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Physicochemical Characterisation and Determination of the Effect of Temperature on the Amylase Activities of Germinated Sorghum and Maize Grains

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Maize and sorghum are staple foods in many African countries. Along with rice, they constitute the bulk of the cereals consumed by the populations of northern Côte d'Ivoire. These cereals are used either directly or indirectly in malted and/or fermented form in the preparation of several traditional

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foods and the production of alcoholic beverages such as *dolo* a local beverage. This study aims at the physicochemical characterization and the determination of the effect of temperature on the amylase activities of germinated cereal grains of corn and sorghum collected on the market of the city of Korhogo. To do this, the analysis of cereals germinated over 6 days revealed that amylase activities are optimal on the second day of germination with a diastatic power of 110.29 ± 1.8 IU / g of protein and 114.17 ± 2, 4 IU/g of protein respectively for sorghum and maize. The optimal temperatures are mesophilic and are between 40 and 50°C. Beyond these temperatures, amylase activities are denatured until they reach 20% relative activity after exposure to 65°C. During germinationsand at an acid pH, the levels of reducing sugars, polyphenols and tannins increased with the duration of germination. The results of this study revealed that certain physicochemical characteristics and the effect of temperature condition the obtaining of a malt likely to influence the quality of a traditional beer. The germination time set at 2 days makes it possible to obtain fermentable sugars during malting for the preparation of alcoholic beverages according to the production conditions of traditional vendors. For sugars, they increase from 0.39±0.035 mg/100g DM (Dry Matter) before germination to 0.86± 0.003 mg/100g DM on the 6th day of germination for sorghum. As for maize, these levels increase from 0.11± 0.014 mg/100g DM to 0.43± 0.02 mg/100g DM. The diastatic power is optimal on the 2nd day, 110.29 ± 1.8 IU/g of protein for sorghum and 114.17 ± 2.4 IU/g of protein for maize.

Keywords: Germination; amylase activity; maize; sorghum; malting.

1. INTRODUCTION

Cereals are species generally cultivated for their grain, whose starchy albumen, reduced to flour, is edible for humans or animals. Most cereals belong to the Poaceae or graninea family [1]. Africa is the center of origin and a major producer of various cereals such as sorghum, millet, maize, rice etc. Cereals are a source of energy and micronutrients, then the most consumed staple food in the world [2,3]. They are cultivated plants whose grains serve as food for humans and animals. Maize and sorghum are the staple cereals in the diet of several million people in arid and semi-arid areas of Africa and Asia [4,5,3]. These cereals are used directly or indirectly in malted and/or fermented form in the preparation of several traditional foods including porridge (especially for children), and alcoholic and non-alcoholic beverages [6,7,3]. In some West African countries, such as the Ivory Coast, the production of traditional beer is very important. Indeed, the production of this beer has a remarkable socio-economic character and constitutes an important source of income for the women who produce it locally using traditional technology. This beer is widely used during traditional and initiation ceremonies [3]. However, the production process for this traditional beer requires an essential step, which is malting. This old technique is a mechanism of biochemical transformation which aims to transform the macromolecules of cereals into rich malt by enzymatic hydrolysis such as amylases and proteases substances secreted during germination. In addition, this process enriches cereals with hydrolytic enzymes, sugar, free amino acids, and vitamins and improves technological and nutritional quality [8-10]. Traditional malts have a great variability in diastatic and thinning power [11]. The malt intended for the production of beverages must have high diastatic power. This allows complete starch hydrolysis during brewing. The malting of sorghum and maize is a process traditionally practiced in many African countries for the manufacture of alcoholic beverages [12]. In Côte d'Ivoire, the traditional malting of cereals, as carried out by the producers of dolo (traditional local beer), includes the stages of soaking the grains in water, a phase of germination, maturation (during which the grains are piled up and protected from light) and drying in the sun. At the end of the process, the quality of the drink obtained is a major concern for traditional vendors because of its variability. This variability is due to the lack of mastery of malting technology and the diversity of processes according to ethnicity and agroecological environment [13]. In this context, the optimization of malting processes remains a necessity. Therefore, this study aims to determine the physicochemical characteristics of germinated maize and sorghum grains as well as the effect of temperature on amylase activities during the germination of maize and sorghum grains.

