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Research article

Optimisation of the production of corn amylase flour from corn *Atp* and *Kassaï* varieties for the fluidification and energy density increase of cassava gruel

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ABSTRACT

The energy density of the complementary gruels can be increased by the use of sprouted flours. This led us to determine the conditions for obtaining and using sprouted corn flour for optimal fluidification of fermented cassava flour gruels. To do this, the germinated corn flour (GCF) varieties *Atp* and *Kassaï* was produced according to the response surface methodology and the Doehlert plan was used with factors such as soaking (12–48 h) and germination (24–96 h) times. Once obtained, these GCF were used to increase the energy density of cassava flour gruels with flow velocity as response. The Doehlert plan was also used with fermented cassava flour concentration (17.5–30 %) and optimised sprouted corn flour mass (1–5 g) as factors. The experimental design that had been performed indicated that the effectiveness of corn amylase flour to fluidify the gruels depends not only to the conditions of production (soaking and germination) ($p < 0.05$) but also their incorporation during the preparation (corn amylase–fermented cassava flour) ($p < 0.05$). The rich corn amylase flour can be obtained by soaking *Kassaï* and *Atp* varieties for 20 h and 28 h respectively and germinated for 90.82 h. Application of these GCF during the preparation of fermented cassava flour gruels has shown that to obtain gruels with flow velocities between 100-160 mm/30s, it was necessary to couple 1.16 g of sprouted corn flour variety *Kassaï* for 26.23 % of fermented cassava flour and 1.12 g of corn flour variety *Atp* for 25.94 % of fermented cassava flour. The use of these couples has made it possible to multiply the energy density of the gruels by 6.55 and 6.49, respectively. In view of these results, it is therefore advisable to use the germinated corn flours produced under the conditions obtained to fluidise and increase the energy density of the fermented cassava gruels.

1. Introduction

Child malnutrition is a pathological problem affecting several developing countries, including Cameroon. According to FAO (2018), it affects more than 821 million people worldwide, 90% of them in developing countries. According to this report, 1 in 9 people suffer from malnutrition including more than 22.2 % (150 million) of children under five years in the world (FAO, 2018). It is caused by a deficiency of one or more nutrients. In Cameroon, more than 29 % of children under five years suffer from undernutrition with 18.4 % in severe form (INS , 2018). This problem occurs mainly after 6 months, normally corresponding to the end of exclusive breastfeeding and the introduction of gruels who are semi-liquids breastfeeding foods (Sodipo and Fashakin, 2011; Zannou-Tchoko et al., 2011). These gruels are generally cooking using cereals (corn), tubers (yam) and roots (cassava) (Toulsoumdé et al., 2016; Yaredi et al., 2016). But during cooking, under the effect of heat and excess water, starch fixed water molecules, swells which give the pores a viscous character, difficult to swallow by the child. Mothers are therefore forced to obtain a sufficiently fluid and acceptable consistency by the child's digestive system to limit the proportion of flour relative to water. The infant food obtained have low energy and nutrient density; then reduce the growth of the child (Phuka et al., 2015). The most effective solution to increase the energetic intake of children thus seems to be the implementation of enzymatic treatments that reduce the viscosity of the gruel with a high concentration of flour dry matter. This is made possible by the use of sprouted flours, whose flowability depends

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on the production conditions. In this approach, the fluidification of the gruel has been the subject of many studies having opted for the germination of cereals (corn in particular) for the production of amylase flours (Yaredi et al., 2016; Tambo et al., 2018; Tambo et al., 2019a,b).

Previous works have been carried out in recent decades to produce amylase concentrates from local sources capable of improving the fluidity of the gruel while maintaining high concentrations of dry matter. The corn amylase flour contains enzymes that hydrolyze amylose and amylopectin to dextrin and maltose, thus reducing the viscosity of the thick gruels without dilution with water while simultaneously enhancing their energy and nutrient densities (Helland et al., 2002; Klang et al., 2020; Nguemguo et al., 2020). However, the effectiveness of this source of amylase to fluidify the gruel depends not only on the conditions of their production (soaking and germination) and variety but also their incorporation during the preparation (corn amylase flour-dry matter combination) for optimal consistency which is between 100-160 mm/30 s (Zannou-Tchoko et al., 2011; Klang et al., 2020). Soaking consists of hydration of the seed allowing the embryo to reach water content necessary for the initiation of germination (Guillot et al., 2008). The time associated with this operation must be controlled so that the grain reaches a sufficient moisture content favoring germination. High water content causes a slowdown in the germination process (Patane et al., 2016). Effective germination, which results in the activation of enzymes (amylase, proteases, and lipases) in all parts of the seed, still requires control. Cassava (*Manihot esculenta*, Crantz), a plant belonging to the Euphorbiaceae family, is cultivated for its roots and leaves (Rocksloh-Papendieck, 1989). It is consumed in many forms by adults and is also used in the preparation of porridge for children. The use of fermented cassava flour for the preparation of infant formula causes a real problem because of its consistency. The objective of this research was to optimise the production of corn amylase flour from corn *Atp* and *Kassaï* varieties for fluidification and increasing the energy density of cassava gruel.

2. Material and methods

2.1. Material

Yellow (*Atp* variety) and white corn (*Kassaï* variety) were obtained from IARD (Institute for Agriculture Research and Development), Foumbot, West-Cameroon. Cassava roots (bitter cultivar) were also obtained from IARD, Santchou, West-Cameroon.

2.2. Methods

2.2.1. Fermented cassava flour production

The cassava roots obtained were transformed into flour according to the method described by Bindzi (2012) with slight modifications. They were washed and peeled. They were cut into slices (5–7 mm thick) using a stainless steel knife. The slices obtained were introduced into a bucket containing 20 L of water for 4 days (96 h). The retting was carried out at a ratio of 1 kg–1.3 l of water (w/v). After fermentation, the fermented cassava roots were removed from the water and then washed, wrung out by hand, spread on grills and dried in an electric oven at 45 ± 0.5 °C during 48 h. The dried samples were then ground using an ordinary mill (Hamilton Germany®) and the flour obtained was sieved (400 μm). The flour obtained was preserved in a polyethylene bag before use.

2.2.2. Chemical and mineral composition

Previous work (Tambo et al., 2019a,b) showed the chemical and mineral composition, functional and physical properties of the cassava flour used in this study.

2.2.3. Optimisation process of germination of corn for obtaining flour rich in amylase

2.2.3.1. Experimental design. The optimum germination conditions of corn for obtaining flour rich in amylase were evaluated using the Doehlert design. The intervals of the independent variables (soaking time, germination time) used were: 12–48 h for soaking time and 48–96 h for germination time. These parameters were determined on the basis of preliminary works and literature review (Tambo et al., 2019a,b). A total of 09 experimental runs with two replications at the center point (Table 1) were completed and evaluation of the consistency of gruel using amylase-rich flours expressed as response were determined. The number of tests were determined by applying the formula $k^2 + k + 1$ with k representing the number of factors.

Data were fitted to a second-degree polynomial model (Y) of the form presented in Eq. (1):

$$Y = I + aX_1 + bX_2 + cX_1^2 + dX_2^2 + eX_1X_2 \quad (1)$$

where Y represent the response; X_1 and X_2 are the levels of the independentvariables; a and b are the linear terms; e is an interaction term; c and d are the quadratic terms and I is a constant.

