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Research article

DETOXIFICATION OF ANTI NUTRIENTS (TANNINS) PRESENT IN FRUIT EXTRACTS FOR PROBIOTIC YEAST PRODUCTION

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ABSTRACT: Injured/spoiled fruits like Banana, Orange, Grapes and Pomegranates were collected locally from fruit market. *Prosopis juliflora* pods were collected from university campus and all the fruit substrates were dried in a hot air oven at 60 ^{0}C for 8 hours. The amount of Tannin (Catechin) present in different fruit substrates were estimated by standard AOAC method. Tannins acting as anti nutrients were detoxified by using 5% Ca $(OH)_2$ method. 100 g of each detoxified and non detoxified fruit substrate were boiled in 1 liter distilled water to obtain 10 per cent fruit extract followed by centrifugation at 5000 rpm for 5 minutes to remove sediments and impurities. 100 ml of each fruit extract medium was taken into 250 ml conical flask, sterilized by autoclaving and inoculated with 1 per cent probiotic yeast *Saccharomyces cerevisiae* (OBV9) and incubated. After incubation, optical density (OD_{660}) and cell packs were determined. Based on the yield of yeast, availability of substrates and cost, among all the fruit extracts *Prosopis* juliflora was selected for further studies.

Key words: Tannins, detoxification, fruit extracts, probiotic yeast.

INTRODUCTION

Yeast culture "*Saccharomyces cerevisiae*" is used in monogastrics and ruminants to stimulate and stabilize the processes that occur in the gastro-intestinal tract and help to increase competitive exclusion of undesired organisms in the digestive tract thereby enhancing the performance of livestock (Bhukya Bhima et al., 2010). Since the media available in market for the production of probiotic yeast is not cost viable, the present study concentrates on development of economically viable medium using damaged/spoiled fruits which are going waste in fruit markets. Tannins-the anti nutrients common in fruits, leaves, flowers,twigs, tree bark (Reed et al., 1985)Tannic acid is an important gallotannin belonging to the hydrolysable class, while catechin belongs to the non-hydrolysable class. Tannins have been reported to be bacteriostatic or bactericidal agents (Hisanori Akiyama et al.,2001). Tannin can be toxic to filamentous fungi, yeasts, and bacteria. Their main characteristic is that they bind and precipitate proteins. They can have a large influence on the nutritive value of many foods eaten by humans and feedstuff eaten by animals. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity (Scalbert, 1991).

The antimicrobial properties of tannins present in many plant foods have been well documented (Howell et al., 2010). Since the anti nutrients interfere with mineral absorption by microorganisms (Ochanda et al., 2010) this study concentrates on one aspect that has led to low utilization of the nutrients by probiotic yeast. Several detoxification methods are available because detoxification is vital to enhance the nutrient value. Detoxification methods include physical methods like autoclaving (Jose Serrano et al., 2009.) and chemical methods like $Ca(OH)_2$ treatment (S.Anandan et al.,2005) and alkali treatment (Banda-Nyirenda and Vohra, 1990). Among these methods, physical (autoclaving) and chemical ($Ca(OH)_2$) treatments were employed in this study for detoxification of different fruit extracts for mass cultivation of probiotic yeast.

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MATERIALS AND METHODS

Damaged/spoiled fruits like Banana, Orange, Grapes and Pomegranates were collected locally from kothapeta fruit market, Hyderabad. *Prosopis juliflora* pods were collected locally from university campus.

Estimation of tannins in fruit substrates: Tannins present in different fruit substrates were estimated by standard AOAC method. Condensed tannins or its monomeric components are extracted from duplicate samples of dried fruits using 1 per cent HCl in methanol. The clear extract is mixed with vanillin reagent or 4% HCl in methanol (Blank) and the resulting soluble complex is measured calorimetrically (kannan,R., BabuUV.,2011). Amount of tannin in the original sample is calculated as D-catechin equivalents/g.

All the fruit substrates were dried in a hot air oven at 60 ^{0}C for 8 hours and 200 mg of each dry powdered sample were taken in 2 screw capped test tubes and 10 ml HCl in methanol was added and mixed the contents. All the test tubes were kept in a shaking water bath for 30 min. at room temperature. Samples were then centrifuged and supernatants were taken. For each of the samples two test tubes were taken and to each of the tube 1 ml of aliquot of the supernatant was added. To one of the test tubes 5 ml vanillin reagent was added and mixed well. To the second tube 5 ml 4% HCl in methanol was added and mixed well. After 5 min. of incubation at room temperature, absorbance (OD) was read at 510 nm.(Price et al.,1978).

