Ravi [67] showed that amylolytic activity dropped after 4 days of germination. Hassani et al. [68], showed that the optimum of alpha and beta-amylase activities was 6 and 5 days respectively. The drop in amylase activity on either side of these peaks is thought to be due to a partial lifting of the seed dormancy state, inhibition of hydrolases beyond a certain germination time and inhibition of the process of translation of RNA into proteins responsible for catabolic reactions [69]. Amylolytic activity also increases up to 29.75 h of maturation before

dropping off beyond that in both varieties. The improvement of the different responses with ripening time up to a peak at 29.75 h is thought to be due to an activation of excess soluble proteins with catalytic activity and the inhibition of hormones responsible for dormancy. Beyond this time, there is a lowering of the various responses. This would be the consequence of an inhibition of the catabolic phase in favour of anabolism.

3.5. Contour plot defining the optimal zones of amylolytic activity according to the significant interactions

The definition of the optimal conditions using Minitab 18.1 software allowed us to draw the contour plot below. These curves were drawn from the interactions of the factors that significantly ($p<0.05$) influenced the said responses. The hatched areas represent these areas of interest. They define the optimal values of the responses obtained under specific conditions for the two selected factors. It can be seen from Fig. 2a and e showing the effect of soaking time and plant salt concentration on the amylolytic activity of *Atp-Y* and *Coca-sr* sprouted flours respectively, that the optimum activities were 14 UI [2a] and 25–30 UI [2e] respectively. These values were observed for salt concentrations below 0.2% [2a] and soaking times below 10 h [2and2e]. The variation in salt concentration did not affect the activity in the *Coca-sr* variety. It also follows that increasing the soaking time induces a decrease in activity in both varieties. A loss of substrates and soluble proteins with activity as a result of long soaking times would explain these observations. The low salt concentrations required for the *Atp-Y* variety are further explained by its richness in cupric ions (see Table 5), an inhibitor of amylase activity [32]. Fig. 2b and f shows the optimal activities of *Atp-Y* and *Coca-sr* were respectively at a long soaking time for a low soaking temperature (less than 20 °C) and a short soaking time (less than 10 h) for soaking temperatures ranging from 10 to 60 °C. This could be explained by the nature of the amylases present and their cellular localization. In addition, Patane et al. [70] reported that a short soaking time associated with a temperature of 45 °C provided sufficient hygrometry to promote good seed germination. Fig. 2c and g shows optimal activities of 16 UI and 20 UI for *Atp-Y* and *Coca-sr* respectively. They also show a decrease in the amylolytic activity of the *Atp-Y* variety with the increase of the soaking temperature-plant salt concentration couple. The same observations were noted with the *Coca-sr* variety on both sides of the salt concentration and the optimal activity temperature. The definition of the optimal conditions according to the salt concentration-germination time couples shows an activity around 10 UI for *Atp-Y* and 20 UI for *Coca-sr*. Both varieties required salt concentrations close to 1% while the *Atp-Y* variety required a much longer germination time. The *Coca-sr* variety presented the best optimal conditions regardless of the chosen interactions.

3.6. Validation test of optimal conditions

Table 4 below shows the optimal conditions for salt concentration, soaking temperature, soaking time, germination and maturation of *Atp-Y* and *Coca-sr* maize kernels to obtain optimal amylolytic activities. These values were obtained from the manipulations performed on the Minitab 18.1 software. The predicted optimal values obtained from the software were compared to the experimental optimal values obtained in the laboratory. The results show that a soaking time of 25.12 h at a temperature of 25.54 °C in the presence of 0.52% vegetable salt followed by germination for 144.37 h and maturation for 37.65 h maximized the amylolytic activity of the *Atp-Y* maize grains.

For the *Coca-sr* variety, soaking for 1.608 h at a temperature of 36.63 °C in the presence of 1.10% vegetable salt was required for germination of 144.37 h and maturation of 27.07 h. It was found that a long soak at a lower temperature was necessary to activate and facilitate amylolytic activity in the *Atp-Y* variety. Klang et al. [18] and Milala and Addy [79] also reported a variation in the optimal germination conditions of *Atp-Y* and *Kassaï* maize seeds. They further reported a correlation between liquefying power and steeping time for the *Atp-Y* variety. They attributed this to a difference in epidermal composition, genetic variability, the nature of the predominant amylases and a difference in the area of enzyme localization in the two varieties. Desirability represents the percentage reproducibility of a system and its robustness. Validation of a model requires a desirability greater than 0.5. From this table it can be seen that the amylolytic activity showed a good fit with the mathematical model, demonstrating that the factors, the response and the ranges of variation of each factor were well delineated. The desirability of the *Coca-sr* variety shows that the equations and the optimal conditions are 84% and 94% reproducible for the *Atp-Y* variety. It also appears from the amylolytic activity optimization trials that no significant difference ($p>0.05$) was observed with that predicted by the system for the two varieties. However, this activity was more marked with the *Coca-sr* variety (26.25 IU). Klang et al. [18] also reported a strong liquefying capacity with the white maize variety in contrast to the yellow variety. The inactivation of catabolic processes for anabolism due to the high maturation time, the dilution of reagents with high water retention during the high soaking time, the loss of soluble proteins with activity during soaking and finally the low activator concentration as well as the high inhibitor content brought by salts during soaking would be at the origin of the observed differences and the low diastatic power of the *Atp-Y* variety. An antagonistic effect of proteases on amylases overexpressed in this variety would also be one of the causes [79]. Evaluation of α-amylolytic activity shows that this parameter was significantly ($p<0.05$) higher with the *Atp-Y* variety. Tambo et al. [32]; Tsopbeng et al. [11] also reported a higher α-amylolytic capacity with the *Atp-Y* variety compared to the *Kassaï* and *Coca-sr*. They attributed this to an overexpression in this variety of the gene responsible for α-amylolytic protein synthesis, germination time and grain architecture [56,79,80]. Gujjaiah and Kumari [56] reported in their work a high α-amylolytic activity up to 200 h of germination while the β-amylase activity was inhibited after 96 h of germination. These observations suggest a high β-amylase activity in the *Coca-sr* variety. These results indicate that both varieties could be used in beer production as a substitute for barley malt, as a sweetener in cake formulation, as an anti-thickening agent in the preparation of energy-dense supplementary foods and as a source of enzyme in bread making. The soluble proteins are mainly the enzymes with enzymatic activity. The *Coca-sr* variety showed a significantly ($p<0.05$) higher content (12.11 mg) than *Atp-Y* (10.60 mg). Indeed, Dongmo et al. [7] reported the loss of nutritional and bioactive substances with soaking time. In addition, the high proteolytic activity marked in *Atp-Y* would have induced the hydrolysis of these into amino acids used as energy source for the newly growing plant. The FAN content is respectively 103.20 and 293.40 mg/100 g in *Atp-Y* and Coca-sr. The results observed are contrary to the proteolytic activity reported in both varieties (0.06 UI for *Atp-Y* and 0.02 UI for *Coca-sr*). Indeed, the strong presence of peptidases in the *Coca-sr* variety, although not evaluated in this work, would be at the origin of this difference. An extracellular localization (outside the aleurone layer) of these enzymes in the *Atp-Y* variety would also be one of the consequences. The FAN content obtained with *Coca-sr* is close to that of Djameh et al. [81] which ranged from 232.72 to 291 mg/100 g and similar to that of Embashu and Nantanga [82] which ranged from 111.80 to 162.70 mg/100 g for *Atp-Y*. Malt loss and yield of the two varieties were also assessed. From Table 4 below, it can be seen that the mass loss is 42.66 and 28.23% respectively for an observed yield of 57.34 and 71.77% for *Atp-Y* and *Coca-sr*. This difference can be explained by the long soaking time of the *Atp-Y* variety, which made the grain more friable, resulting in losses [59]. Moreover, the values obtained are far higher than those of Embashu and Nantanga [82] which varied between 11.00 and 30.40%. The *Coca-sr* variety appears to be more suitable for future applications, especially in malting. The Kolbach index is positively influenced by proteolytic activity and FAN content [83]. Nevertheless, no significant difference ($p>0.05$) was observed

