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Effect Of Lactic Acid Bacteria In Development Of Papaya Juice Using Response Surface Methodology

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ABSTRACT:

The Objective of the study was to determine the suitability of papaya juice for production of probiotic juice by Lactic acid bacteria. Fruit juices are the suitable media for cultivating probiotics. Probiotics are live microbial food supplements that beneficially affect an individual by improving intestinal microbial balance. The ability of probiotics to withstand the normal acidic conditions of the gastric juices and the other microorganisms, allow them to be established in the intestinal tract. The juice was fermented at different time intervals (24-72hrs) and inoculum size (1-3%). The optimization can be done using response surface technology. The microbiological and chemical analyses such as pH, acidity, total sugar and absorbance, etc were studied. The optimum conditions for production of papaya juice are at 48hrs of fermentation time and 3% of inoculum size. At 48hrs maximum number of viable cells survive. *L. plantarum* showed a more rapid drop in pH than *L. acidophilus*. When pH decreases acidity increases, then it will minimize the influence of spoilage bacteria. Probiotic papaya juice could serve as a healthy beverage for vegetarians and diabetic patients and this juice is mainly aiming for lactose intolerance patients. If probiotics are fed to lactose intolerance patients, then milk lactose is hydrolysed by probiotic strains and lactose is assimilated.

Keywords: papaya juice, probiotics, response surface methodology, lactose intolerant

INTRODUCTION

Now a days the market for functional foods or foods that promote health benefits is developing. Probiotics are a type of functional food in which it is defined as live microbial food ingredients that exerts health benefits in humans. Probiotics affects an individual by improving the intestinal microbial balance and is also used as a growth promoter for antitumour, lactose intolerance and hypocholestrolaemic (Suvarna *et. al*., 2005). For the production of fermented functional foods one of the most important method is probiotication. In order to exhibit the probiotic health benefits it should be viable at a high concentration, typically 10^6cfu/g of product (Shah, 2001).

For cultivating probiotics, fruit juices serves as the best medium (Mattila-Sandholam *et al*., 2002). Since the fruit juices contain potassium salt, bioflavones and vitamins etc, it have good results in prophylaxis and even in treating cardiovascular diseses(Dana *et al*., 2007). This probioticated fruit juices does not contain dairy allergens so this will be helpful to lactose intolerant patients (Lucknow and Delahunty, 2004). *Carica papaya L*. is regarded as an excellent source of ascorbic acid, a good source of carotene, riboflavin and a fair source of iron, calcium, thiamin, niacin, pantothenic acid, vitamin B6 and vitamin K. (Krishna *et. al*). Christopher Columbus called papaya the "fruit of angels" because of its sweet delicious flavor. Papaya fruits are rich in enzymes called papain and chymopapain that break down the proteins from the food a person eats into amino acids and therefore helps digestion. *Lactobacillus acidophilus* and *Lactobacillus plantarum* possess functional properties such as the ability to synthesis various vitamins and antitumor activity. *Lactobacillus acidophilus* colonizes the intestinal tracts of man and animals and suppresses the pathogenic microorganisms. Soluble and insoluble fibres present in the fruits may contribute positively to growth and viability of probiotic strains and recent studies indicated that the positive effect of plant ingredients on the viability of probiotic bacteria and the high applicability of apple juice and pomace as matrix for probiotication (Ana Paula do Espirito Santo-PhD). The commonly consumed juices like watermelon, sapodilla, grape and orange were taken for the study as a proper medium for the development of probiotic juices, which is a healhy beverage for consumers who are allergic to dairy products (Anita *et al*., 2013).

The response surface methodology (RSM) is a effective tool which uses quantitative data in an experimental design to optimize a process (Vieira *et al*.,). It has been used for optimizing processes in fruit and vegetable juice production (Rai et al., 2004; Sin et al., 2006; Sun et al., 2006. A central composite rotatable design (CCRD) is an experimental design to define empirical models or equations for describing the effect of test variables and their interactions on the responses (Sun et al., 2006).

The Objective of this study was to establish optimum conditions for the development of probioticated papaya juice and to study the chemical and microbiological analysis of the probioticated papaya juice using response surface methodology

MATERIALS AND METHODS

PAPAYA JUICE PREPARATION

Ripened papaya (*Carica papaya L*.) with 80-90% maturity and free from visual blemishes were purchased from the local market of Coimbatore.

The juice was prepared in the ratio fruit : water in 1:1 ratio. Commercial enzyme (pectinase) from the source organism *Aspergillus niger*, was procured from

HIMEDIA with activity of 8000-12000 U/g proteins was used for clarification of papaya juice.