2. MATERIALS AND METHODS

2.1 Plant Materials and Chemicals

The plant material used consists of red sorghum (*Sorghum bicolor* (L.)) and yellow maize (*Zea mays*), which purchased at the cereal market in the town of Korhogo located in the north of the Ivory Coast (632 Km from Abidjan, Côte d'Ivoire) at the start of the dry season. All other chemicals and reagents used were analytical grade.

2.2 Preparation of Malting

The preparation of the malting consisted of alternating soaking followed by germination at low temperatures.

2.2.1 Soaking

Maize and sorghum grains were soaked (immersed) in buckets of water with lids, for better thermal insulation for 24 to 48 hours.

2.2.2 Germination

At the end of the period under air all the grains are washed and placed in a layer of about 5cm on a tiled surface. They are watered on average 4 times a day at 6-hour intervals with 2 liters of water. After every 24 hours (every day) the green malt is taken at 150g, put in a bag and kept in a freezer for up to 9 days.

2.3 Dosage of Amylase Activities

2.3.1 Preparation of the crude enzymatic extract

Fifty (50) grams of each cereal (maize and sorghum) for each sample (1st day to 9th day) were each ground in a blender in one hundred 100 ml of distilled water in the presence of 0.9g of sodium chloride (NaCl 0.9% w/v). The ground material obtained is centrifuged at 12,500 revolutions/min for 15 min at a temperature of 4° C in a JENWAY-7315 Spectrophotometer brand centrifuge. The supernatant obtained constituted the crude enzymatic extract of the cereal seeds.

2.3.2 Measurement of amylase activity

The quantity of reducing sugars released during the enzymatic hydrolysis of starch was determined according to the method of [14] using 3,5-dinitrosalicilic acid (DNS). Under standard conditions, the cellulase and xylanase activities were determined using the reaction medium composed of: 125 µl of 20 mM acetate buffer pH 5.0; 50 µl of enzymatic extract or distilled water for the control; 75 µl of starch (0.5%, w/v). This reaction medium was incubated in a water bath for 30 min at 45°C. Then, 300 µl of a DNS solution was added thereto to stop the enzymatic reaction. It was then homogenized and heated in a boiling water bath for 5 min then cooled for 10 min at room temperature (25°C). The absorbance was measured at 540 nm on a spectrophotometer against a control (containing all the products with the exception of the enzymatic solution) after addition of 2 ml of distilled water.

2.4 Diastatic Power

The diastatic power was determined according to the method proposed by [15]. It consists of causing an enzymatic extract to act on a starch solution. The reducing sugars and the reducing functions thus released are assayed by colorimetry (540 nm) in the presence of 3-5-dinitrosalicylic acid. A range of maltose standards is produced and dosed under the same conditions as above. Absorbance is measured at 540 nm. The enzymatic activity is determined by referring to the calibration curve and is expressed in mill equivalent glucose/min/g of malt.

2.5 Influence of Temperature on Amylase Activities

2.5.1 Determination of optimum hydrolysis temperatures

The influence of temperature on amylase activities was studied in 20 mm acetate buffer pH 5.0 at temperatures between 30 and 80°C. Enzyme activity was determined under standard conditions. It was expressed as a percentage of maximum activity.

2.5.2 Thermal denaturation

The thermal denaturation of the enzyme was determined after a 15 min pre-incubation at temperatures between 30 and 80°C followed by a 10 min cooling at room temperature (25°C). The carboxymethylcellulose and xylanase activities were determined under standard conditions. They were expressed as a percentage relative to the maximum activity.

2.6 Proximate Analysis

The determination of moisture and dry matter was carried out according to the AOAC method (1990). The Hydrogen potential (pH) of the sample was determined according to [16]. The total titratable acidity was deduced according to the method of [16].

2.7 Extraction of Phenolic Compounds

The phenolic compounds were extracted with 70% (v/v) methanol. Thus, 1 g of sprouted sorghum or maize grains was homogenized in 10 mL of 70% (v/v) methanol. The resulting mixture was centrifuged at 1000 rpm for 10 min. The pellet was recovered in 10 ml of 70% (v/v) methanol and centrifuged again. The supernatants were combined in a 50 Ml flask and adjusted with distilled water to the gauge line. The extract obtained constituted the phenolic extract for the determination of phenolic compounds.

