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Short Communication

Enzyme Efficacy of Aero - contaminated Digestive Syrups

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Abstract - *Airborne microbial contamination may affect the functional properties of enzyme in digestive syrups. Present investigation was carried to assess the α-amylase activity of aerocontaminated digestive syrups. The protein content along with enzyme activity of digestive syrups challenged with environmental isolates of proteolytic aero flora. The results obtained show considerable reduction in the efficacy of an enzyme activity indicates the need for post selling quality management of pharmaceutical products.*

Key words: Aero-contamination, proteolytic aeroflora, pharmaceutical products, amylase activity, protein content.

Introduction

Microbial contamination of pharmaceutical products is rapidly becoming a matter of concern worldwide [1]. Pharmaceutical products are often susceptible to contamination by variety of microorganisms during manufacturing and consumption. Due to ubiquity of microorganisms, specific control measures must be adopted to avoid microbial contamination. Air is major source of contamination, during the use of pharmaceutical products contamination with microorganisms irrespective of being harmful or objectionable non-pathogenic microbes can bring about physical and chemical changes [1]. Contaminating microbes may bring about the conversion of syrup formulation into the toxic metabolites [1].

Altah et al. (2004) [1] recognized such products as microbiologically unsafe for consumption and may pose potential health hazards to patients as well as constitute wastage and may yield serious economic losses to the manufacturer. Although, the use of preservative for maintaining the quality of syrup formulations is a commonly practiced, some contaminating microbes may grow even in presence of preservative causing spoilage of the products [1,2]. The digestive syrup formulations often contain amylolytic, proteolytic and lipolytic enzymes.

Present investigation aim to assess the effects of aerocontamination of digestive syrups taking α-amylase as a marker enzyme. *In vitro* evaluation of enzyme activity and protein content of digestive syrup formulations against challenged proteolytic aeroflora isolated from different environment viz. home, hospital and environment, has been carried out.

Materials and Methods

Isolation of proteolytic aeroflora and consortium preparation

The proteolytic aeroflora was isolated on milk agar. The pates were exposed to different aerial environment viz. home, hospital and environment for a period of 10 min. and incubated at 28^0C for 24 hrs. Bacterial colonies showing clear zone of hydrolysis around them were selected and enriched in nutrient broth and cell density of $3X10^6$cfu/ml was adjusted as per McFarland standards [2].

Challenging syrups with proteolytic consortium

Locally available and frequently used digestive syrups were purchased and labelled as sample A and B, further used as model. Total 10 ml quantity of each sample was transferred separately to six different test tubes and numbered sequentially. Each sample tube was then challenged with enriched proteolytic consortium @ 10 ml ($3X10^6$cfu/ml). Separate tubes containing 10 ml of enriched consortium was maintained as control. All tubes were incubated at 28^0C for different incubation period viz. 24, 48, 72, 96 and 120 hrs. The growth rate of challenged proteolytic consortium was monitored by evaluating standard plate count (SPC) after respective incubation period [2].

Estimation of enzyme activity and protein content

Enzyme activity and protein content of test and control was determined during the incubation period at an interval of 24 hrs as described by Rao and Deshpande, (2005)[2]. Enzyme activity was assayed

spectrophotometrically (Systronics make) estimation of amount of reducing sugar (maltose) was done by by 3,5-dinitrosalicylic acid (DNSA) method.Further Enzymeunits were calculated adopting standard definition viz: One unit of enzyme will liberate 1.0 mg of maltose from 1 % starch solution in 5 min under defined conditions. Protein content was estimated by Biuret method.

Enzyme units were calculated by following formula:

Enzyme Units/ml = Maltose (mg) released/Vol. of enzyme used (ml) X 5.

Results and Discussion

Five different proteolytic isolates were isolated from different aerial environment and were used for the preparation of consortium. Syrup samples (A and B) both were challenged with proteolytic consortium. The analysis of challenged syrup samples revealed that as incubation period increases, the α-amylase activity decrease significantly along with the reduction in protein content.

Protein content profile

Table 1 depicts the effects of aero bacterial consortium on the reduction of the protein content in both challenged digestive syrup samples as compared to uninoculated control. It was observed that as incubation period increases there is gradual reduction in protein content in the samples tested. For sample A, the initial protein was 4.6 mg/ml where as it was only 2.63 mg/ml after 120 hrs. of incubation which corresponds to 42.82 % reduction in syrup protein content. In case of sample B, initial protein was 7.2 mg/ml and was found to be 4.03 mg/ml after 120 hrs. of incubation corresponding to 44.02 % reduction in syrup protein content.

