Unstructured kinetic modeling of growth and lactic acid production by *Lactobacillus plantarum NCDC 414* during fermentation of vegetable juices

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ABSTRACT

Unstructured Gompertz and Logistic models were developed to describe growth, substrate utilization and lactic acid production by *Lactobacillus plantarum* NCDC 414 in juices of bitter gourd (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*) and carrot (*Daucus carota*). These vegetable juices have considerably good quantities of total sugars that allowed the growth of *L. plantarum* for 24 h at 37 °C, after which the cell numbers begin to decline. Kinetics of cell growth, lactic acid production and sugar consumption were evaluated. The viable cell counts increased from 4×10^5 CFU/ml to 7×10^{10} CFU/ml after 24 h of fermentation. The lactic acid increased by about 4.5 times in 24 h and about 44% w/v reduction in sugar content was observed during growth of *L. plantarum*. Significant lactic acid production occurred during the growth phase as well as stationary phase. With the kinetic model proposed by R. Luedeking and E.L. Piret for lactic acid production rate, the growth associated and non growth associated coefficients were determined. The model was demonstrated for batch growth of *L. plantarum* in vegetable juice.

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1. Introduction

Lactic acid fermentation is a product inhibited process. High acid tolerance is a distinctive feature of *Lactobacillus plantarum*, and this allows the fermentation process to proceed almost free of contamination. Therefore, the homofermentative lactic acid bacterium, *L. plantarum*, was used in this work to study the production of lactic acid from sugars in vegetable juice. *L. plantarum* is probably the most advantageous of the more commonly used bacteria for the conversion of lactose to lactic acid. *L. plantarum* is often used as a starter culture in the production of fermented commodities such as sausage, cucumber pickles and silage (Gupta, Abu-Ghannam, & Scanell, 2011). However, very few studies have been conducted on the fermentation kinetics of *L. plantarum* in vegetable substrates. This work examines in detail the batch kinetic behavior of *L. plantarum* in the fermentation of vegetable juice under aerobic conditions.

The purpose of fermentation modeling is to design large-scale fermentation processes using data obtained from small-scale fermentations. The mathematical models that are used to simulate a bioprocess can generally be classified as unstructured or structured. In unstructured models the biomass is considered as one entity described only by its concentration. These models do not take into account any changes that could take place in the inner cells. In structured models the biomass is defined and includes intracellular components, such as the RNA content, enzymes, reactants and products. Although the structured models provide a better understanding of the modeled system, unstructured models are mainly used to describe bacterial kinetics in complex natural substrates. This is mainly due to the complexity of the substrates and to the difficulties in obtaining large sets of experimental data for the intracellular components. In food microbiology, mathematical modeling has been mainly applied to predict growth or inactivation of spoilage of bacteria and foodborne pathogens (Charalampopulous et al., 2008).

In the last 10 years, there has been an increasing interest in modeling the kinetics of beneficial microorganisms in food systems. Mathematical modeling techniques have been used more often to predict growth or inactivation of spoilage bacteria and pathogens however; recently attention has been paid to biokinetics of beneficial food grade microorganisms, such as lactic acid bacteria. Lactic acid bacteria are used as starter cultures to many food products such as milk, meat, vegetables, cereals, for processing,

fermentation, extending shelf life of foods and offering unique organoleptic qualities (Leroy and De Vuyst, 2003). Their major advantages are excellent competitiveness, desirable acidification of food, production of antimicrobial substances, which preserves the food from undesirable spoilage and pathogenic microflora. For this reason modeling focuses on cell growth and acidification power (Bello & Sanchez-Fuertez, 1995; Ganzle, Ehmann, & Hammes, 1998; Passos, Fleming, Ollis, Hassan, & Felder, 1993). Growth or nongrowth related models are also applied to describe the changes of other biochemical compounds and physical properties in these food systems. These changes include primary or secondary metabolites concentrations, volatile production as well as rheological and textural properties (Bouguettoucha, Balannec, Nacef, & Amrane, 2007). The aim of these models is to mathematically relate the biochemical properties (response variables) to environmental factors (controlling factors), such as temperature, pH, water activity and substrate composition. This contributes to a better understanding and control of the fermentation process. In general, modeling is performed in two stages; in the first stage the primary models are applied to the experimental data describing the change of a response variable over time, in the second stage, secondary models are developed expressing the bio-kinetic parameters derived from the primary models as a function of a single environmental factor. It must be noted that both primary and secondary models are built using data from experiments in synthetic media under carefully controlled conditions. The predictability of the models is then assessed in the complex food systems. LAB research has focused so far in modeling the dependence of the growth rate on temperature and pH at pH-controlled conditions. Very little research has been done in the secondary modeling of growth when pH is not controlled, or taking into account other bio-kinetic parameters, such as lactic acid and bacteriocins production (Vázquez & Murado, 2008).