INOCULUM PREPARATION

Inoculum were prepared by transferring a glycerol stock culture of *L. acidophilus* and *L. plantarum* to a 250 ml Erlenmeyer flask containg 100 ml of sterile MRS broth. Cell cultivation is carried out at 37^0 C for 24 hrs. This culture was used as an inoculum to the juice fermentation.

FERMENTATION OF PAPAYA JUICE AND EXPERIMENTAL DESIGN

To determine the optimum fermentation conditions, response surface methodology was used. CCRD was used to study the combined effect of these independent variables and a two-variable, eight combinations was employed to study the combined effect of these independent variables. The inoculum size (%) and time (hr) was chosen as response variables. Chemical and microbiological analyses have been done. The factors chosen are Absorbance, TSS, ph, acidity, total sugar, reducing sugar, ascorbic acid and microbial viability.

Fermentation experiments were conducted in 250 ml Erlenmeyer flasks, each containing 100 ml of sterile papaya juice. Inoculum size ranges from 1-3 (%) was added to the sterilized juice and kept for incubation at 37^0 C at 100 rpm in a orbital shaker. Samples were taken every 24 hr for chemical and microbiological analyses. The datas obtained have to be recorded in the design table obtained from the software.

CHEMICAL AND MICROBIOLOGICAL ANALYSES

Samples were taken at every 24 hr interval for chemical and microbiological analyses. pH was measured with a pH meter. Total acidity was expressed as total lactic acid percent, was determined by titrating with 0. 02 N NAOH. Sugar content was analyzed as glucose by phenol sulphuric acid method (Dubois *et al*., 1956). Absorbance can be found out using spectrophotometry at 590 nm. TSS can be found out using Refractometer and ascorbic acid can be determined using redox titration method using iodine solution. Viable cell counts (cfu/ml) were determined by the standard plate method with MRS agar after incubation at 37^0 C.

STATISTICAL ANALYSIS

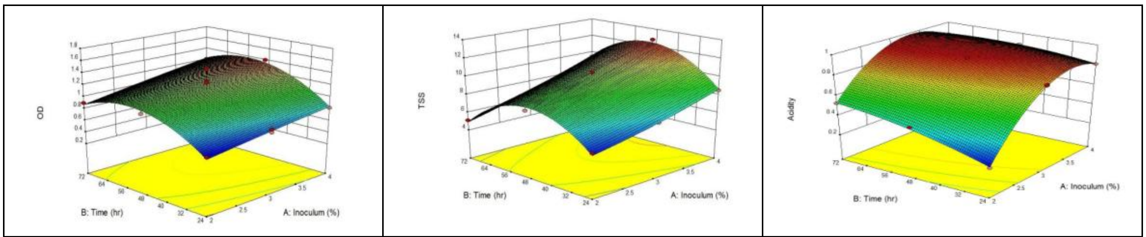
The experimental design and statistical analysis were performed using Design Expert Software Versio9. 0. 4. Analysis of variance (ANOVA) were conducted to determine whether significant effect existed for the factors.

RESULTS AND DISCUSSION

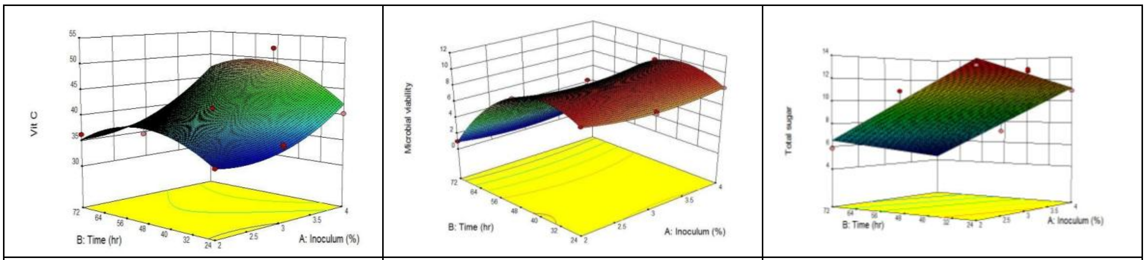
Both *L. plantarum* and *L. acidophilus* were found capable of growing well in sterilized papaya juice. It was reported that the probiotic fermentation of indigenous food mixtures containing papaya pulp using *L. casei* and *L. plantarum* showed a decrease of pH, increase of acidity, and improvement of the digestibilities of starch and proteins (Sindhu and Khetarpaul, 2001). Although the papaya juice had an initial pH of 5. 3, the *L. acidophilus* and *L. plantarum* actively fermen the juice and lowered he pH to as low as 3. 2 after 72 hr fermentation. When compared to *L. acidophilus*, *L. plantarum* showed a more rapid drop in pH. It was reported that acid production ability by lactic acid bacteria, especially post-incubation affected the cell viability of probiotic bacteria including *L. acidophilus* and *Bifidobacterium bifidum* (Ishibashi and Shimamura, 1993; Shah *et al*., 1995). *L. plantarum* consumed the sugar at a much faster rate than *L. acidophilus*. Extending the fermentation time from 48 to 72 hr did not result in a significant increase in viable cell counts. This could be due to the low pH and high acidity in the fermented juice (Yong *et al*., 2004). It is important to have a significant number of viable lactic acid bacteria present in the probiotic products for maximum health benefis (Shah, 2001). Probiotic cultures are commonly used in the dairy industry, and some products produced during lactic acid fermentation such as lactic acid, diacetyl, and acetaldehyde could be associated with the loss of viability of the added probiotic bacteria (Post, 1996). In general the cell viability depends on the strains used, interaction between the species present, culture condition, oxygen content, final acidity of the product and the concentration of the lactic acid and acetic acid (Yong *et al*., 2004).