To confirm or validate the optimum conditions of germination, two experimental replicates were performed under optimized conditions. The experimental and predicted values of flow velocity were compared. In addition to the coefficient of determination (R^2), bias factor (BF) and absolute average deviation (AAD) have been used to verify the validity and robustness of the predictive model.

2.2.3.2. Production of germinated corn flour. The corn kernels were soaked in distilled water at a temperature of 30 °C and germinated at different period as shown in Table 1. The germination consisted in spreading the grains on a cotton cloth and placed in a room sheltered from the solar rays. During this process, the grains were watered (15 mL/100 g of grain) twice a day at the intervals of 12 h per watering. After germination the seeds were matured (put the seeds for germination in a polyethylene and stored in the dark for 11 h). The grains were dried in an electric oven for 36 h at 45 °C. After degerming, the grains were crushed

Table 1. Experimental design for the process optimization of production of corn amylase flour.

N°	Coded levels		Real levels		Experimental flow velocities (mm/30s)	
	X_1	X_2	Soaking time (h) (X_1)	Germination time (h) (X_2)	*Atp*	*Kassaï*
1	1.000	0.000	48.00	72.00	443.33 ± 21.79	185.00 ± 27.83
2	-1.000	0.000	12.00	72.00	236.00 ± 8.54	336.67 ± 26.08
3	0.500	0.886	39.00	92.78	151.00 ± 30.55	470.67 ± 27.68
4	-0.500	-0.886	21.00	51.22	469.00 ± 28.68	223.33 ± 5.77
5	0.500	-0.886	39.00	51.22	277.67 ± 7.33	350.00 ± 79.37
6	-0.500	0.886	21.00	92.78	271.67 ± 5.56	530.00 ± 60.82
7	0.000	0.000	30.00	72.00	287.67 ± 19.39	375.67 ± 19.13
8	0.000	0.000	30.00	72.00	443.33 ± 8.50	360.67 ± 26.10
9	0.000	0.000	30.00	72.00	236.00 ± 36.07	398.33 ± 52.51

and sieved before being stored in dessicator for analysis (Traoré et al., 2003).

2.2.3.3. Evaluation of the consistency of gruels. The richness of the different germinated flours of corn in amylase was evaluated by their ability to fluidify fermented cassava gruel. The incorporation of the amylase flour and the preparation of the different gruel were carried out according to the method described by Tambo et al. (2019a,b). To the fermented cassava flour contained in beakers, was added distilled water (17.5 g of fermented cassava flour dry matter/100 mL distilled water). The beakers were then placed in a water bath calibrated at 99 ± 1 °C and the contents stirred regularly for 10 min. These beakers were then cooled to 60 °C and placed in a water bath at this (60 °C) temperature for 20 min for pre-incubation. At the end of this time, the various samples of germinated corn flour (2 g) were added to the gruel and the incubation was carried out for 10 min. The reaction was stopped by re-introducing the beakers into a boiling water bath (95 °C) for 10 min. The consistency of the gruels was estimated by measuring the Bostwick flow velocity (Elenga et al., 2012). When the gruel reached 45.0 ± 0.5 °C, 100 ml were poured into the first chamber of the Bostwick Consistometer (ZXCON-CON3, France). The trigger (guillotine) of the apparatus was actuated to release the gruel which was then passed into the second compartment. The flow velocity of the gruel was evaluated over 30 s and the results were expressed in mm/30s.

2.2.4. Improvement of the energy density of the cassava gruel: effect of the mass of sprouted flour and cassava flour on the flow velocity of fermented cassava gruel

The fermented cassava flour dry matter-mass of corn amylase flour combination, which makes it possible to obtain a flow velocity between 100-160 mm/30s (standard) was determined. The amylase source used was the two corn amylase flour variety prepared under the optimum conditions defined above.

2.2.4.1. Experimental design. The Doehlert design was used to evaluate the effect of process parameters (mass of fermented cassava flour and corn amylase flour) on the flow velocity of fermented cassava gruel. The intervals of independent variables used were: 1–5 g for corn amylase flour and 18.34–29.16 g for dry matter (fermented cassava flour). They were determined on the basis of previous works (Tsopbeng et al., 2018; Tambo et al., 2019a,b) and preliminary tests. The design consisted of 09 runs with two replicates at the center point and the flow velocity expressed as the optimization response was determined (Table 2). The number of tests was determined by applying the formula $k^2 + k + 1$ with k representing the number of factors.

Full quadratic model for fitting of data and the predictive model verification is presented in Eq. (2):

$$Y = I + aX_1 + bX_2 + cX_1^2 + dX_2^2 + eX_1X_2 \quad (2)$$

where Y represent the response; X_1 and X_2 are the levels of the independent variables; a and b are the linear terms; e is an interaction term; c and d are the quadratic terms and I is a constant.

Two experimental replicates were performed under optimized conditions in order to validate the empirical model. The experimental and predicted values of flow velocity were compared. In addition to the parameter announce above were also used to verify the validity and robustness of the predictive model.

2.2.4.2. Incorporation of corn amylase flour into fermented cassava gruel and determination of the flow velocity. Amylase-rich flours were incorporated at 60 °C during the preparation of gruel as described above as part of the evaluation of the consistency of gruel using amylase-rich flours. The quantities of fermented cassava flour and amylase flour used have been prescribed in the experimental design (Table 2). Flow velocities of the different gruels were subsequently measured using a Bostwick consistometer (ZXCON-CON3, France) as described above.

2.2.4.3. Expression of the energy value of the formulated gruel. The effect of addition of corn amylase flour on the energy density of fermented cassava gruel was also evaluated. The energy density (ED) was assessed by a precise method (equation 3) by combining all the ingredients providing energy and using the coefficients of Atwater and Benedict (1899).

$$ED = [(4\times \% \text{ Carbohydrate}) + (9 \times \% \text{ Lipid}) + (4 \times \% \text{ Protein})] \quad (3)$$

2.3. Statistical analysis

Minitab software version 17.0 was used for the experimental design and statistical analysis of the data. All dependent variables were determined in duplicate, and the power of the model was assessed by evaluating the coefficient of determination (R^2) obtained from the analysis of variance (ANOVA). Statistical significance of the model variables was determined at 5% probability level. Main effects and contour plots were plotted using Sigma Plot v11.0 (c) systat. The energy density was performed in duplicate, and results were represented as means ± standard deviations. The Dunnett and Student-Newmann-Keuls tests were used to compare mean(s) using the software GraphPad-InStat, version 3.05 for Windows.

3. Results and discussion

3.1. Optimisation of the production of amylase flour from corn Atp and Kassaï

The optimal conditions of production of amylase flour from corn (*Atp* and *Kassaï*) as well as the determination of different associated factors

Table 2. Experimental design for the study effect of corn amylase flour and fermented cassava flour on the flow velocity of gruel.

