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BATCH FERMENTATION OF BLACK TEA BY KOMBUCHA: A CONTRIBUTION TO SCALE-UP

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Local domestic Kombucha was used in fermentation of 1.5 g L^{-1} of black tea (Indian tea, "Vitamin", Horgoš, Serbia and Montenegro), sweetened with approximately 70 g L^{-1} of sucrose. Inoculation was performed either with 10% or 15% (v/v) of fermentation broth from previous process. The fermentation was conducted in geometrically similar vessels with 0.4 L, 0.8 L, 4 L and 8 L of substrate, at 22±1 ^{0}C for 28 days. The samples were analysed after 3, 4, 5, 6, 7, 10, 14 and 28 days, so that their pH values, content of total acids, sucrose, glucose and fructose contents, as well as contents of ethanol and vitamin C were determined. Based on the experiment design, the response surface for the product pH, as a function of time, beverage volume and inoculum concentration, was defined in the form of a second-order polynomial. From the obtained response surface, a formula for scaling-up of the process was derived.

KEYWORDS: Kombucha; fermentation; scale-up; metabolites; vitamin C

INTRODUCTION

A beverage possessing characteristics of a functional food is prepared by sugared tea fermentation caused by activity of a symbiotic association of bacteria and yeasts, is well known as Kombucha (1, 2). The yeast cells hydrolyze sucrose to glucose and fructose, producing ethanol (3, 4), while acetic acid bacteria convert glucose to gluconic acid and ethanol produced by yeasts convert to acetic acid. Apart from two main metabolites (ethanol and acetic acid), Kombucha beverage contains most of tea ingredients and other compounds that appear as a result of numerous reactions (5-7). Fermentation process in-

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fluences the content of B-vitamin complex and folic acid, as well as the synthesis of ascorbic acid - vitamin C (8-10).

On the other hand, production of vitamin C and other valuable compounds is related to the working conditions. It has been reported (3, 11, 12) that optimal conditions are: i) seven-day fermentation possesses, ii) interval of working temperatures of 22-28^{0}C, iii) composition of fermented tea and concentration of inoculum adjusted to the particular Kombucha association iv) interval of sucrose concentration of 50-100 g L^{-1}, and v) pH value of the product 3.8-3.7. However, all the data were determined at lab-scale processes, performed in flasks, with less than 0.5 L of liquid. In order to determine impact of liquid volume to the characteristics of the obtained product, as well as to define the system response to the increase of the volume, experiments in this study were conducted in vessels with substrate volumes varying from 0.4 L to 8 L. Some of the reported scaling-up strategies (13-16) were applied on the acquired data, giving a solution for Kombucha beverage processing on a large scale.

EXPERIMENTAL

Kombucha culture and fermentation

Local domestic Kombucha determined by Markov et al. (17) was used for the fermentation.

Tap water was boiled, sweetened with approximately 70 g L^{-1} of sucrose and 1.5 gL^{-1} of black tea (Indian tea, "Vitamin", Horgoš, Serbia and Montenegro) was added and removed by filtration after 15 min. After cooling to room temperature, the tea was inoculated with 10% or 15% of fermentation broth from the previous Kombucha fermentation. Geometrically similar vessels (bioreactors) with 0.4 L, 0.8 L, 4 L and 8 L of substrate were covered with cheesecloth and incubated at constant room temperature of 22±1^{o}C for 28 days. Samples of the products were taken after 3, 4, 5, 6, 7, 10, 14 and 28 days to measure the following quantities: pH value, sucrose, glucose and fructose content, total acids, ethanol concentration and content of vitamin C.

Methods of analysis

pH values were measured using an electric pH meter.

Sucrose, glucose and fructose contents were determined using the test of Boehringer Mannheim (Cat. No. 716260).

Total acids content was determined by the volumetric method with sodium hydroxide and phenolphthalein as indicator.

Ethanol content was measured in accordance with the procedure of Boehringer Mannheim (Cat. No. 176290).

Vitamin C content was determined using the test of Boehringer Mannheim (Cat. No. 409677).

