DEGRADATION KINETICS OF FOOD MACROMOLECULES DURING SORGHUM GERMINATION

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Abstract
Germinated sorghum grains present good replacing attribute for barley used in brewing industries but the carbohydrate, protein and fat content degradation during germination is accentuated by increase in germination days. This research investigated the kinetic variation in composition of these food macronutrients as influenced by germination. Sorghum grains were steeped for 40 h prior to successive germination in an incubator at an isothermal condition of 26°C for 0-7 days, and later dried at 60 °C for 48 h. Each of the samples was analysed for carbohydrate, protein and fat contents. The generated data were subjected to analysis of variance and regression analysis. There was initial increase in all the macromolecules between day 0 and day 1, with subsequent decrease till day 5. Values for all the three nutrients increased by day 6 and decreased subsequently by the 7th day. R^2 values of first-order kinetic were 0.766, 0.876 and 0.912 for protein, fat and carbohydrate respectively. The rate constants were, -0.045, -0.093 and -0.166 for protein, fat and carbohydrate respectively. Based on presented results, the orders of degradation of macromolecules as influenced by germination in sorghum were zero and first-order kinetics which could help in optimising the industrial processes to which germinated sorghum is being used.

Keywords: Brewing, sorghum, macronutrients, regression, germination

Submitted: 16.03.2017 Reviewed: 22.05.2017 Accepted: 02.06.2017

1. INTRODUCTION

Worldwide, the brewing industry is becoming more competitive and is constantly looking for ways to improve beer quality and reduce manufacturing costs (David *et al*., 2004). Barley malt and brewing adjuncts are a main factor contributing to overall production costs; thus, the utilization of alternative raw materials, like sorghum (Bajomo and Young, 1993), and innovative processes can increase profitability where barley cultivation is unsuccessful.

Sorghum, according to Ihekeronye and Ngoddy (1985), is composed of 68 to 80% starch, 10 to 15% protein and about 3 to 5% fat, which made up the food reserve for grains during germination. During germination of seeds, the food reserve which is largely composed of food macromolecules namely carbohydrate, protein and fat provide nourishment to the growing embryo till germination ends. When seeds imbibe water, hydrolytic enzymes are activated which break down these stored food resources into metabolically useful chemicals (Raven *et al*., 2005). The degradation (gradual use up) of these macromolecules during germination is expected to follow a certain order of kinetics which was examined.

It is therefore of importance to study the phenomena taking place during germination of sorghum to the food macromolecules in order for food processors to be able to optimize the process of using sorghum for malt and other products to which germination is being used as a part of the processing techniques. This study is therefore essential for the development of reliable process models for such unit operation. This study is essential if sorghum is to really take the place of barley, found more usage or reduce the production cost of beers and malt drink. How germination affected these food macromolecules namely carbohydrate, protein and lipids in sorghum is the question this research work answered. This research work aims to kinetically investigate the variation in compositions of carbohydrate, protein and fat in sorghum as influenced by germination.

2. MATERIALS AND METHODS

Materials

Sorghum bicolor L. Moenchgrains were purchased from Teaching and Research Farms of Ladoke Akintola University of Technology. All chemicals used were of analytical grade.

Methods

Germination of sorghum grains

Sorghum grains were cleaned manually with water, 700 mL L^{-1} ethanol and hypochlorite solution containing 10 mL L^{-1} available chlorine, then washed several times with sterilized water. This treatment was applied to prevent the growth of moulds. The washed grains were soaked in sterilized water for 40 h and subsequently drained and spread over paper filters on pre-sterilized stainless steel plates. Germination was carried out in an incubator at 26 °C for 7 days. The sorghum grains (malt) were revolved daily (to prevent excessive root malting). Spraying was done twice a day with sterilized water (to prevent drying out) (Thaoge *et al.*, 2003). Harvested sprouted samples were dried in an oven at 60 °C for 48 h after which the sprouts were removed. Finally, the sorghum grains were ground in a disc attrition mill to obtain a sorghum flour of 700 µm granularity.

Chemical analyses

Protein content determination

Crude protein (% total nitrogen x 6.25) was determined by using the Kjeldahl method outlined in AOAC (2010). This was followed by using 2 g dried powdered sorghum sample. This assay was carried out from the onset of germination till the end of the germination.

