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A study of gossypol reduction by choline and ethanolamine using a model system

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The objective of the present investigation was to assess the effect of choline and ethanolamine on the availability of lysine, employing a cottonseed flour-gossypol model system. The model system was developed so that the time, temperature and level of gossypol used produced a 33–38% decrease in lysine availability. Heating cottonseed flour with gossypol at a ratio of 16:1 at 90°C for 30 min. reduced the available lysine from 21–22 to 12–14 mmol/100 g protein. Different amounts of choline and ethanolamine were added to the model system to compete with lysine for the formyl groups of gossypol. A significant ($p < 0.05$) increase in available lysine was evident in the presence of 0·8 and 1.6×10^{-1} M choline or ethanolamine. Of the bases examined, ethanolamine was almost twice as effective as choline in restoring the level of available lysine in cottonseed flour.

INTRODUCTION

The potential of cottonseed protein for human nutrition is limited by the presence of gossypol, a toxic polyphenolic pigment. This pigment is located in the glands of the cottonseed kernel and has been shown to interact with proteins during heat treatment (Clark, 1928). Cottonseed protein is limited in lysine so that any reduction in its availability through interaction between the formyl groups of gossypol and the ϵ-amino groups of lysine would have an adverse effect on protein quality (Lyman *et al.*, 1953; Conkerton & Frampton, 1959; Martinez *et al.*, 1961; Rolz *et al.*, 1972). The development of glandless cottonseed by plant breeders to overcome this problem was limited due to the poorer yields and the increased susceptibility of this crop to insects and diseases. Consequently glandless cottonseed accounts for less than 0·5% of the total crop in the USA. A number of methods have been developed for removing gossypol from cottonseed including solvent extraction of free gossypol,using aqueous acetone followed by dry acetone (Damaty & Hudson, 1975), a butanol–hydrochloric acid solution (Canella & Sodini, 1977), a methylene chloride extraction of a liquid cyclone process product (Cherry & Gray, 1981) and a 1:1 mixture of isopropanol and hexane followed by acetone (Rahma & Narasingo Rao, 1984). The reduction of gossypol using solvent systems suffers from the difficulty of totally removing residual solvents that have potential harmful as well as detrimental effects on flavor.

A novel approach was reported by Yannai & Bensal (1983) in which cottonseed was heat-treated with soybean gums. This method incorporated 3–5% gums into the cottonseed meals which were then heated at 100°C for 5 min. A superior quality meal was obtained as evidenced by a lower level of free gossypol and higher level of available lysine. These researchers attributed this improvement to the competitive binding between the phospholipid amino groups with free gossypol, thereby protecting the available lysine ϵ-groups. Since the major amino group phospholipids in soybean are phosphatidylcholine and phosphatidylethanolamine this study examined the respective roles that their bases, choline and ethanolamine, play in binding gossypol and protecting lysine in model systems containing cottonseed protein.

MATERIALS AND METHODS

Materials

Cottonseed flour, crystalline choline bitartrate and ethanolamine (2-aminoethanol) bases, and Crocein orange (G) dye were purchased from Sigma, St. Louis, MO. All other chemicals used in this study were of reagent grade. Gossypol acetate was generously provided by Dr K. J. Carpenter, Department of Nutritional Sciences, University of California, Berkeley.

Model system

The basic model system developed in the laboratory consisted of 3·5 mg cottonseed flour to which was added 600 μl ethanol (containing 0, 50 and 100 ppm gossypol, respectively) and 1·0 ml acetate buffer (pH 8·0). The mixtures were then placed in small screw-top vials and heated at 70, 80, 90 and 100°C for 15, 30 and 60 min. To this system were added three levels of choline or ethanolamine (0·4, 0·8 and 1·6 × 10^{-1} M).

Gossypol extraction and determination

Free gossypol in the cottonseed flour was extracted following the procedure of Pons & Guthrie (1949) and measured using the aniline method of Pons *et al.* (1958). All measurements were carried out in triplicate.

Protein

The protein content of flour was measured by the microKjeldahl method (AOAC, 1984). A factor of 6·25 was used to convert nitrogen to protein.