2.8 Determination of Total Phenols Content

The method described by [17] was used for the determination of total phenols. A volume of 0.5 mL of methanolic extract was added to 0.5 mL of Folin ciocalteu's reagent in a test tube. The mixture was well homogenized by manual stirring. After 3 min, 0.5 mL of an aqueous solution of sodium carbonate 20%, (w/v) was added and the volume was adjusted to 3.5 ml with distilled water. Then the tube was placed in the dark for 30 min. The absorbance was read using a spectrophotometer (UV/VIS spectrophotometer) at 725 nm against white. Finally, a calibration curve was produced using a gallic acid concentration range from 0 to 1 mg/mL. The results were expressed in equivalent mg of gallic acid (GAE)/100g of dry matter (DM).

2.9 Determination of Tannins Content

The dosage of tannins was carried out according to the method described by [18]. A volume of 1 mL of methanolic extract was taken and to this volume was added 5 mL of vanillin reagent (50 g of vanillin + 4 mL of hydrochloric acid in 100 mL of distilled water). Then the tube was left to rest for 20 min in the dark and the reading of the absorbance with the spectrophotometer (UV/VIS spectrophotometer) was read at 500 nm against the blank. Finally, a calibration curve was produced using a range of tannic acid concentrations ranging from 0 to 2 mg/mL. The results were expressed in mg equivalent of tannic acid (EAT)/100g of dry matter (DM).

3. RESULTS

3.1 Hydrogen Potential of Water during Soaking

The hydrogen potential of cereal grains increased from 6.97±0.023 to 5.49±0.012 for sorghum and from 6.84±0.068 to 4.86±0.029 for maize after 24 h of soaking and at 6.05±0.017 for sorghum and 5.40±0.054 for maize at the end of soaking, after 48 h (Table 1).

3.2 Physicochemical Characterization

The average values during the biochemical characterization obtained for each sample of maize and germinated sorghum are recorded in (Table 2).

The moisture of maize and sorghum kernels after soaking were 6% to 8% respectively. During the 6 days of germination, this humidity increased reaching 40% and 46% respectively for maize and sorghum grains (Table 2).

The hydrogen potential of germinated maize grains went from 6.74±0.023 on the first day of germination to 5.29±0.01 after 6 days. In the same order, that of germinated sorghum grains went from 6.88±0.010 to 5.4±0.01 after 6 days of germination. The contents of sprouted cereal grains acidify (Table 2).

The acidity of maize and sorghum grains after soaking increased respectively from 3.2±0.02% and 3.88±0.06% to 13.8±0.06% and 14±0.02% after 6 days from germination. Acidity increased with germination time (Table 2).

Table 1. Evolution of the hydrogen potential during soaking

	Cereals	Time (Hour)		
		0	24	48
Hydrogen potential (pH)	Sorghum	6,97±0,023	5,49±0,012	6,05±0,017
	Maize	6,84±0,068	4,86±0,029	5,40±0,054

Values are the mean ± standard deviation of three measurements (n = 3)

Table 2. Evolution of biochemical parameters during germination

Parameters	Cereals	Contents						
		D0	D1	D2	D3	D4	D5	D6
Moisture(%)	Sorghum	8	38	36	44	48	58	46
	Maize	10	36	38	38	46	36	40
Dry matter (%)	Sorghum	92	62	64	56	52	42	54
	Maize	94	64	62	62	54	64	60
Hydrogen	Sorghum	6,88±0,10	6,57±0,15	6,01±0,08	5,68±0,02	5,67±0,01	5,42±0,07	5,4±0,01
potential	Maize	6,74±0,23	6,625±0,025	6,38±0,02	5,715±0,035	5,72±0,02	5,365±0,05	5,29±0,01
Titratable	Sorghum	3,88±0,6	4±0,2	8,4±0,2	10,5±0,1	12,3±0,3	12,8±0,2	14±0,2
acidity (%)	Maize	3,2±0,2	3,4±0,2	4,7±0,5	11,2±1,2	13,3±0,3	12,2±0,2	13,8±0,6
Reducing	Sorghum	0,39±0,035	0,22±0,01	0,25±0,0035	0,18±0,014	0,45±0,01	0,71±0,015	0,86±0,003
Sugars	Maize	0,11±0,014	0,12±0,008	0,09±0,003	0,118±0,007	0,18±0,01	0,37±0,015	0,43±0,02
mg/100g DM								
diastatic power	Sorghum	48,15± 3,6	65,64± 4,1	110,29± 1,8	48,76± 5,1	27,16± 9,2	35,73± 2,6	40,16± 4,9
IU/g protein	Maize	52,43± 6,7	60,39± 0,9	114,17±2,4	44,65± 1,7	39,11± 6,2	45,18± 5,5	36,15± 1,3

