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#### Analytical Methods

# Effects of cold extrusion process on thiamine and riboflavin contents of fortified corn extrudates

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#### ABSTRACT

In this study, corn extrudates were produced from fortified corn flour by conventional and cold extrusion techniques at different barrel temperatures of 80, 110, 130 and 80 °C, respectively, and feed moisture contents. Thiamine and riboflavin contents of extrudates were determined by HPLC. Thiamine contents of the samples produced at feed moisture contents 20% and 25% decreased as temperature increased. There was no significant difference between riboflavin contents of conventional extrudates produced at both feed moistures at 80 and 110 °C barrel temperatures. However, riboflavin content of extrudates produced at 20% feed moisture was higher than the one produced at 25% feed moisture at 130 °C. In cold extrusion, there was no significant difference between riboflavin contents of samples. The samples produced by  $CO_2$  injection had the lowest expansion index and uniform air cells. However, the samples produced by conventional extrusion had higher expansion index and size distributions of air cells were not uniform.

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#### 1. Introduction

#### Extrusion cooking is a high-temperature, short-time processing technique, which has been increasingly used for the production of breakfast cereals, puffed snacks and various other cereal products by cooking and puffing corn grits and wheat flour (Athar et al., 2006; Ryu & Ng, 2001). Since cereal products are among the most important sources of B vitamins and comprise an important part of the diet, retention of micronutrients like B group vitamins during processing has a considerable interest (Athar et al., 2006; Cheftel, 1986). Recent research suggests inadequate vitamin status for great part of population in many countries (Agostini, Scherer, & Godoy, 2007). Vitamins are crucial for maintaining good health in humans, thus lack of a sufficient amount of any of them can cause serious diseases (Combs, 1992; Zafra-Gómez, Garballo, Morales, & García-Ayuso, 2006). In order to solve the vitamin deficiency problem, fortification of cereal products by increasing the level of consumption of deficient micronutrients plays an important role in terms of public health (Ranum, 2000). Therefore, it is very

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important to determine the vitamin levels precisely in enriched extrusion products.

Although, significant improvement was accomplished in reduction of the B-vitamin deficiencies in the world, there are periodic reports of outbreaks of B-complex vitamin deficiencies, linked to insufficiency of B vitamins in populations especially under suffering conditions (FAO/WHO, 2001). There are a lot of publications on B-complex deficiency but they probably underestimate the extent of the deficiency. Many cases are not reported in the literature and also these deficiencies are not publicised by governments since these conditions may be considered as politically sensitive information (FAO/WHO, 2001). Harper (2006) reported that there is an increase in the prevalence of Wernicke-Korsakoff syndrome (WKS) due to thiamine deficiency in almost every country in the world, thiamine deficiency in infants is a major problem in third world countries and is still prevalent in elderly groups in many countries. Another report by WHO and FAO indicated that prevalence of riboflavin deficiency is alarmingly high (WHO/FAO, 2006). Therefore, fortification of cereal products by these micronutrients is a choice in the solution of the vitamin deficiency problem.

Thiamine (vitamin  $B_1$ ) and riboflavin (vitamin  $B_2$ ) are two of the major water-soluble vitamins used to enrich cereal products (Mauro & Wetzel, 1984). Determination of these vitamins in foods is generally carried out by microbiological, fluorimetric, spectrophotometric and HPLC methods. Official methods that use



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microbiological procedures show high specificity and reliability but are time consuming whereas the specificity of the fluorimetric methods is low. Therefore, high performance liquid chromatography (HPLC) methods become popular in terms of providing short analysis times, high specificity and selectivity. Also HPLC methods allow determination of thiamine and riboflavin together from the same extract (Eitenmiller & Landen, 1999; Esteve, Farré, Frígola, & García-Cantabella, 2001). Most HPLC methods use either UV or fluorescence for detection of thiamine (Lynch & Young, 2000). Liquid chromatography with fluorescence detection has been preferred using either the natural fluorescence of the vitamins such as riboflavin or with derivatization to form a suitable fluorescent complex like thiochrome from thiamine (Finglas, 1993). The HPLC method of Finglas and Faulks (1984) is still being used in current publications dealing with thiamine and riboflavin analysis (Caglarirmak, 2009; Erkan, Selcuk, & Ozden, 2010; Ersoy & Ozeren, 2009).

