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Research Paper

Effect of a Natural Phenolic compound, Thymol on Alcoholic Fermentation

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Abstract

In fermentation with herbs as the base medium, it was observed that certain phytoconstituents affect the pace of fermentation. *Trachyspermum ammi* is a common ingredient in many herbal based fermentation products especially in Ayurvedic system of medicine. Thymol constitutes 40-50% of the volatile oil in this herb. Thymol is known to be nihibitory lactic acid fermentation. Evaluation of the effect of Thymol in alcoholic fermentation was undertaken Varying concentrations of Thymol 0.3, 0.1 and 0.05% were added to the sugar medium and kept for fermentation. The result of the study showed a complete inhibition of alcoholic fermentation conducted by yeast and *Woodfordia* fruticosa flowers at Thymol concentrations of 0.3 and 0.1% but a retarded pace was observed in case of lower concentration (0.05%).

Keywords: Alcoholic fermentation, inhibition, thymol, *Woodfordia fruticosa, T rachyspermum ammi*

Fermentation, the degradation of sugar or other organic nutrients into small amounts of ATP is one of the oldest biotechnological methods practiced by our progenitor's millennia ago. During this process, the six-carbon sugar glucose is broken down into two molecules of the three-carbon organic acid, pyruvic acid, coupled with the transfer of chemical energy to make adenosine triphosphate (ATP). In alcoholic fermentation, the pyruvic acid from glycolysis gets converted into acetaldehyde, which is reduced to ethyl alcohol by NADH (David *et al*., 2005; Soni and Chawla 1992).

Fermentation has wide applications in many industries like food, brewing, pharmaceuticals biofuel, starch processing, enzyme production etc. Ayurvedic industry produces self fermented medicines, like Arishtas or Asavas, which forms a major category of medicines (Murthy.,1984). Medicinal plants form the base of these preparations. *Arishtas* or *Asavas* have water or water extract of herbs as the base. To this base, sweetening agents like jaggery or sugar is added. A combination of powdered herbs known as *Prakshepa choornam* is added to this mixture which mostly contains aromatic herbs. The whole mixture is subjected to fermentation. (The Ayurvedic Formulary of India., Sekhar *et al*., 2008). Often in certain combination, fermentation has been observed on retared pace. Subsequent to this issue in fermentation, Ajamoda one of the ingredients in the *Prakshepa choornam* (Krishna *et al.*, 1996) was separated from the initial fermentation mixture and was later added in the course of fermentation. This step improved the pace of fermentation. In fact, observation basically evoked the need for such a study. Flowers of *Woodfordia fruticosa* (Wf), an inclusion in most of the

arishtas or *asavas* is supposed to be fermentation initiator in the arishtas preparation (Ramachandra Reddy, 2004; Sivarajan *et al*.,2004).

Woodfordia fruticose are used to enhance sucrose, hydrolysis. Hydrolysis of sucrose is mediated by endogenous invertease flesent in the flowers (Weerassa ooooriya *et. al.,* 2001)

Ajamoda botanically known as *Trachyspermum ammi* belongs to the family Apiaceae. Phytochemical studies on its seeds show the presence of Thymol which constitutes about 40-50% of the essential oil (Dwivedi *et al.,* 2012; Rafiul *et al*., 2012; Quality Standards of Indian Medicinal Plants., 2005, The Ayurvedic Pharmacopoeia of India., 2001., Nair., 2003; Kirtikar *et al.,* 2006; Nesamony.,1998). It is a phenolic monoterpene compound, which possesses strong antimicrobial and antifungal activity (Bahnaz *et al*., 2011; Falcone *et al*., 2005). Survey revealed that Thymol has an inhibitory action on lactic acid fermentation (Laura *et al*., 1983). The present work aims at evaluating the effect of the natural phenolic compound, Thymol on *Woodfordia fruticosa* induced fermentation and yeast fermentation as a control.

Materials and Methods

Samples and chemicals

The samples of Jaggary and Woodfordia were collected from The Arya Vaidya Pharmacy (Coimbatore). All the chemicals used were of analytical grade.

Preparation of Sugar Media

Sugar solution (20%w/v) (Rajkumar, 2012) was prepared by dissolving 200g jaggery in 1 L distilled water and filtered through a muslin cloth. The medium was then, sterilized by autoclaving at 121^{o} C, 15 psi for 15 min. and allowed to cool. Eight media in all were prepared in the similar manner in conical flasks which were then divided into two sets for performing the study.

Inoculation of yeast cells and addition of Thymol

20g of yeast cells were made into a suspension with 400ml sterile water at 40^{0}C.This was then, transferred equally into four conical flasks. Varying concentration of Thymol 0.3% (Sample 1), 0.1% (Sample 2) and 0.05% (Sample 3) were added to different conical flasks. One sample was kept without Thymol as a control (C1).