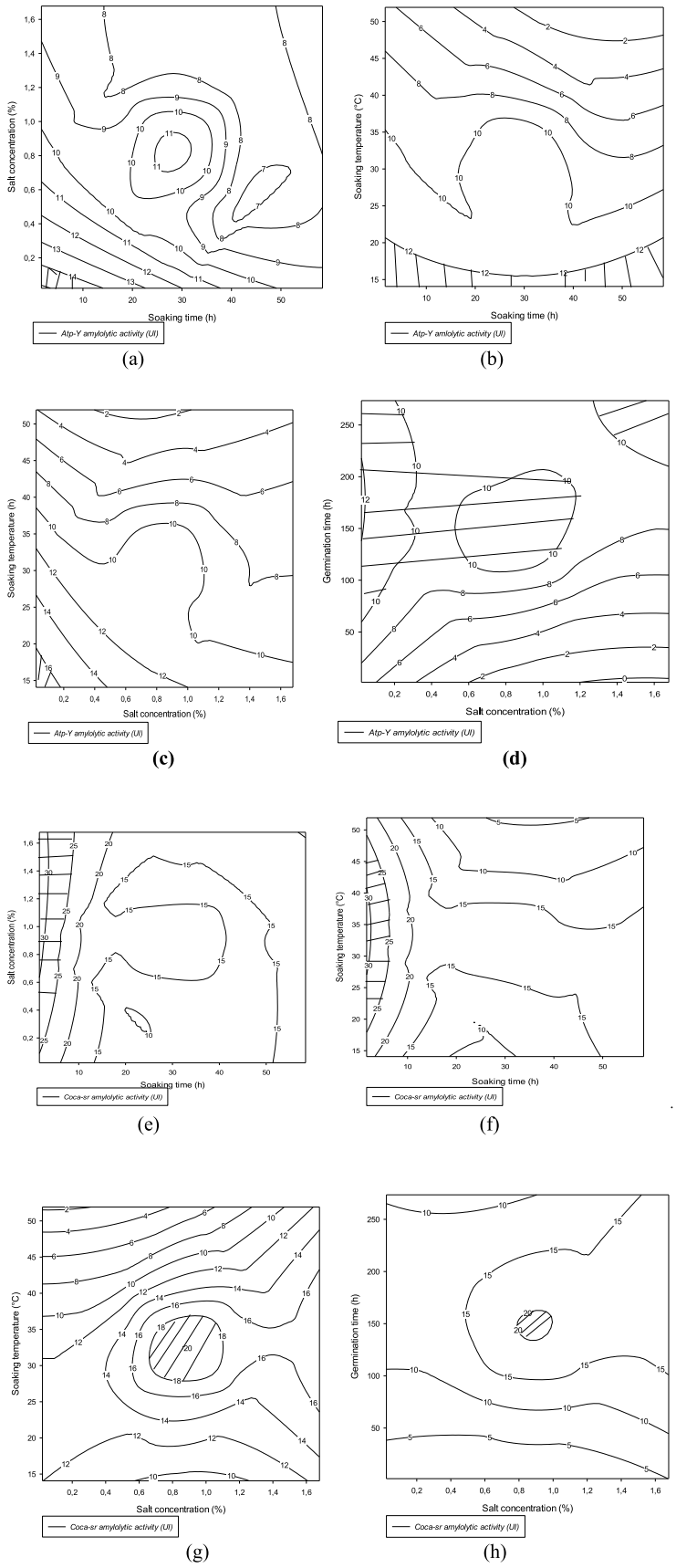


Fig. 2. Contour plot showing the optimal activity zones obtained from the interactions that had a significant effect (2a, 2b, 2c and 2d: *Atp-Y*; 2e, 2f, 2g and 2h: *Coca-sr*).

Table 4
Validation trial of optimal conditions.

Samples	*Atp-Y*	*Coca-sr*
Optimal conditions		
Soaking time (h)	25.12	1.608
Soaking temperature (°C)	25.54	36.63
Salt concentration (%)	0.5238	1.1093
Germination time (h)	144.37	144.37
Maturation time (h)	37.65	27.07
Desirability	0.94	0.84
Experimental optimal value	15.13 ± 1.82^{aB}	26.25 ± 1.53^{aA}
Predicted optimal value	15.28 ± 0.53^{aB}	26.22 ± 2.12^{aA}
Soluble protein (mg)	10.60 ± 0.06^{b}	12.11 ± 1.82^{a}
Free amino nitrogen (mg/100g of DM)	103.20 ± 4.10^{b}	293.40 ± 14.25^{a}
Proteolytic activity (UI)	0.06 ± 0.01^{a}	0.02 ± 0.00^{b}
α-amylase activity (UI)	14.54 ± 0.50^{a}	11.39 ± 1.44^{b}
Malt loss	42.66 ± 0.00^{a}	28.23 ± 0.02^{b}
Malt yield	57.34 ± 0.00^{a}	71.72 ± 0.02^{b}
Kolbach Index (%)	0.15 ± 0.00^{a}	0.15 ± 0.02^{a}
Germinative energy (%)	85.33 ± 6.43^{b}	97.33 ± 1.15^{a}
Moisture content (%)	33.89 ± 1.65^{a}	23.09 ± 1.58^{b}
Water absorption capacity (%)	34.92 ± 0.97^{a}	22.02 ± 0.40^{b}
Water soaking pH before	9.46 ± 0.00^{a}	9.58 ± 0.00^{a}
Water soaking pH after	6.67 ± 0.04^{b}	8.58 ± 0.05^{a}
pH variation	2.78 ± 0.05^{a}	1.00 ± 0.04^{b}

