

Research Application Summary

Effect of amylase activity in germinated maize flour on viscosity, energy and nutrient density of complementary porridge

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Abstract

This study aimed at determining amylase activity in different maize varieties during germination and developing an energy and nutrient dense maize complementary porridge using different amounts of germinated maize flour. Two varieties of maize grains were collected, cleaned, and soaked for 24 hours at room temperature ($25\pm 2^{\circ}\text{C}$). Each variety was germinated for 168 hours (7 days) separately at soaking temperature, dried using a conventional oven at 60°C for 24 hours, and milled into flour. Standard procedures were used to determine the amylase activity of the two varieties. Results showed that amylase activity in the two varieties increased consistency until the fourth day and then decreased thereafter. The yellow maize flour had average amylase activity of 12.41CU/g compared to the white maize flour (10.73CU/g). Addition of germinated maize flour at 5%, 10%, 15% and 20% rates yielded porridges with viscosities of 2130, 1860, 1326 and 1230 cP, and energy and nutrient density of 376.59, 375.35, 372.24 and 377.22 kcal/100g, respectively. Since these porridges were developed targeting children in the age range of 6-24 months, the porridges have appropriate, viscosities, energy and nutrient densities which will supply the needs of the target group especially children aged 9-11 months, both for milk breastfed and complementary fed.

Key words: Amylase activity, complementary food, energy and nutrient density, germination, maize, viscosity

Résumé

Cette étude visait à déterminer l'activité de l'amylase dans différentes variétés de maïs lors de la germination et à développer une bouillie complémentaire de maïs énergétique et nutritive à base de différentes quantités de farine de maïs germé. Deux variétés de grains de maïs ont été choisies, nettoyées et trempées pendant 24 heures à la température ambiante ($25 \pm 2^{\circ}\text{C}$). Chaque variété a été germée séparément pendant 168 heures (7 jours) à la température de trempage, séchée à l'aide d'un four conventionnel à 60°C pendant 24 heures et moulu en farine. Des procédures standard ont été utilisées pour déterminer l'activité de l'amylase dans les deux variétés de maïs. Les résultats ont montré que l'activité de l'amylase dans les deux variétés a augmenté constamment jusqu'au quatrième jour, avant de chuter par la suite. La farine de maïs jaune avait une activité d'amylase moyenne de 12,41CU / g comparée à la farine de maïs blanc (10,73 CU / g). L'addition de farine de maïs germée à 5%, 10%, 15% et 20% ont permis de produire des bouillies de viscosités 2130, 1860, 1326 et 1230 cP, et de densités énergétiques et nutritives respectifs de 376,59 ; 375,35 ; 372,24 et

de 377,22 kcal / 100 g . Étant donné que ces bouillies ont été développées pour des enfants âgés de 6 à 24 mois, elles ont des densités appropriées, des viscosités, d'énergie et des éléments nutritifs qui répondent aux besoins du groupe ciblé, en particulier les enfants âgés de 9 à 11 mois, nourris au lait maternel et ou avec un complément alimentaire.

Mots clés: Activité d'amylase, alimentation complémentaire, densité énergétique et nutritive, germination, maïs, viscosité

Background

Complementary feeds are introduced when human breast milk is insufficient to meet the nutritional needs of children (WHO, 2005). Complementary feeding therefore involves introduction of semi-solid and liquid foods to children aged 6-24 months along with breast milk. The first two years of life are a critical window for ensuring optimal child growth and development. Inappropriate complementary feeding increases risk of protein energy malnutrition, illness and mortality (Senarath *et al.*, 2012). Protein energy malnutrition leads to physical, mental and motor development retardation. Complementary foods in developing countries are mainly made from starchy staples; starch is the major constituent of flour made from maize endosperm. The starch of the ungerminated grains allows these foods to bind water yielding a thick porridge. The high viscosity of the porridge limits adequate amount of nutrient intake by the children 6-24 months old. Dilution of porridges decreases viscosity but causes decrease in energy and nutrient content per gram.

