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Detoxification Treatments of Free Gossypol in Cottonseed Meal by Microbial Treatment of Mixed Cultures and Biochemical Evaluation on Rabbits

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ABSTRACT

Detoxification of free gossypol (FG) in cottonseed meal (CSM) by *Saccharomyces cerevisae* and *Aspergillus niger,* as a mixed culture, was carried out in solid state fermentation (SSF). Experiments were adopted to optimize the fermentation conditions. Maximum detoxification efficiency (90.2%) occurred after 48h of incubation at 30°C in a 250ml conical flask containing 15 g of CSM supplemented with 1% (w/w) $(NH_4)_2SO_4$ at the optimal conditions including the initial moisture content 55% (w/w) and inoculum level at 5% (v/w). The detoxification of FG was a growth-associated process, which was highly correlated with the dry matter weight loss. Moreover, high activities of hydrolytic enzymes were also produced in solid state fermentation, which enhanced the nutritive value of the detoxified cottonseed powder.

A total number of 48 white New Zealand male rabbits were used to biologically examine the feeding of treated (detoxified) CSM without any adverse effects. Hematological and biochemical relevant parameters of white New Zealand male rabbits as affected by feeding treated meal were in the normal physiological range without no obvious change. No significant changes in liver and kidney functions of the rabbits weight gain, feed conversion and efficiency did not significantly change among experimental groups. The study showed that the feeding of the detoxified CSM by *S. cerevisae* and *A. niger* as a mixed culture in this research without any adverse effects on rabbits.

Key words:*Detoxification; Free Gossypol; Cottonseed Meal; Saccharomyces cerevisae; Aspergillus niger; Solid State Fermentation; Biochemical Evaluation.*

INTRODUCTION

Cottonseed meal (CSM), one of the cheapest agricultural residues containing high level of protein, are produced in many countries. However, because of high content free gossypol (FG), only a little of CSM is used as feedstuff and most of it is simply added to soil as a fertilizer, which means a huge economic waste [1]. FG, polyphenolic binaphthyl dialdehyde, is a toxic pigment found primarily in the pigment glands of cottonseed. The intake of FG causes a decrease of animal growth and feed conversion, depression of fertility in bulls, and reduction of viability of gametes in cattle [2,3]. A number of methods including chemical, physical and biological treatments have been developed to reduce the content of FG by removing it from CSM or turning it into bound gossypol (BG), which is relatively safe to animals [4]. Chemical treatments to reduce FG by adding some chemicals such as ferrous sulfate and calcium hydroxide may affect the protein nutritive value and reduce the biological activity of vitamins [5,6]. Physical treatments include organic solvents extraction and heat treatments, but the residual solvents may be potentially harmful to animals and the high temperature can reduce the nutritive value of the CSM [7,8]. Moreover, most of the chemical and physical treatments have low detoxification efficiency and are not in commercial use now. However, as a biological treatment, microbial fermentation is a detoxification method of great promise, which has high a detoxification efficiency and also can enhance the nutritive value of the CSM [9].

Various microorganisms have been tried to detoxify FG, including *Candida tropicalis*, *Torulopsis candid*a, *Aspergillus flavus* and *Aspergillus niger* [9-13]. Among these microorganisms, *Candida tropicalis* had the highest detoxification efficiency of 96.367% when the fungus was inoculated into CSM supplemented with wheat bran and inorganic salts in solid state fermentation [12]. Up to now, the detoxification mechanism of FG by microorganism unknown because of the limited data of isolation of FG biodegrading microbes and the optimization of detoxification technology.

Rabbits production plays a considerable role in solving the problem of meat shortage in Egypt, particularly on the level of the small-scale farmers and new reclaimed areas. But the most obvious limitation to rabbit production in hot climate area is the susceptibility of the species to heat stress and availability of clean healthy and good quality drinking water and feeding diet, which lead to the impairment of production and feed efficiency [14 -16]. Also, the cost of buying

clean and healthy diet in rabbit farms can affect the feasibility of rabbit production.

In this paper, *Saccharomyces cerevisae* was adopted together with *A. niger* to detoxify FG in CSM for the first time. Experiments were adopted to optimize the fermentation conditions. Moreover, a linear correlation equation between detoxification efficiency and the growth of fungi was established, and the enzyme composition in the detoxified CSM was examined also. Also, the objective of this study was to biologically evaluate the quality of the detoxified CSM by the present microbial culture using white New Zealand male rabbits.