N°	Coded levels		Real levels		Experimental flow velocities (mm/30s)	
	X_1	X_2	Corn amylase flour (g) (X_1)	Fermented cassava flour (g) (X_2)	*Atp*	*Kassaï*
1	1.000	0.000	5	23.75	204.66 ± 22.50	121.00 ± 24.43
2	-1.000	0.000	1	23.75	182.33 ± 83.81	94.00 ± 14.73
3	0.500	0.886	4	29.16	140.33 ± 21.57	332.33 ± 10.39
4	-0.500	-0.886	2	18.34	380.00 ± 45.82	416.33 ± 58.00
5	0.500	-0.886	4	18.34	358.33 ± 18.01	116.67 ± 13.06
6	-0.500	0.886	2	29.16	108.33 ± 15.27	203.15 ± 16.86
7	0.000	0.000	3	23.75	181.00 ± 17.08	123.33 ± 34.21
8	0.000	0.000	3	23.75	180.33 ± 25.00	111.66 ± 7.00
9	0.000	0.000	3	23.75	123.00 ± 10.81	123.33 ± 13.57

Table 3. *P* values, contribution of factors (CF), coefficient of multiple determinations (R^2), absolute average deviation (AAD) and bias factor (BF) for optimized production of amylase flour following Doehlert design.

	Kassaï		*Atp*	
	P	CF (%)	P	CF (%)
X_1: Soaking time (h)	0.295	8.07	**0.003***	10.87
X_2: Germination time (h)	**0.029***	28.58	**0.000 ***	39.18
X_1X_1	0.097	24.12	**0.003 ***	16.86
X_2X_2	0.313	14.87	**0.002***	24.25
X_1X_2	0.183	24.35	**0.047***	8.85
Model validation				
R^2		0.75	0.99	
AAD		0.00	0.00	
BF		1.01	1.01	

*Independent variable that significantly ($p < .05$) affects the response.

responsible for that optimal activity has been done using Doehlert design. The flow velocities of fermented cassava gruel enriched with different amylase flours produced (by varying germination and soaking time) are presented in Table 1. The amylase flours were produced by varying the independent variables (soaking and germination times) and the quality of the product was evaluated by measuring the ability to fluidise the fermented cassava gruel. Thus, the higher the flow velocity, the better the amylase flour. The Table 1 also shows that the *Kassaï* variety had the best flow speeds whatever the soaking and germination times. This could be explained by the nature as well as the rate of amylases synthesised in this variety of corn. In fact, Tambo et al. (2018), had shown that *Kassaï* variety are more amylolytic than *Atp* due to it high proportion in α-amylases.

3.1.1. ANOVA, contribution of independent variables and validation of the model

Table 3 present ANOVA, contribution of independent variables and validation of the model of this study. These parameters were obtained by comparing the experimental values obtained in the laboratory with the predicted values of the software. From this table, all the factors (linear, interaction and quadratic terms) significantly affect ($p < 0.05$) the flow velocity of *Atp* variety with linear effect of soaking and germination time who have more affect the quality of the amylase flour produced. The germination time of the corn *Atp* for the production of amylase flour is the factor that most affects the flow velocity of the gruel because it has a major impact on the evaluated response (CF = 39.18 (X_2) + 24.25 (X_2X_2) = 63.43 %). For the *Kassaï* variety, its production is significantly affected by the linear effect of germination time. Indeed, the dormant state of amylases in cereals is lifted during germination. Like the *Atp* variety, the germination time contributes most to amylolytic activity.

The mathematical model of relationship for flow velocity of fermented cassava gruel enriched with amylase flour (Y1) with soaking time (X_1) and germination time (X_2) for the production of amylase flour is given by Eqs. (4) and (5):

$$Y_{Atp} = 279.00–46.44\ X_1 + 167.30X_2 – 72.00X_1X_1 + 103.6X_2X_2 + 37.8X_1X_2 \tag{4}$$

$$Y_{Kassaï} = 378.20–39.30\ X_1 + 136.10\ X_2 - 117.40\ X_1X_1 + 72.40\ X_2X_2 – 118.50\ X_1X_2 \tag{5}$$

The results showed that the mathematical model represented the experimental data. In fact, the coefficient of determination (R^2) for the response is 0.75 and 0.99 for *Kassaï* and *Atp* respectively and is within the range of a good set (more than 0.75) (Joglekar & May, 1987). This means that the observed model is able to explain respectively 75 and 99 % of the results. In addition, the values of the absolute average deviation (AAD) and the bias factor presented in the same table confirm the suitability of the models because they are included in the normalized ranges (0 for AAD and $0.75 < Bf < 1.25$ for BF).

3.1.2. Analysis of main effect of contour plot

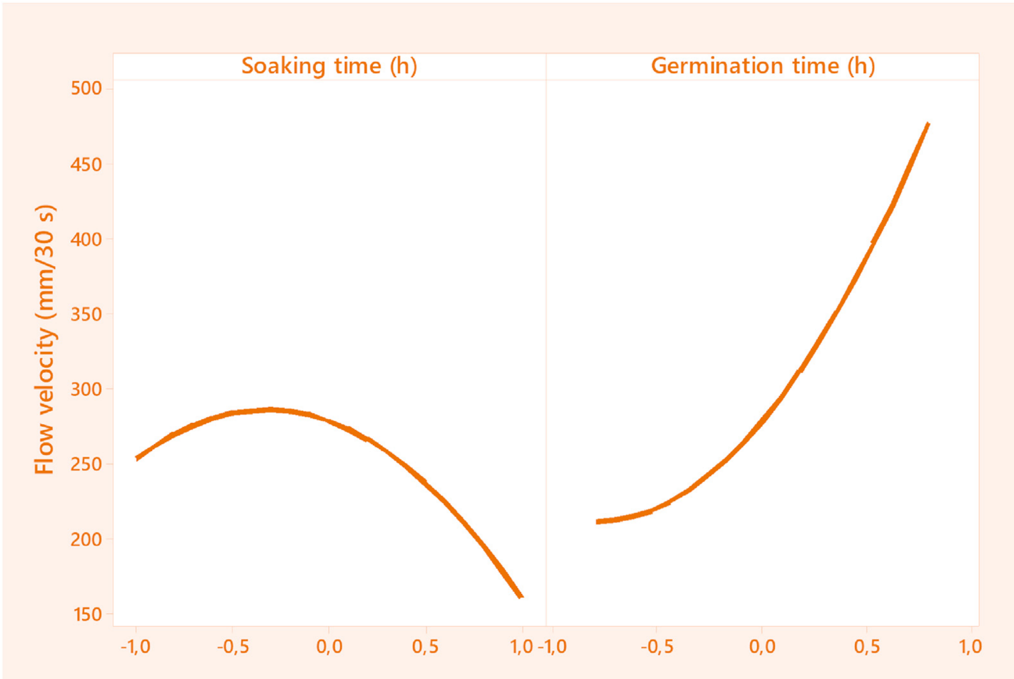
The main effects of the soaking time and germination time of corn of the *Kassaï* (a) and *Atp* (b) varieties for the preparation of germinated flours capable of liquefying gruels based on fermented cassava flour are presented in Figure 1. It can be seen from this figure that the flow velocity of the gruels increases proportionnaly with the germination time regardless of the variety used. It also increases with the soaking time and decrease above the peak time (20 h and 28 h respectively for germinated corn flours *Kassaï* and *Atp*). Increasing the fluidity of the gruel with the germination time indicates a production of amylase-rich corn flour. Germination activates respiration, protein synthesis (amylase) and other metabolic activities (Milala and Addy, 2014). These results are in accordance with those reported by Helland et al. (2002) who have found a positive correlation between germination time, alpha-amylase content and gruel viscosity. Increasing germination time led to increased production of amylase, which has the ability to hydrolyse starch and reduce its viscosity capacity. However, it is noted that, the variation observe with soaking time can be explained by the presence of water and oxygen who allows the activation of respiratory and mitotic processes. Water makes the water-soluble phytohormones stored in the grain mobile and active (Hariri, 2003). This is the case of gibberellins, which are thus transported to the aleurone layer where they will activate the synthesis of hydrolases, including amylases necessary for the degradation of reserves (Hariri, 2003; Anzala, 2006).

