NOTE

Distribution of Fungal Biomass Among Fine Bran, Coarse Bran, and Flour from Wheat Stored at Four Different Moisture Levels

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ABSTRACT

The fungal content of flour, fine bran, and coarse bran from wheat stored at moisture contents of 12, 16, 20, and 25% was determined as numbers of colony-forming units and as ergosterol levels. The highest values were found when wheat was stored at high moisture contents. In the sound wheat, 97% of the fungal biomass (ergosterol) was found in the bran fraction, which constituted only 31% of the kernel weight. At higher storage moisture, a more even distribution of fungal biomass was found. A weak correlation was found between the number of fungal propagules and ergosterol content. The high concentration of fungal biomass in the bran indicates that a potential mycotoxin hazard is associated with the use of bran to increase the content of dietary fiber in various foods.

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In recent years, the demand for cereal products with high levels of dietary fiber has grown. Bran from wheat and oats is being used to an increasing extent in bread and breakfast cereals. Therefore, the microbiological quality of this bran needs to be evaluated.

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Fungi are the most important spoilage organisms in cereal grains. Although one can assume that the early stages of infection by field fungi (such as *Fusarium* and *Alternaria* spp) occur mainly in the outer layers, little quantitative data exist on the spatial distribution of fungal biomass in cereal grains. However, using an immunofluorescent technique, Warnock (1971) observed that fungal hyphae were predominant in the outer layers of the seedcoat of barley grain. Young et al (1984) found that the highest levels of the fungal membrane-component ergosterol and the mycotoxin deoxynivalenol were in the bran fraction after milling of soft

winter wheat.

Determinations of the numbers of colony-forming units (CFU), which are routinely used to estimate mycological quality, do not accurately represent the fungal biomass in a sample (Pitt 1984). CFU values mainly reflect the amount of sporulation that has taken place on the outside of the grain. The ergosterol assay (Seitz et al 1977) has instead become an increasingly used alternative for determining the mycological quality of cereals (Müller and Lehn 1988). Ergosterol is a fungal-specific membrane component (Weete 1980) well correlated with other markers of fungal growth, such as chitin (Seitz et al 1979). A primary objective of the present study was to use the ergosterol assay to quantify fungal biomass in bran fractions and flour from wheat stored at different moisture contents. A secondary objective was to compare the results from the ergosterol assay with those from the time-consuming traditional determination of CFU values.

MATERIALS AND METHODS

Wheat Grain

Winter wheat of breadmaking quality with 12% moisture content was obtained from a commercial mill in Uppsala, Sweden. The wheat came from south-central Sweden and was harvested in 1989, a year with little rainfall during the later part of the growing season and during the harvest. The resulting wheat grains were of good hygienic quality. Subsamples (5 kg) were wetted to 16, 20, and 25% moisture content and incubated at 24°C for one week. On day 7, mould was visible at the highest moisture content. The grains were then spread on trays and dried for three days at room temperature to 14% moisture content.

Milling

Before milling in two laboratory mills (Bühler Laboratoriums, Mahlautomat model MLU-202, Gebrüder Bühler Maschinenfabrik, Uzwil, Switzerland), all samples were moistened to 16–17% water content for 24 hr. The milling produced three fractions: flour (70–75%), coarse bran (12–16%), and fine bran (9–19%) (Table I). The pericarp was the main component of the coarse bran, whereas the aleurone layer and the germ predominated in the fine bran.

Ergosterol Assay

The procedure used was adapted from Seitz et al (1977). Samples of whole kernels, flour, or bran (20 g) were refluxed with 80 ml of methanol at 80°C for 2 hr. After filtration through a glass filter, 10 ml of 4% potassium hydroxide in 96% ethanol was added to hydrolyze sterol esters, and the refluxing was continued for 30 min. After cooling to ambient temperature, sterols were removed from the alcoholic base by partitioning into pentane.

