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Change in water activity and fungal counts of maize-pigeon pea flour during storage utilizing various packaging materials

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Abstract

Maize-pigeon pea fortified flour has been processed with the potential to address protein-malnutrition, especially among children. This combination is preferred since cereals lack amino acids, such as methionine, and legumes lack lysine. When mixed together, the amino acid concentrations can be complemented. However, stored maize is an excellent substrate for *Aspergillus* spp., especially under warm (20-30^{0}C) and humid conditions (70-90^{0}C). This study investigated the changes in water activity and fungal counts in maize-pigeon pea flour stored for up to 8 weeks using different packaging materials. Maize pigeon flour was processed at different concentrations of 90:10 to 70:30 from fermented, dried, and milled maize and blanched, dehulled, and milled pigeon pea seeds. The flour samples were packaged into four different packaging materials: low and high density polythene bags, as well as plastic and aluminum containers. These containers were stored under simulated tropical conditions of 28$\pm$2^{0}C and 83$\pm$2 % relative humidity in an incubator. Water activity (a_w) of the flours was determined and fungi were enumerated using Petrifilm. Initial (day 0) a_w of samples ranged from 0.15 to 0.17; after 8 weeks, a_w ranged from 0.20 to 0.32 in low density polyethene, while lower a_w was recorded for samples stored in the plastic and aluminum containers. Initial fungal counts ranged from 1.69 to 2.31 $\log_{10}$ CFU/g which increased from a range of 2.45 to 2.78 $\log_{10}$ CFU/g after 8 weeks of storage, with higher counts in samples stored in low density polythene bags. These results indicate that a_w and fungal counts of the flours increased slightly over time in the different packaging materials, but values appear to be within tolerable limits. Further research will evaluate the stability of amino acids, as well as the level of fungi and resulting aflatoxin production up to 11 months under the storage conditions, formulations, and packaging materials described above.

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Keywords: maize-pigeon pea flour, storage, keeping quality, food safety;

1. Introduction

Maize-pigeon pea fortified flour has been processed with the potential for addressing protein- malnutrition, especially among children under five years old (Fasoyiro *et al,* 2013). The flour can be processed into different convenience foods, ranging from main meals to various snacks. This complementation is built on the basis that cereals lack amino acids, such as methionine and legumes lack lysine, which complement each other when mixed together (Potter and Hotchkiss, 1998). Fortified foods made from a maize-legume blend can serve as cheaper, nutritious, and healthy alternatives for people who cannot afford animal

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proteins, while also serving as a good protein source for vegetarians. Food products under different processing technologies must not only be nutritious, but safe (Fasoyiro *et al,* 2010). Stored maize is an excellent substrate for mold growth, such as *Aspergillus* spp. under warm (20-30^{0}C) and humid conditions (70-90^{0}C) (Popoola, 2003). Previous studies have reported storage properties of pigeon pea seeds in different containers (Bankole *et al,* 1996). Unacceptable levels of mold growth in flours can not only cause health risks, but also nutritional and economic losses of the product. Monitoring the final water activity (a_w) of these types of foods may be used for process control in Hazard Analysis and Critical Control Point (HACCP) programs. In such cases, samples of the food product are taken periodically and tested to ensure a_w values are within a specified range for food quality and safety. Therefore, it is important to understand what conditions will allow for the safety and quality of maize-pigeon pea flour utilized for human consumption. To our knowledge, very little research has addressed this issue. The findings from this research may provide alternative methods for the formulation of and storage conditions for pigeon-pea flours.

Objective: This study will investigate the changes in water activity and fungal counts in various formulations of maize-pigeon pea flour stored for up to eight weeks under tropical conditions, using different packaging materials**.**

2. Materials and Methods

2.1 Flour preparation

Maize was obtained from the Department of Plant Sciences, Penn State University, while pigeon pea seeds (*Cajanus cajan*) were obtained from Barry Farms (Wapakoneta, Ohio). Maize-pigeon pea flour was processed using a modified method of Fasoyiro *et al* (2013). Maize seeds were weighed, washed, and water was added at a ratio of 1:3 (w/v). The maize was fermented by soaking at 25$\pm$2^{0}C for two days. The fermented water was removed and resulting seeds were washed and drained (30 min), spread thinly on an aluminum tray, and dried at 65^{0}C for 8 hr in a convection oven. The dried maize seeds were milled into flour using a grinder (Thomas-Wiley, USA, Model 4) with a 2 mm sieve. Pigeon pea seeds were weighed, washed, and water was added at ratio of 1:3 (w/v). The pigeon pea seeds were blanched (30 min) in boiling water and washed under running water to cool down the seeds to 25-30^{0}C. The seeds were dehulled mechanically by shredding 200 g of the blanched seeds in 200 ml of water for 30 sec in a blender (Hamilton Beach, USA, Model 56250). Hulls were removed by washing the pigeon pea seeds with water and sieving. Dehulled pigeon pea seeds were allowed to drain for 30 min, spread thinly on an aluminum tray, and dried at 65^{0}C for 8 hr in a convection oven.

Flour mixtures were made as follows: milled maize flour was mixed with pigeon pea flour at ratios of 90:10, 80:20, and 70:30. Maize flour only and pigeon pea flour only were used as controls. The resulting flour products were stored using four different packaging materials: low density polythene bag (3" x 7.25", 0.057 mm thickness; with closure tape) (Nasco WHIRL-PAK, USA), high density polythene bags (6" x 10", 2 mm thickness; Associated Bag Co., USA), plastic jars (2.6"x 3.5'', 8 oz white polypropylene plastic jar with white screw top), or aluminum containers (2.8" x 1.7'', 6 oz silver seamless tin with slip cover top) (Freund Container and Supply, USA) for a period of eight weeks. The flour products were stored under simulated conditions of 28$\pm$2^{o}C in an incubator at 83$\pm$2% relative humidity (RH) using saturated potassium chloride salt (50%).

2.2 Water activity

An Aqua Lab 4TE water activity meter (Decagon Inc., USA) was used to measure water activity of the flours (APHA method 2.532; 2015). Prior to sampling, the flour was first shaken to ensure even distribution in the various packages. A representative flour sample (3 g) was taken from the center of the package and transferred into a disposable sample container (15ml 1.53" x 0.447" white plastic cup) (Decagon Inc, USA). The water activity meter was first calibrated with 0.760 a_w (NaCl 6molar in water and 0.984 a_w KCl 0.5 molar in water) and sample readings were taken at 23 $\pm$2^{0}C. All flour samples were analyzed in duplicate and readings averaged.

2.3 Microbiology

Yeast and Mold Petrifilm (3M; Minneapolis, MN) was used for the enumeration of fungi and plates were stored at 4^{0}C until used. Flour samples were prepared for microbial evaluation by mixing 10 g with 90 ml of Buffered Peptone Water (BPW; BBL, Cockeysville, MD). Samples were homogenized in a stomacher (Stomacher 400 Circulator, Seward, USA). Resulting stomachates were serially diluted in 9 ml of BPW and plated directly on the Petrifilm using a sterile pipette. The sample was spread across the Petrifilm using a plastic spreader following instructions by the manufacturer. Plates were incubated at 25^{0}C for 5 days and enumerated manually. The platings were performed in duplicate. For Petrifilm, samples were enumerated in totality and also based on morphology of the colonies (brown, green, black colonies). Samples were transferred to fresh agar slants after growth by peeling back the Petrifilm and picking the colonies with a sterile loop. Slants were incubated for 5 days at 25°C prior to visual inspection and confirmation.