Lysine availability

Available lysine was measured following the dye binding procedure of Anderson *et al.* (1984). All measurements were performed in triplicate.

Statistical analysis

A one-way analysis of variance (ANOVA) was conducted on the cottonseed flour model systems to determine the effect of gossypol level, time, temperature and base on the availability of lysine.

RESULTS AND DISCUSSION

The amount of free gossypol in the cottonseed flour was found to be 241 ppm (Table 1). In order to check the efficiency of the extraction procedure, 50 and 100 ppm gossypol were added to the flour and the percentage recovery was determined (Table 1). It is evident from the results that the extraction method used for free gossypol resulted in the complete recovery of added gossypol.

To establish optimum conditions for the reaction between cottonseed flour and gossypol, the cottonseed flour model systems containing a flour:gossypol ratio of 16:1 were heated at 70, 80, 90 and 100°C for up to 1 h. A rapid decline in available lysine was observed during the first 15 min irrespective of the temperatures used (Fig. 1). No significant differences ($p < 0·05$) in lysine availability were observed between 70 and 80°C, although systems heated at the higher temperatures (90 or 100°C) had significantly ($p < 0·05$) lower levels of lysine. The system heated at 100°C, however, was significantly ($p < 0·05$) lower in lysine availability as compared to the system heated at 90°C. Based on these results a combination of 90°C and 30 min was selected for studying cottonseed flour model systems as this produced a 34·2% decrease in available lysine.

Table 1. Percentage recovery of free gossypol in cottonseed flour

Sample	Free gossypol (ppm)	Recovery (%)
Cottonseed flour	241 ± 10	
Cottonseed flour + 50 ppm gossypol	292 ± 10	100
Cottonseed flour + 100 ppm gossypol	342 ± 12	100

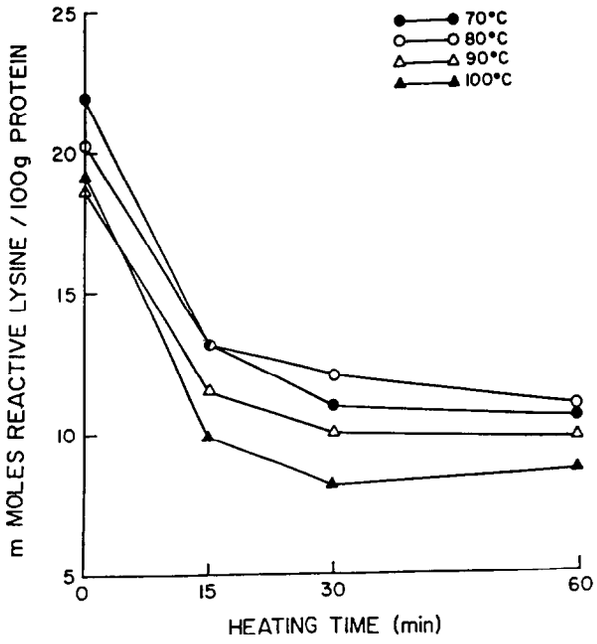

Fig. 1. Decline of lysine with time.

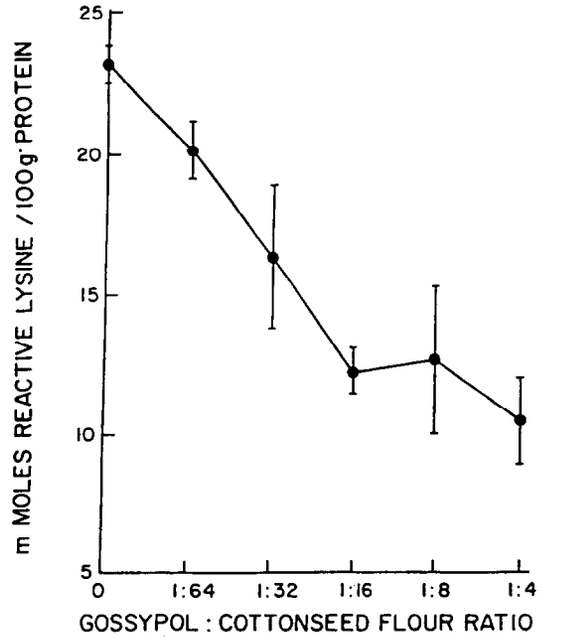

Fig. 2. Effect of different levels of gossypol on availability of lysine in model systems heated for 30 min.