MATERIALS AND METHODS

Microorganisms and inoculums preparation

The strains of *S. cerevisae* and *A. niger* were bred and collected by Microbiology Department (NCRRT). They were maintained on potato dextrose agar slants at 4^{o}C.

Stock culture of *S. cerevisae* was inoculated into 100 ml potato dextrose liquid medium in 250 ml conical flasks and incubated at 30 ^{o}C for 48 h at 200 revolutions per minute, and stored for the study. Stock culture of *A. niger* was cultivated in potato dextrose agar slants and incubated at 30 ^{o}C for 4 days. The spores of the 7-day-old culture were transferred into a 250 ml conical flask, mixed well with 50 ml sterile deionized water. Then the mixture was diluted to 1×10^{6} spore/ml with sterile deionized water. Direct microscopic counts were taken using a blood counting chamber.

The culture solution of *S. cerevisae* and the spore solution of *A. niger* were mixed well at the ratio of 3:1 (v/v) in a sterile 250 ml conical flask, and then used as inoculums in the present study.

Basic fermentation medium materials

The CSM was obtained from a local market in Cairo. The ground CSM was used as basic fermentation medium. The moisture content (MC), total protein content and crude fiber content of CSM were 7, 36 and 14%, respectively. The content of FG and total gossypol (TG) in CSM were 442 μg/g and 1150 μg/g, respectively.

Screen of the optimal combination of test strains

Solid state fermentation (SSF) was carried out in a 250 ml conical flask containing 15 g CSM. The initial moisture content (IMC) was adjusted to 55%

(w/w) and the pH of the medium was under natural (pH5.5). The conical flask was closed with cotton and was autoclaved at 121°C for 20 min. The pH of the medium did not change after autoclaving.

The culture solution of *S. cerevisae* and the spore solution of *A. niger* mentioned above were mixed well at different ratios. One milliliter of the different mixtures was inoculated into the culture medium, respectively. After mixing well, the inoculated medium was incubated at 30 °C for 48 h. Each conical flask was gently tapped intermittently for mixing well during incubation period. After the fermentation was completed, the fermented substrates were dried at 50 °C for 24h and were subsequently processed into flour for related analysis.

Experiment design

To improve the detoxification efficiency, an experiment was adopted to optimize the IMC, substrate load, percentage of $(NH_4)_2SO_4$ and KH_2PO_4, incubation temperature, and initial pH value of substrate. The levels of the seven factors are shown in Table 1. The initial pH of the substrate, which was determined by pH meter, was adjusted by the solution of 1M NaOH or 1M HCl. The methods of sterilization, inoculation, fermentation and subsequent processes were the same as described above.

Table 1. Factors and levels of experimental treatments.

Factors		Levels		
		1	2	3
A	Substrate load (g)	10	15	20
B	Initial moisture (%, w/w)	50	55	60
C	KH_2PO_4 (%, w/w)	0	0.3	0.6
D	$(NH_4)_2SO_4$ (%, w/w)	0.5	1.0	1.5
E	Temperature (°C)	25	30	35
F	Inoculation levels (%, v/w)	3	5	10
G	Initial pH	4.5	5.5	6.5

Relationship between dry matter weight loss and gossypol detoxification

Direct determination of biomass in solid medium is very difficult because it is impossible to separate the organism from the substrate. Terebiznik and Pilosof [17] found that dry matter weight loss was highly correlated with the biomass, and the biomass in SSF system can be estimated by determining the dry matter weight loss [17]. In this experiment, the dry matter weight loss and the level of FG and TG were measured at different fermentation time. The

optimized conditions obtained from the experiment were adopted in this test, and the methods of sterilization, inoculation and fermentation were the same as described above.

Determination of FG and TG

FG was determined by the official method of the American Oil Chemists Society [18]. To determine TG, the same method was adopted to turn BG into FG by hydrolyzing 1 g sample in 30 ml 0.1M oxalic acid-butanone-water solution (the ratio of butanone to water was 11:1) at 78 ^{o}C for 6 h [19].

Assay of enzyme activity

The activities of proteinase, cellulase, hemicellulase, glucoamylase and pectinase were assayed as previously described [20]. One international unit (IU) of enzyme activity was defined as the amount of enzyme required to release 1 μmol of tyrosine or reducing sugar (glucose for cellulase and glucoamylase, xylose for hemicellulase, and galacturonic acid for pectinase) per minute from the substrate under assay conditions.

