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Soaking of Maize Determines the Quality of Aflata for Kenkey Production

P. F. Nche*, G. T. Odamtten†, M. J. R. Nout*‡ and F. M. Rombouts*

*Department of Food Science, Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands, †Department of Botany, University of Ghana, P.O. Box 55, Legon/Accra, Ghana

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ABSTRACT

Aflata is a gelatinised maize paste, serving as intermediate in the manufacture of kenkey, a traditional cooked fermented maize product of Ghana. The effect of water uptake during soaking of whole or dry-milled maize, the extent of starch damage, dough pH, fermentation time, and of endogenous and added enzymes on pasting and set-back viscosities of aflata dough were studied. Water uptake by coarsely dry-milled maize (grits) reached 0.63 mL/g dry matter in just 1 h, compared with 0.50 mL/g in 3 days for whole grain. High endogenous activity of proteases and carbohydrases were recorded in both grits and whole maize when soaked at 4 °C or 25 °C. These were significantly reduced after soaking at 60 °C. Soaking of grits at 60 °C with a heat-stable protease, or wet fine-milling of fermented grits resulted in significant ($P < 0.05$) increases in pasting viscosities. Peak viscosities increased with fermentation time up to 24 h. Pasting viscosities decreased with repeated wet milling of fermented dough.

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Keywords: aflata, kenkey, maize, dry-milling, wet-milling, viscosity.

INTRODUCTION

Kenkey, a popular Ghanaian staple is produced from fermented maize dough. During traditional kenkey production^{1,2}, whole maize is soaked for 1–2 days at ambient temperature (25–30 °C), milled and made into a dough (moisture content approx. 500 g/kg dry matter), which is then allowed to ferment naturally at ambient temperature for several days. Part of the fermented dough (usually half) is mixed with water to give a slurry with a dry matter content of 250 g/kg, and cooked to gelatinisation to give a thick and sticky paste, called the aflata. This paste is then mixed with the uncooked portion of the dough and serves as a binding agent³ as well as moisturiser. When kneaded together, the aflata holds the uncooked dough together into a dumpling which can then be shaped, wrapped and boiled to give kenkey.

The whole process is tedious and can take up to 6 days to complete.

A dough with a high starch gelatinisation index³ (during cooking) and a high set-back viscosity, on cooling, is required to give an aflata of adequate binding and moisturising capacity. This is very important in determining the desired textural characteristics of cooked kenkey, which is a cohesive mass of coarse particulate matter cemented by the aflata. Good kenkey is neither too sticky nor crumbly. The choice of ingredients and the pre-treatment of maize and dough for aflata production are, therefore, crucial to the achievement of these properties. Local women kenkey producers prefer maize with a high swelling index.

In an attempt to reduce process time, it was suggested earlier^{4,5} to omit the soaking stage and substitute it with a lactic starter-fermentation of a suspension of dry-milled flour. In addition, household-scale kenkey making could be made less tedious by supplying a mix containing dehydrated fermented flour and dehydrated, pre-gelatinised

‡ To whom correspondence should be addressed.

aflata. It has, however, been reported that significant starch damage in dry-milled maize resulted in a dough of very low pasting and set-back viscosities⁶, although poor release of starch during dry milling would also be an important factor. Soaking of maize, on the other hand, is known to facilitate smooth milling⁷, and is also considered crucial to the production of dough with high pasting and set-back viscosities⁵ necessary for making aflata with the desired binding potential. Soaking usually takes 24–48 h, and omission or shortening of this step would be desirable if production time is to be reduced for the purpose of industrial-scale kenkey manufacture. However, given the importance of soaking, it is necessary to understand which changes occur during this step and how they affect the desirable properties of aflata. These changes may be (bio)chemical (enzymatic, pH, acidity), microbiological (microbial proliferation) or physical (water uptake). The aim of this work is to investigate the individual and/or collective roles of such changes in determining the quality of aflata for kenkey production.

MATERIALS AND METHODS

Dry local white maize (*Zea mays* L cv. Obataanba) was supplied by the Crops Research Institute, CSIR, Kwadaso, Ghana. This is a cultivar commonly used by traditional kenkey makers and had been stored dry at -20°C in our laboratory prior to being used.