However, the decrease in flow velocity with soaking time could be due to the fact that the seed has reached a sufficient moisture content to promote germination. On this subject, the work of Patane et al. (2016) showed that not only the germination efficiency was due to the moisture content of the grain but also that high moisture content causes a slowing down of the germination process. Furthermore, long soaking times lead to poor seed growth and germination due to excess water (Vieira et al., 2004). Indeed, high water contents lead to asphyxiation of the grain due to lack of aeration (Ali et al., 2011).

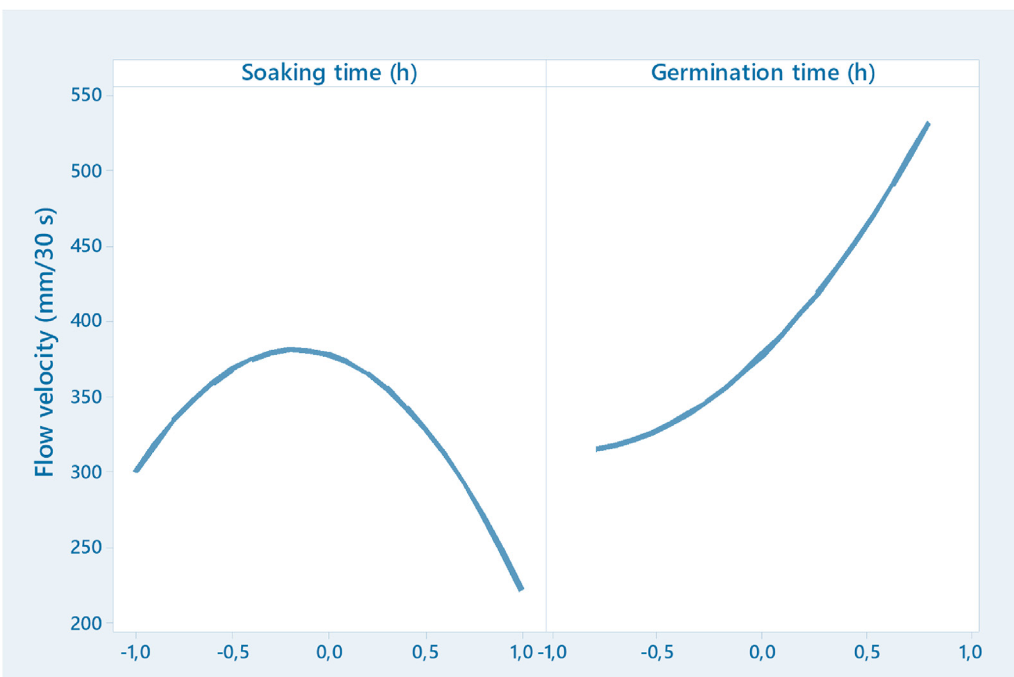
Figure 2 is a contour plot showing relation between process parameters and evaluated response. On the basis of the maximum flow velocity which reflects high amylase content in corn flour, the areas of interest have been delineated. This area of interest (hatched) defines the experimental domain in which the application of the soaking and germination times will lead to the production of amylase-rich *Atp* and *Kassaï* corn flours capable of maximum fluidification of the fermented cassava gruels.

3.1.3. Effect of soaking time on moisture content and germination capacity of corn grains (Atp and Kassaï varieties)

Figure 3 (a and b) shows the evolution of the moisture content of the grains during the soaking of *Atp* and *Kassaï* corn varieties. From this figure, one notes a progressive increase in moisture content over time until a constant time is reached. After 20 h and 28 h of soaking of the corn grains of varieties *Kassaï* and *Atp* respectively, one notes a water content



(a)



(b)

Figure 1. Main effects plot showing effect of soaking and germination times of corn *Atp* (a) and *Kassaï* (b) on the flow velocity of fermented cassava gruel.

of 46.30 % and 37.00 % respectively. Beyond these soaking times, a gradual increase in the water content of the grains with the soaking time whatever the variety of corn have been noted. The variation in time and moisture content of the different varieties is said to be related to the variety and the composition of the seed coat. Bryce et al. (2010), demonstrated that the germination process is directly related to the moisture content of the kernels. According to the same author the germination process is important for moisture contents between 42-45 % important. These results are in agreement with ours since we obtained values of 46.39 % and 37 % for the corn varieties *Kassaï* (a) and *Atp* (b) respectively. These results also show that the soaking time required for a good germination is between 15-20 h and 25–30 h for the *Kassaï* and *Atp* varieties respectively.

3.1.4. Process optimisation and validation of the optimal conditions

Experimental validation tests of the optimal soaking and germination conditions as predicted by the mathematical model were carried out to confirm the adequacy between theory and experiment. These predicted and experimental values were compared in order to validate the optimal conditions defined by the models. In general, it appears that the values obtained after experimentation do not differ significantly ($P > 0.05$) from the predicted results. Similarly, the comparative study of predicted and

