Optimisation of food-grade bacterial cellulose production in raisin finishing side-stream extracts and synthetic media: Effect of citric, gallic, and ascorbic acid addition

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Introduction

Bacterial cellulose (BC) is an extracellular microbial polysaccharide that can be produced by a large group of microorganisms with the bacterium *Komagataeibacter sucrofermentans* being established as the model microorganism for its production because of its high BC yields and the ability to utilize a variety of C- and N-sources. Despite its numerous applications, the high cost of BC production in synthetic substrates still makes its applications ineffective. For that reason, researchers and industries are in seek of efficient production methods featuring low-cost substrates (like agri-food wastes, by-products and side-streams), the use of which requires short-scale fermentation process, optimized culture conditions (temperature, pH, substrate composition, etc.) and efficient microbial strains (Bekatorou et al., 2019). The finishing of Corinthian currants (black raisin variety cultivated in Greece) generates a large amount of side-stream (5-6% of the raw material), with similar nutritional quality with the marketable currants. This finishing side-stream (FSS) has been proposed as substrate for food-grade BC production, plain or in mixtures with cheese way (Bekatorou et al., 2019). Apart from tartaric acid which is the main acid in FSS, common organic acids and phenolic compounds in food wastes and side-streams, are citric acid (citrus wastes) and gallic acid (FSS, grape wastes, tea extracts, and a variety of other plant sources). These compounds are known to affect the production of BC (Fernandes et al., 2020). Specifically, citric acid is considered to enhance BC production due to its assimilation as C-source, the buffering of substrate pH, and its involvement in the Krebs cycle. Vitamins also appear to enhance the production of BC. Ascorbic acid specifically, is believed to limit the inhibitory factors D-gluconic acid and D-2-keto-gluconic acid of BC production (Keshk, 2014). In this study, the combined effect of ascorbic, citric, and gallic acid on BC biosynthesis in FSS extracts was studied, in comparison with synthetic media. Optimization of BC production in the substrates was effected by Response Surface Methodology (RSM) based upon the Central Composite Design (CCD) combining the above agents, in order to predict the optimum composition of a low-cost natural substrate made by mixing various agri-industrial side-streams or wastes.

Materials and Methods

Komagataeibacter sucrofermentans DSM No. 15973 was supplied by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. A stock culture was prepared by inoculation in a Hestrin–Schramm medium (HS) consisting of (% w/v): glucose 2.0, bacterial peptone 0.5, yeast extract 0.5, Na_2HPO_4 0.27, and citric acid 0.115, in water. The pH was adjusted to 6.0 by the addition of acetic acid glacial, and the medium was sterilized for 15 min at 120 ºC and 1–1.5 atm. Growth was carried out in 250 mL Erlenmeyer flasks containing 200 mL of the HS-glucose medium for 4 days at 30 ºC (Bekatorou et al., 2019). For BC production, the FSS was extracted with warm water (1:1) at 70 ºC (to pasteurize and avoid fermentation or caramellization of the contained sugars), until an extract of about 4 ºBe density (Baume scale hydrometer) was obtained. Then yeast extract and bacterial peptone (0.5 % w/v each), were added and the whole was pasteurized for 1 min at 120 ºC and at 1.0–1.5 atm. When the temperature of the HS-medium or pasteurized extract decreased to 30 ºC, ascorbic, citric, and gallic acid were added. Growth and BC production was carried out in petri dishes with 15 mL of substrate for 7 days at 30 ºC. To find the maximum yield, the RSM/CCD methodology was used. The independent variables were the concentrations of ascorbic, citric, and gallic acid in the substrates and the dependent variable was the BC yield. Table 1 shows the independent variables and their corresponding coded values. A total of 20 experiments of different independent variable combinations were performed. Each experiment was performed in triplicate.

Table 1. Independent variables and their coded values, for RSM/CCD optimization of BC production.

Independent Variable		Symbol	Coded Values		
			-1	0	1
Ascorbic acid	g/L	X_1	0.00	5.00	10.00
Citric acid	g/L	X_2	0.00	0.50	1.00
Gallic acid	g/L	X_3	0.00	1.00	2.00

Results and Discussion

For the optimization of BC production by RSM/CCD, a large similarity between the experimental and the predicted

values was observed after the mathematical processing, which implies that the model has great credibility. The 2nd-order linear regression equations were obtained, which describe the relation between the dependent variable and the independent variables for each substrate: For HS medium: BC (g/L) = $5.110520 - 0.278873X_1 + 0.164758X_2 + 1.431640X_3 - 0.017833X_1X_2 - 0.020250X_1X_3 + 0.044167X_2X_3 + 0.022327X_1^2 - 0.047475X_2^2 - 0.611818X_3^2$