Values are the mean ± standard deviation of three measurements (n = 3)

Table 3. Evolution of phytochemical parameters during germination

Germination day (D)	Polyphenols (mg/100 g DM)		Tanins (mg/100 g DM)	
	Sorghum	Maize	Sorghum	Maize
D0	661,7±12,9	534,22±48,1	214,6±0,5	182±7,7
D1	350 ± 2,5	503,96±45,6	107,07±2,9	81,51±1,76
D2	537,71±16,86	566,53±53,6	133,75±0,65	105,78±4,46
D3	650,79±23,81	703,86±15,4	135,85±0,8	112,85±3,5
D4	766,86±23,81	145,33±10,6	145,33±10,6	140,85±5,8
D5	859,60±23,3	811,01±12,4	166,26±1,3	175,34±6,4
D6	963,29±24,8	924,16±16,4	191,64±2,2	185,85±1,7

Values are the mean ± standard deviation of three measurements (n = 3), DM: Dry matter

The reducing sugars contained in cereal grains after soaking were 0.11±0.014 mg/100g DM (dry matter) and 0.39±0.035 mg/100g DM respectively for maize and sorghum. After the first day of germination (D1) the reducing sugar content went from 0.12±0.0008 mg/100g DM and 0.22±0.01 mg/100g DM respectively for maize and sorghum to 0, 43±0.02 mg/100g DM for maize on the sixth day (D6) of germination (Table 2).

The diastatic power of germinated grains of sorghum and maize reached a peak on the second day of germination with respective contents of 110.29 ± 1.8 IU/g of protein and 114.17 ± 2.4 IU/g of protein (Table 2).

3.3 Phytochemical Parameters

The results of the study of phytochemical parameters during the germination of maize and sorghum in Table 3 show a significant increase in polyphenols (mg/100g) and tannins (mg/100g). These levels went from 350 ± 2.5 mg / 100g DM and 503.96 ± 45.6 mg / 100g DM increase to 963.29 ± 24.8 mg / 100g DM and 924.16 ± 16.4 mg / 100g DM respectively for sorghum and maize, while for tannins, these levels from 107.07±2.9 mg/100g DM and 81.51±1.76 mg/100g DM increase to 191.64±2.2 mg/100g DM and 185.85±1.7 mg/100g DM respectively for sorghum and maize.

3.4 Effect of Temperature on the Amylase Activities of Germinated Maize and Sorghum Kernels

3.4.1 Optimal hydrolysis temperature

The optimum temperatures of germinated maize kernels after soaking were 40, 45 and 50°C for Days (M0), 5th day (M5) and 2nd day (M2) respectively (Fig. 1). As for germinated sorghum kernels, the optimum temperatures are respectively 40°C for Day (S0) and 50°C for the 2nd day (S2) and 5th day (S5) (Fig. 2).

3.4.2 Thermal denaturation of hydrolysis

The amylase activities of germinated maize kernels stable at temperatures of 45°C for days (M0) and 5th day (M5). Beyond this temperature, the activity gradually decrease to less than 20% at 80°C, thus reflecting their thermal denaturation. The amylase activities tested on the 2nd day (M2) of germination also stable up to their optimum temperature. At 80°C only 20.63% of relative activities obtained, reflecting their denaturation (Fig. 3).

The amylase activities of germinated sorghum kernels stable at temperatures of 40, 50 and 45°C respectively for days, Day (S0), 2nd day (S2) and 5th day (S5). At 80°C, all amylase activities denatured (Fig. 4).