The conditions used in conventional extrusion of cereals would be inconvenient to heat labile ingredients such as vitamins, functional proteins, flavours that may be incorporated in the formulation (Jeong & Toledo, 2004). There have been several studies examining the effects of extrusion process on the retention of B group vitamins (Athar et al., 2006; Camire, Camire, & Krumhar, 1990; Cha, Suparno, Dolan, & Ng, 2003; Cheftel, 1986; Ibanoglu, Ainsworth, & Hayes, 1997; Killeit, 1994; Schmid, Dolan, & Ng, 2005). Björck and Asp (1983) and Schmid et al. (2005) suggested that one of the disadvantages of extrusion process carried out at high temperatures and low moisture contents was the loss of vitamins because of breaking of chemical bonds during mixing or the loss of vitamin stability due to heat. Investigations of an increase in thiamine loss with an increase in temperature in extruded products have been reported (Cha et al., 2003; Cheftel, 1986; Guzman-Tello & Cheftel, 1987; Ilo & Berghofer, 1998; Schmid et al., 2005).

The objective of this research was to produce fortified corn extrudates by conventional and cold extrusion techniques at different barrel temperatures and feed moisture contents and study the effects of process variables on thiamine and riboflavin contents by high performance liquid chromatography (HPLC) as well as expansion index, bulk density and SEM properties of these extrudates produced from fortified corn flour.

#### 2. Materials and methods

#### 2.1. Materials

Commercial corn flour was obtained from Shin Dong Bang CP, Ansan, Korea and vitamin premix from DSM Nutritional Products Europe Ltd., Switzerland.

#### 2.2. Chemicals and reagents

Thiamine hydrochloride and riboflavin were obtained from Labor Dr. Ehrenstorfer-Schlosser (Augsburg, Germany). Methanol (HPLC grade) and potassium hexacyanoferrate were purchased from Sigma-Aldrich (Steinheim, Germany). Water (HPLC grade) and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Sodium acetate trihydrate was purchased from Riedel-de Haën (Steinheim, Germany). Sulphuric acid was obtained from J.T. Baker (Daventer, Holland). Takadiastase enzyme was from Fluka (Buchs, Switzerland).

#### 2.3. Methods

#### 2.3.1. Chemical analysis

Moisture contents of extrudates were determined according to AACCI Method No. 44-01 (2000).

#### 2.3.2. Extrusion process

2.3.2.1. Sample preparation. Corn flour was enriched with B vitamins using a vitamin premix (DSM Nutritional Products Europe Ltd., Switzerland). The vitamin premix was diluted in several steps to achieve reasonable and uniform level of mixing/blending without concentrated pockets of premix (Wesley, 2005).

2.3.2.2. Conventional extrusion process. Corn flour was extruded in a co-rotating twin-screw extruder (THK 31, Incheon Machinery Co., Incheon, Korea) with a 25:1 screw length to diameter ratio. The extruder was equipped with a circular die of 3.0 mm diameter. The screw configuration is shown in Fig. 1. Water was injected into the barrel around the feed section to adjust the moisture content at 20% and 25%. Barrel temperature was adjusted to 80, 110 and 130 °C. When the extrusion system reached a steady state as indicated by constant torque, pressure and temperature, extrudate samples were collected. All collected samples were oven-dried at 50 °C to adjust the moisture content to less than 12% before grinding in a laboratory mill followed by passing through a 212  $\mu$ m sieve. The ground extrudates were stored in air-tight plastic containers and held at 4 °C until analysis.

Twin-screw extruder was used to produce the extrudates at different processing variables (Fig. 1). The extrudates were produced in two replicates which were produced at two different times.

2.3.2.3. Cold extrusion process with  $CO_2$  gas injection. The same extrusion equipment was also used in the cold extrusion process. Barrel temperature was adjusted to 80 °C.  $CO_2$  was injected into extruder barrel through a valve at the pressure of 1 MPa. The point of  $CO_2$  injection was 180 mm before the die. The feed moisture contents were 20% and 25% as in conventional extrusion.

#### 2.3.3. Scanning electron microscopy (SEM)

Extrudate samples were cut into 4-5 mm thick slices perpendicular to the longitudinal axis and mounted on aluminium stubs with carbon paste. A thin layer of conductive carbon paste brushed on the side of each sample to conduct electrical charge from the coated specimen surface to the stub to eliminate charging from the coated surface during scanning. The mounted samples were

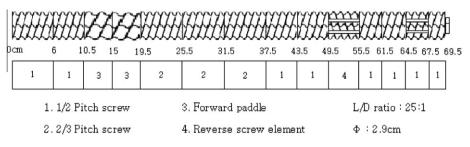


Fig. 1. The screw configurations of twin-screw extruder.

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