4% Concentrated HCl in methanol: 4 ml of HCl in 90 ml methanol in 100 ml volumetric flask and volume was made up to 100 ml with methanol.

D-Catechin standards (3 mg/ml in methanol): Accurately weigh 9 mg D-Catechin and dissolve in 3 ml methanol. Carefully pipette 0.05, 0.20, 0.50 and 1.0 ml of D-Catechin standard in to screw capped test tubes (Table 1).

Dilutions were made with Catechin stock as follow:

Absorbance at 510 nm was taken in a spectrophotometer (*Systronics-UV-Visible*) for both standards and samples.

Table1. Method of Tannin standard preparation

S.No.	D-Catechin std. (ml)	Catechin (mg)	Methanol (ml)	Absorbance
1	0.05	0.15	1.00	0.0162
2	0.20	0.60	0.95	0.0646
3	0.50	1.50	0.80	0.1625
4	1.00	3.00	0.50	0.3242
				Mean=0.1419

Tannin is calculated by: Tannin (mg Catechin equivalents/g) = Mean X Sample absorbance X 10 X 5. Since tannins present in fruits act as anti nutrients and inhibit/minimize the absorption of micro nutrients, these anti nutrients must be detoxified using appropriate methods.

Detoxification of Tannins

Chemical Method: Dried and mashed *prosopis* pods and other injured fruits like grapes, bananas, orange, pomegranates were taken and mixed with 5% $Ca(OH)_2$ separately. The mixed contents were kept under sun light for 8 h for reduction of tannins (S.Anandan et al., 2005).

Method of extract preparation and cultivation of probiotic yeast

***Prosopis juliflora* pods extract medium:** Locally collected 100 g each of ground detoxified and undetoxified *prosopis* pods were boiled in 1 liter distilled water separately for 15 min to obtain 10 per cent extract. The contents were filtered through double layered muslin cloth followed by centrifugation at 5,000 rpm for 5 minutes to remove sediments and impurities. 100 ml of each extract medium was taken into 250 ml conical flask and the flasks were sterilized by autoclaving at 15 lbs/15min. After sterilization, medium flasks were inoculated with 1 per cent probiotic yeast *Saccharomyces cerevisiae* (OBV9) inoculum and incubated at 32 ^{0}C for 48 h in shaker incubator at 150 rpm speed. After incubation, optical density (OD_{660}) and cell packs were determined and cell packs were represented in grams/liter on dry weight basis (Bhima 2009).

Injured/spoiled orange extract medium: One hundred grams each of injured detoxified and undetoxified orange fruits were boiled in 1 liter distilled water separately for 15 minutes to obtain 10 per cent extract. The contents were filtered through double layered muslin cloth followed by centrifugation at 5,000 rpm for 5 minutes to remove sediments and impurities. 100 ml of each extract was dispensed into 250 ml conical flask and the flasks were sterilized by autoclaving at 15 lbs for 15minutes. After sterilization, medium flasks were inoculated with 1 per cent probiotic yeast inoculum and incubated at 32 ^{0}C for 48 hours in shaker incubator at 150 rpm. After incubation, optical density (OD_{660}) and cell pack were determined.

Injured/spoiled banana extract medium: Injured and spoiled banana fruits, which were collected locally from fruit market were peeled and sliced and detannified. 100 g of each detoxified and non detoxified banana were taken in 1 liter distilled water separately for preparation of 10 per cent medium and boiled the contents for 15 minutes. Contents were filtered through double layered muslin cloth and filtrate was spinned at 5,000 rpm for 5 minutes. One hundred ml of each extract medium in 250 ml conical flasks were sterilized by autoclaving at 15 lbs/15 minutes and inoculated with 1 per cent inoculum. After inoculation, flasks were incubated at 32 ^{0}C and 150 rpm for 48 hours in shaker incubator. OD and cell packs were determined and represented as described in the above methods.