Means with the same lower and uppercase letter along the line are not significantly different at 5%.

between the two varieties. The values obtained are lower than those of Hassani et al. [68] which were between 30 and 32% in sorghum malt. It is also lower than that of Narziss and Back [84] on germinated barley (30–35%). Hassani et al. [68] report that long steeping combined with high temperatures leads to loss of soluble protein and consequently lower Kolbach index. Germination energy represents the percentage of germinated seeds when soaking conditions followed by germination are applied. This parameter can be used to boost crop production and combat grain loss. In this study, 85.33% and 97.33% of the maize kernels of the *Atp-Y* and *Coca-sr* varieties respectively were found to have sprouted. This difference is believed to be related to the long soaking times and insufficient ion content to activate the enzymes of the plant catabolic reactions in the *Atp-Y* variety. The values obtained are lower for the *Atp-Y* variety but similar for the *Coca-sr* variety to those of Ebbah et al. [38] which ranged between 94 and 96% with red sorghum. The application of the present soaking conditions to the *Coca-sr* variety could lead to improved field production yields. The malting process is mainly influenced by steeping and moisture content. From Table 4 below it can be seen that the moisture content and water holding capacity of the kernels were 33.89 and 23.09% and 34.92 and 22.02% respectively for the varieties *Atp-Y* and *Coca-sr*. The high values for *Atp-Y* could be explained by the high soaking time and the skeleton of its skin. Klang et al. [18] also reported a high propensity of *Atp-Y* maize kernels to retain in contrast to *Kassaï*. In the same vein, Rahman et al. [60] observed a gradual moisture intake of a hybrid maize variety with increasing soaking time and temperature. Several authors including Kolawole and Kolawole [12] have shown that the use of plant ash during soaking improves germination while reducing soaking time. They provide certain metallic elements (Ca, Mg, Na, P, K, Cu, Fe, Zn, Se …) that can more or less influence enzymatic activities [12,32]. A diffusion of these elements in the seed is influenced by the soaking time, the composition of the epidermis and the soaking temperature. This will lead to a variation in pH as observed with both varieties (Table 4 below).

3.7. Nutritional, mineral, colour, bioactive and antioxidant composition of Atp-Y and Coca-sr sprouted maize meal

3.7.1. Nutritional composition

Table 5 below shows the nutritional composition of the optimal germinated maize flours of the *Atp-Y* and *Coca-sr* varieties. The water

Table 5
Nutritional, mineral, color, bioactive and antioxidant composition of *Atp-Y* and *Coca-sr* optimal germinated maize flours.

Samples	*Atp-Y*	*Coca-sr*
Nutritional composition		
Moisture (%)	5.39 ± 0.25^{a}	5.80 ± 0.51^{a}
Proteins (%)	6.88 ± 0.20^{b}	8.09 ± 0.06^{a}
Lipids (%)	6.23 ± 0.07^{a}	6.19 ± 0.06^{a}
Fibers (%)	3.37 ± 0.25^{a}	4.41 ± 0.30^{a}
Ash (%)	1.00 ± 0.00^{b}	2.00 ± 0.00^{a}
Digestibles Glucids (%)	78.06 ± 0.59^{a}	74.33 ± 0.20^{b}
Starch (%)	32.86 ± 2.03^{a}	28.26 ± 0.90^{b}
Amylose (% of starch)	22.31 ± 3.24^{a}	17.73 ± 0.76^{b}
Amylopectin (% of starch)	77.69 ± 3.24^{b}	82.27 ± 0.76^{a}
Amylose/Amylopectin	0.29 ± 0.06^{a}	0.21 ± 0.01^{a}
Reducing sugar (% of glucids)	28.35 ± 2.02^{a}	21.18 ± 1.15^{b}
Energy content (Kcal)	395.81 ± 2.69^{a}	386.99 ± 0.35^{b}
Mineral composition (mg/100 of DM)		
Ca	787.00 ± 1.41^{b}	826.50 ± 0.71^{a}
Mg	69.80 ± 0.28^{a}	70.90 ± 0.14^{a}
Na	124.90 ± 0.58^{a}	124.90 ± 0.58^{a}
K	418.41 ± 2.54^{a}	427.77 ± 0.76^{a}
Cu	0.37 ± 0.03^{a}	0.18 ± 0.02^{b}
Ca/Mg	11.27 ± 0.02^{a}	11.66 ± 0.01^{a}
Na/K	0.30 ± 0.00^{a}	0.29 ± 0.00^{a}
Oxal/Ca	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
Oxal/Mg	0.16 ± 0.05^{a}	0.06 ± 0.00^{b}
Oxal/Cu	29.73 ± 5.68^{a}	24.48 ± 2.81^{b}
Phy/Ca	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}
Phy/Mg	0.08 ± 0.01^{a}	0.00 ± 0.00^{b}
Phy/Cu	14.60 ± 0.92^{a}	0.00 ± 0.00^{b}
Color parameters		
l*	78.87 ± 0.06^{a}	83.23 ± 1.89^{a}
a*	1.19 ± 0.01^{a}	0.59 ± 0.05^{b}
b*	17.34 ± 0.03^{a}	6.93 ± 0.14^{b}
Browning Index (BI)	31.05 ± 0.51^{a}	5.65 ± 0.45^{b}
Antinutritional and bioactive compounds		
Phytates (mg/100 g of DM)	5.49 ± 0.86^{a}	0.00 ± 0.00^{b}
Oxalates (mg/100 g of DM)	5.62 ± 1.59^{b}	7.87 ± 1.59^{a}
Trypsin inhibitors (mg/100 g of DM)	2.13 ± 0.29^{a}	2.92 ± 0.08^{a}
Cyanide (mg/100 g of DM)	2.97 ± 0.00^{a}	2.68 ± 0.00^{a}
Condensed tannins (mg CE/g of sample)	3.27 ± 0.10^{a}	2.84 ± 0.30^{a}
Hydrolyzed tannins (mg CE/g of sample)	9.54 ± 0.59^{a}	5.44 ± 0.12^{b}
Phenols (mg GAE/g of sample)	48.77 ± 10.30^{b}	84.08 ± 7.93^{a}
Flavonoids (mg CE/g of sample)	39.55 ± 6.21^{b}	89.53 ± 12.43^{a}
Saponins (mg/100g of DM)	0.58 ± 0.06^{a}	0.56 ± 0.03^{a}
Anti-oxydant properties		
ABTS (% of inhibition)	16.46 ± 2.33^{b}	21.06 ± 2.51^{a}
DPPH (% of inhibition)	12.13 ± 1.60^{b}	23.04 ± 2.59^{a}
FRAP (μM of Trolox/g)	12.16 ± 1.02^{b}	35.70 ± 3.25^{a}