Crude fat determination

Crude fat was obtained by exhaustively extracting from 3g of sample in a Soxhlet apparatus (soxtec HT2 Tecator system) using hexane (boiling point range 68-69°C) as the extractant (AOAC, 2010). This assay was carried out daily from the onset of germination till the end of the germination.

Carbohydrate determination

One gram (1g) of macerated sample was weighed into a 25 mL bottle, 10 mL of distilled water was then added and shaken vigorously followed by addition of 15 mL of 52% perchloric acid. This was stirred continuously for 30 minutes and the mixture was later filtered using Whatman no1 filter paper. One millilitre (1 mL) of the filtrate was mixed with 4 mL of Anthrone reagent in a test tube and the absorbance of the mixture was measured using spectrophotometer at a wavelength of 620 nm. The total soluble carbohydrate was then estimated using the standard curve of glucose (Pearson *et al.*, 1976). This assay was carried out from the onset of germination till the end of the germination.

Application of kinetic model

Analysis was based on kinetic principles for zero or first-order so that a coherent evaluation of the data of carbohydrate, protein and fat obtained could be made. For zero-order, the rate of loss of compound, A, is constant at condition i.e.

$$A = A_0 - K_z t \quad (1)$$

While for first-order it is exponential

$$A = A_0 e^{-K_f t} \quad (2)$$

Where: A_0 = Initial concentration

A = Concentration of measured nutrient (carbohydrate, protein or fat) at time t

K_z = Constant rate of zero-order equation

K_f = Constant rate of first-order equation

The change in quantity indices, dC of a parameter both positively and negatively at a constant temperature, T (expressed in absolute) over a period of time can be described by a relationship according to Bello and Bello (2008).

$$\frac{dC}{dt} = \pm k(T).C^n \quad (3)$$

Where k = specific rate constant for the change

T = temperature in absolute value, K

n = order of reaction

Therefore, the integral of the differential form of equation (3) can be used directly to generate the n^{th} order of reactions. Based on first-order reaction for the degradation changes of any compound in any food or plant matrix reported by Bello and Bello (2008),

$$\ln\left(\frac{C}{C_o}\right) = -kt \quad (4)$$

Where C = concentration at process time, t

C_0 = initial concentration (at t = 0); and
k = degradation rate constant

Thus equation (4) allows the logarithm plots of experimental value of C/C_0 against process time, t in which the closeness R^2 value obtained to 1 is an indication of the validity of first-order reaction kinetics for the food system under consideration.

Statistical analysis

The statistical significance of the existing differences among the means of the experimental readings were subjected to analysis of variance using Statistical Package for Social Sciences version 14.0. Means were separated using Duncan's multiple range test at 95% confidence level (Odunlade *et al*., 2016).

3.RESULTS AND DISCUSSION

Effects of germination on sorghum macro-nutrients

Carbohydrate

As presented in Table 1, the carbohydrate content of the sorghum after steeping was 28.72%. This value increased to 29.74% after 24 h of germination. Degradation occurred from the first day of germination till the fifth day of germination. The carbohydrate content after the fifth day of germination was 12.01%. The carbohydrate content increased to 12.98% after the sixth day of germination and later decreased to 10.27% after the seventh day. There were significant differences ($p< 0.05$) among the values of carbohydrate measured on each germination day.

The increase in the content of carbohydrate after the first day of germination might be as a result of altered metabolic activity and respiration changes in the grain that can be influenced by environmental changes, that is, steeping and germination conditions that the sorghum grains were subjected to.

Fat

As presented in Table 1, the fat content of the sorghum grain after steeping was found to be 7.52%. This value increased to 7.71% after the first day of germination and later followed the degradation pattern for nutrient use up by embryo till the fifth day of germination (4.29%). After the sixth day of germination, the fat content was found to increase to 4.78% which later decreased to 4.23% after the seventh day of germination. The measured fat contents were significantly different ($p< 0.05$) from one another except for days 4 and 6 and days 5 and 7.