Scaling-up method

The method for scaling-up, whose algorithm is given in Fig. 1, is based on a series of particular processes in a small-scale reactor to obtain the information for scale-up (15).

The effect of process variables and their variation on a selected controlled characteristic of product (here, pH value of the beverage) is determined and decision is made concerning the scale-up of the process.

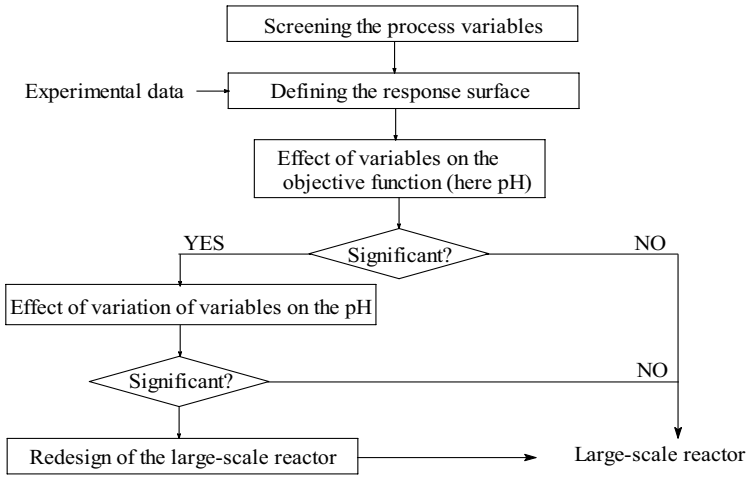

Fig. 1. Algorithm of the scaling-up method (15)

RESULTS AND DISCUSSION

Screening the process variables

In order to investigate the influence of various working conditions on Kombucha fermentation process, experiments were performed and the results presented in Figs. 2-8.

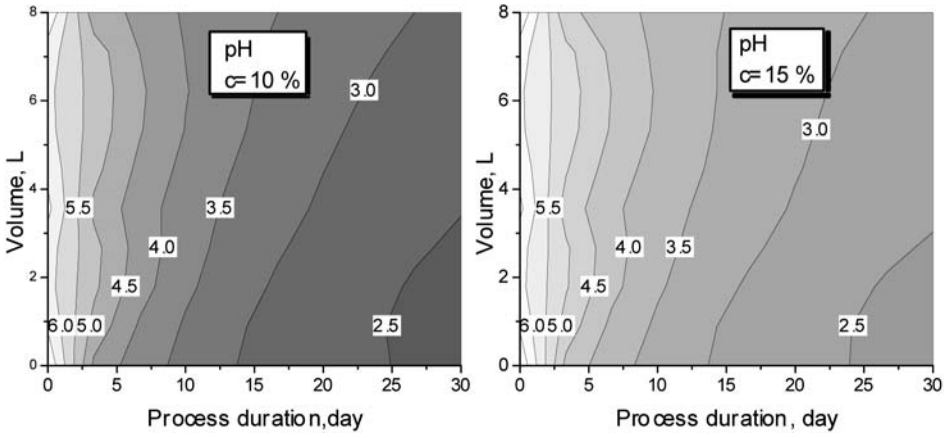

Fig. 2. pH as a function of process duration, vessel volume and inoculum concentration