Injured/spoiled grapes extract medium: Injured and spoiled grapes were collected locally and crushed to make fine pulp and the same was detannified. Both detoxified and non detoxified fine grape pulp were taken for preparation of 10 per cent extract medium. Contents with distilled water were boiled for 15 minutes, filtered through muslin cloth and spinned at 5,000 rpm for 5 minutes. 100 ml of each extract in 250 ml conical flasks were sterilized at 15 lbs for 15 minutes and inoculated with 1 per cent probiotic yeast culture inoculum. Flasks were incubated at 32 ^{0}C and 150 rpm for 48 hours in shaker incubator. Cell packs and OD_{660} were determined after incubation.

Injured/spoiled Pomegranates extract medium: Conical flasks of 250 ml capacity containing 100 ml of each 10 per cent de-skinned & detannified and non detannified pomegranates extract in distilled water were sterilized at 15 lbs for 15 minutes and inoculated with 1 per cent probiotic yeast culture inoculum. The flasks were incubated at 32 ^{0}C for 48 hours and cell density was measured in terms of OD and cell pack.

RESULTS AND DISCUSSION

Estimation of Tannins: Tannin (Catachin) content of *Prosopis* pods, Banana, Orange, Grapes and Pomegranates were estimated and represented as 1.11, 0.42, 0.21, 0.70 and 0.31 mg/g of dry fruit substrate, respectively. The percentage of tannin (Catachin) present in *Prosopis* pods, Banana, Orange, Grapes and Pomegranates are 0.11, 0.04, 0.02, 0.07 and 0.03, respectively (Table 2). The tannin percentage present in *Prosopis* pods is in accordance with Makkar et al.,1990.

Method of detoxification: Mashed *Prosopis* pods and other fruits like Grapes, Banana, Orange, Pomegranates were taken separately and mixed with 5% $Ca(OH)_2$ and kept under sunlight for 8 h later tannins were estimated. There were no tannins found in all the fruit substrates.

This indicates the chemical method used was effectively detoxified/removed the tannins from all the fruit substrates and it is appropriate for detoxification of tannins.

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Table 2. Tannin content in different fruit substrates

S.No.	Sample	Absorbance$_{510nm}$	Tannins (mg/g)	Per cent
1	*Prosopis* pods	0.1574	1.11	0.11
2	Banana	0.0598	0.42	0.04
3	Orange	0.0302	0.21	0.02
4	Grapes	0.0996	0.70	0.07
5	Pomegranates	0.0446	0.31	0.03

The biomass yields on dry weight basis obtained in different detoxified substrate extract media *Prosopis* pods, Banana, Orange, Grapes and Pomegranates are 5.16, 4.36, 4.72, 2.17 and 0.95 g/l, respectively. The biomass yields on dry basis obtained in different non detoxified 32 substrate extract media *Prosopis* pods, Banana, Orange, Grapes and Pomegranates are 5.07, 4.02, 4.53, 1.96 and 0.89 g/l, respectively. The optical density values obtained for detoxified *Prosopis* pods, Banana, Orange, Grapes and Pomegranates extract media are 6.21, 5.12, 5.83, 3.41 and 2.25 respectively. The optical density values obtained for non detoxified *Prosopis* pods, Banana, Orange, Grapes and Pomegranates extract media are 6.21, 5.12, 5.83, 3.41 and 2.25 respectively (Table 3). In all the above media, except orange and pomegranates extract media, there was comparable and considerable growth at 10 per cent levels. There is not much difference in yeast growth between chemical detoxified and non detoxified fruit extract media. Based on the growth of yeast, availability of substrate and cost effectiveness of substrate, *prosopis juliflora* pods extract medium was selected for further studies.

Table 3. Effect of different detoxified and non detoxified fruit substrate extracts on the growth of probiotic yeast (*Saccharomyces cerevisiae OBV9*).

S.No.	Substrates	Detoxified		Non detoxified	
		OD660	Cell pack d/b(g)/l	OD660	Cell pack d/b(g)/l
1	Prosopis Pods	6.21	5.16	6.04	5.07
2	Grapes extract	5.12	4.36	5.03	4.02
3	Banana extract	5.83	4.72	5.43	4.53
4	Orange extract	3.41	2.17	3.37	1.96
5	Pomegranates extract	2.25	0.95	2.16	0.89

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