Biochemical Changes Occurring During Germination and Fermentation of Millet and Effect of Technological Processes on Starch Hydrolysis by the Crude Enzymatic Extract of Millet

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Abstract: Millet grain was steeped before germinated and fermented (0 to 8 days). Increasing germination and fermentation time led to increased production of amylase and corresponded to increased content soluble sugars (reducing and total sugars) from days 1-4 for germination and days 1-2 for fermentation. Since germination provokes increasing the content in protein from days 1-3, contrary fermentation decreases the content in this one during the processing. The study of inter-relationship of crude enzyme extract of millet seed to technological traitment of cereals revealed that cooking, germination, roasting and fermentation affected differently the digestibility of a flour and consequently flour from germinated millet could be added to different flour to initiate starch degradation and thereby reduce viscosity.

Key words: germination, fermentation, digestibility, amylolytic activity, technological traitment

INTRODUCTION

Malnutrition in infants and young children is one of the most serious health problems in developing world. Thirty-two percent of children under 5 y old suffer from being underweight and 39% from stunting[17]. There are considerable differences between the different regions, with the highest prevalences in South Asia and sub-saharan Africa where 53% and 41%, respectively, are stunted[24]. Generally a cereal-based porridge is the main complementary food in these most developing countries. Typically it is prepared from maize, rice, sorghum or millet but in some areas also from starchy roots or tubers[17]. In tropical Africa, cereal grains are milled and used to produce thick porridges, which are known by various names in different parts of the continent[12]. These porridges play an important role in the weaning diets of children but their energy density is often low because large volumes of water are added during preparation to achieve a thin, drinkable consistency[26]. The low energy density (40 kcal/100 g) and fluid consistency of these porridge applied as infant may have an effect on baby’s health during the first period. It is generally recognised that the insufficient energy density of complementary foods is an ethiological factor of protein energy malnutrition in young children[29]. Infants may not be able to consume sufficient quantities of these porridges to meet their energy requirements because gastric capacity of infants was limited (30-40 ml/kg body weight)[24]. Increasing the energy density of weaning foods is therefore considered a high priority in may health and programm nutrition[23]. The simple traditional household technologies have been used to process the cereal in order to improve the nutritional quality[21,22]. These include roasting, germination, fermentation, cooking and soaking. The application of such technological processes provokes changes physico-chemical characteristics of the components. Roasting is a dry heating of the cereal. It can improve protein digestibility if it is not excess, but has little or no effect during later preparation[22]. As far as it concerned germination, when grains are hydrated in ambient conditions, endogenous enzymes start to modify the grains constituents[15] in particular, changes in soluble sugars, protein and activities in enzymes. Germination has a profound on nutritional quality of the cereal[6]. As a consequence, starch (amylose and amylopectin) is degraded, resulting in significant reduction of the viscosity when preparing porridge. Protein bioavailability is increased. Flour from germinated...
Cereals can be added to ordinary flour to initiate starch degradation and thereby reduce viscosity\cite{17}. Fermentation by lactic bacteria and yeast of cereals for complementary food has many potentially positive effects. The fermentation causes degradation of grain components, especially starch and soluble sugars, by both grain and fermented media enzymes\cite{5}. Fermentation improves the nutritional quality of the porridge and protein digestibility might be enhanced. However, the effect on viscosity of the prepared porridge is only marginal. Since the consumption of the cereals based flour food is very popular among weaning children in Côte d'Ivoire, there is necessary to study of various traditional methods of improving the nutritive value of cereal-gruels.

The present investigation reports firstly the changes in amylolytic activity, soluble sugars and protein during germination and fermentation on millet seed and secondly the inter-relationship of crude enzyme extract of millet seed germinated to technological traitment of cereals.

**MATERIALS AND METHODS**

**Cereals Seeds:** Seeds of millet (**Pennisetum glaucum**), maize (**Zea mays**) and sorghum (**sorghum bicolor**) were used for this study were bought from local retailers in Abobo (Côte d'Ivoire). The seed of millet were used to study the effect of germination and fermentation. Maize (**Zea mays**) and sorghum (**sorghum bicolor**) were thoroughly cleaned and divided into six equal parts for study interation between crude extract enzymatic of millet and technological treatment.