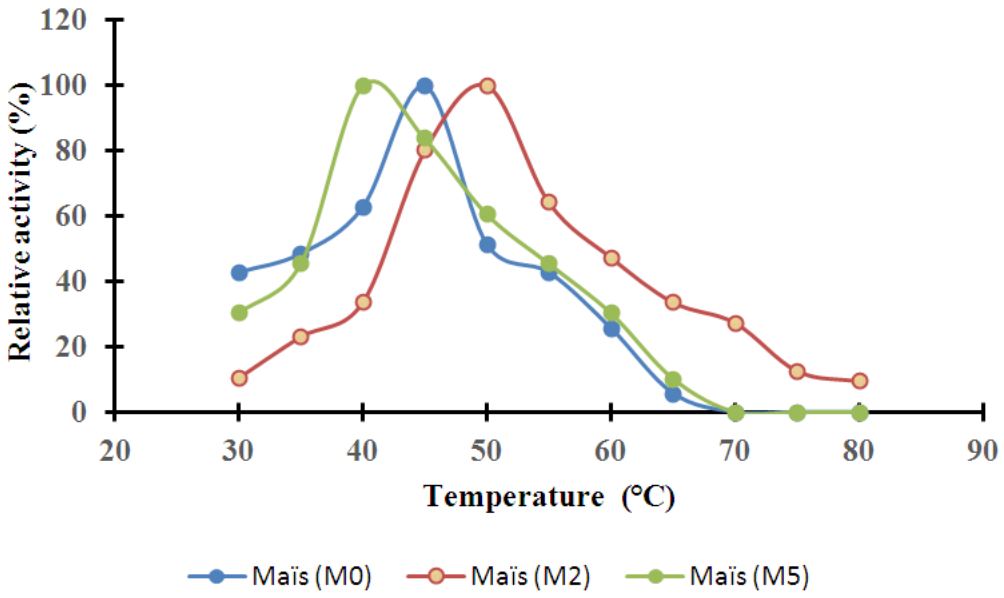

Fig. 1. Optimal temperature of amylase activities of germinated maize kernels

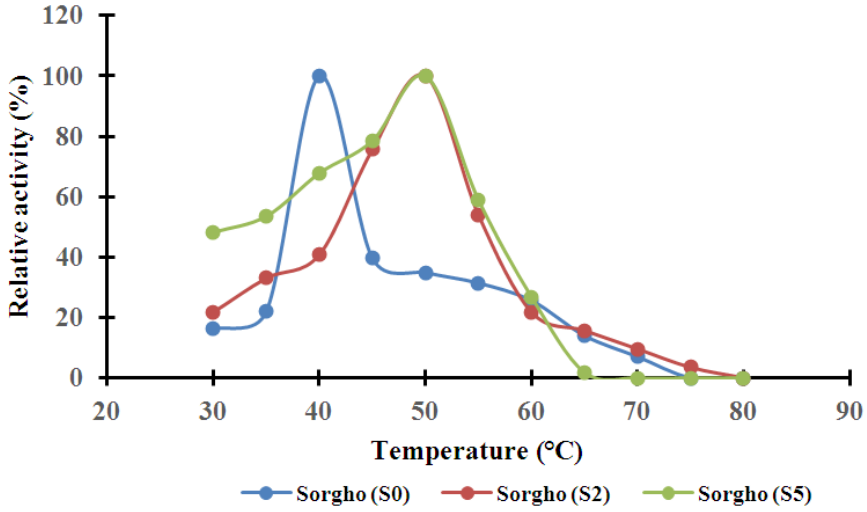

Fig. 2. Optimal temperature of amylase activities of germinated sorghum kernels

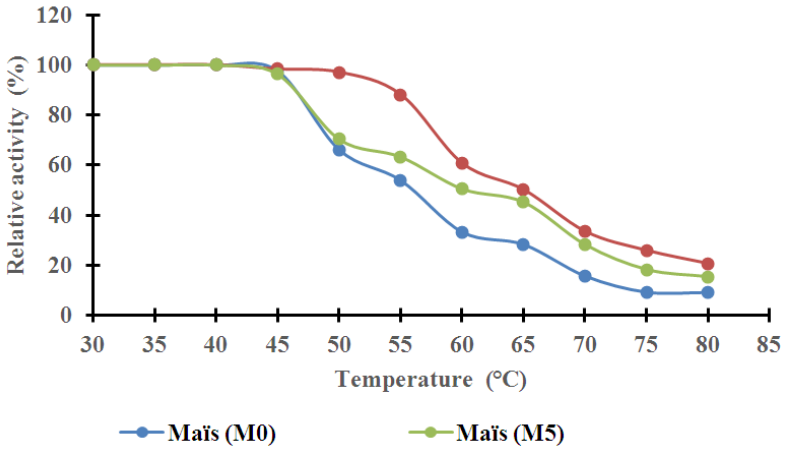

Fig. 3. Thermal denaturation of amylase activities of germinated maize kernels

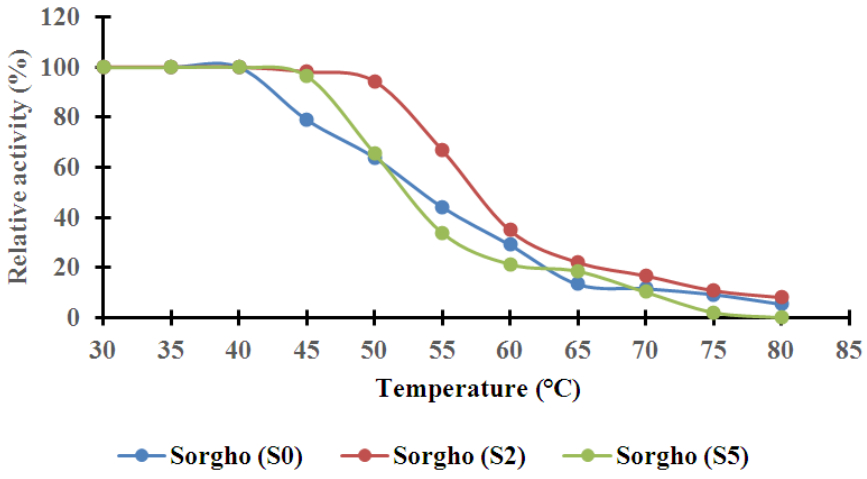

Fig. 4. Thermal denaturation of amylase activities of germinated sorghum kernels

4. DISCUSSION

In the cereal processing sector, the quality of germination is a decisive criterion because it determines the type of use and the suitability for processing. Thus, our study focuses on the physicochemical characterization and the effect of temperature on the amylase activities of sorghum and germinated corn grains during malting. According to [19], his survey carried out in Kaya, a locality in Burkina Faso, consumers are in perpetual quest for a traditional alcoholic drink (*Dolo*), as natural as possible (without the addition of external alcohol or synthetic molecules), with a pleasant taste (neither acidic nor bitter), without any unpleasant odor and which does not make consumers sick. To meet these aspirations, an optimization of the production process is necessary, hence the initiation of this study. According to [20], malting aims to transform cereals into malt rich in enzymes such as amylases and proteases essential for the degradation of macromolecules into simple molecules necessary to obtain alcohol. As a result, the cell walls of the albumen of cereals are weakened, thus promoting the attack of reserved constituents, which are degraded into molecules of low molecular weight. It promotes interesting biochemical modifications but can also lead to considerable losses of grain material as well as an increase in the content of nitrolosides, in particular toxic cyanogenic β-glycosides such as durrhine. Malting is therefore an important step that determines the quality of the drink obtained at the end of the process. Its mastery is essential for a quality drink. From one producer to another, the number of days of germination, one of the stages of malting varies, thus influencing the quality of the drink. In this study, after soaking, six (6) days of germination were tested under conditions similar to those of traditional producers in the city of Korhogo (Côte d'Ivoire). For Wang and Fields (1978) compared to the improvement of nutritional value through malting, it takes 4 days of germination at 25°C or 2 days at 30°C or 3 days at 35°C for maize and 5 days at 25 °C or 6 days at 30°C or 3 days at 35°C for sorghum in order to have the highest values. The results after 6 days of germination show that a set of physical, biochemical, phytochemical characters, the