Rabbits feeding

A total number of 48 white New Zealand male (NZW) rabbits aging 6 weeks old were used. The animals were housed during the experimental period (70 days) in metal batteries with automatic drinkers. All experimental rabbits were kept under normal healthy conditions and fed commercial pellets diets prepared by Atmeda, Dakahlia, Egypt as the normal control diet Table 2.

Table 2. **Experimental normal rabbit ration composition.**

Ingredients	**Composition**
Berseem hey	33.00 %
White corn	28.13 %
Barley	20.00 %
Soya bean meal	13.00 %
Molasses	1.30 %
CaCO3	1.00 %
*Vitamins minerals mixture	3.00 %
NaCl	0.50 %
DL-methionine	0.07 %

*Vitamins: A, D, E and K (K, diydrogen phosphate). Minerals: Mg SO4, CaCl2 and ZnSO4.
*Vitamins and minerals per 1 ml diluted in one liter drinking water contains: Vit. A (50000000 IU), Vit D_3 (5000000 IU), Vit. E (4000 mg), Vit. C (100000 mg), Mn (6000 mg), Zn (7200 mg), Fe (1500 mg), Cu (500 mg), I_2 (120 mg), Se (100 mg), Co (100 mg), Mg (1000 mg), Na (14000 mg), K (7500 mg) and P (10000 mg).

Feed allowances of rabbits were calculated according to NRC requirements [21]. Water and diet was provided *ad libitum*. After feeding on the normal diet for one week (adaptation period) rabbits were divided randomly into three groups (16 rabbit each) according to the following scheme to evaluate the effect of the treated detoxified CSM as following:

First animal group: Control group, which provided normal diet.
Second group: Rabbits provided untreated CSM.
Third group: Rabbits provided treated detoxified CSM.

During the experimental period (10 weeks), the consumed diet and body weights were recorded every week and at the same time, blood was collected using a heparenized vaccutainer tubes from the ear marginal vein. Whole blood hemoglobin (Hb, g/dl) was quantified immediately then blood was centrifuged at 3000 rpm to obtain the plasma and keep it at -20ºC till further analysis. Body weight gain, feed intake and feed efficiency were recorded and calculated. Blood plasma AST (aspartate transaminase) and ALT (alanine transaminase) activities were determined also. Total protein, albumin, glucose, urea, creatinine were determined. Globulin was calculated by subtracting albumin from total protein. Table 3 represent the methods and kits of blood analysis.

Table 3. **Methods and kits used to quantify the relevant blood biochemical.**

parameters	Methods	Company	References
Hemoglobin, g/dl	Colorimetric	Stanbio Lab (Antonio, Texas, 78202 USA)	22
Total protein, g/dl	Colorimetric	Stanbio Lab (Boerne, Texas, 78202 USA)	23
Albumin, g/dl	Colorimetric	Stanbio Lab (Boerne, Texas, 78202 USA)	24
Glucose, mg/dl	Enzymatic Colorimetric	Diamond diagnostics	25
Bilirubin, mg/dl	Colorimetric	Bio Adowic	26
AST and ALT, U/l	Colorimetric	Quimica Clinica Applicada S.A. (Ampposta, spain).	27
Urea, mg/dl	Colorimetric	Bio Adowic	28
Uric Acid, mg/dl	Colorimetric	Bio Adowicc	29
Creatinine	Colorimetric	Bio Adowic	29

Statistical analysis

Data set was analyzed using the general linear model of SAS [30]. Differences among means were tested using Duncan [31].