DM: Dry Matter; GAE = Gallic Acid Equivalent; CE = Catetchin Equivalent; Means with the same letter along the line are not significantly different at 5%.

content influences the development of microorganisms and the preservation of foodstuffs. It is respectively 5.39 and 5.80% for *Atp-Y* and *Coca-sr* without significant effect ($p>0.05$). Nevertheless, the *Coca-sr* variety presented a higher content than the *Atp-Y* variety. This is related to the soaking temperature and the salt content used during soaking which favoured the absorption of water followed by the formation of strong intramolecular bonds. The observed contents are lower than those recommended (less than 12%) for preservative and stability over a long period [85]. The values obtained are similar to those of Tambo et al. [2,3], which ranged from 6 to 8% in maize flours. Proteins values of *Coca-sr* variety was significantly ($p>0.05$) higher (8.09%) than that of *Atp-Y* (6.88%). This difference would be linked to genetic variability, as well as the destruction of the antinutrients-proteins complex under the effect of temperature [79]. In addition, it would also result in the removal of these antinutrients by leaching [86]. The high maturation time for the *Atp-Y* variety would also explain this difference in that the proteins released during the germination process are used as a source of energy and as a precursor for the new growing plant. Nevertheless, the levels observed are higher than those of Tambo et al. [2,3] on maize meals and lower than those of Obilana et al. [87] on millet. The values

obtained are in line with the 6–15% recommended by Codex Stan [88] for infant flours suggesting germination of cereals in order to improve its nutritional value. The values of lipids obtained were not significantly ($p>0.05$) affected by variety. The results are higher than the 2% obtained by Raimi et al. [69] on guinea corn after 7 days of germination. Overexpression of lipases followed using of the released fatty acids as an energy source in the growing plant would explain these observations [89]. The values obtained in both varieties are below the 9% recommended for supplemental flours [88]. Fibers constitute the non-digestible carbohydrate fraction and facilitate digestion. They are the main constituents of plant cell walls. The values obtained are 3.37 and 4.41% for the *Atp-Y* and *Coca-sr* varieties respectively. The low value in the *Atp-Y* variety can be explained by a genetic difference, the overexpression of fiber digestion enzymes (glucanases, cellulases, etc.) and finally the destruction of the wall for the synthesis of the new plantlet due to the longer maturation time [90]. The values obtained do not comply with the standards of less than 3 g for supplemental flours [88]. The highest ash content was obtained with *Coca-sr* (2%) with a significant effect ($p>0.05$). The high content of vegetable ash used during soaking as well as a loss in the *Atp-Y* variety during soaking which proved to be longer [91] would be the causes. Obilana et al. [87] reported a similar content in sprouted millet. The digestible carbohydrate, starch and amylose contents were significantly ($p<0.05$) higher in the *Atp-Y* variety. This could be explained by an increased degradation of carbohydrates in the endosperm resulting in improved protein, fiber and lipid content as well as a higher amylolytic activity in the *Coca-sr* variety [87]. Genetic variability would also explain these differences. These values are lower than those of Dongmo et al. [7] for starch (89.50%) and Obilana et al. [87] for digestible carbohydrates (85.41%). The activation of enzymes (amylases) responsible for the digestion of carbohydrates and especially endospermic starch is the main reason for these differences [92]. The amylopectin content was significantly ($p<0.05$) low (77.69%) in *Atp-Y* variety compared to *Coca-sr* variety (82.27%). This difference is related to the higher α-amylase activity in the *Atp-Y* variety. Nguemguo et al. [93] reported a reduction of the consistency of potato-based gruels by germinated maize meal rich in α-amylase. The amylose/amylopectin ratio influences the functional and rheological properties of flours by determining their application areas [93]. The results obtained show a high retrograde ability of the *Atp-Y* variety due to a high ratio. These results show that the *Atp-Y* variety would be more applicable in bread making. The evaluation of the reducing sugar content shows that it is significantly ($p<0.05$) higher in the *Atp-Y* variety than in the other. This difference could be explained by the reduced α-amylolytic activity in the *Coca-sr* variety. Indeed, Tambo et al. [32] reported improved reducing sugar content in sprouted cereals compared to tubers due to their high α-amylolytic activity. The reducing sugar contents obtained in *Atp-Y* and *Coca-sr* are respectively 27 and 20 times higher than the values of Tambo et al. [2]. This implies a possible application of these sprouted cereals in the sweetening of porridge, cakes and as a substrate source for fermentation in the brewing industry. In addition, the energy density was evaluated and found to be 395.81 and 386.99 Kcal/100 g of *Atp-Y* and *Coca-sr* optimal sprouted flours, respectively. This difference is mainly related to the chemical composition of the two varieties. These results are lower than those of Obilana et al. [87] which was 421.81 Kcal in sprouted millet. This difference could be explained by a loss of solubilization of the reserve substances during soaking under the effect of salts and temperature, and by their use as a source of energy during the development of the new plant.

3.7.2. Mineral composition and nutrient ratios

The effect of variety and malting conditions on mineral composition (Ca, Mg, Na, K and Cu) and nutrient ratios were evaluated and presented in Table 5 below. It follows from this table that this parameter is very high (826.50 mg/100 g) in the *Coca-sr* variety. This could be explained by a high uptake of this mineral from the plant ash used during soaking, a high phytic activity resulting in the degradation of insoluble phytate-ion complexes and the thermal destruction of oxalates due to a slightly higher soaking temperature [94,95]. Tambo et al. [32] further reported an increasing activating effect with increasing Ca concentration on amylase activity. These observations explain the differences observed in the amylolytic power of the two varieties. The contents of Mg, Na, K and Cu vary respectively from 69.80 to 70.90 mg/100 g, 124.90 mg/100 g, 418.41–427.77 mg/100 g and finally from 0.18 to 0.37 mg/100 g. A significantly ($p<0.05$) high effect of copper was observed in *Atp-Y*. Tambo et al. [32] reported a negative effect of this metal on the amylolytic activity of germinated maize and sweet potato, which would explain the low amylolytic activity reported in *Atp-Y*. The results obtained are far superior to those of Ponka et al. [96] for magnesium (2.78–5.80 mg/100 g), Ndagire et al. [97] for sodium (6.8 mg/100 g) and Anigo et al. [99] for potassium (171.32 mg/100 g). These differences can be explained by the reduction of metal-chelating antinutrients during germination, the destruction and leaching of these antinutrients under the effect of temperature, and the contribution of ions contained in the plant ashes used during soaking [100–102]. The bulk ratios of minerals and anti-nutrients were also evaluated to detect the effect of treatments on the availability of these ions. It was found that Ca/Mg, Na/K, Oxal/Ca and Phy/Ca ratios were not significantly ($p>0.05$) affected by variety. The Ca/Mg ratio is higher than 1 while the Na/K ratio is lower than 1. These results suggest an availability of these ions in the different flours. Phytates and oxalates are electronegative and can therefore chelate divalent cations present in the food, thus reducing their availability [7]. The values obtained are lower than the standards (1 for phy/Mg and 0.17 for phy/Ca). These results suggest the availability of these ions and the contribution of the soaking-sprouting combination in improving the availability of ions and the destruction of antinutrients. The results obtained are also lower than those of Dongmo et al. [7] which ranged from 1.24 to 2.16 and 9.31–19.32 for phy/Ca and phy/Mg ratios respectively. The phy/Cu and oxal/Cu ratios ranged from 0.00 to 14.60 and 24.48 to 29.73 respectively. The variety *Atp-Y* showed the highest values. The reduced soaking temperature would be responsible for this variation.