For FSS extract: BC yield (g/L) = $15.359950 - 0.707555X_1 - 8.554550X_2 + 2.529230X_3 - 0.210000X_1X_2 + 0.088500X_1X_3 + 0.050000X_2X_3 + 0.012545X_1^2 + 6.994550X_2^2 - 2.051360X_3^2$

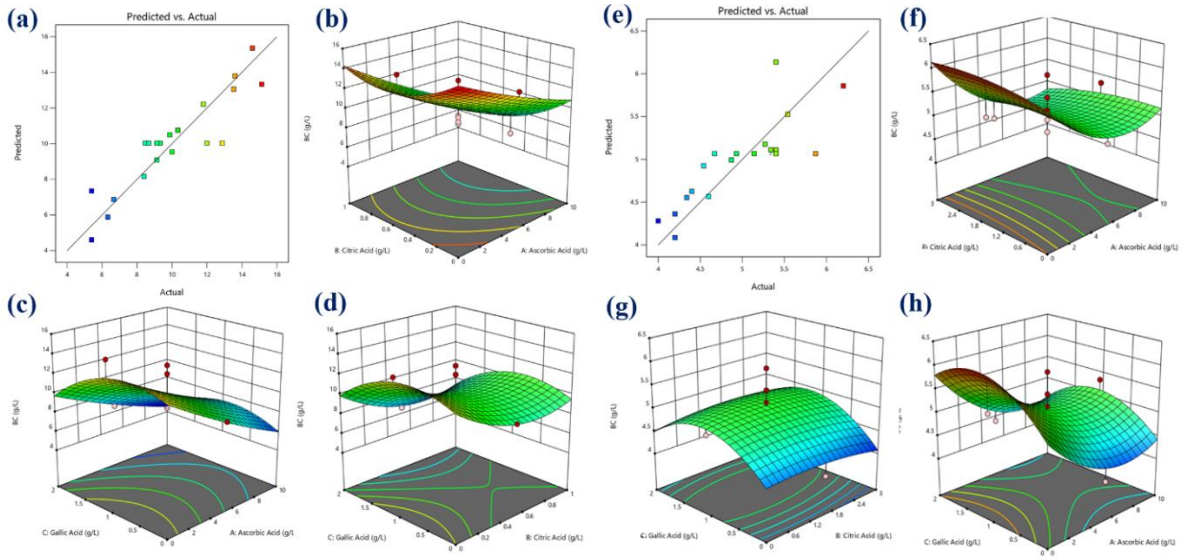

Figure 1. (a) Predicted values against experimental data of BC yield in FSS extract (g/L) according to the experimental design, and 3D-imaging of the BC yield response surface at varying concentrations (g/L) of: (b) citric acid and ascorbic acid, (c) gallic acid and ascorbic acid, and (d) citric acid and gallic acid. (e), (f), (g) and (h) are the corresponding graphs for HS-medium).

The positive coefficients for the model mean that they act synergistically on BC production, while the negative factors mean they have an adverse effect. The model's statistical significance was determined by the F-test for the analysis of variance (ANOVA), where the regression was shown to be significant at the 5% significance level ($p < 0.05$). The probability p was small (<0.0065 for FSS; <0.0276 for HS), showing the importance of the model. The F value of the model (5.57 for FSS; 3.96 for HS) implies that the model is statistically significant with a high degree of confidence. The predicted values of the BC yield using the above optimal combination of factors (citric acid 0.5 and gallic acid 1.0 g/L for FSS, and 1.0 and 2.0 g/L for HS, respectively) in the mathematical model were 13.33 g/L for FSS and 5.86 g/L for HS. Confirmation of these values was done by repeating the experiment with the best obtained factor values. Specifically, 3 experiments were performed and the obtained BC yields were found to be even higher (15.13±0.05 g/L for FSS and 6.20±0.01 g/L for HS). The yield of BC production in plain FSS extract was 11.60±0.00 g/L, and in plain HS medium was 5.40±0.01 g/L. The results will help towards the development of low-cost substrates for efficient food grade BC production from FSS with suitable additions of waste citrus juice and/or tea extracts, or other waste biomass sources containing the studied factors. [Authors acknowledge support of this work by the project "Research infrastructure on Food Bioprocessing Development and Innovation Exploitation–Food Innovation RI" (MIS5027222), implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Enterpreneureship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development fund)].

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