RESULTS AND DISCUSSION

Effect of test strains on the detoxification efficiency

As shown in Fig. 1, the detoxification efficiency of FG varied by the different types and proportions of test strains. When adopted alone, the detoxification efficiency of *S. cerevisae* was much higher than that of *A. niger,* although both of them not satisfying. But when *S. cerevisae* was adopted together with *A. niger*, the detoxification efficiency was improved, and the maximum value was obtained when the ratio of the two strains was 3:1 (v/v). This can be explained as follows. Because the activity of proteinase secreted by *C. tropicalis* was very low when adopted alone (Fig. 1), the growth of *S. cerevisae* was limited by its poor abilities to utilize protein nitrogen, and so did the detoxification efficiency of FG. The growth of *S. cerevisae* as well as the detoxification efficiency of FG was improved when it was adopted together with *A. niger* at a suitable ratio, because the proteinase secreted by *A. niger* could hydrolyze cottonseed protein to produce enough amino acids to meet the need of the two fungi. The ratio of the two fungi had significant influence on the detoxification efficiency. The detoxification efficiency together with the proteinase activity was increased or stimulated when the proportion of *A. niger* in inoculums increased from 0% to 25%. However, progressive increase of the proportion of *A. niger* gave benefit to the detoxification process as well, because the growth of *S. cerevisae* was reduced by the immoderate (overmuch) growth of *A. niger*, although the activity of proteinase still increases or stimulates. Besides high activities of proteinase, *A. niger* can also secrete other enzymes, such as cellulase, hemicellulase, glucoamylase and pectinase [20]. Perhaps all the enzymes secreted by *A. niger* can benefit the growth of *S. cerevisae* as well as the detoxification efficiency of FG more or less. But proteinase played a key role, because supplementation of 1% (w/w) peptone to the culture medium could improve the detoxification efficiency remarkably from 76.8% to 81.2% when *S. cerevisae* was adopted alone, while supplementation of 2% (w/w) glucose or galactose did not benefit the detoxification efficiency remarkably (data not shown).

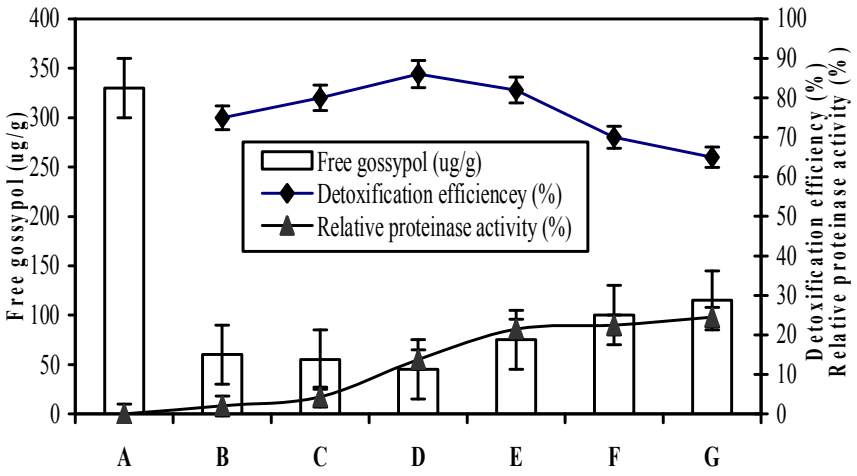

Fig. 1. Effect of the types and proportions of test strain on the detoxification efficiency and proteinase activity. The 100% relative proteinase activity was 24.2 IU/g.
A: Control; B: *S. cerevisae*; C: *S. cerevisae* & *A. niger* (6:1, v/v); D: *S. cerevisae* & *A. niger* (3:1, v/v); E: *S. cerevisae* & *A. niger* (1:1, v/v); F: *S. cerevisae* & *A. niger* (1:3, v/v); G: *A. niger* .

Analysis of the residual FG levels in experiment

The data in Table 4 showed that factors A, B and E had an extremely significant influence on the residual FG level, and the second levels were the best for the three factors, which suggested that the optimum substrate load, initial moisture and incubation temperature was 15g dry substrate /250 ml flask, 55% (w/w) and 30 ^{o}C, respectively. The supplementation of $(NH_4)_2SO_4$ (Factor D) had a significant influence on the detoxification efficiency, and the optimum level was 1% (w/w). As an extra inorganic salt, $(NH_4)_2SO_4$ can act as start-up nitrogen source, which was often necessary for the growth of microorganism before proteinase was secreted [20]. But this was not consistent with the previous reports that $(NH_4)_2SO_4$ has no influence on the detoxification efficiency of FG by *C. tropicalis* ZAU-1 in SSF [1]. The difference was caused by the different medium and microorganism adopted in SSF, and even the different source of cottonseed powder. Since KH_2PO_4, inoculation levels and initial pH of substrate (factor C, F and G) had no significant influence on the detoxification efficiency, the inoculation level of 5% (v/w), the natural pH of substrate (pH 5.5) and no KH_2PO_4 were adopted in the subsequent experiments for convenience and saving.