Table 1:Compositional changes of carbohydrate, protein and fat content in *Sorghum bicolor* L. Moench as influenced by germination

Germination time (day)	Carbohydrate (%)	Fat (%)	Protein (%)
0	28.72 ± 0.40^{b}	7.52 ± 0.05^{b}	12.08 ± 0.04^{b}
1	29.74 ± 0.02^{a}	7.71 ± 0.02^{a}	13.38 ± 0.02^{a}
2	27.28 ± 0.04^{c}	6.41 ± 0.02^{c}	11.29 ± 0.01^{c}
3	16.77 ± 0.03^{d}	5.68 ± 0.03^{d}	11.20 ± 0.03^{d}
4	15.24 ± 0.37^{e}	4.83 ± 0.02^{e}	10.72 ± 0.04^{e}
5	12.01 ± 0.01^{g}	4.29 ± 0.04^{f}	10.00 ± 0.01^{f}
6	12.98 ± 0.03^{f}	4.78 ± 0.01^{e}	10.71 ± 0.04^{e}
7	10.27 ± 0.02^{h}	4.23 ± 0.04^{f}	8.76 ± 0.01^{g}

Values are expressed as mean± standard deviation. Means in the same column with similar letters are not significantly different ($p< 0.05$).

Table 2: Application of zero-order kinetic models

Nutrients	Line equation	K value	R^2 value
Carbohydrate	$y = 29.972 - 3.099x$	-3.099	0.879
Protein	$y = 12.723 - 0.487x$	-0.487	0.760
Fat	$y = 7.552 - 0.534x$	-0.534	0.882

Table 3: Application of first-order kinetic models

Nutrients	Line equation	K value	R^2 value
Carbohydrate	$y = 0.096 - 0.166x$	-0.166	0.912
Protein	$y = 0.057 - 0.044x$	-0.044	0.757
Fat	$y = 0.018 - 0.093x$	-0.093	0.876

Protein

The protein content of the sorghum on successive germination days were presented in Table 1. The protein content of the sorghum after steeping was 12.08%. This value was increased to 13.38% after the first day of germination and latter followed the degradation expected for nutrient use up by the sprouting embryo till the fifth day of germination (10.00%). The protein content was found to increase to 10.71% after the sixth day of germination and later decreased to 8.76% after the seventh day. The degradation trend observed was found to be in support of the work by Correia *et al*. (2008) who reported increase in free amino acids as germination is increased. There were significant differences ($p< 0.05$) among the values of protein measured on each germination day except for days 4 and 6.

Kinetics of carbohydrate, protein and fat degradation in Sorghum grains during germination

The kinetic data presented in Tables 2 and 3 showed all the parameters necessary for determination of the order to which the reactions occurring to the food macro- nutrients during germination followed. Table 2 showed the equations and the R^2 (a statistic that gives information about the goodness of fit of a model) values for zero-order kinetic model simulated for carbohydrate, protein and fat. Table 3 showed the equation and the R^2 values for first-order kinetic models for carbohydrate, protein and fat. From the regression analysis (Tables 2 and 3) between individual food macro- nutrients and the length of germination, R^2 values were 0.912, 0.757 and 0.876 for carbohydrate, protein and fat respectively based on first-order kinetics and 0.879, 0.760 and 0.882 based on zero-order kinetics.

The R^2 values for first-order kinetics model were somewhat lower than those of zero-order kinetic model for protein and fat and hence zero-order kinetic model was satisfactory in describing the depletion of protein and fat in *Sorghum bicolor* L. Moench grains during germination. The degradation of carbohydrate can be satisfactorily described by first-order kinetics because of the relatively higher R^2 value when compared to that of zero-order kinetic model.

As expected, increase in germination days largely promoted macronutrients degradation. The reaction rate constants, K, determined

from the slope of the line obtained by least squares regression analysis for the best kinetic model that fits carbohydrate degradation was -0.166 while that of protein and fat were -0.487 and -0.534, respectively. The K values showed that there were decreases in concentration over time for each of the macronutrient. Steeper curve was obtained for carbohydrate which indicates a high degradation when compared to that of protein and fat. This thus depicts that increase in germination days negatively affects the concentration of food macro-nutrients in sorghum but has more profound effect on the carbohydrate more than protein and fat.

4. CONCLUSION

In this study, the compositional variation of macronutrients existing in sorghum grains during germination and the order of kinetics to which each of the food macronutrients followed were presented. The carbohydrate of the grains was found to degrade following first-order kinetics while protein and fat which made up part of the food reserve for embryo during germination degraded following zero-order kinetic model. The kinetics of degradation was high for carbohydrate due to the fact that it supplies the largest portion of the needed energy required for growth metabolism. The degradation model presented and the parameters obtained for germination of sorghum will enable the optimisation of industrial processes to which germinated sorghum is being used. Further work is required to characterise and quantify the breakdown compounds of each micronutrient as influenced by germination. Moreso, the microstructural changes of these food macronutrients during germination should also be evaluated.

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