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Combined effects of fermentation and germination on nutritional compositions, functional properties and volatiles of maize seeds

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ABSTRACT

Nutritional compositions, functional properties and volatiles of maize seeds affected by combined processes of fermentation and germination were evaluated. After maize seeds were germinated, they were fermented with 4 microbial strain; *Lactobacillus plantarum*, *Lactococcus lactis*, *Bacillus subtilis* and *Bifidobacterium longum*. The presence of *B. longum* could enhance the maximum nutritional compositions of fermented germinated maize seeds. Protein and vitamin E content were increased up to two-fold, total phenolic content up to three-fold, vitamin B1 up to four-fold, GABA content up to five-fold, and antioxidant activities; DPPH• and ABTS•$^{+}$ up to two-fold after germination and fermentation processes. Total phenolic content and its positively correlated antioxidant activities (DPPH• and ABTS•$^{+}$ radical scavenging activity), and α-glucosidase were the highest in germinated maize fermented with *B. longum* for 72 h. Twelve volatile compounds were generated in fermented germinated maize samples which major volatiles were pyrazine and 3-hydroxymandelic acid (sweet, corn, creamy odor).

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1. Introduction

Recent epidemiological studies have indicated a protective role of cereal grain foods against several diseases, associated with westernized societies such as cardiovascular disease and certain cancers (Jacobs and Gallaher, 2004). The bioactive components of maize are being intensively studied for their effects on health with respect to their chemical structure and function, and many postulated possible mechanisms (Slavin et al., 2001). Maize (*Zea mays* L.), a pseudo cereal food and commonly known as corn, has gained a great deal of attention due to its important components of the daily diet, providing carbohydrates, protein, fat, fiber, minerals, vitamins, and antioxidant activities.

However, the amount of nutritive compositions which has high antioxidant activities and biological effects, is very small and, therefore, the enhancement of nutritional compositions is by germination and fermentation processes. During germination (i.e. hydrothermal treatment in ambient conditions), the biosynthetic potential of grains is exploited and a number of hydrolytic enzymes are synthesized. The reactions in germinating seeds lead to structural modification and the synthesis of new compounds (Kaukovirta-Norja et al., 2008). Apart from changing the level of nutrients, the biochemical activities occurring during germination can also generate bioactive components; gamma amino butyric acid (GABA) (Nagaoka, 2005; Watcharatparpaiboon et al., 2010), phenolics (Donkor et al., 2012) and α-glucosidase inhibitor (Girish et al., 2012). Other bioactive components resulting antioxidant activity not only from phenolics but also tocopherols, ferulic acid (Iqbal et al., 2005), are increased during germination (Donkor et al., 2012; Frias et al., 2005). During germination, activated hydrolytic enzymes also hydrolyzed large molecular substances, such as starch, non-starch polysaccharides and proteins, to small molecular compounds. These processes result in an increase of simple sugars, peptides, amino acids and vitamins (Frias et al., 2005) of germinated seeds, as reported in germinated wheat (Nagaoka, 2005).

Fermentation is also a very interesting process used in plant foods to increase nutritional qualities and remove undesirable compounds (Sodhi et al., 2005). Fermentation of food involves the action of microorganisms or enzymes that cause desirable biochemical change and significant modifications in flavour and texture (Heinio et al., 2003). In cereal fermentations, endogenous enzymes, bacteria, yeast and moulds play important roles either singularly or in combination, and contribute to the creation of a great variety of products. Many biochemical changes occur during fermentation, leading to altered ratio of nutritive components of cereals, which affect product properties such as bioactivity and

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some chemical compounds (Katina et al., 2007). Various fermented cereals by *Lactobacillus plantarum* and *Lactococcus lactis* for biosynthesis of γ-aminobutyric acid (GABA), have been studied in chickpea, amaranth, quinoa and buckwheat (Coda et al., 2010). Moreover, Nout (2009) reported that fermented rice, sorghum and millet with two probiotic strains: *Lactobacillus* sp. and *Pendiococcus* sp. had functional and organoleptic properties. In year 2008, Moktan et al. have revealed that soybean fermented with *Bacillus subtilis* to kinema enhanced free radical-scavenging activity, metal-chelating ability, reducing power and lipid peroxidation inhibitory activity. Fermented soybean with *Bifidobacterium infantis* and *B. longum* individually, and in combination resulted in the change in the content of various isoflavones (aglycones, glucoside, acetyl and malonyl-glucosides) and β-glucosidase activity (Lee and Lee, 2001).

However, the combination of these two processes (germination and fermentation) on nutrition, functional properties, and volatiles has not yet been studied in maize. Some works have reported the biochemical compounds and activities occurring during germination (Nagaoka, 2005; Watchararparpaiboon et al., 2010), and fermentation-induced changes in the nutritional value of germinated cereal (Katina et al., 2007). In this study, the effects of germination and fermentation on nutrition compounds, phenolics, functional properties (anti-oxidative properties and α-glucosidase inhibitor) and volatile profile change of maize seeds were studied. In order to improve the antioxidant activities and to obtain high nutritional compositions and good volatile flavor from germinated maize fermented with four microbial strains (*L. plantarum*, *L. lactis*, *B. subtilis* and *B. longum*.) at various fermentation times were conducted.