To confirm these optimal conditions obtained in experiments, a validation experiment was performed. Under these optimized conditions, the residual level of FG was 30.12 μg/g, which was 1.61 μg/g lower than the lowest one (31.91 μg/g) in the experiments. The residual FG in fermented substrate was much lower than legislated level 100 μg/g in growing chicken feed and 60 μg/g in swine feed[9]. Thus, the detoxified CSM is safe to feed animals. The detoxification efficiency in this paper (90.2%) was higher than that of *C. capsuligena* (73.5%), *S. cerevisae* (88.5%), *A. terricola* (82.9%), *A. oryzae* (67.5%), *and A. niger* (85.2%), but a slightly lower than that of *C. tropicalis* (96.367%), which was the microorganism reported up to now with the highest detoxification efficiency [11,12].

Table 4. Range and variance analysis of the residual free gossypol levels in experimental treatments.

No.	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*FG (μg/g)*
1	1	1	1	1	1	1	1	63.60±4.4
2	1	2	2	2	2	2	2	45.25±3.6
3	1	3	3	3	3	3	3	67.54±4.8
4	2	1	1	2	2	3	3	39.39±2.2
5	2	2	2	3	3	1	1	35.91±1.3
6	2	3	3	1	1	2	2	49.78±3.8
7	3	1	2	1	3	2	3	57.82±4.2
8	3	2	3	2	1	3	1	35.53±1.6
9	3	3	1	3	2	1	2	60.55±4.6
10	1	1	3	3	2	2	1	49.02±3.8
11	1	2	1	1	3	3	2	48.69±3.5
12	1	3	2	2	1	1	3	60.25±4.7
13	2	1	2	3	1	3	2	36.79±1.8
14	2	2	3	1	2	1	3	31.91±1.1
15	2	3	1	2	3	2	1	42.40±2.9
16	3	1	3	2	3	1	2	49.53±3.4
17	3	2	1	3	1	2	3	41.82±3.1
18	3	3	2	1	2	3	1	47.35±3.9

A: Substrate load (g); B: Initial moisture (%, w/w); C: KH_2PO_4 (%, w/w) ; D : $(NH_4)_2SO_4$ (%,w/w); E: Temperature (^oC); F: Inoculation levels (%, v/w); G: Initial pH.
Each value represents the mean ± SD.

Relationship between dry matter weigh loss and gossypol detoxification

The relationship between fungi growth and gossypol detoxification under the optimized conditions above were studied. The time course profiles of FG, TG and dry matter weight were shown in Fig. 2. The dry matter weight loss was used to assess the growth of the fungi, because the biomass was highly correlated with the loss of dry matter weight in SSF [22]. At the beginning (0-12 h), the loss of dry matter weight was in a micro scale and the change of the level of FG and TG was very small. During the fermentation period of 12-48 h, the fungi grew fast, resulting in a rapid decrease of dry matter weight and FG level. But at the same time, the increase of TG concentration was obvious. This was because the increase effect caused by the dry matter weight loss was higher than the decrease effect caused by the biodegradation. During the next fermentation stage (48-72 h), the growth of the fungi slowed down, thus changing in the dry matter weight loss and the level of FG and TG. This suggested that both the detoxification of FG and the increase of TG were growth-associated processes. In other words, the detoxification of FG and the increase of TG were highly correlated with dry matter weight loss. These results were consistent with the previous reports which denoted that FG concentration together with the dry matter weight dropped during SSF by *C. tropicalis,* and the biodegradation of FG was highly correlated with the dry matter weight loss [9].

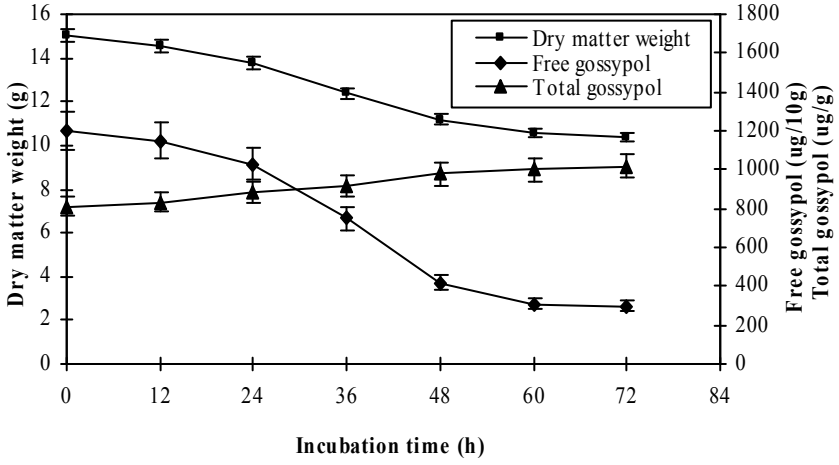

Fig. 2. Time course profile of dry matter weight (g/250 ml conical flask), free gossypol (ug/10g) and total gossypol (ug/g) in SSF.

