Optimisation of food-grade bacterial cellulose production *by Komagataeibacter sucrofermentans* in raisin finishing side-stream extracts and synthetic media: Effect of vitamins and phenolic compounds

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Introduction

Bacterial cellulose (BC) is an extracellular microbial nano-fibrous product (highly polymerized β-1→4 glucan), with versatile applications as a gelling agent in foods or cosmetic products, textile material, wound dressing, drug delivery matrix, etc. It is produced by a variety of microorganisms, with *Komagataeibacter sucrofermentan*s being the model bacteria for both scientific studying and large-scale synthesis. BC chains are held together by inter- and intra-molecular H-bonds. Specifically, BC consists of sub-fibrils 1.5 nm wide, packed into nano-fibrils of 2-4 nm that form nano-films (BC sheets) 40-60 nm wide. The porosity of the BC matrix along with its relatively simple cleaning and modification procedures make it an ideal material for biotechnological applications (Bekatorou et al., 2019; Lestari et al., 2014). However, its production cost in synthetic media is high, and studies to increase its yield focus on the optimization of media composition by adding factors that either promote its synthesis or eliminate the effect of limiting factors of bacterial growth or BC synthesis (such as D-gluconic and D-2-keto-gluconic acids).

Among low cost raw materials for BC production, the finishing side-stream (FSS) of Corinthian currants (black raisins cultivated in Greece) has been successfully proposed as substrate for food-grade BC production (Bekatorou et al., 2019). Also, vitamins such as ascorbic acid and thiamine and phenolic compounds such as gallic acid, have been found to enhance BC production and affect its textural properties (Fernandes et al., 2020; Keshk, 2014). Their combination in FSS extracts or in synthetic media for BC production by *K. sucrofermentans* has not been reported, and is the aim of the present study. Optimization was effected by Response Surface Methodology (RSM) based upon the Central Composite Design (CCD), in order to predict the optimum mixing of low-cost substrates based on agri-industrial side-streams or wastes (containing these factors) for efficient, low-cost BC production.

Materials and Methods

K. sucrofermentans DSM No. 15973 was supplied by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. The Hestrin–Schramm medium (HS) that was used as substrate for BC production, consisted 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 ˚C and 1–1.5 atm. 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 media were cooled down to 30 ˚C, ascorbic acid, thiamine, and gallic acid were added at various combinations. Growth was carried out in petri dishes with 15 mL of the HS medium for 7 days at 30 ˚C. To predict the maximum BC yield, the RSM/CCD methodology was applied. The independent variables were ascorbic acid, thiamine and gallic acid concentrations. Table 1 shows the concentrations of the independent variables and their coded values. A total of 20 experiments of different independent variable combinations were performed (in triplicate) in both synthetic media and FSS extracts.

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

Independent Variable		Symbol	Coded Values		
			-1	0	1
Concentration of ascorbic acid	g/L	X_1	0.00	5.00	10.00
Concentration of thiamine	g/L	X_2	0.00	0.04	0.08
Concentration of gallic acid	g/L	X_3	0.00	1.00	2.00

Results and Discussion

For the production of BC using the RSM/CCD methodology, 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) = $2.081230 - 0.003727X_1 + 5.159090X_2 - 0.955636X_3 + 0.212500X_1X_2 - 0.011500X_1X_3 + 1.437500X_2X_3 - 0.005727X_1^2 - 151.988640X_2^2 + 0.426818X_3^2$

For FSS extract: BC (g/L) = $15.087410 + 0.172591X_1 + 136.361360X_2 - 0.497045X_3 - 1.750000X_1X_2 - 0.033500X_1X_3 - 23.750000X_2X_3 - 0.056509X_1^2 - 1048.579550X_2^2 + 0.587273X_3^2$

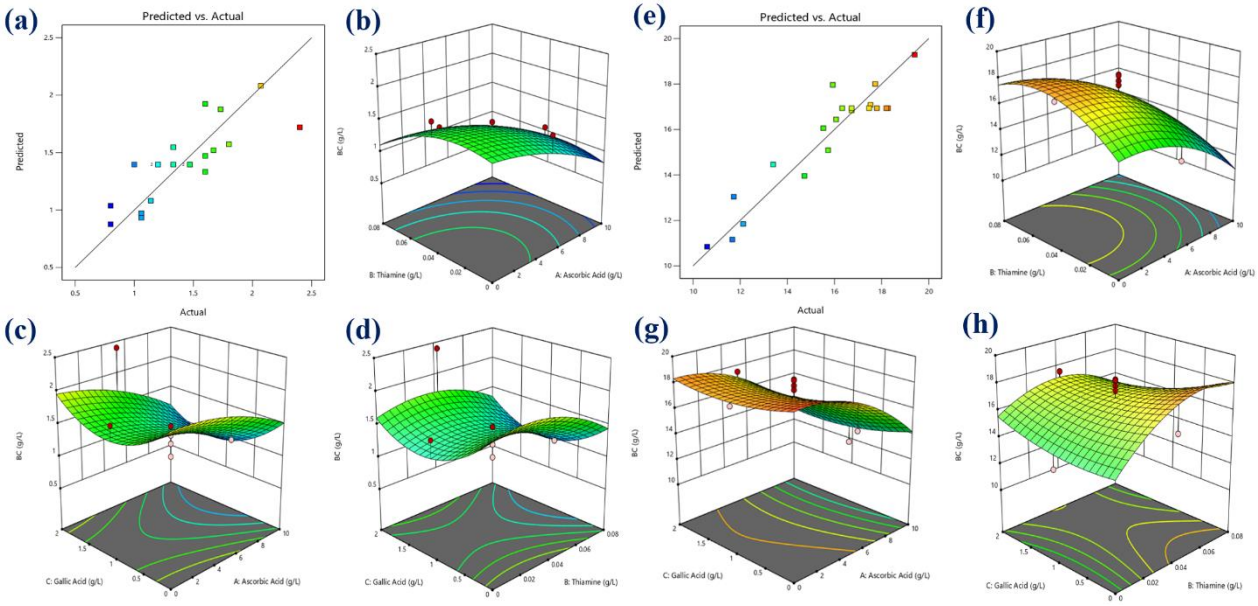

Figure 1. (a) Predicted values against experimental data of BC yield (g/L) in synthetic HS-media, according to the experimental design, and 3D-imaging of the BC yield response surface at varying concentrations (g/L) of (b) thiamine and ascorbic acid, (c) gallic acid and ascorbic acid, and (d) gallic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

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 (<0.0184 for HS medium; <0.0012 for FSS extract) was small, showing the importance of the model. The F value of the model (4.47 for HS; 8.57 for FSS) implies that the model is statistically significant with a high degree of confidence. The predicted value of the BC yield using the above optimal combination of factors in the mathematical model (ascorbic acid 0.5, thiamine 0.04, and gallic acid 2.0 g/L for HS, and 0.0, 0.08, and 0.0 g/L for FSS, respectively) was 2.08 g/L for HS and 19.29 g/L for FSS 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 (2.40±0.00 g/L for HS and 19.40±0.01 g/L for FSS). On the other hand, the yield of BC production in the plain HS medium, without the addition of the above factors, was 1.07±0.01 g/L, and in plain FSS extract it was 15.73±0.02 g/L. The results will help towards the development of low-cost substrates for efficient BC production based on agri-industrial wastes, side-streams and by-products rich in vitamins and phenolic compounds. [Authors acknowledge support of this work by the project "Research infrastructure on Food Bioprocessing Development and Innovation Exploitation–Food Innovation RI" (MIS 5027222), which is 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 he European Union (European Regional Development fund)].

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