3.7.3. Color of samples

The effect of variety and malting conditions on the color attributes show that l* (lightness), a* (redness), b* (yellowness) and the whiteness index (IB) are significantly ($p<0.05$) influenced by variety and malting conditions for the last three parameters. The high whiteness index in the *Atp-Y* variety is related to its reduced content of antioxidant compounds [101,102]. Ye et al. [103] reported an increase in lightness and Browning Index with the destruction of total phenols, the lowering of enzymatic browning, the increase of Maillard reaction, lipid oxidation and the content of reducing sugars. The high values of a* and b* in the *Atp-Y* variety are thought to be related to the Maillard reaction developed between reducing sugars (high content) and proteins during drying and a lowering of brightness [104,105]. These colorimetric attributes are crucial in the choice and acceptability of foods. Kayitesi et al. [106] reported an attraction of consumers to naturally colored foods. These observations would suggest that malting of cereals should be done to improve sensory attributes. The values obtained are higher for brightness (l*) than those of Kayitesi et al. [106] on sorghum but not lower for the other two parameters. The Browning index was reduced in *Coca-sr* variety and higher in *Atp-Y* variety compared to those of Hatamian et al. [107] in Chia seeds flour. This could be explained by the richness of oxidizable vitamins (A, D, E and K) in the colored *Atp-Y* variety and the decrease in protein content due to the Maillard reaction.

3.7.4. Anti-nutrient properties and bioactive compounds of sprouted flours

The contents of anti-nutrients and bioactive compounds were evaluated and reported in Table 5 below. The phytates, oxalates and trypsin inhibitors contents, were found to be 5.49 and 0.0 mg/100 g, 5.62 and 7.87 mg/100 g, 2.13 and 2.92 mg/100 g in *Atp-Y* and *Coca-sr* maize

varieties respectively. On the other hand, cyanides, condensed tannins, hydrolysable tannins, phenols, flavonoids and saponins were respectively 2.97 and 2.68 mg/100 g, 3.27 and 2.84 mg EC/g, 9.54 and 5.44 mg EC/g, 48.75 and 84.08 mg GAE/g, 39.55 and 89.53 mg EC/g and finally 0.58 and 0.56 mg/100 g for *Atp-Y* and *Coca-sr* maize varieties. A significant effect ($p < 0.05$) was observed on phytates, oxalates, hydrolysable tannins, phenols and flavonoids. The zero value of phytates observed with the *Coca-sr* variety is believed to be due to high phytase activity, leaching and destruction under the effect of the high soaking temperature [97]. The values obtained are below the recommended threshold (250 mg for phytates and 450 mg for oxalates) [108]. Inhibitors of protein digestibility, especially trypsin, show that malting conditions significantly reduce this parameter, contrary to those of Fotso et al. [108] in soybean. These observations are due to the presence in the plant ash of competitive inhibitors of these compounds. It can be also explain by destruction trypsin inhibitor by temperature and leaching during soaking. The values are below the threshold (80 mg). Cyanide is the most used defense mechanism by cereals at the beginning of germination [29]. The data collected in this study show an increase in this parameter with the maturation time of the *Atp-Y* variety. Moreover, the results are not in line with the observations of Tambo et al. [2,3] who reported a positive correlation between cyanide content and the liquefying power of germinated maize varieties *Atp-Y* and *Kassaï*. This would be related to the presence of other activating compounds and the nature of the amylases. The values obtained are nevertheless lower (10 mg) than the recommended threshold. Condensed tannins form high-energy bonds with nutrients, thus reducing their digestibility, in contrast to hydrolysable tannins, which are linked by low-energy bonds [109]. Condensed tannins are removed by the combined effect of soaking, temperature and enzymes. The high levels of hydrolysable tannins in the *Atp-Y* variety can be explained by the low soaking temperature. The values obtained are lower than those of Embashu and Nantanga [82] in sorghum. Phenols and flavonoids are powerful antioxidant compounds with health benefits. The *Atp-Y* variety showed low proportions of these compounds. This result is related to the destruction of phenols released by the polyphenol oxidases over activated in this variety during germination, the loss during long soaking of this variety, the existence of phenol-protein complexes hence the low protein content observed in this variety and the low release of phenolic compounds from the cellulosic wall [107]. These results suggest a possible use of the *Coca-sr* variety in beer production and hop economics.

3.7.5. Antioxidant properties

The antioxidant properties (DPPH, FRAP and ABTS) were evaluated. They are 16% (*Atp-Y*) and 21.06% (*Coca-sr*) for ABTS, 12.13% (*Atp-Y*) and 23.04% (*Coca-sr*) for DPPH and 12.16 (*Atp-Y*) and 35.70 (*Coca-sr*) μM of Trolox/g for FRAP. The *Coca-sr* variety presented significantly ($p < 0.05$) higher values for all antioxidant properties. This variation is thought to be related to the high content of phenols and flavonoids with antioxidant properties in this maize variety [87,110]. The loss of phenols through leaching and use in the formation of the skeleton of the new plant during development due to the long ripening time explains the low properties in the *Atp-Y* variety. Azeez et al. (2021) reported an improvement in the antioxidant capacity of brown millet with germination up to six [6] days followed by a lowering beyond that. The results obtained are superior to those of Obilana et al. [87] and Azeez et al. [111].