Since a great amount of spores would be produced after 72 h of fermentation, which turned the color of the substrate into black and did harm to animals, the fermentation should be terminated at 48h.

Analysis of the detoxification effect during autoclaving and fermentation processes

During the process of autoclave treatment, the TG and FG in a conical flask reduced by 115.2 µg and 2918.8 µg respectively (Table 5). This indicates that autoclave treatment could only turn the FG into inactive state, but can hardly degrade it. The result of the positive effects of autoclave treatment on the detoxification of FG was consistent with the previous report that FG can be turned into BG by combining with protein and amino acid, especially lysine, when heated [32]. However, the detoxification efficiency caused by heat treatment was very low. It was reported that when the cottonseed powder containing 1649 µg/g of FG was cooked at 110 ^{o}C for 15 min, the detoxification efficiency reached only 58.6% [32]. In this study, the detoxification efficiency caused by autoclave treatment at 121 ^{o}C for 20 min was only 61.85%, and the residual level of FG in autoclaved substrate was 119 µg/g, which was higher than legislated level 100 µg/g in growing chicken feed and 60 µg/g in swine feed [9]. This indicated that only heat treatment was not enough for the detoxification of FG in CSM, and it was necessary to reduce the residual FG level further by microbial fermentation.

Table 5. Analysis of the detoxification effect during the process of autoclave treatment and fermentation.

	Control	**Autoclaved substrate**	**Fermented substrate**
Dry matter weight (g)	15.0±0.03	14.8±0.02	11.2±0.03
FG (µg/g)	312±18.5	119±4.3	30.7±1.9
TG (µg/g)	798±39	801±35	968±45
Calculated			
Total amount of FG (µg)*	4680±219	1761.2±62.5	343.84±21
Total amount of TG (µg)**	11970±582	11854.8±511	10841.6±482

Each value represents the mean ± SD
*Total amount of FG (µg) = FG (µg/g) × Dry matter weight (g).
** Total amount of TG (µg) = TG (µg/g) × Dry matter weight (g).

During the process of fermentation, the TG and FG reduced to 1013.2 μg and 1417.36 μg, respectively, which can be figured out from the data shown in Table 3. This indicated that there was 1013.2 μg gossypol biodegraded by the fungi, and there was 404.16 μg (1417.36 μg-1013.2 μg) FG turned into BG, namely inactivation. The biodegradation of gossypol was catalyzed by some enzymes secreted by microorganism, which can be confirmed by the previous reports that enzyme extracts of the mycelium of *Pleurotus florida* demonstrated its biodegradability when incubated with FG, and the increase in enzyme concentration showed enhanced gossypol degradation [13]. However, which enzymes answer for the biodegradation reaction of FG is still not clear until now. The inactivation of FG may be caused by binding it with proteins or amino acids secreted by microorganism.

Enzyme formation of the fermented substrate

As was shown in Table 6, almost all the necessary hydrolytic enzymes, such as proteinase, cellulase, hemicellulase, glucoamylase and pectinase, were produced by the fungi in the process of SSF.

Table 6. Enzyme composition of the fermented substrate.

Enzyme	Activity（IU/g)
Protease	11.6
Cellulase	27.0
Hemicellulase	485.0
Glucoamylase	292.0
Pectinase	304.0

Each value represents the mean

The nutritive value of the detoxified CSM was enhanced greatly by these enzymes, which can help animals to hydrolyze the complex substrates of feedstuff effectively. In fact, it is not economically feasible to detoxify FG by fermentation on industrial scale without improving the nutritive value of CSM, due to the higher production cost and dry matter loss than that of chemical and physical treatments. However, almost all the reported microorganisms adopted to detoxify FG did not secrete high activities of hydrolytic enzymes [(9-13)]. In this study, in order to improve the growth of *S. cerevisae* as well as the detoxification efficiency, *A. niger*, an enzyme-producing strain ever used in feed enzyme industry, was adopted, and as a result, high activities of hydrolytic enzymes were secreted. When the fermented substrate was adopted as protein

source to produce complete feed, no extra enzymes needed to be added, thus the production cost was reduced. The combination of the detoxification of FG and the production of multienzyme bio-feed made the biological treatments economically feasible on industrial scale.