TABLE I
Relative Distribution of Mass and Ergosterol Among Flour, Fine Bran, and Coarse Bran from Wheat Stored at Different Moisture Contents

Moisture Contents	Mass (%)	Ergosterol (%)
12%		
Flour	69	3
Fine bran	19	43
Coarse bran	12	54
16%		
Flour	75	25
Fine bran	10	21
Coarse bran	15	54
20%		
Flour	71	19
Fine bran	18	45
Coarse bran	11	36
25%		
Flour	75	37
Fine bran	9	28
Coarse bran	16	35

The pentane fractions were evaporated under nitrogen at room temperature, and the dried sterols were redissolved in 2 ml of methanol. Ergosterol was quantified using a high-performance liquid chromatography system (Waters Associates, Milford, MA) with a Novapak C18 column and methanol, at 2 ml/min, as the mobile phase. Ergosterol was detected at 280 nm about 8 min after injection.

Mycological Analyses

Portions (20 g) of each fraction and of whole kernels were soaked in 180 ml of 0.2% peptone water with Tween 80 (0.02%) for 30 min before homogenizing for 2 × 30 sec in a stomacher (Lab Blender Model 400, Seward Medical Ltd., London), as recommended in King et al (1986). After making a dilution series, 0.1 ml of the mixture was spread on the surface of 2% malt extract agar (MEA) (Oxoid Ltd., Basingstoke, Hampshire, England) with 30 ppm of streptomycin and 30 units of penicillin per milliliter, and on 2% MEA with 8% sodium chloride (MSA). The agar plates were incubated at 24°C for seven days before the colonies were counted.

RESULTS

CFU Determinations

CFU values in the kernels used as starting material were 3×10^2g (dwb) on MEA and 8×10^1 on MSA. The number of fungal propagules on MEA increased with increasing moisture content (Fig. 1). Maximum numbers, 1×10^7/g (dwb), were found in whole kernels stored at 25% moisture content. On MSA, CFU values also increased with increasing moisture (Fig. 2). The highest value, 5×10^6, was found in kernels stored at 25% moisture. The flora in treatments with higher moisture contents was dominated by *Aspergillus* spp, mainly *Aspergillus candidus*. In

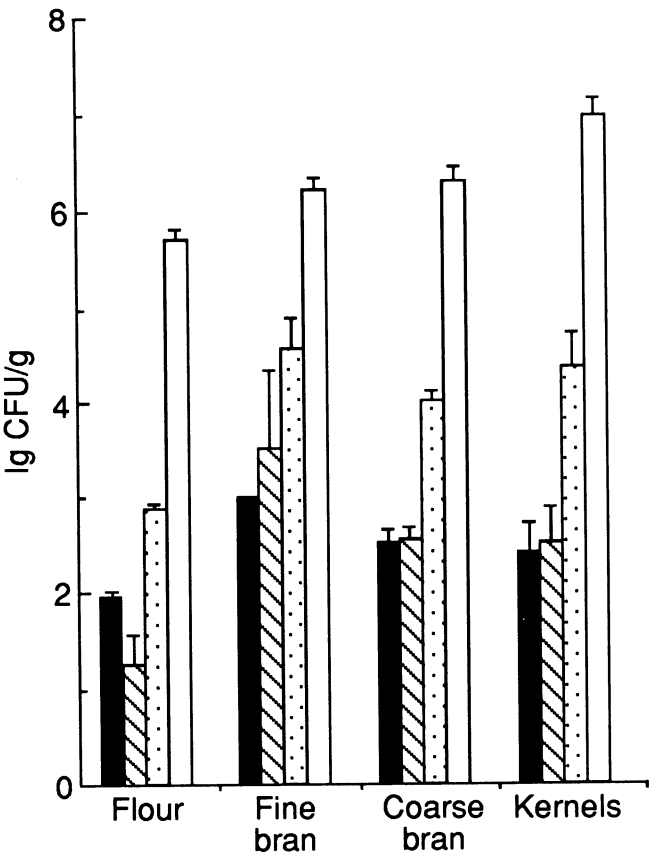

Fig. 1. Logarithms of numbers of colony-forming units (CFU) on 2% malt extract agar. Wheat stored at moisture contents of 12 (black), 16 (diagonal lines), 20 (dots), and 25% (white). Bars indicate one standard deviation; n = 3.

samples stored at 12% moisture, field fungi (such as *Fusarium* and *Rhizopus* spp) were prominent on MEA.

