DEVELOPMENT OF AN ENZYMATIC PROCEDURE TO PRODUCE HIGH-PROTEIN AMARANTH FLOUR

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SUMMARY

A basic procedure was developed to produce high-protein amaranth flour (HPAF) using a commercial preparation of heat-stable alpha-amylase. Slurries (20%, w/v) of gelatinized whole flour were liquefied at 70 and 90°C, pH 6.5, 0.1% (w/v) enzyme concentration and 30 min hydrolysis time. Protein content of raw flour was increased from 15 to 29.6 or 39.3% at liquefaction temperatures of 70 or 90°C, respectively. Some physicochemical and functional properties of HPAF were assessed. HPAF might be used as a dry milk extender.

INTRODUCTION

Microbial enzymes have contributed to the success of various enzymatic conversion processes at a commercial scale by meeting process cost requirements and demands for activity under unique manufacturing conditions (Sheppard, 1986; Matsumara et al., 1988). Hansen et al. (1981) reported a procedure for preparation of high-protein rice flour using fungal alpha-amylase to hydrolyse the starch followed by separation of the soluble carbohydrate by-product. Other workers (Chen and Chang, 1984; Brooks and Griffin, 1987) reported increased yields of solubilized starch while simplifying the overall process of Hansen et al. (1981). They proposed to use the liquefied starch as sweetener and the insoluble residue as high-protein flour.

On the other hand, amaranth is an ancient crop of Mexico, Central and South America. Seeds contain relatively high levels of lysine and adequate amounts of tryptophan and sulfur amino acids. Unfortunately, present consumption of this cereal-like is practically negligible (Paredes-López et al., 1989).
The study reported here provides information concerning a process developed to produce high-protein amaranth flour (HPAF) by the enzymatic hydrolysis of whole flour.

**MATERIALS AND METHODS**

**Commercial preparation of alpha-amylase.** A heat-stable alpha-amylase (Taka-Therm II L170) was provided by Enmex, S.A., México, D.F. It was produced by Bacillus licheniformis, with an activity of 170,000 modified Wohlgemuth units (MWU)/g, with one MWU being defined as that activity which will dextrinize one mg of soluble starch to a defined blue value in 30 min.

**Amaranth and dry whole milk samples.** Mature seeds of Amaranthus hypochondriacus were harvested in the experimental station of INIFAP, Chapingo, México. Samples were passed through a centrifuge grinding mill (Brinkman Inst. Co., Westbury, NY) using an 80 US mesh sieve and kept at 4°C until used. Dry whole milk, imported by CONASUPO, a government organization, was purchased in the local market.

**Liquefaction of amaranth flour slurry.** Amaranth slurries of whole flour were prepared with distilled water (20%, w/v), pH adjusted to 6.5 and gelatinized in boiling water for 5 min. Sample flasks were placed into a shaking water bath at 70 or 90°C, then amylase was added (0.1% v/w), and kept at these conditions for 30 min to liquefy the starch. After incubation, samples were cooled rapidly in ice water and centrifuged at 9,000xg for 30 min at 4°C. The freeze-dried precipitate, termed HPAF, was kept at 4°C for subsequent analysis.

**Analytical methods.** Moisture, protein (%N x 5.85), fat, crude fiber, ash and starch were determined according to AOAC (1984) procedures. Nitrogen conversion factor for dry whole milk was 6.38. For carbohydrates (sugars) determination, 0.25 g of sample were extracted in hot aqueous EtOH (80% conc.) (Hassid and Neufeld, 1964) and then extracts were quantitated according to the method of Dubois et al. (1956). Surface color of samples was measured using a Hunter-Lab model D25-2 Color Difference Meter (Hunter Associates, Inc., Reston, VA). L, a, and b values were recorded as compared to a white standard with the following values: L, 91.2; a, -1.0; and b, -1.7. Total color difference (ΔE) was calculated from the previous Hunter parameters. Water hydration capacity (WHC) was determined according to the 88-04 method (AACC, 1984). Flour dispersibility was measured suspending 1 g of sample with 10 ml distilled water into a graduated conical tube. Sample was stirred for 1 min and layer volume with suspended particles registered after a 30-min rest.

**RESULTS AND DISCUSSION**

The flow diagram of process developed is given in Fig. 1. After enzyme digestion, HPAF was recovered by centrifugation. At the liquefaction temperature of 70°C protein content of HPAF (29.6%) was almost twice as high as raw material, and at 90°C this value was even higher (39.3%) (Table 1). As a result of this hydrolysis, a remarkable