Biochemical evaluation

Blood biochemistry profile was used for the diagnosis of certain problems and defects that may be encounted with feeding the treated diet. Values of hematological parameter of experimental rabbits were in the normal physiological range except the animals were fed on untreated CSM. Blood metabolites values such as plasma proteins, albumin, (A), globulin (G), A/G ratio and glucose content (Table 7) were also in normal physiological range reported by kaneko *et al.* [33]. These findings reflect that the detoxified treated diet was efficient to make this diet feeding.

Table 7. **Blood hematological and biochemical parameters (M±SD) of experimental white New Zealand male rabbits.**

Blood Parameters	Normal diet	Toxified diet of CSM	Detoxified diet of CSM
Hb (g/dl)	9.50^{a}±1.70	5.62^{c}±0.39	7.80^{b}±0.38
Total protein (g/dl)	7.00^{a}±0.16	5.03^{b}±0.46	7.10^{a}±0.43
Albumin (g/dl)	3.90^{a}±0.10	2.16^{b}±0.11	4.10^{a}±0.19
Globulin (g/dl)	3.10^{a}±0.14	2.97^{a}±0.10	3.00^{a}±0.11
A/G ratio	1.26	0.73	1.37
Glucose (mg/dl)	100.0^{a}±2.00	130.9^{b}±0.11	100.12^{a}±2.03

Means having different superscript letters differ significantly ($P < 0.05$)

Values of liver and kidneys function are presented in Table 8. Activities of ALT and AST and ALT/AST showed that there were insignificant differences among control and detoxified group. The activities of liver enzymes were in the normal physiological range [33, 34]. Other kidneys function tests were also in the normal physiological range [33]. The liver and kidneys functions showed that there were harmful effects on rabbit liver and kidneys due to toxified diet but not for detoxified diet relative to normal control group.

Feed consumption throughout the experimental period (offered feeds-residual feeds) are presented in Table 9. Rabbits consumed feeds throughout experimental period about ranged between 16.00 and 17.00 Kg for one group.

Slight increase in feed consumption was observed in rabbits feeding detoxified diet and slight decrease in feed consumption was noticed with group feeding toxified diet. Total feed consumption is with in the normal requirements and allowances [21].

Table 8. **Some liver and kidneys function parameters (M±SD) of the experimental white New Zealand male rabbits.**

Blood parameter	Normal diet	Toxified diet of CSM	Detoxified diet of CSM
AST (U/l)	12.40^{a}±0.91	24.11^{b}±2.22	12.72^{a}±1.00
ALT (U/l)	14.10^{a}±0.90	30.0^{b}±2.09	14.5^{a}±1.02
AST/ALT ratio	0.88	0.80	0.88
Bilirubin (mg/dl)	0.71^{a}±0.04	2.22^{c}±0.19	0.81^{b}±0.05
Urea (mg/dl)	13.92^{a}±0.89	17.72^{b}±0.13	12.99^{a}±0.90
Uric Acid (mg/dl)	4.11^{a}±0.32	7.84^{b}±0.07	4.07^{a}±0.30
Creatinine (mg/dl)	0.85^{a}±0.05	1.64^{c}±0.13	1.00^{b}±0.06

Means having different superscript letters differ significantly ($P < 0.05$).

Table 9. **Some feed parameters (M ± SD) of the experimental white New Zealand male rabbits.**

Feed parameters	Normal diet	Toxified diet of CSM	Detoxified diet of CSM
Feed consumption, Kg	16.90±1.09	13.36±1.13	17.86±1.42
Weight gain, K	3.18±0.21	2.00±0.12	3.10±0.14
Feed conversion	5.31	8.18	5.76
Feed efficiency	0.19	0.12	0.17

Means having different superscript letters differ significantly ($P < 0.05$).

