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Acidic solvent extraction of gossypol from cottonseed meal

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ABSTRACT

To expand the use of cottonseed protein in animal feeding, cottonseed meal was extracted with acetone- and ethanol-based solutions to remove gossypol. Phosphoric acid and water were included in the solutions to catalyze the hydrolysis of protein-bound gossypol. Both solvents were effective at reducing the total gossypol level in meal to between 5% and 10% of its initial value. Gossypol extraction occurred much faster in the ethanol-based extractions than it did in the acetone-based extractions. Treated meals tended to retain phosphorus but most of this could be removed by conducting a final water wash. Water washing also removed hydrophilic components resulting in reduced product yields but increased protein levels. Other acids, e.g., oxalic, citric, or sulfuric acid, were also effective at reducing meal gossypol. In contrast, extractions conducted without acid were not effective. The process can be used to produce low-gossypol cottonseed meals that should be useful in a broader range of feed applications.

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1. Introduction

Gossypol is a polyphenolic terpenoid that exists in the cotton plant as a defense agent and is known to be responsible for toxicity issues associated with the over feeding of cottonseed and cottonseed meal to animals (Berardi and Goldblatt, 1980). In addition to animal toxicity, the compound is studied for its anti-cancer, anti-viral, and male infertility effects (Wang et al., 2009). Ruminant animals tend to handle the effects of gossypol better than non-ruminant animals. Consequently, the meal is used almost entirely as a ruminant feed ingredient. Because of the presence of gossypol, the potential for expanding the use of cottonseed meal as a feed ingredient is limited.

Recent experiments have shown that cottonseed proteins derived from glandless varieties (named because they lack the glands that store gossypol and have only very low levels of the compound) can be substituted for much of the fish meal used in aquaculture diets (Siccardi et al., 2012). These experiments, conducted with meals, concentrates, and isolates prepared from glandless seed, were undertaken in the hope that recently reported RNAi-engineered cotton varieties (Sunilkumar et al., 2006), designed to eliminate gossypol only in the seed, might become commercial. Despite the potential advantages of these modified seeds, there are questions as to when these varieties will become available because of regulatory issues associated with planting a genetically modified organism and the intellectual property rights associated with their development. Hence, it may be some time for these varieties to become commercial.

This then suggests that it might be useful to consider the chemical removal of gossypol to produce meals that might be used favorably in other feeding applications, such as in aquaculture feeds. Because a substantial portion of the

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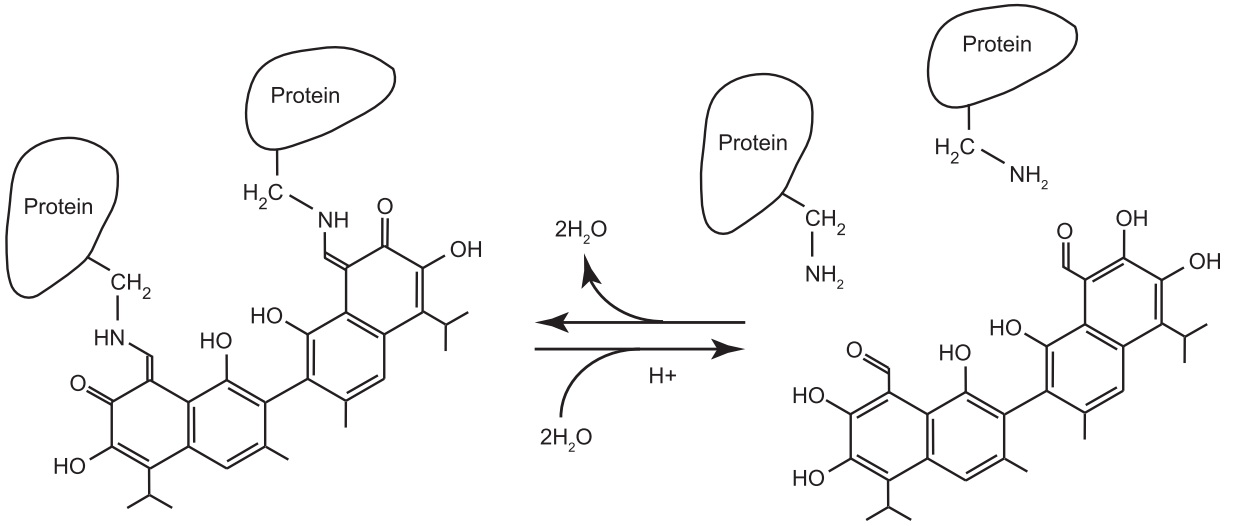

Fig. 1. Structure of gossypol and the reversible chemistry associated with the binding and hydrolysis of gossypol and protein.

gossypol present in cottonseed meal is bound to protein (as Schiff's bases formed with amino groups of lysine), the extractions need to be conducted under conditions that favor hydrolysis of bound gossypol (Fig. 1). Acid has been used to hydrolyze gossypol Schiff's bases formed with simple amines (Matlin et al., 1987; Dowd and Pelitire, 2006). To test for the possibility of extracting gossypol from cottonseed meal, commercial meal was extracted with solvent, water, and acid. The solvents used were acetone and ethanol, both GRAS (generally considered as safe) for use in food processing and capable of dissolving gossypol. The acid used was phosphoric acid, which is commonly used in vegetable oil processing. In addition, a few experiments were conducted with other solvents and acids to test the flexibility of conditions needed to achieve gossypol reduction.

2. Materials and methods

2.1. Materials

The meal used in the study was donated by Cotton Inc. (Cary, NC). The meal was made at the Food Protein Research Center (College Station, TX) primarily as a control material for aquaculture feeding experiments. It was produced under typical expander-solvent extraction conditions, except that the usual addition of hull material back to the kernels was limited to increase the meal's protein content.

Acetone, methanol, ethanol, phosphoric acid, and citric acid were purchased from Thermo-Fisher Scientific (Fair Lawn, NJ, USA). Oxalic acid was from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA) and sulfuric acid was from EM Sciences (Gibbstown, NJ, USA). All chemicals used were analytical grade or better.

2.2. Extractions

Twenty-five grams of meal was weighed into a 500-mL flat-bottom flask with 250 mL of an extraction solvent. Extraction solvents consisted of 80:20, 90:10, or 95:5 (v/v) acetone/water or 80:20, 90:10, or 95:5 (v/v) ethanol/water each with phosphoric acid added to give a 1.4 M solution. A stir bar was added to provide mixing. The flask was fitted with a condenser, and the mixtures were heated to reflux. Extraction times varied from 0.5 to 5.0 h for the acetone-based solutions and from 0.5 to 2.0 h for the ethanol-based solutions. After the extraction period, flasks were separated from the condensers and cooled to room temperature in an ice-water bath. Each sample was vacuum filtered over a Buchner funnel on #4 Whatman paper. After separation of the solvent, the retained meal was washed on the Buchner funnel to eliminate the gossypol contained in the hold-up volume. Two wash conditions were considered. For one set of experiments, the meal was washed with 250 mL of the same solution used for the extractions but without the acid. For the second set of experiments, the meal was first washed with 250 mL of the same solution used above but was then washed with an additional 250 mL of water. After the wash treatments, the meals were air dried under the hood to remove the bulk of the solvent and water and were then dried in a convection oven overnight at 50 °C. After cooling in a desiccator, each sample was weighed. Dry mass yields were determined from these weights and measured moisture levels (discussed below). A few extractions were also conducted with methanol, with the other acids listed above, or without the addition of acid.