Cleaning, disinfection and soaking of whole maize

Whole maize kernels were first rinsed (2×2 min) with sterile distilled water and then disinfected for 5 min at ambient temperature with a 10 g/L solution of sodium hypochlorite. Treated kernels were then rinsed (2×2 min) with sterile distilled water before soaking in sterile distilled water at 4°C or 25°C for 72 h. Untreated maize soaked under the same conditions served as a control. Samples of steep water were analysed on a daily basis for microbial load. Sampling, dilution and enumeration of total aerobic mesophilic bacteria, lactic acid bacteria (LAB), *Enterobacteriaceae* and fungi were as described earlier⁸.

Dry-milling

Dry maize kernels were coarsely milled in a hammer mill (Fritsch Pulverisette Type 14.702, Marius

Instruments, Utrecht, The Netherlands) using a 12 tooth rotor and a 4 mm screen to obtain grits. Rotor speed was set at 20000 rpm.

Wet-milling

Soaked maize kernels were first coarsely milled using a 12 tooth rotor and a 4 mm screen. Subsequently, a 24 tooth rotor was used in combination with either a 1 mm or 0.5 mm screen for fine-milling of fermented dough from coarsely milled grits. Rotor speed was maintained at 20000 rpm.

Endogenous enzyme activity in dry and soaked maize

Maize samples were soaked at 4°C , 25°C and 60°C . Dry milled maize (DM) was used as a standard and the test samples were either dry maize milled before soaking (DMS) or whole maize soaked before milling (WMS). API-ZYM kits (API Products, Montalieu, Vercieu, France) were used according to the manufacturer's instructions to evaluate endogenous enzyme activities of dry and soaked maize. The enzymes tested included: phosphatases (alkaline, acid, naphthol-AS-BI-phosphohydrolase); esterases (C4, C8, C14); peptidases (leucine-, valine-, and cystine arylamidases); carbohydrases (α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase). Aliquots of 65 μL 1:10 maize suspension in distilled water (pH 5) were incubated at 37°C for 4 h after which the enzyme activity was estimated visually using a standard API-ZYM colour chart. This was on a scale of 0–5 indicating the amount of substrate hydrolysed by the enzymes; where 0, 1, 2, 3, 4, 5 represented 0, 5, 10, 20, 30 and ≥ 40 nmoles of degraded substrate, respectively.

Water uptake

Pre-weighed (100 g) whole kernels were soaked in 300 ml of distilled water at 4°C and 25°C for 12, 24, 48 and 72 h, after which the water was drained and the weight increase measured as water uptake. Samples were taken of dry milled maize grits soaked under similar conditions as above for 1, 2, 4, 6 and 8 h, weighed and then dried for 48 h in a hot air oven maintained at 80°C to determine

their moisture contents. Water uptake was determined by subtracting the moisture content of maize flour from that of an equivalent amount of soaked grits.

Viscosity of maize dough

A Brabender Visco-Amylograph was used to measure the pasting characteristics of variously treated maize doughs. Dough suspensions containing 90 g dry matter/L distilled water were used and conditions were as described earlier⁴.

Treatment with proteolytic enzyme

Some (45 g) of the dry-milled maize was also soaked at 25 °C and 60 °C in 500 mL of distilled water containing 5 mL of a mixture of heat-stable acid, neutral and alkaline bacterial proteases (Protease-L 660, Solvay Enzymes, Hannover) to determine the effects of proteolytic enzyme activity on the pasting characteristics of the resulting dough.

Effect of pH on pasting characteristics

Grits were slurried (90 g/L in distilled water) and pH adjusted prior to gelatinisation with 1 M HCl to 6.0 (representing the initial pH of dry-milled maize), 5.6 (representing the pH of wet-milled maize) and 3.6 (representing the pH of a 3 days' fermented dough). The pasting viscosity of each slurry was determined using a Brabender Visco-Amylograph as described above⁴.

Fermentation time

Grits (100 g) were made into a dough by adding 80 mL of distilled water, and the dough fermented by an accelerated natural process⁴, which required an inoculation of fresh dough with a LAB-enriched starter dough prepared as described by Nout *et al.*⁹. Fermentation was carried out at 30 °C for 0, 4, 8, 12 and 24 h. At the end of each fermentation period, the dough was milled to pass through a 1 mm sieve, and slurried in distilled water as described above. The slurry was adjusted to pH 6.0 with 1 M NaOH to eliminate pH effects before measuring the pasting characteristics of the dough.