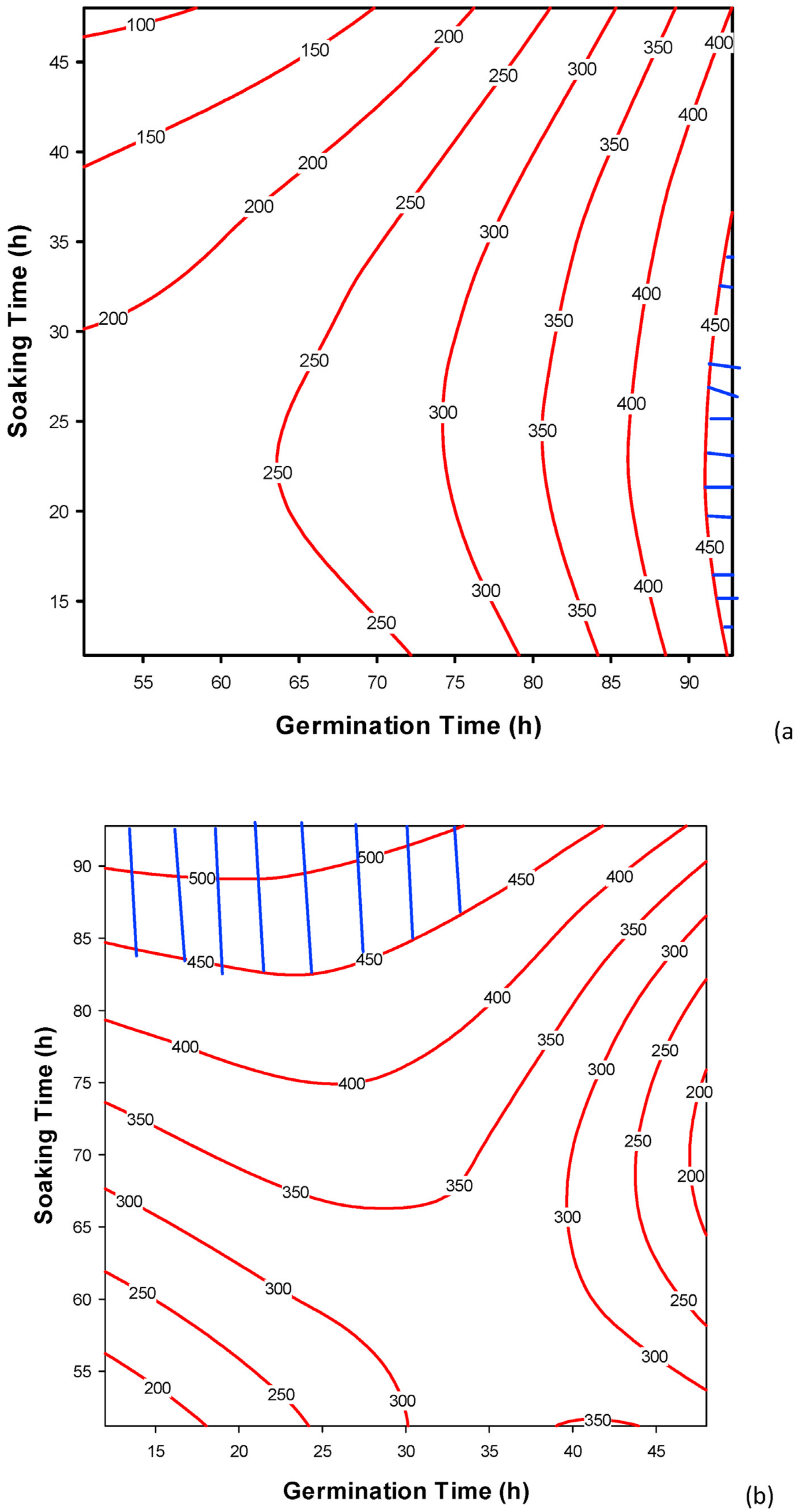


Figure 2. Contour plot showing effect of soaking time and germination time of corn (*Atp* (a) and *Kassaï* (b)) on the flow velocity of fermented cassava gruel.

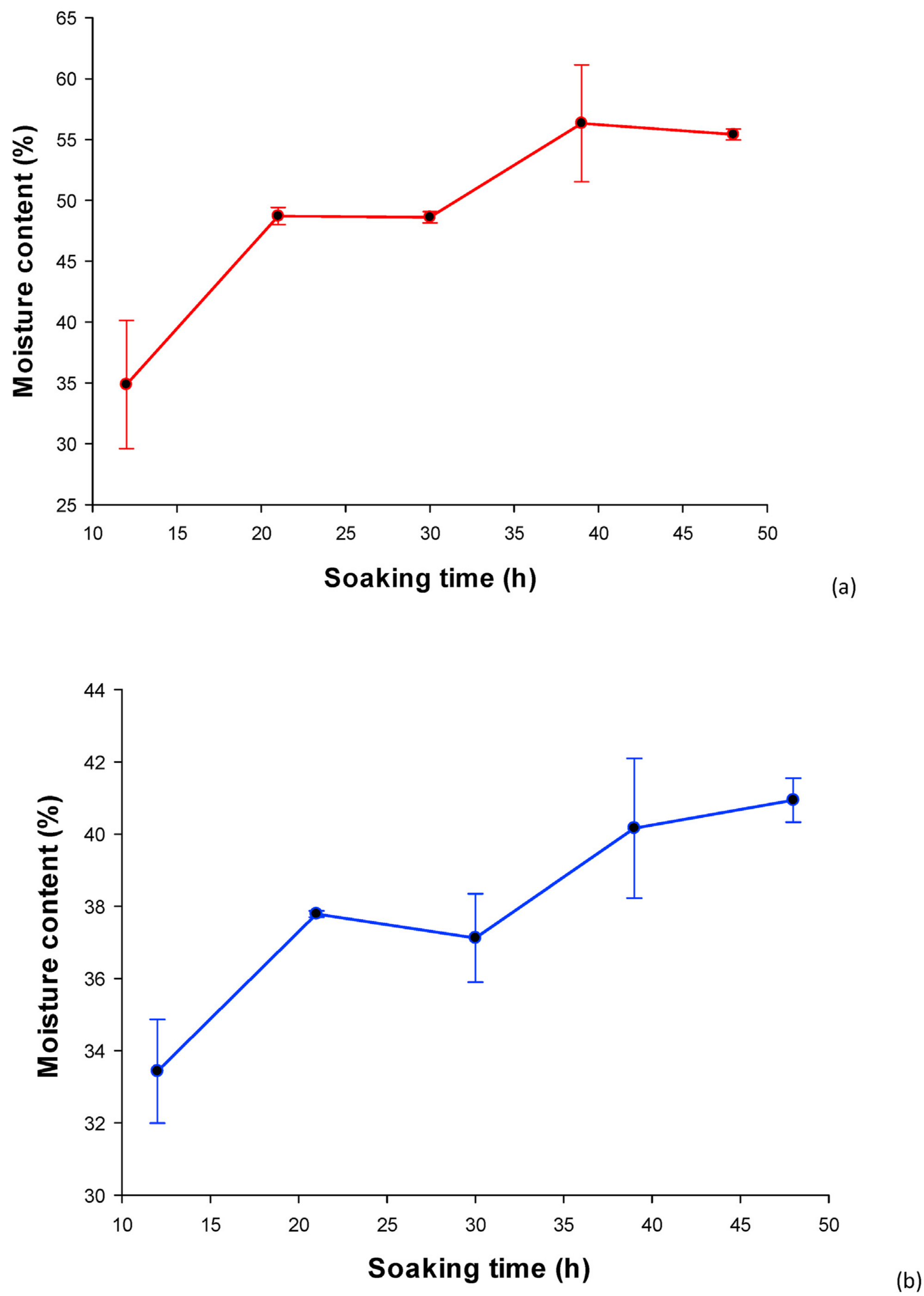


Figure 3. Moisture content in corn grains of *Kassaï* (a) and *Atp* (b) varieties during soaking.

experimental optimal values between corn varieties shows that there is no significant difference between them.

These results show that obtaining a maximum flow velocity requires a germination time of 90.82 h or 3 days 18 h 49min for both varieties and a soaking time in *Atp* slightly longer (28 h) than that of the *Kassaï* variety (20 h). These results are close to those of Muhammad et al. (2017) which shows that the optimum soaking time for corn is 24 h to have a high germination percentage and lower than those of Helland et al. (2002), which shows that the activity of α-amylases is maximal after 4 days of corn germination. The variations observed in the soaking time are in agreement with the previous data and once again shows that the variety and in particular the amylase composition influence the conditions of activation or production. Moreover, both varieties of corn showed evidence of desirability, that prediction and experimentation are in perfect adequacy and that our model is robust.