Reduction in protein content may be attributed to the proteolytic activity of challenged consortium due to secretion of various proteolytic exoenzymes. Significant accumulation of various metabolites including organic acids may also contribute to proteolysis and the denaturation of protein structure, there by reducing the protein content.

α-amylase activity profile

Table 2 summarizes the effects of aero bacterial consortium on per cent reduction in α-amylase activity of challenged digestive syrup samples. Significant reduction was observed in enzyme activity of both test samples over control.

It was observed that as incubation period increases there is gradual reduction in enzyme units. For sample A, initial enzyme units were 92 Units/ml and minimum enzyme units were found to be 42.50 Units/ml after 120 hrs. of incubation which corresponds to 53.08 % reduction in syrup enzyme activity. For sample B, initial enzyme units were 21.0 Units/ml and minimum enzyme units recorded were only 11.50 Units/ml after 120 hrs. of incubation corresponding to 46.42 % reduction in syrup enzyme activity.

Decrease in enzyme activity in both the samples might be due to the alteration in optimal conditions (pH, ionic concentration) required by enzyme. The bacterial metabolic products generated may alter the reaction environment for enzyme as well may interfere with the enzyme structure and stability so posing limitations on enzyme activity.

Growth profile

The growth profile was studied and express as colony forming units per ml. The growth profile of aerobacterial proteolytic consortium in digestive syrup and in nutrient broth, as control, is represented in Table 3. It was observed that as incubation period increases there was increase in growth upto 72 hrs. Thereafter it starts to decrease gradually as evident from their cfu status. The decrease in growth after 72 hrs. incubation could be due to the several reasons. Depletion of the nutrients, accumulation of toxic metabolites as result of metabolic activity of bacterial consortium and may be due to alterations is growth parameters for ex. pH, ionic concentration etc.

Our results are in accordance with experimental findings of Altah et al. 2004 [1], that unhygienic handling of pharma products and lack of microbiological control may contribute to high microbial load. They also observed high counts $2X10^8$ cfu/ml in chloroquine syrup formulation and injection preparations. Parker (1984) [1] recognized that spoilage of medicines could involve initial activity of pioneer invaders biodegrading microbes, which by degrading complex nutrients, altering surrounding pH, moisture etc. may make ways easier for later degraders.

Conclusion

The experimental findings indicate that increase in bacterial loads in protenious pharmacy products decreases the protein quantum which ultimately affects by reduction in the enzyme activity. Hence, present studies reveal that aero-contamination may deteriorate the quality of pharmaceutical formulations specially having microbial nutrition values. Aero- contamination of digestive syrups may lead to serious functional and economic loss. Thus, research and development related to the preservatives in pharmaceutical formulations should be maximized, to avoid the spoilage and subsequent losses of pharmaceutical products especially in use with the consumers.

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Table 1: Effect of aerobacterial consortium on protein content of digestive syrup

S. No.	Incubation period (Hours)	Digestive Syrup Sample A		Digestive Syrup Sample B	
		Protein Content (mg/ml)	Reduction in Protein Content (%)	Protein Content (mg/ml)	Reduction in Protein Content (%)
1	Control	4.6	-	7.2	-
1	0	4.48	2.60	6.98	3.05
2	24	3.71	19.56	8.11	29.02
3	48	3.33	27.60	4.33	39.86
4	72	2.66	42.17	3.96	45.00
5	96	3.26	29.13	4.56	36.66
6	120	2.63	42.82	4.03	44.02

Table 2: Effect of aerobacterial consortium on amylase activity of digestive syrup

S. No.	Incubation period (Hours)	Digestive Syrup Sample A		Digestive Syrup Sample B	
		Enzyme Activity (Units/ml)	Reduction in Enzyme Activity (%)	Enzyme Activity (Units/ml)	Reduction in Enzyme Activity (%)
1	Control	92	-	21.0	-
1	0	90	2.17	20.75	1.19
2	24	65	29.34	17.0	19.04
3	48	60	34.78	13.95.	33.57
4	72	57.2	37.78	13.25	36.90
5	96	45	52.08	12.25	41.66
6	120	42.5	53.08	11.25	46.42

Table 3: Growth profile of proteolytic aerobacterial consortium in digestive syrup
(SPC X 10^6 cfu/ml)

S. No.	Incubation period (Hours)	Digestive Syrup Sample A	Digestive Syrup Sample B	Control
1	0	2.91	2.81	2.91
2	24	6.2	5.6	6.3
3	48	8.9	9.8	11.7
4	72	7.8	7.1	9.8
5	96	5.8	5.1	7.3
6	120	4.2	3.8	5.3