RESULTS AND DISCUSSION

During soaking of whole maize at 4 °C and 25 °C increases of total aerobic counts in soak water,

observed after 48 h incubation, reached levels of $>10^5$ cfu/mL in non-disinfected samples. On the other hand, a solution of sodium hypochlorite (10 g/L) was very effective in keeping the microbial load of disinfected kernels below 10^4 cfu/mL, and was subsequently used in cases requiring kernel disinfection.

Water uptake at 4 °C and 25 °C by soaked whole kernels reached 0.42 mL/g dry matter after 24 h, and 0.50 mL/g after 72 h of soaking. Water uptake was faster when the maize was coarsely milled before soaking, reaching 0.63 mL/g dry matter after 1 h of soaking.

A high endogenous enzyme activity was recorded in both dry and soaked maize at 4 °C and 25 °C (Fig. 1). Following soaking at 4 °C and 25 °C, α -galactosidase and α -mannosidase were stimulated. β -galactosidase, α - and β -glucosidase, N-acetyl- β -glucosaminidase, leucine arylamidase and valine arylamidase activities remained unchanged, whilst cystine arylamidase, trypsin and chymotrypsin were suppressed. With the exception of β -galactosidase and α -mannosidase, all peptidases and carbohydrases were suppressed following soaking at 60 °C.

Dry-milling resulted in a dough with very low pasting and set-back viscosities (Fig. 2), confirming earlier reports^{6,7} that ogi flour from dry-milled maize has very poor pasting properties. Soaking of grits at 60 °C also did not improve on the low pasting and set-back viscosities. The gelatinisation temperature (estimated as the temperature at which viscosity increase starts on a Visco-Amylogram) was 85 °C and no peak was obtained during the heating phase, suggesting incomplete swelling of granules and incomplete gelatinisation¹⁰.

When a heat-stable proteolytic enzyme was added to soaking grits at 60 °C, it resulted in significantly higher pasting and set-back viscosities (Fig. 2). The index of gelatinisation, peak viscosity and set-back viscosity of 610 B.U., 1325 B.U. and 1260 B.U. respectively, were comparable to those reported for traditional fermented maize dough⁵ and ogi from soaked maize⁶. The gelatinisation temperature was also reduced from 85 °C to 73 °C. Soaking of grits with protease at 25 °C, on the other hand, further reduced the pasting and set-back viscosities of the resulting dough (Fig. 2). Dry-milling has been reported⁶ to cause high levels of mechanical starch damage, making any exposed starch granules susceptible to attack by either endogenous amylolytic enzymes or hydrolytic enzymes of fermenting microorganisms⁷. The result

Figure 1 Endogenous enzyme profiles of soaked and dry maize as determined with an API-ZYM kit: DM = dry-milled maize; DMS = dry-milled maize soaked for 2 hours; WMS = whole maize soaked for 24 h before milling. The assayed enzymes are indicated at the bottom by 1–20 where: 1 = control; 2 = alkaline phosphatase; 3 = esterase (C 4); 4 = esterase lipase (C 8); 5 = lipase (C 14); 6 = leucine arylamidase; 7 = valine arylamidase; 8 = cystine arylamidase; 9 = trypsin; 10 = chymotrypsin; 11 = acid phosphatase; 12 = naphthol-AS-BI-phosphohydrolase; 13 = α -galactosidase; 14 = β -galactosidase; 15 = β -glucuronidase; 16 = α -glucosidase; 17 = β -glucosidase; 18 = N-acetyl- β -glucosaminidase; 19 = α -mannosidase; 20 = α -fucosidase.

would be a dough with very much reduced pasting and set-back viscosities as the amount of starch to be gelatinised is significantly reduced.

The effects of endogenous or added proteolytic enzymes on the viscosity of either whole or milled maize during soaking may depend on the composition of the material embedding the starch granules within the endosperm. In the endosperm of wheat grains, for example, starch granules are surrounded by a composite of proteins, minerals

Figure 2 The effect of soaking, with and without protease L-660, on the pasting properties of dry-milled maize grits: 1 = dry-milled grits + water; 2 = dry-milled grits soaked for 2 h at 25 °C with protease L-660; 3 = dry-milled grits soaked for 2 h at 60 °C; 4 = dry-milled grits soaked for 2 h at 60 °C with protease L-660.