3.2. Improvement of the energy density of the fermented cassava gruel: effect of the mass of corn amylase flour and fermented cassava flour on the flow velocity of the gruels

After determining the optimal conditions for soaking and germination to produce germinated corn flour capable of maximizing the fluidity of fermented cassava flour gruels, it was discussed to use these flours in order to find the combination of the fermented cassava flour dry matter-mass ratio of germinated flour that would allow to obtain a flow velocity meeting the standard (100–160 mm/30s) for the preparation of gruels for

Table 4. Regression coefficients (RC), *P* values, contribution of factors (CF), coefficient of multiple determinations (R^2), absolute average deviation (AAD) and bias factor (BF) for optimized combination of cassava flour and amylase following Doehlert design.

	Kassaï		*Atp*	
	P	CF (%)	P	CF (%)
X_1: Corn amylase flour (g)	0.352	7.20	**0.003***	2.98
X_2: Fermented cassava flour (g of DM)	**0.007***	42.23	**0.000 ***	44.82
X_1X_1	0.969	0.44	**0.003 ***	10.41
X_2X_2	**0.043***	33.38	**0.002***	31.89
X_1X_2	0.282	16.76	**0.047***	10.41
Model validation				
R^2		0.87	0.92	
AAD		0.01	0.00	
BF		1.00	1.00	

*Independent variable that significantly ($p < .05$) affects the response.

children between 6 and 36 months of age (OMS, 2010). The experimental values of the response measured in the different experiments prescribed by the experimental design are presented in Table 2. From this table, the flow velocity of the gruels is higher when germinated flours of the *Kassaï* variety are used. Thus the flow velocity of the gruels is between 94.00 and 416.33 mm/30s (experiment 2 and 4 respectively) when the *Kassaï* variety is used and between 140.33 and 380 mm/30s (experiment 3 and experiment 5) for the *Atp* variety. It can be noted that, the combination between *Kassaï* germinated flour-fermented cassava flour presented best the fluidising capacity. The best ratio (29.16 %/2 g) was obtained with experiment 6 for the 2 germinated corn varieties.

3.2.1. Analysis of variance, contribution of factors, regression equations and validation of model

Table 4 below who shows the analysis of variance, contribution of factors, regression equations and validation of model revealed that, the linear effects (X_1 and X_2), quadratics (X_1X_1 and X_2X_2) and the interaction (X_1X_2) between the two factors significantly impacts ($P < 0.05$) the flow velocity of the gruels with variety *Atp*. The flow velocity in the presence of the *Kassaï* variety was influenced by the linear and quadratic effect of the concentration of cassava flour. Tambo et al. (2019a,b) also showed that cassava flour concentration significantly affected the flow velocities of fermented cassava gruels. In the same vein, Nguemguo et al. (2020), reported in their study that flow velocity of blanch and unblanch Irish potato reduce with their increasing concentration. Results also showed that the coefficient of determination (R^2 value) of the flow velocity are within the range of a good set (>0.75) (Joglekar & May, 1987), indicating that the empirical model could explain adequately up to 92.1 % of fluidity of the gruel for *Atp* variety and 87 % for *Kassaï* variety. This shows that these models can be accepted and that the variations in the different responses are significantly related to the independent variables (fermented cassava flour-GCF) chosen for the study. The absolute average deviation (AAD) values obtained (0.00 and 0.012) confirm the suitability of the models. The same is true for the values of the bias factor, which is 1 for the two varieties of amylase source provided. The contributions of the two factors presented in Table 4 show that the variation of the flow velocity of the gruel is preferentially explained by the fermented cassava flour concentration (42.23 and 44.82 % for *Kassaï* and *Atp* respectively) and by its quadratic effect (33.38 and 31.89 % for *Kassaï* and *Atp* respectively). On the other hand, the linear effect of the mass of corn amylase flour (*Atp* variety) has the lowest participation (2.98 %) and the quadratic effect of the same factor for the *Kassaï* variety.

Knowing the fermented cassava flour concentration and the mass of corn germ meal, the gruel flow rate (Y) can be predicted by the regression Eqs. (6) and (7) presented below. From these equations, it can be seen that the linear effect of the germinated corn flour mass and the quadratic effect of the fermented cassava flour have a positive influence on the response regardless of the amylase-rich flour variety used. On the other hand, the flow velocity decreases with linear and quadratic effects of fermented cassava flour dry matter factor; and the interaction between fermented cassava flour and germinated corn flour of the *Kassaï* variety. On the other hand, only the linear effect of fermented cassava flour dry matter decreases the response when *Atp* flour is used. The positive correlation between flow velocity and germinated flour concentration is explained by the reduction in the viscous capacity of the starch due to its hydrolysis by amylases and, on the other hand, the opposite observed with the concentration of fermented cassava flour results from a reduction in the availability of water (indispensable for the action of the amylases) following the fixation by the starch molecules contained in the flour (Nguemguo et al., 2020).

$$Y_{Kassaï} = 146.1 + 25.9\,X_1 - 151.8\,X_2 - 1.6\,X_1X_1 + 120.0\,X_2X_2 - 60.2\,X_1X_2 \quad (6)$$

$$Y_{Atp} = 161.4 + 9.2\,X_1 - 138.3\,X_2 + 32.1\,X_1X_1 + 98.4\,X_2X_2 + 30.5\,X_1X_2 \quad (7)$$

3.2.2. Analysis of main effect plot and contour plot

Figure 4 (a and b) below describes the main effects of the fermented cassava flour dry matter and germinated corn flour mass of the two varieties on the flow velocity of fermented cassava gruels. It can be seen from this figure that the flow velocity of the gruels decreases with increasing fermented cassava flour concentration regardless of the germinated corn flour used. This decrease in response can be attributed to the hydrating power of the starch molecule during cooking. Indeed, starch swells by water retention and causes thickening of the gruels hence the low flow velocity values observed (Zannou-Tchoko et al., 2011). In addition, in excess of water and at temperatures above 60 °C, starch granules open up as a result of the breakdown of hydrogen interactions and their replacement by water molecules. This phenomenon leads to a swelling of the granules up to a threshold value, beyond which they hydrolyse and return to the starting structure with a consequent increase in viscosity (Klang et al., 2020).