Vegetables are one of the most suitable substrates for the development of foods containing probiotic microorganisms (in most cases lactic acid bacteria or bifidobacteria) and may also have prebiotic properties. Traditionally, probiotic microorganisms have been introduced in dairy products, meat and fruit juices. The definition and development of new functional vegetable-based foods combining the beneficial effects of vegetable juices and health promoting bacteria is a challenging issue (Balannec, Bouguettoucha, & Amrane, 2008; Guerra, Agrasar, Macias, Bernardez, & Castro, 2007). The major biochemical properties affecting the functionality and quality of a probiotic formulation are the cell population, lactic acid concentration and pH (Charalampopoulos, Vazquez, & Pandiella, 2009). The cell concentration in the end product is an indicator of probiotic functionality. The lactic acid influences the organoleptic properties and also acts as a preservative agent, while the pH is the main factor determining the stability and safety of the product during storage (Kedia, Vazquez, & Pandiella, 2008; Rozada-Sanchez, Sattur, Thomas, & Pandiella, 2008). Since sugar is the main carbon source used by the cells, knowledge of the sugar kinetics contributes to a better understanding of cell growth and product formation.

The aim of this study was to develop a model that would be able to simulate the kinetics of cell growth, lactic acid production and sugar consumption in vegetable-based fermentations with *L. plantarum*. The kinetic parameters of the primary models for these dependent variables were expressed as a function of the initial sugar concentration of the vegetable juice. The models were built using data from fermentations in synthetic media. Finally, the predictability of the models was evaluated in the vegetable-based fermentations. Thus, the parameters obtained from the models allow for the characterization of these cultures and could be a preliminary step in the formulation of novel probiotic foods.

2. Materials and methods

2.1. Microrganism and inoculums

The strain *L. plantarum* NCDC 414 was procured from National Dairy Research Institute Karnal, (Haryana) India. It was maintained at 4 °C and subcultured monthly on MRS agar (Hi Media). Isolated colonies from MRS agar were pre-cultured twice in MRS broth (Hi Media) for 24 h at 37 °C. The cells were collected by centrifugation at 10,000 rpm and 4 °C for 10 min, washed twice with sterile distilled water and resuspended in the same solution. The bacterial suspension used as inocula for the fermentation studies 1%, (v/v) was obtained from 12 h pre-cultured cells.

2.2. Culture media and microbiological methods

The synthetic media used in the studies on *L. plantarum* growth was modified MRS broth (modified by addition of selected antibiotic, ciprofloxacin) and vegetable juice substrate consisted of a blend of bitter gourd (Total solids: 3.5%), carrot (Total solids: 5.7%) and bottle gourd (Total solids: 1.8%) juices in the proportion of 29:47:24, respectively (previously optimized blend of bitter gourd, carrot and bottle gourd juices, obtained by maximizing polyphenolic content and probiotic cell counts). Vegetables were procured from the local market and their juices, extracted using juicer in food processor (Kenstar Swift Plus, Juicer, Kolkata). The juices were then, heat treated at 80 °C for 20 min, with complete absence of any detectable bacteria or fungi, before fermentation. Shakeflask fermentations were performed in triplicate using 250 ml screw-capped Schott-Duran glass bottles without oxygen control. Bottles were inoculated with 1% (v/v) of *L. plantarum* and incubated at 150 rpm and 37 °C for 24 h. Cell growth was monitored by measuring the optical density of the media at 600 nm. The samples for measurement of optical density were collected from the bottles by transferring them from incubator to a pre sterilized laminar air flow, using sterile micropipette. Sterilized MRS broth, without inoculation was used as blank for synthetic media. The absorbance was measured after a 1:10 dilution of vegetable juice with distilled water, for vegetable juice media. The distilled water was used as blank.

The biomass of *L. plantarum* was quantified by measuring the optical density measured at 600 nm. Growth was expressed as dry mass concentration (g/L) calculated according to equation, obtained from a calibration curve, built using *L. plantarum* dry cells.

$$\text{Biomass(g/L)} = (\text{Absorbance 590 nm} - 0.00818)/$$
$$3.395 \ (\text{Rodrigues, Lona, \& Franco, 2003})$$

In the fermentation samples pH, reducing sugar (as glucose) and lactic acid content were analyzed.

2.3. Analytical methods

The dinitrosalicylic acid (DNS) assay was used to measure the reducing sugar concentration in the supernatants of the fermented vegetable extracts. A standard curve was made using glucose at various concentrations. Lactic acid was measured by titrimetric method using 0.01 N NaOH.

2.4. Numerical and statistical methods

Fitting procedures and parametric estimations calculated from the results were carried out by minimization of the sum of quadratic differences between observed and model-predicted values. Origin 8.0 software was used to evaluate the significance