Validation of a RP-UPLC-PDA method for gossypol determination in cottonseed meal during biodetoxification process by fungi

Aparecido A. Conceicao[1], Ana Paula F. Araújo[1], Thais D. D. Santana[1], José Antônio A. Ribeiro[1], Simone Mendonça[1], Felix G. de Siqueira[1]*

Background

Cottonseed shows as a potential animal feed since it is a great source of protein, fiber and minerals. However, the use of cottonseed meal is mainly directed to ruminants and restricted to monogastric animal feed due to the high occurrence of gossypol in the glands of cotton plants (*Gossypium* spp.). Gossypol is a yellow pigment that confers resistance to cotton plants against natural predators. Also, it has harmful effects to livestock leading to immunotoxicity, liver damage, reducing weight gain, respiratory distress, anemia, weakness and infertility (GADELHA et al., 2014). In order to neutralize the gossypol actions and expand the use of cottonseed meal for all kind of animal, several research have been done such as supplementation with iron (GABER et al. 2012), microbial fermentation (LIM, 2011), and use of fungi strains (ZHANG et al. 2006). Treatment using fungi is a potential and efficacious method to eliminate gossypol from the cottonseed meal since it is a cheap, sustainable and an accessible method. Considering that most of used methods to determine gossypol is mainly for sources containing high concentration of the molecule, it makes necessary to develop and validate a method to extract and determine small amounts of gossypol is sources such as treated and untreated cottonseed meal. Thus, this work aimed to validate a methodology of extraction and determination of gossypol during the cottonseed meal biodetoxification process by fungi.

Methods

Sample extraction – Several solvents (chloroform, acetonitrile 80% (BENSON et al. 2001), acetone 100% and acetone 70%) and extraction conditions (with or without sonication, cold or ambient temperature; with or without solvent evaporation) were tested. The optimized extraction procedure was accomplished as following: One gram (1 g) of the cottonseed meal were weighed into centrifuge tubes (15 mL) and extracted by sonification (10 min) in ice with 10 mL of acetone 70%. The samples were centrifuged (5 min at 9000 rpm and 8°C), an

1 Embrapa Agroenergia, PqEB, Av. W3 Norte (final), Brasília/DF, Brasil, 70770-901;
*aparecido.conceicao@colaborador.embrapa.br; felix.siqueira@embrapa.br

aliquot of the supernatant was transferred to a tube (1.5 mL) and centrifuged again (10min at 14000 rpm and 8°C) and finally an aliquot of the supernatant was transferred directly into an auto sample vial to be analysed at UPLC (Ultra Performance Liquid Chromatography). Recovery test was evaluated spiking gossypol standard on untreated cottonseed meal.

Standard - A gossypol standard (SIGMA G8761, purity 95%) was prepared dissolving 5 mg of gossypol standard in 5 mL of acetone 70%.

UPLC Analysis – All samples were performed in a Waters UPLC Acquity H-Class equipped with a Photodiode Array (PDA) detector and auto injector. For method development, the following parameters were tested: different reversed phase columns (RP-C18), organic mobile phase (CAN, MeOH or mixture of both), type of elution (isocratic or gradient), and use of mobile phase modifiers (acetic acid, formic acid, or triluoracetic acid). The wavelength for UV detection was 254 nm. A 1 µl of the samples at 8°C were injected at a flow rate of 0.4 mL/min.

Linearity, precision, limit of detection, limit of quantification and recovery tests were evaluated to validate the optimized method.

Results and Conclusions

Gradient elution using a mobile phase starting with 40% Aqueous (TFA 0.1%, v/v) and 60% of Organic (100%Methanol) with a Kinetex C18 column (100 x 2.1 mm, 2 µm) maintained at 35ºC presented the best peak shape and sensitivity for gossypol. With regard to extraction conditions, poor recovery or stability issues were observed when chloroform, acetonitrile 80% or acetone 100% were employed as solvents. The best results were achieved by sample sonication with acetone 70% in an ice bath for 10 minutes.

The optimized RP-UPLC-PDA method showed good linearity (R2>0.999) in the range of 0.5 µg/mL to 100 µg/mL; detection and quantitation limits were 0.2 µg/mL and 0.5 µg/mL, respectively; recovery was greater than 94% in three tested levels, with good precision (RSD<15%).

The proposed method for extraction and UPLC analysis of gossypol is efficient and more sensitive than others methods available in literature (BENSON et al. 2001) allowing for detection and quantitation of small amounts of free gossypol in treated and untreated cottonseed meal.

References

BENSON, C. G.; WYLLIE, S. G.; LEACH, D. N.; MARES, C. L.; FITT, G. P. Improved method for the rapid determination of terpenoid aldehydes in cotton. **Journal Agriculture Food Chemicals**, Washington, DC, v. 49, n. 5, p. 2181-2184, 2001.

GABER, M. M.; ELHALFAWY, M. M.; RAMADAN, A. M. Utilization of cottonseed meal supplemented with iron for detoxification of gossypol in nile tilapia, broodstock and their impact on the hatchability of their progenies. **Journal Aquaculture Research & Development**, v. 3, n. 7, p. 1-5, 2012.

GADELHA, I. C. N.; FONSECA, N. B. S.; OLORIS, S. C. S.; MELO, M. M.; BLANCO-SOTO, B. Gossypol toxicity from cottonseed products. **Scientific World journal,** New York, número do artigo 231635, 2014.

LIM, S.; LEE, K. J. A microbial fermentation of soybean and cottonseed meal increases antioxidant activity and gossypol detoxification in diets for nile tilapia, *Oreochromis niloticus*. **Journal of the World Aquaculture Society**, Malden, v. 42, n. 4, p. 494-503, 2011.

ZHANG, W.; XU, Z.; SUN, J.; YANG, X. Effect of selected fungi on the reduction of gossypol levels and nutritional value during solid substrate fermentation of cottonseed meal. **Journal of Zhejiang University Science B**, Hangzhou, v. 7, n. 9, p. 690-695, 2006.