3.8. Recommended nutritional intake (%) of fat, protein and calorific energy of different malt corn flours in relation to the daily nutritional requirements of children aged 6–59 months

The contribution to energy density, protein and fat from the consumption of 100 g of the two sprouted maize varieties is presented in Table 6 below. It follows that the *Coca-sr* variety due to its nutritional composition presented earlier (Table 5) would contribute most to the daily protein demand while the *Atp-Y* variety contributes most to the energy density and lipid intake. Tambo et al. [2] also reported similar effects with the same maize variety compared to *Kassaï*. The contributions decrease in both varieties as age increases. This is influenced by the physiological change of the young child requiring more nutrients and energy resources [17,18]. The two varieties contribute between 21.50 and 60.89% of the energy demand of children aged 0–59 months, between 28.69 and 62.23% for protein and 26.57–37.30% for fat. The contents obtained in this study, independently of the age group and variety, are higher than the data obtained by Dongmo et al. [7] on two varieties of maize that have undergone soaking, fermentation and roasting. This could be explained by germination, which resulted in improved nutritional value. However, the contributions suggest that these flours should be supplemented with legumes as sources of protein and lipids in the formulation of supplementary food as recommended by FAO/WHO [112].

Table 6
Contribution (%) of energy, proteins and fats content of flour from 100 g of corn single flour toward RDA for children aged 6–59 months.

Variables	Age group (years)	Recommended Daily Allowances (RDA)	Contribution (%) of sample flours to RDA	
			Atp-Y NTB	*Coca-sr* PIB
Energy (Kcal/day)	0–0.5	650[a]	60.89	59.53
	0.5–1.0	850[a]	46.57	45.53
	1–3	1300[a]	30.45	29.77
	4–6	1800[a]	22.00	21.50
Proteins (g/day)	0–0.5	13[a]	52.92	62.23
	0.5–1.0	14[a]	49.14	57.76
	1–3	16[a]	43.00	50.56
	4–6	24[a]	28.67	33.71
Fats (g/day)	0–0.5	–	–	–
	0.5–1.0	–	- -	–
	1–3	16.70[b]	37.30	37.07
	4–6	23.30[b]	26.74	26.57

[a] Food and Nutrition Board [113].
[b] Alasfoor et al. [114].

3.9. Physical and functional properties of optimal flours

Physical and functional properties are important in determining the future applicability of a flour [115]. These properties are presented in Table 7 below. It can be seen that the pH was not significantly ($p > 0.05$) influenced by the variety, although the *Atp-Y* variety had a low value. This is explained by its long soaking time and the transformation of its fermentable sugars into organic acids during this process [116]. The values obtained are lower than those of Klang et al. [6], which were 6.22 in the maize flour variety *Atp-Y*. The production conditions of these

Table 7
Physical and functional properties of optimal flours.

Samples	*Atp-Y*	*Coca-sr*
Physical Properties		
pH	5.66 ± 0.01[a]	5.86 ± 0.01[a]
Titrable acidity (meq of NaOH/100g of DM)	0.75 ± 0.00[b]	1.75 ± 0.35[a]
Bulk Density (g/ml)	0.47 ± 0.02[a]	0.50± 0.00[a]
Tapped bulk density (g/ml)	0.68 ± 0.00[a]	0.71 ± 0.01[a]
Porosity (%)	30.07 ± 6.46[a]	29.50 ± 2.83[a]
Hausner ratio	1.44 ± 0.13[a]	1.42 ± 0.06[a]
Functional Properties		
Water retention capacity (%)	13.00 ± 1.41[b]	14.50 ± 2.12[a]
Swelling capacity (%)	57.77 ± 1.76[a]	57.91 ± 2.50[a]

Means with the same letter along the line are not significantly different at 5%.

flours could be one of the explanations. These pH values further suggest the richness of these maize varieties in acidic amino acids [117]. Titratable acidity results from the synthesis of organic acids during different processing in foods. This parameter is highly determinant in the acceptability of foods [7]. It is significantly ($p < 0.05$) influenced by the variety and the treatments applied. The *Coca-sr* variety had the highest titratable acidity (1.75 meq NaOH/100 g). This can be explained by the loss (evaporation during drying) of low molecular weight organic acids synthesized during soaking and germination in this variety [85]. Dilution due to the high final water content of the *Atp-Y* variety and leaching during soaking of these small molecules of organic acids would also be at the origin of the observed results [116]. Furthermore, the lipolytic activity leading to the hydrolysis and subsequent release of fatty acids in the *Coca-sr* variety also explains this result [118]. Mass density is influenced by protein content and particle size [115]. Low bulk density is associated with good nutrient digestibilities, easy packing and suitability for supplemental food formulation [7,85]. Loose bulk density and packed bulk density ranged from 0.47 to 0.50 g/mL and from 0.68 to 0.71 g/mL, respectively. No significant ($p > 0.05$) effect was observed despite high values in the *Coca-sr* variety. A high protein and digestible carbohydrate content (see Table 5) in this variety would be the reason for these observations. Similarly, hydrolysis of macronutrients leads to the formation of low molecular weight compounds and therefore less density [117]. Atlaw et al. [119] also reported a reduction in particle size and thus mass density of flours from Fenugrek with increasing α-amylolytic activity. Given these results, these flours would appear to be suitable for the formulation of supplementary food and would also reduce packaging costs as they have good dispersibility and ease of transport [120]. Porosity is a parameter associated with mass density in predicting the use of a flour. Porosities above 20% are highly recommended in the formulation of supplementary food [121]. The values obtained are similar to those of Djikeng et al. [85], which were between 15 and 30% in snail meat that had undergone different treatments. These results also corroborate the observations made with the mass density. The Hausner ratio is associated with the internal compression of particles in a sample [122]. The values are 1.44 and 1.42 for *Atp-Y* and *Coca-sr* respectively. These values are higher than 1.25, which suggests a low free flowability of the samples. Djikeng et al. [85] also reported this in snail meat and attributed this to the oil content. Water holding capacity and swelling rate are influenced by polysaccharide content, lipid content, nature of amino acids and protein conformation [121]. They represent the hydration properties of flours and are related to the viscous or non-viscous nature of formulations [2]. The swelling rate is not influenced by variety in contrast to the water holding capacity which was the highest (14.50%) with the *Coca-sr* variety. This could be explained by the high presence of hydrophilic amino acids on the surface of this flour [123]. The results obtained are contrary to those of Klang et al. [6,8,17,18] who demonstrated a significant positive correlation between starch content and water holding capacity. Furthermore, they are lower than those of Tambo et al. [2,3] who ranged between 40 and 90% in maize flours. This could be explained by the enzymatic activity observed in these flours [119]. These results show that these flours are suitable for the formulation of low viscosity infant foods.