- the first part (NT) 1kg was not subjected to any treatment. It served as control for digestion test.
- the second portion (T) 1kg was soaked in water in ratio of 1:3 (p/v) for over 24 h. Then the soaked grains were dried at 45°C and ground to pass a 200µm screen.
- third portion (Gm) 1kg was immersed in water 1:3 (p/v) overnight and then the grains were spread on trays lined with cloth at room temperature (25 °C) for one week. It was kept wet by frequent spraying of water (Fig.1). After germination, the seeds were dried separately at 45°C to constant weight and were ground.
- fourth portion (F) 1kg of seeds were immersed in water in a ratio of 1:4 (P/V) and allowed to ferment by the natural microorganisms present on the seed for one weekend (Fig.2). The
- fifth portion (C) 1kg was cooked at 100°C for 60min. The grains were ground to pass a 200 µm screen for analysis,
- sixth portion (G) 1kg were roasted in a preheated oven at 60°C. The roasted sample were cooled to room temperature then ground using through a 200mm mesh sieve, then packaged in plastic bags and stored until further analysis. The flours were packaged in polystyrene and frozen until analysed.

**Extraction of Soluble Sugars in Sprouted Seeds of Millet:** Sugars were extracted from seed millet as follow: 0.5 g of sample were homogenised with 10 ml of 80 % ethanol solution. The insoluble residue was removed by centrifuging at 3000g for 15 min. The precipitate was re-extracted with 2 ml of 80 % ethanol and recentrifuged. The supernatants were pooled and dried under a stream of hot air, and residue was resuspended in 5 ml of water.

**Preparation of Enzyme Extract of Millet and Protein Essay:** Enzymes were extracted in the following way. Ten grammes of seeds millet degermed were homogenized with 20 ml of NaCl 0,9 % at 4°C. The homogenate was centrifuged at 3000 g for 10 min and the supernatant containing enzyme was used for the essays (Fig. 3). Soluble protein was determined by procedure of \cite{19}.

**Amylolytic Activity:** Amylolytic activity of germinated millet was determined using colorimetric method developed by method of\cite{2}. It consists of hydrolysis with crude enzyme of sample extracts of 1% flour. Amylolytic activity is expressed in U1 per mg of crude protein.

**Determination of Sugar:** Quantitative determinations of total soluble sugars were carried out according phenol – sulfuric acid method\cite{19}. Reducing sugars were estimated by the Dinitrosallylic acid (DNS) method of\cite{2}. Digestibility of Different Flours of Maize and Sorghum: In vitro starch in flours digestibility were determined after digestion with crude enzyme of grains of millet. 100 µl of 1% flour was suspended in 325 µl of buffer acetate (100mM, pH5) containing 25µl of crude enzyme extract. Then the suspension were placed in a water bath at 37°C for 30 min. The released reducing sugar was determined by\cite{2}.

**RESULTS AND DISCUSSION**

Changes in amylolytic activities during germination and fermentation process of millet is presented in Fig. 4. The results indicate a rapid rise in amylolytic activity in millet. The level of amylolytic activity reached a maximum on 2 days and 4 days respectively for fermentation and germination and thereafter a slight decrease was observed. The maximum value of
Fig. 1: Flow chart illustrating preparation of germinated cereal

Fig. 2: Flow chart illustrating preparation of fermented seed millet
Fig 3: Flow chart illustrating preparation of crude enzyme of millet malted

Fig. 4: Changes in amylase activity during natural fermentation and germination of millet from 1 to 8 days at 25°C
Fig. 5: Changes in reducing sugar during natural fermentation and germination of millet from 1 to 8 days at 25°C