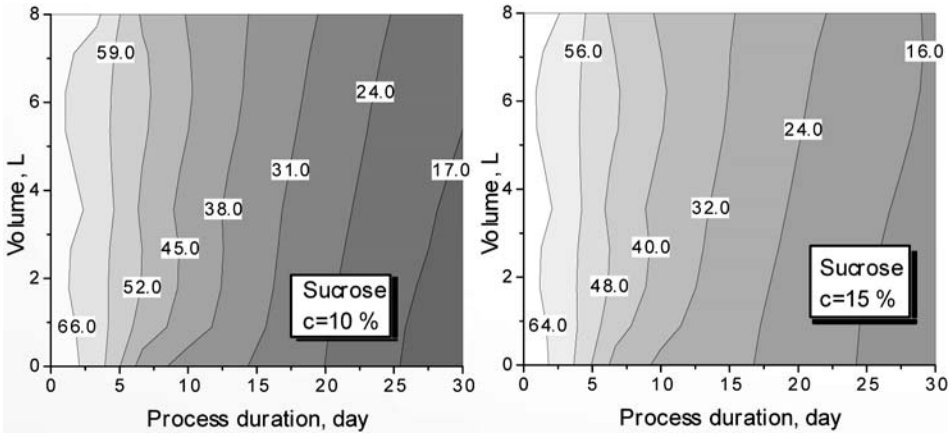

Fig. 3. Sucrose content as a function of process duration, vessel volume and inoculum concentration

Dependence of pH value of the beverage (Fig. 2) on process duration is very significant. The effect of volume on pH is almost negligible at the beginning of the process, and later the influence slightly increases.

Almost the same conclusion follows from Fig. 3, where the changes of sucrose content are presented.

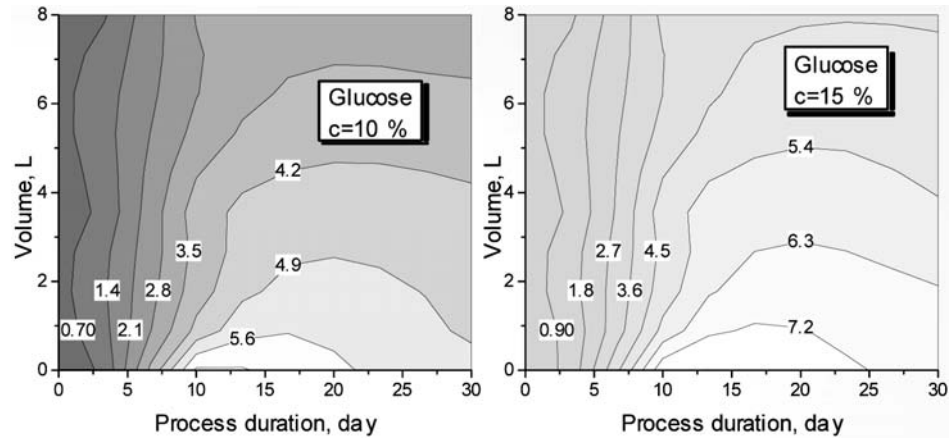

Fig. 4. Glucose content as a function of process duration, vessel volume and inoculum concentration

Changes of glucose and fructose content (Figs. 4 and 5), depend on the change of the process duration and volume, and they follow a more complicated mathematical model. However, this will not be taken into account while scaling-up the fermentation process.

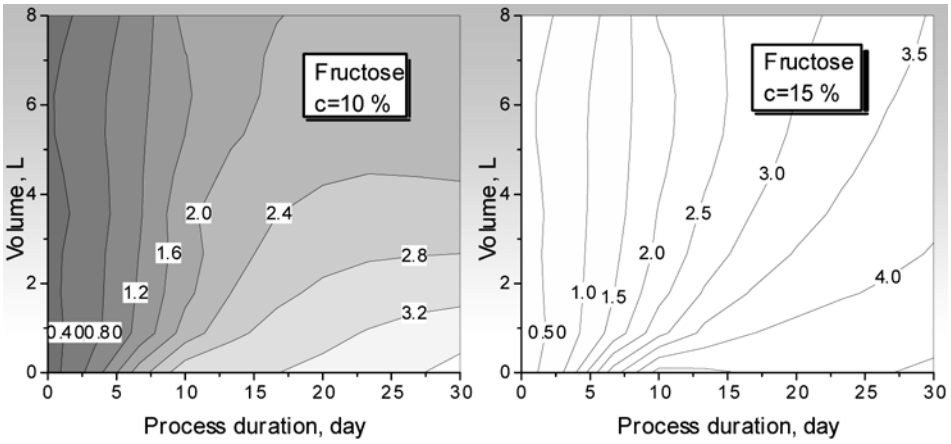

Fig. 5. Fructose content as a function of process duration, vessel volume and inoculum concentration

