CHAPTER 8

Estimation of Protease Activity by Use of the Mixolab

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1. PROTEASE IN WHEAT

It is known that the preharvest attack on wheat by some heteropterous insects (*Eurygaster* spp., *Aelia* spp., and *Nysius huttoni*) reduces the breadmaking quality of the resulting wheat flour (Lorenz and Meredith 1988, Swallow and Every 1991, Harriri et al 2000). The problem is quite common in Mediterranean, Middle Eastern, and East European countries. Before harvest, these insects inject their salivary secretions into maturing wheat ears when they feed. Following attack by the insect, the grain matures normally, but a light-colored opaque patch surrounds the site where the insect pierced the grain. The penetration point is visible as a small dark spot in the middle of this patch. The damaged area is softer and usually collapses when pressed by a fingernail. The secretions contain proteases that break down the gluten structure during mixing and fermentation (Kretovich 1944; Every et al 1998; Sivri et al 1998, 1999).

During the early stages of kernel development (e.g., the milk ripe stage), most of the kernel contents can be sucked out by the insect, resulting in smaller, lighter, and shriveled kernels. Such kernels can be easily separated in the cleaning section of flour mills. Therefore, these kernels do not cause significant problems for flour quality (Koksel et al 2002). However, during the later stages of kernel development, a small part of the kernel is affected by the damage; the kernels retain their normal size and shape and are difficult to separate in the wheat-cleaning section of flour mills. Flour obtained from bug-damaged wheat has weak dough properties and poor baking quality due to enzymatic degradation of gluten proteins (Cressey and McStay 1987; Sivri and Koksel 1996; Sivri et al 1998, 1999; Koksel et al 2001).

Various rheological methods, a modified sedimentation test, a microassay based on the measurement of gluten-gel height, and a baking test have been used for the detection of bug damage in wheat (Kretovich 1944, Greenaway et al 1965, Every 1991). It has been reported that the protease of *N. huttoni* could not be assayed by proteolytic activity methods using conventional substrates such as hemoglobin, azocasein, cytochrome-c, albumin, fibrin, gelatin, or hide powder (Meredith and Best 1985; Every 1989, 1991). Preliminary studies also showed that conventional methods for protease activity were not suitable for determining protease activity of the wheat bug (*Eurygaster* spp.). Sivri and Koksel (2002) have developed two spectrophotometric methods using gluten and glutenin as substrates for determination of bug protease activity. Their results suggested that *Eurygaster* spp. proteases exhibit considerable substrate specificity for gluten proteins.

The Mixolab is a new instrument, and the information related to its utilization on different aspects of wheat flour quality is quite limited. However, the information so far published on the Mixolab indicates that it has a high potential for use in cereal quality research (see Chapter 1). Recently, it was used for the assessment of the quality of different wheat genotypes (Koksel et al 2009). It was also utilized to investigate the effects of hydrocolloids (Rosell et al 2007) and flaxseed on the rheological properties of dough. Bonet et al (2006) investigated the effectiveness of transglutaminase for the formation of heteropolymers of wheat and wheat-exogenous proteins by using a Mixolab instrument. Kahraman et al (2008) and Ozturk et al (2008) tested the possibility of using it to predict the cake-baking and cookie quality of different wheat flours, respectively.

The main purposes of this study were to test the possibility of using a Mixolab to investigate the effects of suni-bug damage on flour samples and to develop a new Mixolab protocol to approximately estimate suni-bug damage in flour.

2. EXPERIMENTAL

2.1 Materials

For the first part of the research, sound and suni-bug-damaged samples of four wheat cultivars (cvs. Bezostaya, Demir, Gerek, and Zencirci) obtained from the Field Crops Research Center (Ankara, Turkey) were used. Wheat samples were milled in a Bühler laboratory mill (MLU 202, Uzwil, Switzerland) to obtain straight-grade flour according to AACC International Method 26-21.02 (AACC International, no date).

For the second part of the research, a durum wheat sample (cv. Svevo) heavily damaged by the suni-bug (*Eurygaster* spp.) was obtained from contracted farmers (Sanliurfa, Turkey). Weak and strong wheat flour samples milled from sound wheats were obtained from CHOPIN Technologies (Villeneuve la Garenne, France). A moderately suni-bug-damaged wheat flour sample was obtained from the Field Crops Research Center.

2.2 Preparation of Suni-Bug Enzyme Extract

Suni-bug enzyme extract was prepared from the heavily bug-damaged wheat sample (cv. Svevo) according to the method of Sivri and Koksel (2002) with some modifications. Bug-damaged wheat samples were ground to pass through a 0.5-mm screen in a Udy cyclone mill (UDY Corp., Fort Collins, CO). The whole meal (300 g) was mixed with distilled water (1,500 mL) and stirred for 16 h on a magnetic stirrer at 4°C. The suspension was centrifuged at 15,000 × *g* for 10 min at 4°C. The resulting supernatant was freeze-dried and ground in a coffee grinder (Moulinex type A505, France) to obtain a crude enzyme extract.

2.3 Analytical Methods

Moisture contents and sedimentation values of the flour samples were determined by AACC International Approved Methods 44-15.02 and 56-60.01, respectively (AACC International, no date). For the sedimentation analyses, portions of the weak and strong flour samples were replaced by the moderately bug-damaged flour or the crude suni-bug enzyme extract at several levels. A modified sedimentation test was made to estimate the level of insect damage in flour samples (Greenaway et al 1965, Koksel et al 2002). Flour samples were incubated for 2 h at 35°C in a sedimentation test tube after addition of the bromphenol blue solution, to allow proteolytic reactions to occur.

Change in the sedimentation value was calculated as follows:

$$\mathrm{CSV}(\%) = \frac{(\mathrm{MSV} - \mathrm{SSV})}{\mathrm{SSV}} \times 100$$

where CSV is change in sedimentation value, MSV is modified sedimentation value (mL), and SSV is standard sedimentation value (mL).

2.4 Mixolab Analysis

The effects of suni-bug enzyme on wheat flours were studied by using a Mixolab. For this purpose, two protocols (Protocols A and B) were created.