and enzymes^{11,12}. Presumably in maize grains, the effect of added or endogenous proteolytic enzymes during soaking will be to break down a similar matrix embedding starch granules, thus allowing them to swell freely and gelatinise faster and better. Addition of the heat-stable proteolytic enzyme, protease L-660, during soaking of dry-milled maize flour at 25 °C, did not affect pasting properties for two possible reasons. First, 25 °C is not the optimum temperature for this enzyme and, second, some of the damaged or freed starch granules may be hydrolysed by endogenous or microbial enzymes. The use of other proteases with optimum activities at ambient temperatures had a similar effect (data not included) to that obtained with Protease L-660. At 60 °C, however, the activity of protease L-660 is highest and the matrix structure holding granules together^{11,13} may be degraded, thus releasing starch granules. Furthermore, at 60 °C, endogenous and microbial carbohydrases are considerably suppressed, hence, very little hydrolysis of damaged or freed starch granules is

Figure 3 The effect of pH on the pasting properties of dry-milled maize grits: 1 = pH 6.0; 2 = pH 5.6; 3 = pH 3.6.

expected to occur. Following such treatment, starch granules may swell freely, when heated, exuding solubilised amylose¹⁴ that will contribute significantly to an increase in the pasting and set-back viscosities of the dough.

Soaking of maize and the subsequent fermentation of dough results in a lowered pH. Figure 3 shows that by lowering the pH of dry-milled flour, the pasting viscosity of the resulting dough was slightly reduced. In all instances, no peak was obtained during the heating phase, again indicating incomplete gelatinisation and consequently low set-back. This suggests that pH may be ruled out as a factor contributing to higher gelatinisation viscosity in fermented wet milled maize doughs.

Wet fine-milling (to pass through a 1.0 mm sieve) of fermented dough from grits, however, resulted in a significant increase in pasting viscosity (Fig. 4), suggesting that more intact starch granules were freed by this process.

Visco-Amylograms of doughs fermented for 0, 4, 8, 12 and 24 h, milled to pass through a 1.0 mm sieve and adjusted to pH 6.0 show increasing

Figure 4 The effect of fermentation time (at 30 °C) on the pasting properties of dry-milled maize grits at 0, 4, 8, 12 and 24 h.

pasting and set-back viscosities (Fig. 4). At 0, 4 and 8 h, no clear peak viscosities were obtained due to incomplete gelatinisation¹⁰, whereas after 12 and 24 h of fermentation, peak viscosities were obtained during the heating phase, indicating a higher degree of gelatinisation. Fermentation for 24 h resulted in a lower gelatinisation temperature, a higher peak viscosity, but a reduced set-back viscosity compared with a 12 h fermented dough. These observations are in agreement with earlier reports^{15,6} that fermentation of maize flour increases the swelling and thickening potential of the maize starch component. After 24 h fermentation, the starch component rapidly swells on heating to 95 °C. At temperatures higher than the gelatinisation temperature, some of the swollen starch granules break up and exude their solubilised contents. This explains the dip in viscosity after a peak has been reached. It can be deduced from Figure 4 that 12–24 h of fermentation of dry-milled maize dough would give an acidified dough with adequate pasting characteristics for aflata production; however, such a dough must be wet-milled before cooking to give aflata.

Figure 5 The effect of milling levels (4 mm, 1 mm and 0.5 mm) on the pasting properties of 24 h-fermented (at 30 °C) dough from wet-milled maize.

Repeated wet-milling of fermented dough, to pass through 1 mm and 0.5 mm sieves (Fig. 5), resulted in doughs with lower pasting and set-back viscosities than dough from coarsely wet-milled (through a 4 mm sieve) maize. Although not determined here, mechanical starch damage could be one reason for lower viscosities.

Various factors have been listed as being important in the formation and determination of characteristics of starch gels. Amongst these factors are: the type, size and previous treatment of starch granules, as well as paste concentration, amylose/amylopectin ratio, and the temperature and time of cooking¹⁰. Results presented here confirm that a complex combination of factors during soaking and milling of maize and fermentation of the resulting dough contribute to the final pasting characteristics of kenkey dough. Endogenous enzyme activity, hydration and grain softening during soaking combine to facilitate the release of starch during milling, thus ensuring better hydration and swelling of granules, achieving a high degree of gelatinisation and set-back necessary for good aflata quality.

Acidification of dough from dry-milled maize by accelerated natural fermentation⁴ and wet-milling of the aflata portion may be more acceptable than the use of proteolytic enzymes which would raise production costs and arouse consumer fears, as this could be interpreted in the same light as the addition of chemical substances to a product that has always been considered as natural. Soaking whole maize before milling, however, remains the best option for developing the necessary dough textural characteristics.

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