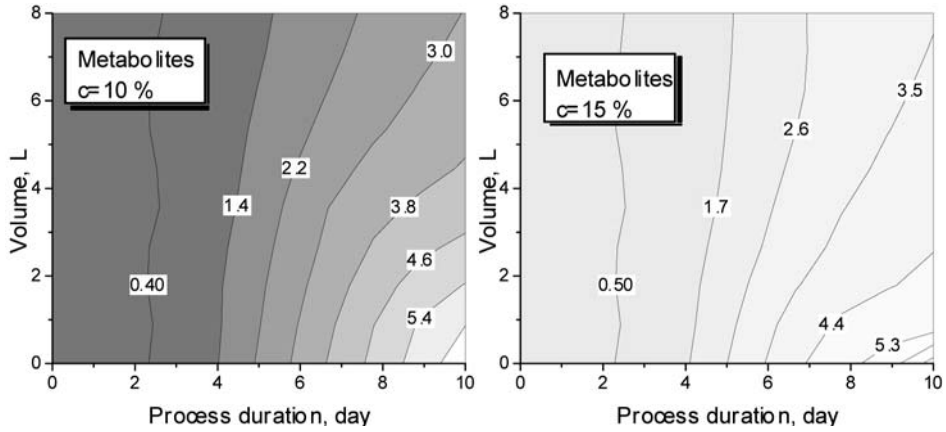

Fig. 6. Quantity of metabolites (sum of quantity of total acids and ethanol) as a function of process duration, vessel volume and inoculum concentration

During sucrose fermentation, two main metabolites - acetic acid and ethanol - appear. However, other acids giving total acidity are also contained in the product and should be taken into account. As for metabolites quantity (Fig. 6), it increases significantly with the increase of time, but decreases slightly with the increase of vessel volume.

Finally, quantity of vitamin C, which is one of the numerous valuable compounds, does not depend of the vessel volume, but increases with the increase of process duration (Fig. 7). This might be important when scaling-up of the fermentation process is concerned.

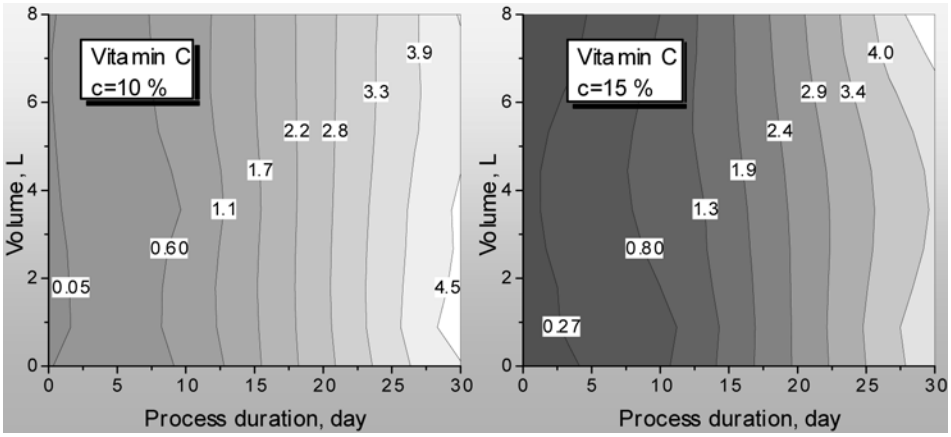

Fig. 7. Vitamin C content as a function of process duration, vessel volume and inoculum concentration

Defining the response surface

Based on the plan of experiments, as well as on the adopted independent variables, the following form of response surface for pH of the beverage was chosen:

$$y = b_1 + b_2x_1 + b_3x_2 + b_4x_3 + b_5x_1x_2 + b_6x_1x_3 + b_7x_2x_3 + b_8x_1^2 \qquad [1]$$

where y denotes pH, x_1 is time, x_2 is the volume of beverage in a vessel and x_3 denotes the inoculum concentration. Polynomial [1] appeared to be the best function when compared with several alternative functions, based on the following criterion:

$$\delta = \frac{\sum_{i=1}^{N} \left(y_{exp} - y_{cal} \right)_i^2}{N} \qquad [2]$$

where y_{exp} denotes the experimental value, y_{cal} represents the calculated value while N corresponds to the total number of experiments. It is obvious that the function [1] consists of the linear and non-linear terms, whereas non-linear terms represent the interaction of two variables (as their product), as well as quadratic time term.