Feed conversion (daily feed intake / daily weight gain) and feed efficiency (daily weight gain / daily feed intake) is a good indicator of any stress that the animal weight suffers due to the quality of feeding diet. Values of both feed conversion and feed efficiency indicate that there are insignificant differences among normal and detoxified diet group but not for toxified diet group. Weight gain of control rabbits were within the range but not for toxified diet group reported in growing rabbit [35].

CONCLUSION

For the first time, the present investigation reported that *S. cerevisae* could detoxify FG in CSM in SSF. The detoxification efficiency as well as the growth of *S. cerevisae* could be enhanced by *A. niger* when the two strains were cultivated together at suitable ratio (3:1, v/v). Maximum detoxification efficiency (90.2%) occurred after 48h of incubation at 30 ^{o}C in a 250ml conical flask containing 15g of CSM supplemented with 1% (w/w) $(NH_4)_2SO_4$ at the optimal conditions including the IMC 55% (w/w) and inoculum level at 5%(v/w). The detoxification of FG was a growth-associated process, which was highly correlated with the dry matter weight loss during the process of fermentation. Both the autoclaving and fermentation treatments played important roles in the detoxification of FG. Moreover, high activities of hydrolytic enzymes, such as proteinase, cellulase, hemicellulase, glucoamylase and pectinase, were also produced in SSF, which enhanced the nutritive value of the detoxified CSM. It can be concluded that the feeding of detoxified CSM by *S. cerevisae* and *A. niger* as a mixed culture in this study without any adverse effect on rabbits.

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مجلة البحوث الإشعاعية والعلوم التطبيقية

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معاملات ازالة سمية الجيسيبول الحر من جريش بذرة القطن بواسطة المعالجه الميكروبيه بالمزارع المختلطه والتقييم الكيميائى الحيوىلها على الارانب

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فى هذا البحث تم دراسة مقدرة المزارع المختلطة لخميرة كانديدا تروبيكالس و فطر اسبرجيلس نيجر فى ازالة سمية الجيسيبول الحر من جريش بذرة القطن تحت ظروف التخمر شبه الصلبة وتفعيل نشاط العزلة الأكثر اختزالا للجيسيبول من خلال تحديد ظروف التخمر المثالية.

دلت النتائج على أن المعالجة الميكروبية المختلطة بواسطة هذه الفطريات لهذا المخلف أدت إلى اختزال محتوى الجيسيبول الحر به. سجلت أعلى نسبة ازالة للسمية وهى 90% بعد 48 ساعة من بدء عملية التخمر عند درجة حرارة 30 م فى دوارق مخروطية سعة 250 مللى تحتوى على 15 جرام من المخلف المغذى ب 1% من مادة كبريتات ألأمونيوم عند مستوى رطوبة 55% و معدل حقن 5%. كما وجد أن ازالة سمية الجيسيبول الحر من هذا المخلف مر تبط بمنحنى النمو للفطريات المعالجة له و التى بدورها ترتبط بقوة بمعدل الفقد فى المادة الجافة لبيئة النمو. علاوة على ذلك، سجلت النتائج نشاط متزايد لبعض ألأنزيمات الهاضمة فى بيئة التخمر مما يساعد فى رفع القيمة الغذائية لجريش بذرة القطن المتخمر بواسطة المزارع الميكروبية المستخدمة فى عملية التخمر.

كما تم دراسة تأثير التغذية بجريش بذرة القطن منزوع الجيسيبول على نمو عدد 48 ذكر أرنب نيوزيلندى ابيض. وأوضحت النتائج أن جميع القياسات الهيماتولوجية والكيميائية الحيوية لدم ألأرانب التى تغذت على الجريش منزوع السمية كانت فى المدى الفسيولوجى الطبيعى ولم يحدث أى تغيير معنوى فى وظائف الكبد أو الكلى لحيونات التجربة، كذلك لم تحدث اختلافات معنوية فى نمو الجسم (الزيادة الوزنية) كذلك التحول الغذائى و كفاءة استخدام الغذاء بين مجموعة الكنترول ومجموعة التغذية على الجريش منزوع السمية. وتستخلص من هذه الدراسة قابلية استخدام جريش بذرة القطن منزوع سمية الجيسيبول بواسطة المزارع المختلطة لخميرة كانديدا تروبيكالس وفطر اسبرجيلس نيجر فى التغذية للارانب محل الدراسة دون حدوث أى تأثيرات عكسية عليها.