2. Materials and methods

2.1. Maize sample preparation

Maize variety 'High Brix # 3' was purchased from Marketing Organization for Farmers (MOF), Bangkok, Thailand. Maize seeds were cleaned by removing the dirt and then dried with a hot air oven (Memmert, UFE600, Germany) at 55 °C for 10 h, to a moisture level of 10%. The dried samples were kept in a refrigerator (10 °C ± 2) prior used.

2.2. Microorganism

Three lactic acid bacteria; *Lactobacillus plantarum*, *Lactoccocus lactis*, and *Bifidobacterium longum*, and a proteolytic bacteria; *Bacillus subtilis*, were supplied by the Thailand Institute of Scientific and Technology Research (TISTR). The starter (dried inoculated) was inoculated into the nutrients broth and incubated for 36 h at 37 °C. After incubation, 0.1 ml suspension was streaked on nutrient agar media and incubated 24 h at 37 °C until the single colony growth occurred. The single colony was added to the broth media and inoculated to give a final inoculum of 1×10^8 CFU/ml (Fernandez-Orozco et al., 2009).

2.3. Preparation of combined process of germination and fermentation

A hundred grams of maize seeds was soaked in distilled water at 40 °C for 6 h, after that the distilled water was drained. The soaked maize seeds were then incubated in a nylon net and placed in a plastic box in the dark at room temperature (25 °C ± 2). The germinated seeds were dried in a hot air oven at 55 °C for 10 h, then stored in a refrigerator at 4 °C until used (Watchararparpaiboon et al., 2010).

Germinated maize seeds were fermented with three lactic acid bacteria and a proteolytic bacteria. Maize seeds (200 g) were added 10 ml distilled water and sterilized in autoclave for 15 min. The sterile maize samples were then inoculated with *L. plantarum*, *L. lactis*, *B. subtilis*, and *B. longum* (10 ml of inoculum/100 g dried sample). All samples were allowed to ferment at 37 °C for 0 (control), 12, 24, 48 and 72 h. After fermentation, samples were dried in a hot air oven at 55 °C for 10 h. All samples were then stored in a desiccator at 4 °C prior analysis (Katina et al., 2007).

2.4. Determination of nutritional compositions

2.4.1. Proximate analysis

Maize samples; non-germinated maize, germinated maize and fermented germinated maize with 4 microbial strains were grinded by grinding machine (IKA M20, Germany) to a fine powder, and sieved pass a 0.149 mm (sieve size 100 mesh) and stored at 4 °C prior to analysis. Proximate analyses were determined according to AOAC (2005). Moisture content was measured by drying in a hot air oven at 105 °C for 3 h. Fat content was measured by extraction with petroleum ether in a Soxhlet system. Proteins were analysed as total nitrogen content by the Kjeldahl method, and the conversion factor used to transform nitrogen into protein was 6.25. Finally, ash content was determined by incineration of samples at 550 °C in a muffle furnace (Nabertherm B150, Germany) and total available carbohydrates were estimated by difference.

2.4.2. Determination of thiamine (Vitamin B1)

The vitamin B1 was determined by two methods with spectrophotometry (Liu et al., 2002) and HPLC (Plonka et al., 2012). For spectrophotometry method, filtrates of water extracted samples (1 ml) were added with 1.5 ml of NH_4Cl–NH_4OH buffer, plus 1 ml of Triton X-100, and adjusted volume to 10 ml with distilled water. Two drops of dye solution (0.05% phenol red) were added, and then shaken for 10 min. After reaction, the absorbance was measured by spectrophotometer (G-10, UV Scanning, Thermo, Germany) at 420 nm against blank. Standard stock solution of vitamin B1 was prepared by dissolving 25 g of standard thiamine in 50 ml of distilled water. However, vitamin B1 by HPLC was determined by diluting samples with methanol, and filtered with 0.45 μm nylon filter. All samples (20 μL) were analysed by C18 reverse phase silica column (Eclipse XDB- C18, 5 mm, particle size 4.6 × 150 mm) of a HPLC consisting of the diode array detector (Agilent LC-1000, Germany). HPLC grade of methanol, trifluoroacetic acid (TFA), and water used for the mobile phase was purchased from Merck (Darmstadt, Germany). Gradient elution with 0.01% TFA in water (A) and methanol (B) was applied as following: 0–4 min, 95%A+ 5% B (flow rate 0.6 ml/min); 4–13 min, 2%A+98%B (flow rate 0.7 ml/min); 13–25 min, 0%A+ 100%B (flow rate 1.3 ml/min). Wavelength programmed at 253 nm was used for thiamine analysis.

2.4.3. Quantification of α-tocopherols (vitamin E)

The method of α-tocopherols determination was modified according to Kurilich and Juvik (1999) as follows. Three gram sample were placed in a screw-cap tube and 0.25 g ascorbic acid and 7.3 ml saponification solution were added. The saponification solution was 70% ethanol in distilled water (v/v) with 10% potassium hydroxide (w/v). The tubes were placed in ultrasonic bath (S 30 H Elma Sonic, Germany) for 20 min at 80 °C. After the saponification, the tubes were cooled in tap water for 1 min, and 4 ml hexane + 2 ml distilled water were added. The added water increased the polarity of the aqueous phase and improved partitioning of vitamin E into the hexane phase. Tubes were vortexed and the upper layer (hexane) was collected in a screw-cap vial for analysis by C18 reverse phase column HPLC, consisted of diode array detector. Twenty microliters were injected into a silica

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