For the purpose of scaling-up Kombucha fermentation process, the empirical mathematical model [1] should be applied to describe the response of the beverage pH, as a leading variable, to the variation of time, volume and inoculum concentration. After statistical processing of the measured data by the regression analysis method, parameters (b) for the pH-response surface are obtained as follows:

$$\begin{aligned} y = {} & 6.113 - 0.3814\ x_1 + 6.311E-02\ x_2 - 1.246E-02\ x_3 + 1.869E-03\ x_1x_2 \\ & + 3.551E-04\ x_1x_3 - 1.2E-03\ x_2x_3 + 8.749E-03\ x_1^2 \end{aligned} \qquad [3]$$

When significances of all b-parameters are considered, it can be concluded that process duration (time) is the most significant variable. On the other hand, inoculum concentration is the least significant variable, both taken as a single and in the interactions with other variables.

Application of the response surface for scale-up

In view of the previous observations, a leading scale-up formula can be obtained by solving equation [3] for the process duration:

$$(x_1)_1^2 = \frac{-B \pm \sqrt{B^2 - 4AC}}{2A} \qquad [4]$$

where:

$$A = 8.749E-03$$

$$B = -0.3814 + 1.869E-03 \ x_2 + 3.551E-04 \ x_3$$

$$C = 6.113 + 6.311E-02 \ x_2 - 1.246E-02 \ x_3 - 1.2E-03 \ x_2 x_3 - y$$

It can be used for estimation of the process duration (x_1) in a specified (enlarged) vessel volume (x_2), when inoculum concentration is either 10 % or 15 %.

For example, if the vessel volume changes from 8 L to 20 L, the product will achieve optimal pH value (3.8) after the times presented in Table 1. The estimated values of process duration increase with increase of the beverage volume quite expectedly. As far as the inoculum concentration is concerned, it can be noticed that the higher inoculum concentration yields a shorter process duration for the same volume of the beverage and the same pH value.

Table 1. Estimated process duration in enlarged vessels

c=10%, pH=3.8		**c=15 %, pH=3.8**	
Volume, L	Process duration, day	Volume, L	Process duration, day
8	9.20	8	8.74
10	9.91	10	9.35
15	12.12	15	11.21
20	16.05	20	14

CONCLUSION

The method for scaling-up, based on a series of particular processes in a small-scale reactor as well as on the application of response surface (defined from experimental data)

was successfully applied to a batch process of Kombucha fermentation. By this method, the process duration, satisfying the required pH value of the product, was estimated for a series of enlarged batch reactors.

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ШАРЖНА ФЕРМЕНТАЦИЈА КОМБУХЕ НА ЦРНОМ ЧАЈУ: ПРИЛОГ ИСПИТИВАЊУ SCALE-UP ПРОЦЕСА

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За ферментацију 1,5 г/л индијског црног чаја, заслађеног са приближно 70 г/л сахарозе, коришћена је локална домаћа комбуха. Супстрат је инокулисан са 10 и 15% ферментационе течности из претходне ферментације, рачунато на запремину супстрата. У геометријски сличним посудама је култивисано 0,4; 0,8; 4 и 8 литара заслађеног црног чаја, на температури од 22±1°C, током 28 дана. Узорци су анализирани након 3, 4, 5, 6, 7, 10, 14 и 28 дана, односно измерена је вредност pH и одређен садржај укупних киселина, сахарозе, глукозе, фруктозе, етанола и витамина Ц. На основу изведених експеримената и усвојених независних променљивих, времена, запремине супстрата и концентрације инокулума, површина одзива је дефинисана као полином другог реда. Вредност pH производа је усвојена као главна зависно променљива. Изведена површина одзива је предложена као основна формула за scale-up процеса ферментације комбухе.

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