3.10. *In vitro* digestibility study of starch in comparison with pancreatic amylase

The *In vitro* digestibility kinetics of soluble starch is presented in Fig. 3 below. It can be seen that the hydrolysis of starch increases gradually with time for extracts of both maize varieties, unlike pancreatic amylase, which showed a peak at 30 min followed by a gradual decrease until 2 h of digestion. Pancreatic amylase activity was higher than that of the amylases of the two maize varieties between 10 and 90 min of digestion, but lower after that. This could be explained by the purity of this enzyme. Indeed, the crude extracts of the *Atp-Y* and *Coca-sr* varieties contain other enzymes in addition to amylases, such as proteases and lipases, which can interfere with their activity. Gujjaiah and Kumari [56] reported a decrease in proteolytic activity with increasing germination time and amylolytic enzyme activity. The drop in activity observed with pure amylase beyond 30 min is thought to be related to a negative feedback of the digestion products on the amylase. Furthermore, it appears that for times lower than 120 min, the *Coca-sr* variety presented the best amylolytic capacity. This difference would be related to the high soluble protein content and diastatic power in this variety (Table 4). These results suggest the use of the enzymes of these two maize varieties in the digestion of resistant starches present in several cereals for applicability in the sugar industry and in the formulation of supplementary foods.

3.11. Fourier Transform Infrared Spectrum (FTIR) of the two maize varieties (Atp-Y and Coca-sr)

Fourier Transform Infrared Spectrum (FTIR) is a recently introduced tool to assess the influence of processing on the formation of certain compounds whose identification is characterized by the absorption

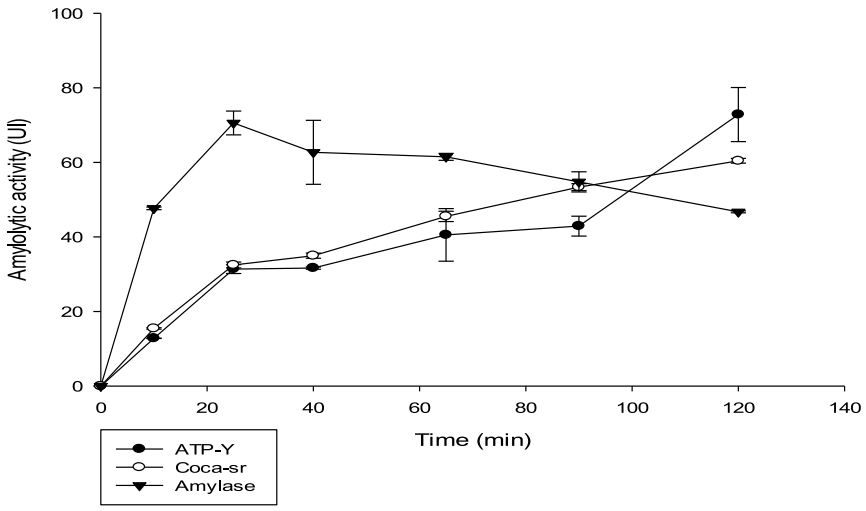


Fig. 3. *In vitro* digestibility kinetics of soluble starch by pancreatic amylase and crude extracts of the two maize malt varieties (*Atp-Y* and *Coca-sr*).

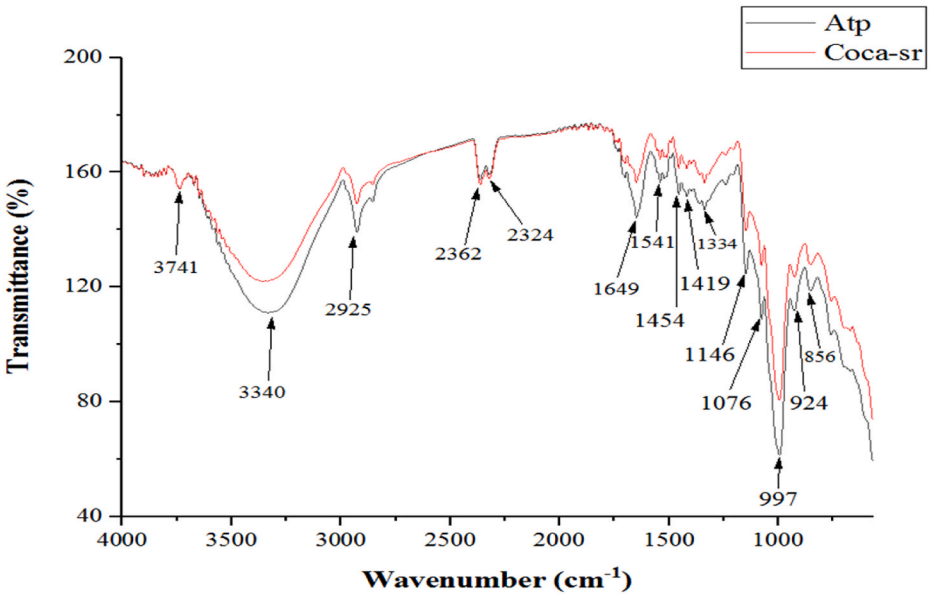


Fig. 4. Fourier transform infrared (FTIR) spectra of two malted maize varieties (*Atp-Y* and *Coca-sr)*.