L. acidophilus and L. plantarum growth and viability were significantly influenced by inoculum size (%) and time (hr). The fitted models were obtained from the CCRD. The fitted models were validated by ANOVA analysis and F-test. All the models were statistically significantly because the calculated F- value (6. 0) were greater than the listed F-value (5. 05) at 95% confidence level. The determination coefficient (R^2) was 0. 95 for all fitted models. Fig 1 and Fig 2 shows the response graphs for *L. acidophilus* and *L. plantarum*. The optimum operating condition for the microbial growth were inoculum size 3(%) at 48hr.

The results presented here in agreement with other studies (Yoon *et al*., 2006 ; Gupta *et al*., 2010), which suggested that different vegetable matrices could serve as good media for growing probiotics by stimulating their growth, resulting in good viable counts. Maximum growth was available at different conditions of viability. Microbial survival in foods is strongly dependent on the food matrix (Shah, 2007).

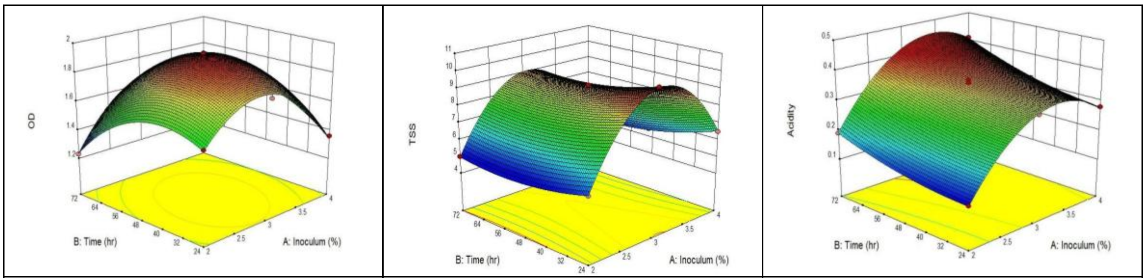


(a) (b) (c)



(d) (e) (f)

FIG 1: Surface response graphs of *L. acidophilus* in papaya juice as function of inoculum size and time (a);Absorbance (b);TSS (c);Acidity (d) Vit C (e);Microbial viability and (f);Total sugar



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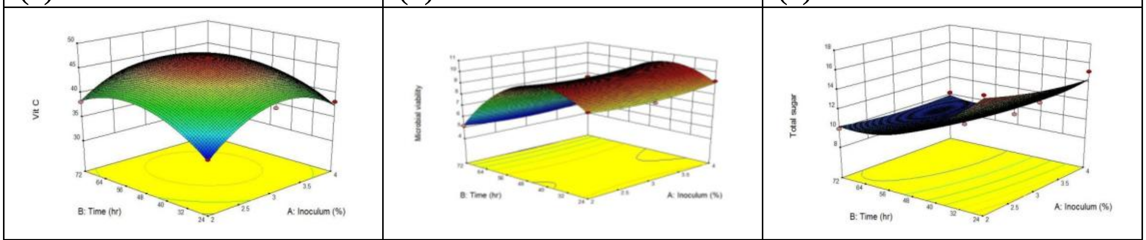

(d) (e) (f)

FIG 2: Surface response graphs of *L. plantarum* in papaya juice as a function of inoculum size and time (a);Absorbance (b);TSS (c);Acidity (d);Vit C (e);Microbial viability (f);Total sugar

CONCLUSION

Despite the great potential for the use of fruit juice as probiotic carriers, little work has been done in this field to consider fermented juices. Most reported studies are based on microbial addition of probiotic strains to fruit juices. This study shows that probiotics can grow well in papaya juice without any external nutrient supplementation. Good viable counts are obtained at 48 hr for 3 % inoculum. From the result of this study, it is concluded that both *L. acidophilus* and *L. plantarum* are suitable for use as probiotic cultures for production of a healthy beverage from papaya pulp for vegetarians or consumers who are allergic to lactose present in probiotic dairy products.

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