Preparation of *Woodfordia fruticosa* (Wt) added media with varying concentration of Thymol

Four conical flasks with 20g of dried flowers of Wf instead of yeast as fermenting agent were also prepared with varying concentrations of Thymol 0.3% (Sample 4), 0.1% (Sample 5) and 0.05% (Sample 6). One sample was kept without Wf flowers as control (C2) in the present study. The temperature during the study was maintained at 26± 2^{0}C. Sampling was done periodically and aseptically for physico-chemical evaluation.

Determination of Alcohol content

The ethanol content of the liquid was expressed in per cent volumes at 25^{o}C. 100ml of the sample was measured accurately at 25 ^{o}C and transferred to a 500ml round bottom flask. 50ml of distilled water was added and neutralized with sodium hydroxide. Connected the flask to a condenser by means of a suitable still-head, and 90 ml of the distillate was collected. It was then, made upto 100ml using distilled water at 25^{o}C. The specific gravity of the distillate was determined at 25^{o}C. The percentage of ethyl alcohol corresponding to the specific gravity was noted from the standard table. [The Ayurvedic pharmacopoeia of India., 2007.]

Estimation of Sugar Content

The reducing sugars in the samples was determined by titration method using Fehling solution A and B [Indian standard specification for extracted honey, 1982]. The percentage of reducing sugars was calculated accordingly.

Results and Discussion

Fig 1 shows the alcohol percentage produced and the sugar utilized during the yeast fermentation. The control showed a gradual increase in the concentration of alcohol and a decrease in the quantity of sugar with passage of time. By the end of the fourth day, the alcohol percentage reached a maximum of 8.54% thereafter maintain a constant value. The sugar concentration was inversely proportional to the alcohol percentage. However, Sample 1 and 2 with Thymol recorded almost negligible amount of alcohol. Thus, complete inhibition of fermentation. The sugar percentage remained same as that of the initial day of fermentation and accordingly the alcohol percentage was also “0” throughout the study. In case of sample 3 with Thymol concentration 0.05%, a complete inhibition of fermentation was not observed, but the rate of fermentation was lower

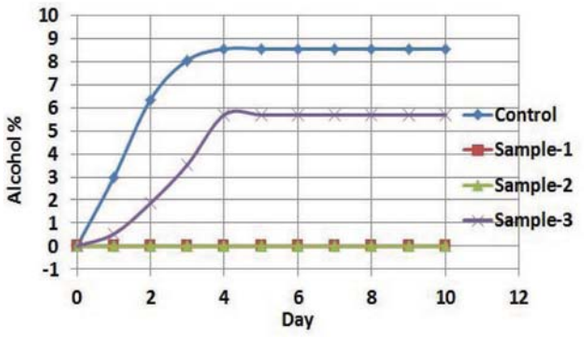

Fig. 1: Production of Alcohol percentage of yeast

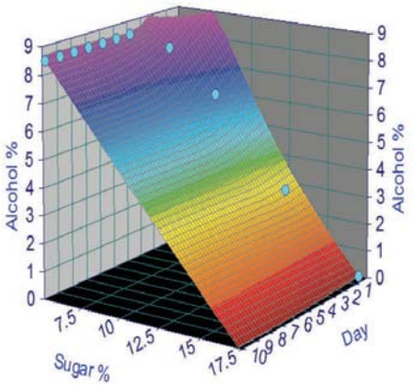

Fig. 2: Showing 3D plot of yeast fermentation as a control

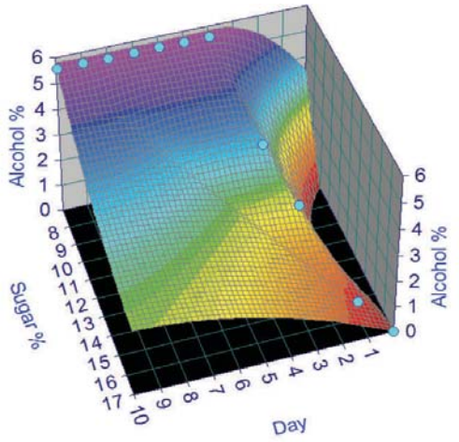

Fig. 3: Showing 3D plot of sample

than that of the control (C1) attaining a maximum alcohol percentage of 5.69 % by the tenth day. The sugar percentage in sample 3 was according to the alcohol level.

The 3 D plots of control (C1) and Sample 3 were made for representing and comparing the three important factors of fermentation such as time, substrate concentration and product concentration (Fig 2 and 3). X axis represents the day, Y axis the alcohol formed and Z axis the sugar % remained in the medium.

The results obtained in the fermentation with WF flowers were more or less similar to that of the yeast fermentation with slight difference in the alcohol percentage and sugar percentage (Fig 4 and 5). The control sample (C2) had started fermenting on the 4th day and the rate of fermentation was relatively slow when compared to the yeast control (C1) attaining a maximum alcohol percentage of 9.97 (V/v), which remained constant in the subsequent days. Sample 4 and 5 as in yeast sample (1 and 2) showed a total inhibition in the fermentation throughout the study. In case of sample 6 with Thymol concentration of 0.05%, complete inhibition of fermentation was not observed as in sample 3. Here the maximum alcohol produced was 3.11%.