Fig 6: Changes in total sugar content during natural fermentation and germination of millet from 1 to 8 days at 25°C
amylolytic activity was 0.04 and 0.01 µg/min/mgs of protein respectively for germination and fermentation. The total and reducing sugar contents, increased from 1 to 2 days and 1 to 4 days respectively for fermentation and germination (Fig. 5, 6) and thereafter started to decrease until at the end of the processes.
The increase in amylolytic activity and sugar contents observed during germination of seeds of millet probably reflected an increase in carbohydrate metabolism, in response to the increased water uptake by germinating seeds. These results are consistent with the findings of [14] who had already found that seed carbohydrate metabolism can be considered a dynamic process involving often concomitantly occurring processes of polysaccharide degradation and synthesis of new compounds. During germination, there was a decrease in stokage carbohydrates and increase in total soluble and reducing sugars due the energy needs of growing plant. The increase in the soluble sugars could be the result of amylolytic activity of the endogenous amylases whereas decrease in soluble sugars might be due their involvement in the synthesis of new polysaccharides, probably for the walls. The results obtained in this study agree well with the results of investigations on other seeds reported by [18] who had found that total soluble sugars, both reducing and non–reducing sugars increased significantly during germination of pearl millet. [9] have studied the malt characteristics of *sorghum vulgare* from Ghana and reported that dextrin, maltose and glucose increased during germination. [10] indicated that starch in the endosperm is degraded slowly during germination and the sugar levels are developed according to degradation of starch. Similar results had been found by [3] in chickpea grain, [14] in mung bean and in pearl millet by [18]. On the other hand, the reduction in sugar levels probably occurs in response to an increase requirement for simple sugar triggered by the increased metabolic activity accompanying germination. As far as it concerned fermentation, the increase in content total and reducing sugars can be derived from carbohydrates hydrolysed on the one hand by fermentations endogenous grains amylases and on the other hand by fermenting microbes which possess both endogenous alpha and beta amylases [8]. These results are consistent with the higher concentrations of fermentation substrates (total and reducing sugars) in the millet seed from 1 to 2 days. The generate fermentable sugars will serve as a source of energy for the lactic acid bacteria [4]. Thus, a sharp decrease in total and reducing sugars after 48h may be due to consumption of the later sugars by bacteria during fermentation.

The Fig. 7 shows the evolution of protein during germination and fermentation. In general, germinating slightly increased protein content from 1 day to 3 days [23] also observed in 2 varieties of millet from Tanzania, an increase in protein after 48h of germination. This increase in protein content is attributed to a passive variation due to a decrease in the carbohydrate compounds used for respiration [23]. Fermentation cause any significant decrease in the total protein content in millet. The protein content ranged from 0,15% to 0,05% in samples fermented. This suggests microorganisms such as lactic acid bacteria and yeasts and endogenous enzymes, are involved in natural fermentation to produce fermented millet seed. A proteolytic system that allows the degradation of proteins is crucial for growth, and the conversion of larger peptides to small peptides and free amino acids and the subsequent utilization of these amino acids is a central metabolic activity in fermentation microorganisms such as lactic acid bacteria [9]. The low amount of protein in millet seed indicate the need for complementing it with proteinaceous foods in order to improve its nutritional quality for use as a weaning food.

The digestibility of different flours of cereals has been analyzed through the amylolytic activity of the crude enzymatic extract of germinated millet (Fig. 8). Every typical of flour has been prepared from grains having undergone a technological treatment. The result exhibit remarkable differences hydrolysis of starch content in flour between treatments groups by crude enzyme extract of millet. From the results, it is clear that the order of quickly digestible starch in the flour is maize white Gm > G > T > C > F > NT. As far as concerned the yellow maize, the order of digestibility starch in the flour is maize yellow G > Gm > F > C > Tr > NT. The order of digestible starch in the flours of sorghum is sorghum C > NT = T = F > G > Gm. The results agree with those of [1]. These workers has been reported that, soaking, germination, ordinary cooking and roasting were increased significatly in vitro starch digestibility of bambara groundnut. This extract can be considered as the one enzymatic source for the improvement of the nutritional quality particularly the energy density of the flour of maize, gotten by germination of the seeds millet.

As far as it concerned yellow maize, the crude enzyme extract of millet was 3 fold more efficient for the digestion of flours way out of yellow maize roasted that for those of the raw flours. For the white maize and sorghum, the crude extract of millet seed was respectively 5 and 2 fold more efficient for the digestion of flours descended of white maize germinated and sorghum cooked that those of the raw flours.

The whole results shows clearly that it is possible to find an enzymatic source to improve the nutritional quality through the increase of the flour digestibility. Thus, the flour from germinated cereals can be added to a different flour to initiate starch degradation and thereby reduce viscosity.
**Conclusion:** The seeds of cereals during their germination and fermentation develop a strong enzymatic activity (amylolytic). This strong enzymatic, induced activity a strong mobilization of the soluble sugars. These two phenomena take place in a parallel way and reach their maximum on the 4th day of germination for the grains of millet, on the 2th day for fermentation. Otherwise, the analysis of the flour digestibility descended of seeds having undergone of the treatments of cooking, germination, roasting and fermentation by crude enzyme extract of grains germinated showed that the digestibility of a flour depends on the process of preparation.

**REFERENCES**