Ergosterol Determinations

The ergosterol level was 1.1 μg/g (dwb) in the starting material and increased with increasing storage moisture (Fig. 3). The highest levels were found in the bran fractions. In wheat stored at 12% moisture, 3% of the ergosterol was in the flour fraction and 97% was in the bran (Table I). In wheat kernels stored at higher moisture levels, the ergosterol was more evenly distributed. Ergosterol values were weakly correlated with the logarithms of CFU values on MEA ($r = 0.67$, $P < 0.01$), and MSA ($r = 0.58$, $P < 0.05$).

DISCUSSION

The wheat used as starting material in this investigation had low numbers of fungal propagules and low ergosterol values. The data agree with those found in American studies of high-quality wheat (Rogers and Hesseltine 1978, Seitz et al 1977). Baseline counts of fungal propagules in English wheat (Seiler 1986) ranged from 10^3 to 10^7/g (dwb), which is 10-100 times higher than those found in the present study. Ergosterol levels in German wheat (Müller and Lehn 1988) have also been somewhat higher, presumably because of the moist climate. The 1989 harvest season was exceptionally good in Sweden. During normal years with more rainfall, the level of fungal infection is more likely to resemble that of other north European grain. As expected, incubation at higher moisture contents resulted in increased numbers of fungal propagules and higher ergosterol levels in flour, bran, and kernels. Variation in ergosterol levels was greater for whole kernels than for milled grain. This indicates that fungal growth was patchy, probably as a result of an uneven moisture distribution. Once the kernels were milled, it was possible to take more representative samples.

Some earlier investigators compared the mycological quality of flour with that of bran and whole kernels. Young et al (1984) reported that ergosterol and the *Fusarium* mycotoxin deoxynivalenol were fractionated during milling of Canadian winter wheat. Increased levels were found in the bran (outer kernel) portion, and decreased levels were found in the flour. Using microscopy, Seitz and Bechtel (1985) observed that hyphae were more prevalent in caryopsis coats than in the central endosperm of scab-infected wheat. Seiler (1986) found that fungal counts in wheat flour were, on average, 17% of those in whole wheat. He also reported that values in bran were in the same range as those found in flour (10^3–10^4/g). However, it is not possible to calculate the actual distribution of fungal biomass between bran and flour from determinations of fungal counts since these mainly reflect the degree of sporulation (Pitt 1984). The ergosterol assay makes it possible to obtain quantitative data on fungal biomass. Using this method, 97% of the fungal biomass appeared to be concentrated in the bran fractions of sound wheat. This agrees with the 85–90% of total ergosterol found in the bran and shorts from Canadian winter wheat, recalculated from Young et al 1984. They found a similar partitioning of deoxynivalenol between outer and inner fractions. Other mycotoxins are also likely to be concentrated into the bran. Thus, in view of the current trend to increase the content of bran in various foods, the mycological quality of such foods should be subject to close scrutiny.

Although the main hazards are associated with different mycotoxins, a rapid screening method to estimate the degree of fungal infection is needed. The traditional plate counts require one week of incubation and provide a poor measure of fungal biomass (Pitt 1984). On the other hand, recent developments have