temperature and the hydrogen potential of germinated grains vary according to the germination time. Sorghum moisture increases with germination time, reaching its maximum on the 5th day (46%) while that of maize reaches its maximum moisture on the 4th day (40%) of germination. This moisture content could be a favorable factor for enzymatic hydrolysis activities in germinated grains. It can then drop with drying, which marks the end of malting [21]. The hydrogen potential of cereal grains (maize and sorghum) subjected to soaking and sprouting are acidic. The longer the germination time, the more the medium becomes acidic. This corroborates the results obtained by [22] who indicate that the bacteria responsible for the natural fermentation of cereals were mainly made up of the original flora of the grains, and would be the basis of the acidity of starches. Previous results on steeping cereal grains for malting or production of fermented products conducted by [23] indicate that a lowering of pH results from uncontrolled natural lactic acid fermentation. This assertion can be justified by the increase in titratable acidity observed in the solution preparations resulting from the germination of sorghum and maize cereals during the 6 days of germination. The reducing sugar content in germinated maize and sorghum cereals after soaking increases with germination time. After 6 days of germination, these rates multiplied by two. This increase can be explained by the principle of malting, which consists of degrading cereal polysaccharides. Indeed, malting leads to an increase in reducing sugar content during germination. Remember that malting aims to transform cereal grain into malt rich in enzymes [21,24,25]. Among these catalysts are alpha amylase and beta amylase. These enzymes hydrolyze starch, the main polysaccharide in cereals, into fermentable simple sugars in malt. The amounts of phytochemicals (polyphenols and tannins) highlighted in this study increased with germination time. This increase can be explained by the ongoing metabolic process in germinating maize and sorghum grains. According to [26], polyphenols are low molecular weight secondary metabolites produced by the plant in response to biotic or abiotic stress and which generally possess antifungal and antimicrobial properties. The ability of a species to produce these molecules is correlated with its resistance to aggression [27,28]. The results obtained are in agreement with those of [29] whose research work focused on sorghum. As for the tannins, antioxidant molecules, they give the beer its color and particular aromas. The tannin content at D0 respectively in sorghum and maize are 107.07±2.9 mg/100g DM and 51.51±1.76 mg/100g DM. After 6 days of germination, these levels increase to 191.64±2.2 and 185.85±1.7 mg/100g DM respectively in sorghum and maize. Thus, tannins represent poisons for bacteria and