characteristic wavelengths by their functional groups [124]. It follows from Fig. 4 below that the two varieties of maize malts exhibited similar absorption bands with different intensities. Gunes and Karaca [125] also reported similar absorption spectra between the proteins of lentils undergoing different treatments with different absorption intensities. These differences can be explained by the content of these different compounds. The different peaks obtained with or without vibration are due to the groups and bonds –C═N, –CH, –OH, –COH, –C═C, –NH, –C═O which are characteristic of the presence of carbohydrates, proteins, nucleic acids, phenolic compounds, lipid oxidation compounds and water [126]. The peak appearing at 3741 cm^{-1} (3800–3584) may be associated with the vibration of a free –OH group. This could be due to the presence of carbohydrates, reducing sugars produced during germination, water, alcohol from the transformation of sugars by endogenous microorganisms and amino acids such as serine and tyrosine [126]. Adebiyi et al. [117] also reported the presence of a stretching vibrational peak around 3500 cm^{-1} in pearl millet biscuits and flours. There is no difference in the peaks between the two varieties. The stretching of the aliphatic primary amine bond observed at 3340 cm^{-1} (3400–3300) and secondary amine (3350–3310) could be explained by the hydrolysis of proteins with the release of basic amino acids (Lysine and Arginine), the presence of proline and peptide bonds [117]. This absorption band is more pronounced with the *Atp-Y* variety, which would be linked to the more marked proteolytic activity in this variety. It also marks the presence of an ether bond between the glycosylation features of the proteins [127]. The amide A region characterised by the vibration of the asymmetric –CH bonds of hydrocarbons at 2925 cm^{-1} as well as the peak at 1454 cm^{-1} reveals the presence of proteins, carbohydrates, lipids, hydrophobic chain amino acids and nucleic acids [125]. The observed peak differences can be explained by the lipid and water richness of the *Atp-Y* variety (Tables 4 and 5). Lu et al. [128] also observed a similar peak at 2921 cm^{-1}. The stretching of the –SH functions represented by the peaks at 2362 and 2324 cm^{-1} suggest the existence in similar proportions of sulphur-containing amino acids (cysteine) and anti-oxidant compounds such as glutathione. The amide II region characterised by the bending of the –NH bond and the stretching of the –C═N bond is located at 1649 cm^{-1}. It also marks the area of vibrational movement of the peptide functions. The amide I region, on the other hand, was located at 1541 cm^{-1}. Gunes and Karaca [125] also reported similar peaks for proteins isolated from chickpeas. These results suggest the existence of beta-sheets as a major secondary structure mainly in the *Atp-Y* variety. In addition to this, the peak at 1649 cm^{-1} also marks the stretching of the –C═C (*cis*) bonds of fatty acids [126]. This implies that the *Atp-Y* variety is rich in unsaturated fatty acids. Similarly, the existence of aromatic compounds such as phenols, tannins and amino acids with aromatic rings (Phenylalanine, Tyrosine and Tryptophan) can be explained by the bending of the –CH functions (2000-1650 cm^{-1}). The existence of these amino acids would further explain the nature of the secondary structure. The bending of the –OH groups of the COOH functions of carboxylic acids (1440–1395), alcohols (1420–1330) and phenols (1390–1310) is marked by vibrations at 1419 and 1334 cm^{-1} respectively. It is related to the presence of acidic amino acids, fatty acids released during germination, phenolic compounds and carbohydrates. Adebiyi et al. [117] further reported peaks between 1158 and 1020 cm^{-1}. The high carbohydrate and starch contents, especially the low overall amylolytic activity, in the *Atp-Y* variety explain the differences observed in the peaks (1146 and 1076 cm^{-1}) of –CH bond bending present in these compounds (Table 5). Aydogdu et al. [129] also reported peaks at lengths close to 1100 cm^{-1} characteristic of starch. The intense peak observed at 997 cm^{-1} represents the bending of substituted double bonds present in hydrocarbons. It follows from this figure that variety has affected the proportions of these compounds. It is contrary to those of Adebiyi et al. [117] who reported instead stretching of the -C-N and –C═O bonds around this peak (1000 cm^{-1}). This could be explained by the treatments applied and the different nature of the samples. Unlike the peak at 997 cm^{-1}, the peak at 924 cm^{-1} rather reveals the presence of *trans*-substituted flexible –C═C bonds. These are characteristic of certain molecules with fingerprints (1000-700 cm^{-1}). They also characterize the presence of *Trans* olefins and trans unsaturated fatty acids such as elaidic acid. The same applies to the vibrations observed at 856 cm^{-1}.

4. Conclusion

At the end of this work, which aimed to evaluate the influence of the variety and malting conditions on the amylolytic activity, digestibility, physico-chemical and functional properties of the sprouted flours from the *Atp-Y* and *Coca-sr* maize varieties, it was found that these parameters were influenced by the factors studied and the maize variety. The *Coca-sr* variety showed higher amylolytic activity and soluble sugar content. On the other hand, α-amylolytic and proteolytic activities were more pronounced with the *Atp-Y* variety. Water content and malting

conditions were reduced with the *Coca-sr* variety for similar germination times (144 h). Chemical properties were improved with the malting conditions of both varieties including protein, lipids, ash and minerals such as Ca, Mg and Na. Both varieties showed relatively low levels of anti-nutritional compounds and the *Coca-sr* variety showed the highest levels of bioactive compounds and better antioxidant capacity. The *Atp-Y* variety would contribute most to the daily energy and fat requirements. With the exception of water retention capacity, all physical and functional properties showed similar and reduced values. Starch digestibility was better with the *Coca-sr* variety except for 120 min. The FTIR spectra show similar peaks with different intensities. The analysis of this FTIR spectra shows that the beta-sheet is the major secondary structure of the enzymatic proteins. This work shows a possible application of these malting conditions and varieties in the production of gluten-free beers, the improvement of the digestibility of nutrients contained in complementary foods as well as their sweetening and the formulation of bakery products such as cakes with improved nutritional value.

5. Originality of work

The authors declare that this work is original and has not been submitted anywhere.

Additional information

No additional information is available for this paper.

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This article does not contain any studies with humans or animals participants performed by any of the authors.

CRediT authorship contribution statement

Stephano Tambo Tene: Conceptualization, sample collecting, preparation and designing of research work, execution of laboratory experiments, formal analyses, Methodology, and data collection.Analysis of data and Interpretation.funding acquisition. preparation of the manuscript and review editing. **Derek Tantoh Ndinteh:** execution of laboratory experiments, formal analyses, methodology and data collection., Analysis of data and Interpretation., preparation of the manuscript and review editing.. **Jean Roger Dongmo:** execution of laboratory experiments, formal analyses, methodology and data collection., Analysis of data and Interpretation., preparation of the manuscript and review editing.. **Oluwafemi Ayodeji Adebo:** Conceptualization, sample collecting, preparation and designing of research work. execution of laboratory experiments, formal analyses, Methodology, and data collection.Analysis of data and Interpretation.supervision of the work. preparation of the manuscript and review editing. **Yusuf Olamide Kewuyemi:** execution of laboratory experiments, formal analyses, methodology and data collection., Analysis of data and Interpretation., preparation of the manuscript and review editing.. **Michael Hermann Kengne Kamdem:** execution of laboratory experiments, formal analyses, methodology and data collection., Analysis of data and Interpretation., preparation of the manuscript and review editing.. **Anthony Olusegun Obilana:** execution of laboratory experiments, formal analyses, methodology and data collection., Analysis of data and Interpretation., preparation of the manuscript and review editing.. **Julie Mathilde Klang:** and Conceptualization, sample collecting, preparation and designing of research work execution of laboratory experiments, formal analyses, Methodology, and data collection. Analysis of data and Interpretation.supervision of the work. preparation of the manuscript and review editing. **Patrick Berka Njobeh:** execution of laboratory experiments, formal analyses, methodology and data collection., Analysis of data and Interpretation., preparation of the manuscript and review editing.. **Hilaire Macaire Womeni:** Conceptualization, sample collecting, preparation and designing of research work execution of laboratory experiments, formal analyses, Methodology, and data collection.Analysis of data and Interpretation.supervision of the work. preparation of the manuscript and review editing.

Declaration of competing interest

all authors declare that they have no conflict of interest.

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