Different approaches are needed to offer families the opportunity to feed their infants on improved formulations using low cost and locally available staples (Muhimbula *et al.*, 2011). Processing techniques such as germination and fermentation have been found to improve the quality of cereals due to chemical changes that enhance organoleptic response (Oluwole, 2012). The techniques for reducing viscosity of complementary porridges is through germination of the cereals (Isaac and Koleosho, 2012). However, alpha amylase activity is increased during germination of cereals. This enzyme hydrolyzes amylose and amylopectin to dextrin and maltose, thus reducing the viscosity of the thick cereal porridges without dilution with water while simultaneously enhancing their energy and nutrient densities (Helland *et al.*, 2002). The alpha-amylase enzymes degrade the starch granules, reduce their water binding capacity and lower the viscosity. The addition of a small quantity of germinated maize flour, rich in the enzyme, alpha-amylase, to thick cereal porridges is known to improve the digestibility of storage proteins and starch, and to dramatically reduce the viscosity of porridges and increase energy and nutrient density of complementary foods.

Literature summary

Maize (*Zea mays*), the American Indian word for corn literally means “that which sustain life” (FAO, 1992). It is a monocotyledonous annual crop belonging to the family of grasses

(Poaceae) with a botanical name *Zea mays* (Stephen *et al.*, 2014). Maize is the world's most widely grown cereal, cultivated across a range of latitudes, altitudes, moisture regimes, slopes and soil types, and it is a main staple food for millions of people in sub-Saharan Africa (Nicole *et al.*, 2010). It serves as a staple food for approximately 400 million people in developing countries and about half of the estimated 603 million tones world production of maize is produced in the developing countries (Igbabul *et al.*, 2014).

Traditional complementary foods in Tanzania are based on cereals such as maize, sorghum and finger millet or non-cereals such as cassava, potato, sweetpotato and plantains because they are cheap, locally available and easy to prepare (Mosha *et al.*, 2000). Most of the maize produced (about 85%) is consumed at the household level, and is the main energy source in the diet accounting for 25% of total caloric intake (Barreiro, 2012). Grains for germination should have at least 95% germination capacity (Ojukwu, 2012). Germination occurs after the radix break up seed tegument and it shows as a young radix. The energy needed for seed germination is provided by sugar from endosperm respiration. Amylase activity is a function of germination time, and the activity of amylase increased with germination time. The alpha-amylases are synthesized within the cells of the aleurone layer, from here, they migrate to the starchy endosperm, where hydrolysis of the starch granules begins. This means that upon cooking, the starch granules will not swell to the same extent, and the amylase which forms the gel network will be broken down (Tizazu *et al.*, 2010). The objective of this paper was to assess the variation in amylase activity in different maize varieties during germination.

Study description

The study aimed at determining amylase activity in different maize varieties during germination and developing an energy and nutrient dense maize complementary porridge using different amounts of germinated maize flour. Two varieties of maize grains were collected, cleaned, and soaked for 24 hours at room temperature ($25\pm 2^{\circ}\text{C}$) and germinated for 168 hours (7 days) separately at soaking temperature, dried using conventional oven at 60°C for 24 hours, and milled into flour. Amylase enzyme activity of the two varieties was determined using standard procedures. Six complementary porridges were formulated using flour from un-germinated seed mixed with soybeans which were added to germinated maize flours in ratios of 70:30:0, 65: 30:5, 60: 30:10, 55: 30:15, 50: 30:20 and 0: 30:70 (w/w). Each formulated porridge was measured before and after addition where by the cooked porridge was poured into the viscometer beaker, cooled to 40°C , and viscosity values (in Centipoises, cP) determined using a viscometer (Model HAAKE viscotester 1 plus/2 plus version 1.5, Germany). The proximate compositions (energy density) of the formulated porridges were determined using standard methods (AOAC,1990).