Table 2. Effect of choline on the reaction between gossypol and cottonseed protein in model systems

Model system	Available lysine (mmol/100 g protein)	Decrease in lysine (%)
Cottonseed flour–gossypol (16 : 1) (unheated control)	22·07 ± 1·41	
Cottonseed flour–gossypol (16 : 1) (heated control) with	13·55 ± 1·57	38·6
0·4 × 10^{-1} M choline	14·37 ± 1·20	34·9
0·8 × 10^{-1} M choline	16·50 ± 1·26	25·3
1·6 × 10^{-1} M choline	15·93 ± 0·99	27·8

The effect of different levels of gossypol on the availability of lysine in model systems heated for 30 min is shown in Fig. 2. Increasing the gossypol–cottonseed flour ratio from 1 : 64 to 1 : 4 significantly ($p < 0·05$) decreased lysine availability with the maximum decrease noted at a ratio of 1 : 16 or below. Consequently, a 1 : 16 gossypol–cottonseed flour mixture was selected in the model systems studied.

The different effects exerted by choline and ethanolamine on this system were examined. The results for choline are summarized in Table 2. Lysine availability was significantly ($p < 0·05$) higher in the presence of 0·8 × 10^{-1} M choline, while 0·4 × 10^{-1} M choline made no difference. The addition of gossypol to cottonseed flour reduced lysine availability by 38·6%. However, in the presence of 0·8 and 1·6 × 10^{-1} M choline, lysine availability was improved to 25·3 and 27·8%. Available lysine increased from 13·55 mmol/100 g protein in the heated control to 16·5 and 15·9 mmol/100 g protein in the presence of 0·8 and 1·6 × 10^{-1} M choline solutions, respectively. This compared to 22·0 mmol available lysine/100 g protein in the unheated cottonseed flour sample.

The effect of ethanolamine on lysine availability is shown in Table 3. A much greater improvement in lysine availability was achieved in the presence of ethanolamine solutions. This corresponded to a decrease in lysine availability of 15·8 and 12·2% for systems containing 0·8 and 1·6 × 10^{-1} M ethanolamine as compared to 32·5% in the heated control. Even in the presence of 0·4 × 10^{-1} M ethanolamine, lysine availability was only reduced by 22·3%. In absolute amounts this translated into a lysine availability of 19·33 mmol/100 g protein in the presence of 1·6 × 10^{-1} M ethanolamine as compared to 22·1 and 14·85 mmol/100 g protein for the unheated and heated cottonseed flour control model systems, respectively.

Table 3. Effect of ethanolamine on the reaction between gossypol and cottonseed protein in model systems

Model system	Available lysine (mmol/100 g protein)	Decrease in lysine (%)
Cotton flour–gossypol (16 : 1) (unheated control)	22·01 ± 0·79	
Cottonseed flour–gossypol (16 : 1) (heated control) with	14·85 ± 1·16	32·5
0·4 × 10^{-1} M ethanolamine	18·16 ± 1·01	17·8
0·8 × 10^{-1} M ethanolamine	18·53 ± 1·13	15·8
1·6 × 10^{-1} M ethanolamine	19·33 ± 1·13	12·2

These results suggest that ethanolamine is far more effective than choline in competing for gossypol. It is possible that the tertiary nitrogen of choline may be less accessible in forming a Schiff's base with the formyl groups of gossypol as compared to ethanolamine. Many of the extraction methods to reduce gossypol toxicity in cottonseed flour are limited by the presence of solvent residues such as acetone, isopropanol or aqueous isopropanol. The use of soybean gums as proposed by Yanai & Bensal (1983), however, provides an attractive alternative method for reducing gossypol. In addition to reducing gossypol toxicity it also allows more of the ϵ-amino groups in cottonseed protein to remain free. This study demonstrated the ability of choline and ethanolamine to protect lysine in cottonseed flour and thus explains the reason for soybean gums improving the nutritional quality of cottonseed flour.

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