From the same figure, we also note that this response increases gradually with the mass of germinated corn flour of the *Kassaï* variety. Indeed, the addition of flours rich in amylases allows the depolymerisation of starch into simpler molecules with a low swelling capacity. Thus starch hydrolysis requires the intervention of several hydrolases synthesized during germination (Heller et al., 2000; Wang et al., 2008). The β-amylase (α-(1,4)-D-glucan maltohydrolase) attacks straight-chain amyloses from the non-reducing end by releasing maltose (Scriban, 1999). Dextrin, on the other hand, cleaves the α (1,6) bonds, degrades starch into maltose and α-glucosidase hydrolyses maltose into two glucose molecules. These results are in line with those of Elenga et al. (2012) and Klang et al. (2019) which showed that the incorporation of barley and corn malt increased the flow velocity of gruels made from yam and corn flour. The good activity of germinated corn flour (*Kassai*) on

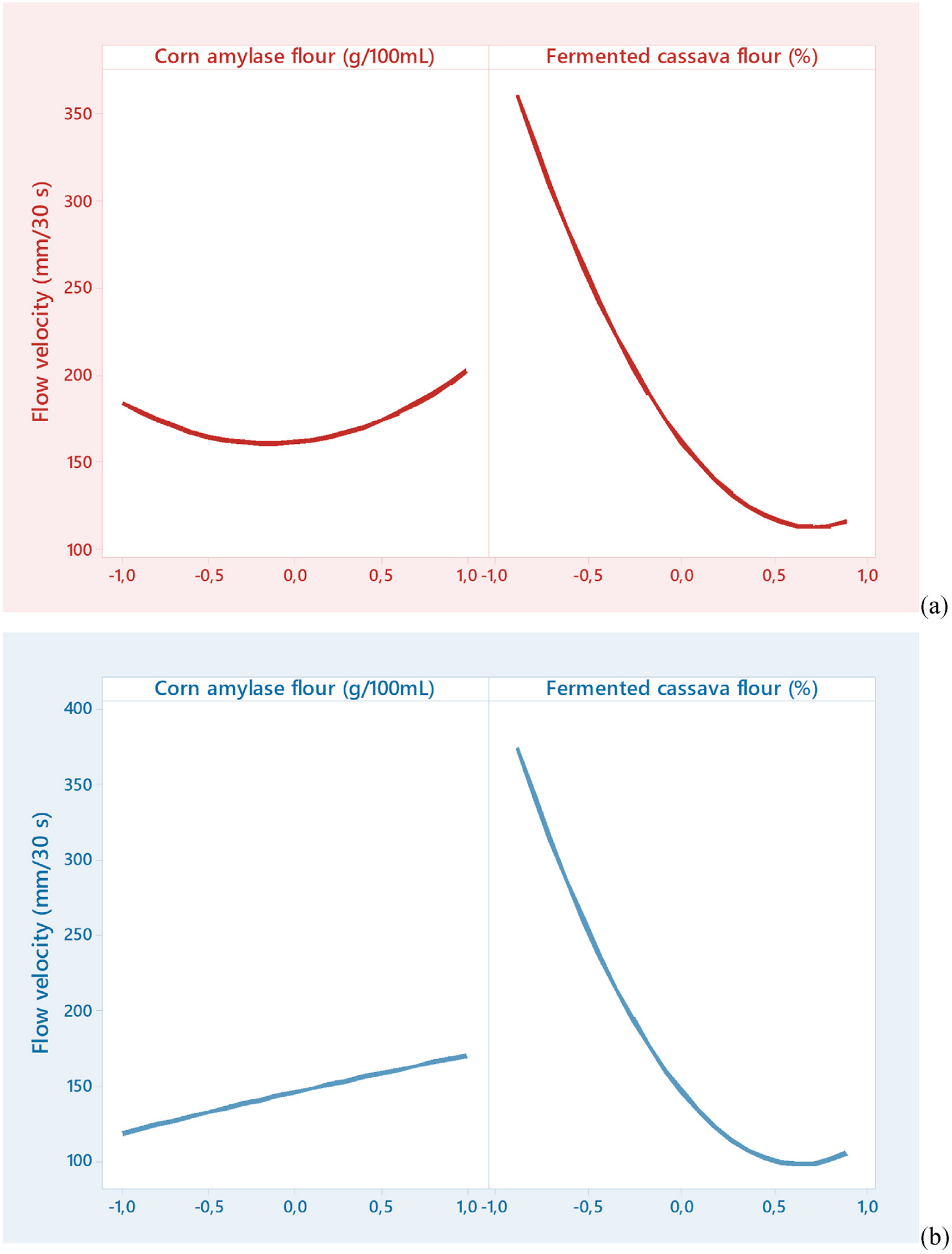


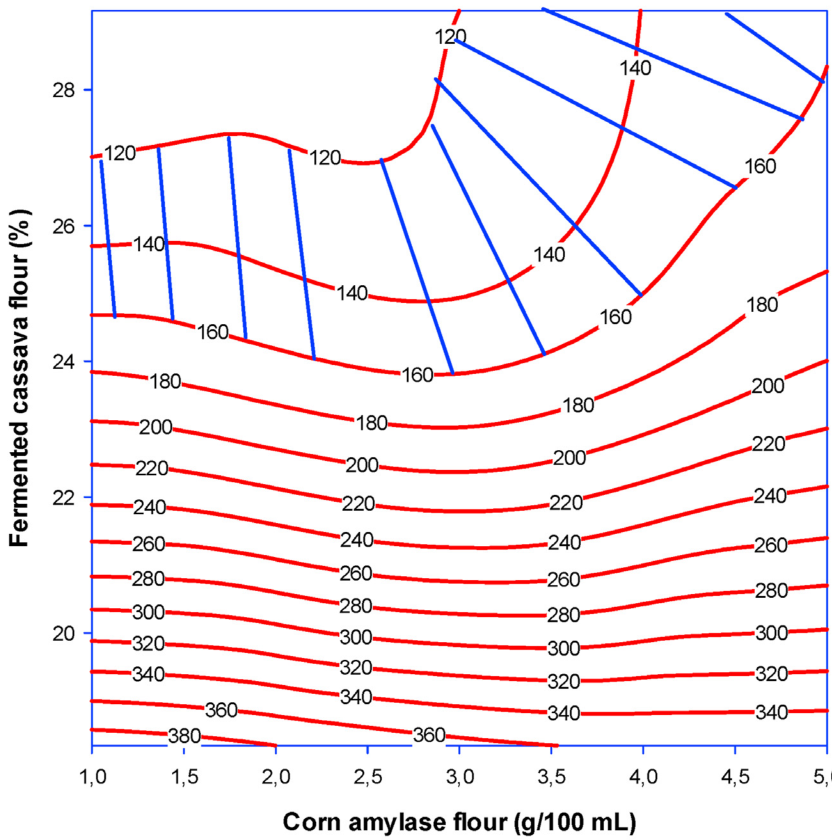
Figure 4. Contour plot showing the main effects of parameters (*Atp* (a) and *Kassaï* (b)).

cassava flour can also be explained by the substrate specificity existing between the starch and the amylase of this flour; it would also be due to the fact that fermented cassava flours are low in lipids and proteins (Tambo et al., 2019a,b; Klang et al., 2020) which would hinder the establishment of amylase–substrate bonds and the hydrolysis of starch bonds leading to the reduction of its consistency.