therefore a means of protecting beers from microorganisms. These results are in line with those of [30] who showed that the duration of germination made it possible to increase the tannin content. Optimum temperatures of amylase activities in germinated grain extracts of sorghum and maize are mesophilic (40 to 50°C). Indeed, low temperatures make it possible to have good enzymatic activities while high temperatures make it possible to accelerate germination and the production of enzymes at the beginning of this stage. Germinations were carried out at room temperature ranging from 25 to 28°C in the shade. These conditions are optimal for good germination coupled with an optimal level of hydrolysis of amylase activities. These enzymatic activities are optimal on the second day of germination. This peak of hydrolytic activity decreases with the duration of germination until the fifth day. This decrease in activity could be explained by the production of polyphenol which increases with the duration of germination due to metabolism. According to studies by [31] Uriyo and Eigel (1999) an interaction between enzymatic activities would lead to a low activity of β-amylase in sorghum malt. For [32,33], polyphenols have a role of inhibitors of enzymatic activities, antioxidants, hormone regulators and complexing of metals. Enzymatic activities are progressively denatured beyond optimum temperatures. These results show that once exposed to temperatures exceeding 50°C, the germination process fades.

5. CONCLUSION

The objective of this study was to investigate the physicochemical characterization and determine the effect of temperature on the amylase activities of germinated maize and sorghum grains during malting. The results of this study revealed that certain parameters such as acidity, reducing sugars, polyphenols, tannins, and temperature condition the obtaining of a malt likely to influence the quality of dolo. Over a total of six days of germination, amylase hydrolytic activities were optimal on the second day. This rate drops gradually until the 6th day. In contrast, polyphenol content and tannins increase during germination due to metabolic activities. This production could be at the origin of an inhibitory effect of amylase activities. As for the pH, the increase in acidity in the medium would favor the obtaining of the necessary fermentable reducing sugars. The germination time set at 2 days makes it possible to obtain fermentable sugars for the preparation of alcoholic beverages according to the production conditions of traditional vendors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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