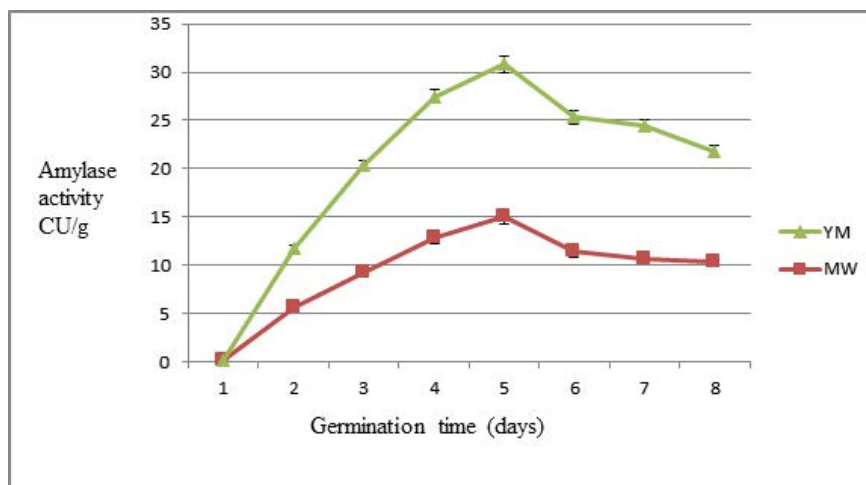
Amylase activity determination

A number of methods are available for the determination of amylase activity, but in this study, amylase activity was determined using a method developed by Bernfeld (Kernels, 1975). About 1g of the soluble starch was weighed into Erlenmeyer flask and distilled water was added to make 100ml mixture (1% starch solution). The mixtures were boiled

for 5 minutes and filtered, about 1% enzyme solution prepared by taking about 1g of germinated samples into 100ml of pH 6.9 phosphate buffers and filtered. About 1ml of substrate (1% starch solution) was transferred into clean 25ml volumetric flask and 1ml extracted enzyme was added. Standard solution of 2 μ g/ml D-Glucose prepared and diluted serially in a range of 0.2-1.2 μ g/ml. About 1ml of the diluted standard was taken into 25ml and about 1ml of pH 6.9 Phosphate buffer (0.02M Sodium Phosphate and 0.01M Sodium Chloride solution in 1:1 ratio) was added followed by the addition of enzyme solution. The mixtures were allowed to be hydrolyzed for 3 minutes in the water bath at 37°C and about 1ml of 3,5-dinitrosalicylic acid in 0.4M NaOH was added to the samples. The Absorbance (yellow colour) was read at 540nm using UV-Visible X-ma 3000 series spectrophotometer (Wavelength range 190-1100nm, spectral bandwidth 2.5nm, optical system single beam, scanning speed Max 3000nm/min, photometric accuracy \pm 0.3%T, stray light \pm 0.05%T, power requirement 220/110V, weight 16kg) The linear regression equation was used to calculate the mg maltose. One unit of amylase activity taken was expressed as the amount of maltose from the starch under pH 6.9 at 37°C for 3 minutes.

Research Application

Results showed that amylase activity of seed of the two varieties of maize increased with germination time and peaked on the 4th day. Thereafter, activity decreased (Figure 1). Maximum amylase activity occurred in Yellow maize variety which had the highest peak 12.41 CU/g of amylase activity compared to white maize variety 10.73 CU/g. The decrease in amylase activity after the fourth day was likely due to reduction of starch concentration in maize seeds.



Days of germination (Time)

Figure 1: The variation in amylase activity in different maize varieties during germination time from 1 to 7 days at 25 \pm 2°C. yellow maize (YM) and white maize (WM)

Addition of germinated maize flour at 5%, 10%, 15% and 20% rates yielded porridges

with viscosities of 2130, 1860, 1326 and 1230 cP, energy and nutrient density of 376.59, 375.35, 372.24 and 377.22 kcal/100g, respectively (Table 1). Viscosity decreased as the amount of germinated maize flour increased.

Table 4.2: Effect of addition of various ratios of germinated yellow maize on the viscosity and energy density of formulated complementary foods (Mean \pm SD)

Sample code	Viscosity (cP) before	Viscosity (cP) after	Energy kcal/100g
100% ungerminated	7298		
95% ungerminated	4201	2130	376.59
90% ungerminated	5867	1860	375.35
85% ungerminated	5012	1326	372.24
80% ungerminated	6113	1230	377.22
100% germinated	1004		

The amylase activity was low but increased until the fourth day when germination was optimum (Fig 1). Yellow maize flour was selected for porridge preparation and determination of the viscosity, energy and nutrient density due to its higher amylase activity. The decrease in amylase activity after the fourth day was due to reduction in starch concentration in the maize seeds (Oluwalana, 2014). The observed reduction of viscosity of porridge during germination was due to starch degradation by the action of amylase developed during germination process (Tizazu *et al.*, 2010). The viscosity of the porridges was significantly reduced by the addition of small amounts of germinated maize flour. Amylase enzymes tend to break down starch granules during the cooking process thus reducing viscosity without dilution with water while simultaneously enhancing the energy and nutrient density (Tizazu *et al.*, 2010). Since these porridges were developed targeting children in the age range of 6-24 months, the porridges have appropriate viscosities, energy and nutrient densities which will supply the needs of the target group especially children aged 9-11 months, both milk breastfed and complementary fed.

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