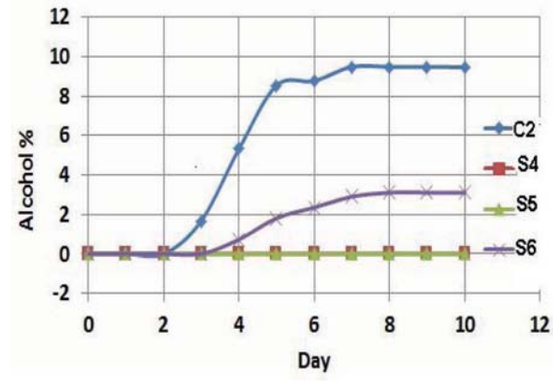

Fig. 4: Alcohol production in WF aided fermentation during different intervals of time.

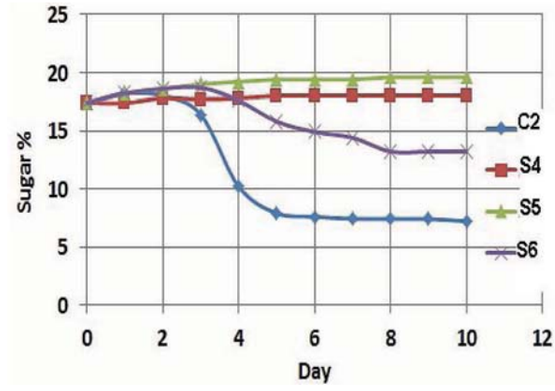

Fig. 6: Sugar percentage of WF aid fermentation during different intervals of time.

The 3 D plots of control (C2) and Sample 6 were plotted for representing and comparing the three important factors such as time, substrate concentration and product concentration (Fig 6 and 7). X axis represents the day, Y axis the alcohol formed and Z axis the sugar % remained in the medium.

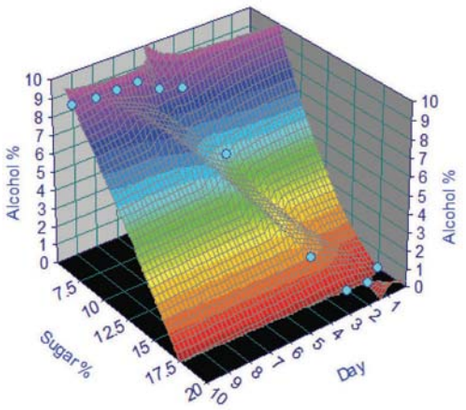

Fig. 6: 3D plot of C2 showing relationship between Alcohol (%), sugar (%) and the time of fermentation.

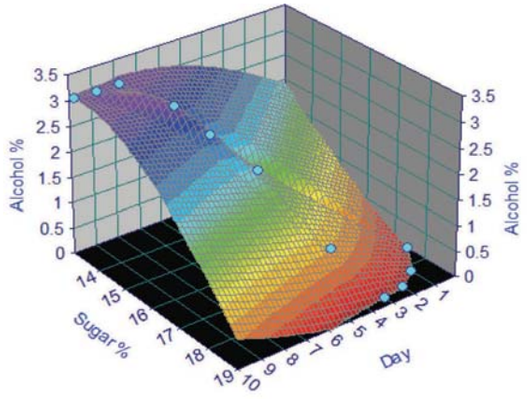

Fig. 7: 3D plot of S6 Showing relationship between sugar (%), alcohol produced and the time of fermentation.

All the samples were kept for 30 days to observe any change in the alcohol percentage and sugar percentage and it was observed that the values remained the same.

From this study, it is concluded that Thymol has a strong inhibitory action on alcoholic fermentation using yeast and Wf flowers even at low concentration. Thymol's action on *in vitro* fermentation of glucose by ruminal microorganisms has been extensively studied. It has been reported that Thymol is a strong inhibitor of lactate production (Jeff *et al*., 2000). Its inhibitory action on alcoholic fermentation using yeast cells would possibly be imparted by its effect on yeast cells. Thymol exerts the action by causing membrane damage accompanied by surface alteration of yeast cells (Bennis *et al*., 2004). The inhibition of alcoholic fermentation mediated by WF flowers by Thymol may also be due to its action on yeast strain present in these flowers. Many reports of isolation of yeasts from Wf flowers have been published and researchers also established that these are acting as the fermentation initiators (Prashant *et al.*, 2013; Jagdish *et al*., 2013). In short, Thymol has a strong effect on alcoholic fermentation preventing the formation of alcohol by inhibiting the yeast cells. This lead may be used to resolve the problems related to slow fermentation, which is often faced in the ayurvedic manufacturing industry.

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