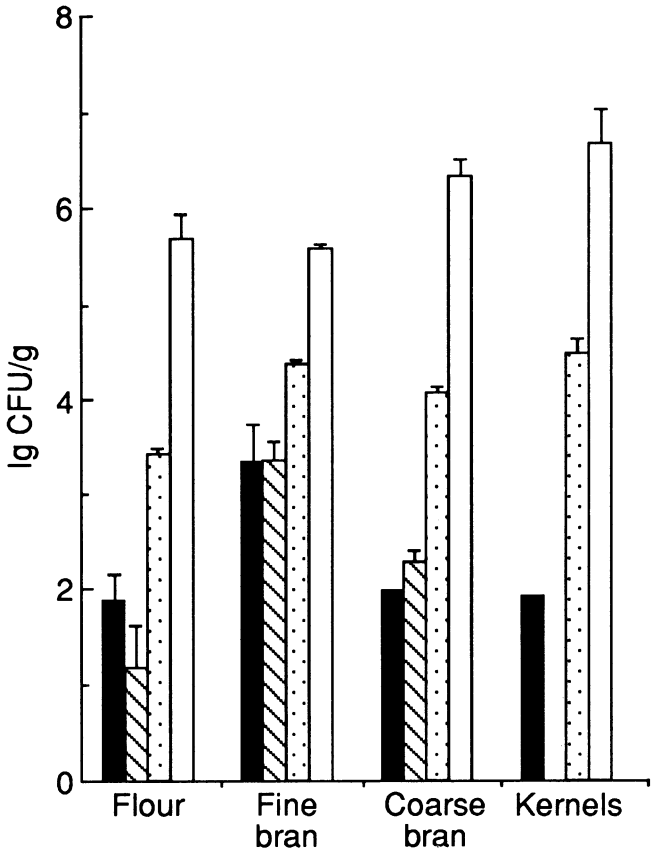

Fig. 2. Logarithms of numbers of colony-forming units (CFU) on 2% malt extract agar with 8% sodium chloride. Wheat stored at moisture contents of 12 (black), 16 (diagonal lines), 20 (dots), and 25% (white). Bars indicate one standard deviation; n = 3.

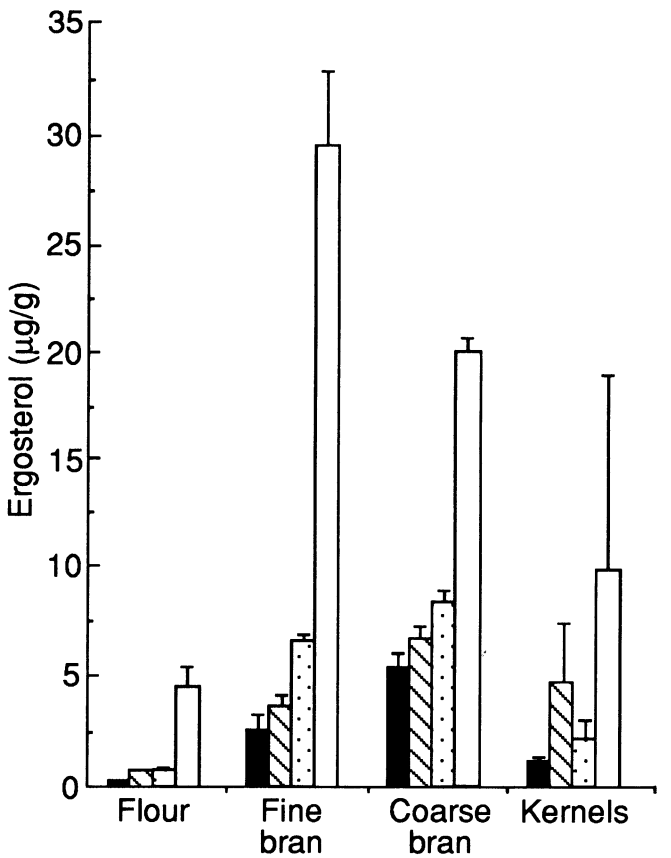

Fig. 3. Ergosterol values for wheat stored at 12, 16, 20, and 25% moisture contents. Wheat stored at moisture contents of 12 (black), 16 (diagonal lines), 20 (dots), and 25% (white). Bars indicate one standard deviation; n = 3.

reduced the total time required for extraction and quantification of ergosterol to less than 30 min (Martin et al 1990). This will make the ergosterol assay even more suitable for quantifying fungal infection in cereal products.

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