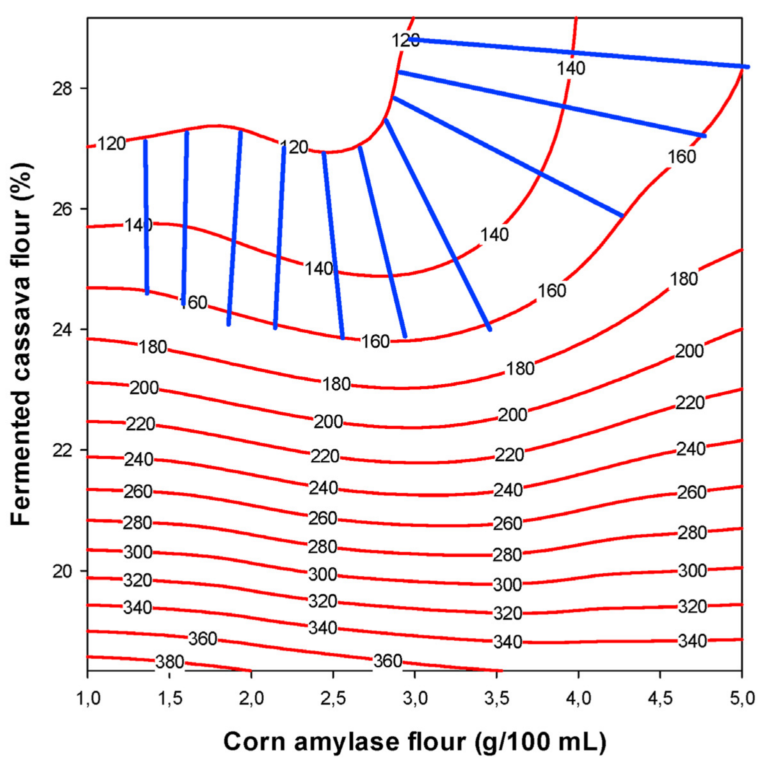
On the basis of the quality standards required for infant gruel (flow velocity between 100 and 160 mm/30 s) according to WHO and UNICEF, the areas of interest have been delineated. This area of interest (hatched) defines the experimental domain in which the application of the mass of fermented cassava flour and mass of corn amylase flour will lead to the production of cassava gruel with acceptable consistency (Figure 5).

3.2.3. Determination and experimental validation of the optimal condition

In order to produce the gruels at normal flow velocities (100–160 mm/30s), mothers need to increase dry matter and in place of water use, use low mass GCF to obtain gruels that are lighter in weight, digestible and high in energy value (Tambo et al., 2019a,b). These conditions as well as the values predicted (flow velocity) by the software are presented in Table 5. To confirm these results, manipulations under the optimal conditions predicted by the Minitab software version 17.0 were carried out in the laboratory and the experimental values were obtained. It appears that the values obtained after experimentation do not differ significantly from the results predicted by the model, which makes it possible to validate the optimal conditions defined by this software (p >



(a)



(b)

Figure 5. Iso-response curves of flow velocities (mm/30s) showing the area of interest (hatched in red line) as a function of the mass of germinated corn flour of the *Kassaï* variety (a) and *Atp* (b); and fermented cassava flour dry matter.

Table 5. Predicted values, dry matter and corresponding flour mass.

	FCF (g)	GCF (g)	Optimal predictive value	Optimal experimental value	Desirability
Kassaï	26.23	1.16	101.569^{aA}	108.00 ± 6.55^{Aa}	1.00
Atp	25.94	1.12	137.330^{bB}	132.33 ± 5.85^{bB}	1.00

Values with the same letter (a,b) in the same row did not differ significantly ($P < 0.05$) and values with the same letter (A,B) in the same column did not differ significantly ($p < 0.05$).
FCF: fermented cassava flour; GCF: optimised sprouted corn flour mass.

Table 6. Fermented cassava flour ratio of gruels with amylase and without amylase; energy value of gruels with a flow velocity between 100-160 mm/30S.

	Without ARF	*Kassaï*	*Atp*
Fermented cassava flour (%)	4	26.23	25.94
Amylase rich flour	0	1.16	1.12
Muliplication factor	1	6.55	6.49
Gruels energy density (kcal/100 mL of gruels)	13.71	89.91	88.91
Supplementary energy gruels (kcal/100 ml of gruels)	0	76.20	75.20

ARF: Amylase rich flour.

0.05). These also show that the combination obtained can be used to prepare cassava gruel having a normal flow velocity for the children. However, the comparative study of the predicted and experimental optimal values between corn varieties shows that there is a significant difference between them ($p < 0.05$) probably related to their different amylase composition and content.

3.2.4. Effect of added corn amylase flour on the energy density of cassava gruel

Table 6 below gives us the fermented cassava flour concentration multiplication factors and the energy value of flour with amylase and without amylase. It shows that, the use of 26.23 % FCF and 1.2 g of germinated corn flour *Kassaï* variety allowed to multiply the dry matter by 6.56 and the use of 25.94 % fermented cassava flour and 1.12 g of germinated corn flour *Atp* variety also allowed to multiply the quantity of dry matter by 6.49. This would be explained by the capacity of amylases to depolymerise the components of starch, thus leading to an improvement in energy and nutritional density (Tizazu et al., 2010). These results are greather than those of Tambo et al. (2019a,b) who observed an increase in energy density of 5.48 and 5.23 of the gruels made from fermented cassava flour following the use of 1 g and 2 g of germinated corn flour varieties, *Atp* and *Kassaï* respectively. The differences observed may be due to the different methodologies used for the production of amylase-rich flours and the preparation of the gruels. Indeed, the soaking and germination conditions significantly improve the quality of the germinated flours (amylase concentration) and their fluidifying capacity. From the same Table 6, it is also noted that the contribution of optimised germinated corn flours during the preparation of the gruels allowed to obtain energy values of 89.91 kcal/100 mL and 88.91 kcal/100 mL respectively for the *Kassaï* and *Atp* varieties improving the energy value of 76.2 kcal/100 mL and 75.20 kcal/100 mL. Indeed, Elenga et al. (2012) have shown that to increase the energy density of gruels prepared from local commodities, it is necessary to proceed by prior fermentation of the main starchy components and to incorporate small quantities of germinated cereal flours particularly rich in amylases (Afoakwa et al., 2010). These results are in line with the recommendations made by Dewey and Brown (2003), who showed that the energy density of the gruels should be 84 kcal/100 mL of gruel for less than 9–11 months old children.

4. Conclusion

The study of optimal soaking and germination conditions showed that the corn variety influences the soaking time. Soaking time was respectively 20 h and 28 h for the *Kassaï* and *Atp* varieties. The optimal germination was 90.82 h for both varieties. The optimal moisture content for good germination was 46.30 % and 37 % for *Kassaï* and *Atp* varieties respectively. The use of these flours to improve the energy density of fermented cassava-based gruels revealed that 1.16 g of *Kassaï* variety in combination with 26.23 % fermented cassava flour and 1.12 g of *Atp* variety in combination with 25.94 % fermented cassava flour resulted in a flow velocity of 108 mm/30s and 132.33 mm/30s respectively. The use of these amylasic flours during the preparation of the gruels made it possible to multiply the energy density between 6.55 and 6.49 for the *Kassaï* and *Atp* varieties respectively. In view of these results, it is therefore possible to consider the use of germinated flours produced under the conditions obtained and the optimal concentration of different flours (fermented cassava flour and amylase rich flours) obtained before in the formulation of a weaning food with a consistency, energy and nutritional value necessary for the proper growth of children.

Declarations

Author contribution statement

Julie M. Klang, Sylvanie-Linda Wouatidem-Nanfack, Stephano T. Tene, Gires T. Boungo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hilaire M. Womeni: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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