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OPTIMIZATION FOR ALCOHOL FERMENTATION CONDITIONS OF SEA BUCKTHORN PRESS RESIDUES USING RESPONSE SURFACE METHODOLOGY

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Abstract: *Sea buckthorn (Hippophae rhamnoides L.) is a unique and valuable shrub cultivated all over the world. Food industry is the most important sector of use of the sea buckthorn, where it is used as a raw material to obtain functional food or food supplements. Sea buckthorn fruits are among the most complex nutritionally of all berries. The aim of the present study was to offer an optimum solution for the valorization by alcoholic fermentation of the press residues obtained after processing the sea buckthorn fruit to obtain juice. The present research was conducted with the application of response surface methodology (RSM) for optimization, using a Box-Behnken design. The determined optimum conditions for a 3 days-fermentation were: a temperature of 25.38 °C, a pH of 4.62 and an inoculum size of 0.18 % to obtain a 3% vol. alc. beverage. This study provides useful information that can help beverage processing industries achieve optimum yield in low alcoholic beverage production using sea buckthorn press residues as sole substrate.*

Keywords: *Hippophae rhamnoides, capitalization, fermentation, optimization, Box-Behnken design*

1. Introduction

Fermentation is a cheap technology and plays an important role as the main biotechnological application of food processing in many developing countries. Benefits associated with fermentation include improving the sensory properties and nutritional value of foods, reducing toxic and anti-nutritional factors, and extending the shelf life of foods [1]. The process of alcoholic fermentation, through its form of manifestation, has been known and used in the technique of preparing beverages, since ancient times [2]. Alcoholic fermentation is a process extensively used in beverage industry, generating value-added, less perishable products, in which ethanol is a major constituent and other minor secondary metabolites are also formed [3]. Yeast plays pivotal role in winemaking and its activity is mainly influenced by factors such as temperature and pH, which play crucial role in controlling the growth and metabolism rate of yeast and in synthesizing or transforming bioactive compounds in wine [4].

Sea buckthorn (*Hippophae rhamnoides* L.) is a unique and valuable species, being cultivated all over the world [5]. In addition to the different areas of importance of all the component parts of the plant, but also of the shrub, as a whole [6], the most important sector of use of the sea buckthorn remains the food industry, where it is used as a raw material to obtain functional food or food supplements [7]. Research carried out in the country and abroad has shown that the leaves, fruits and shoots of sea buckthorn contain a number of biologically active substances with an essential role in regulating metabolism [8]. Sea buckthorn fruits are among the most complex nutritionally of all berries [9]. In Romania, sea buckthorn has an exceptional chemical content, perhaps the richest in the world. Nowhere in the world, where it grows naturally and even the improved varieties do not have a composition at the level of the sea buckthorn grown on the territory of Romania, especially in the sub-Carpathian region [10].

The aim of the present study was to offer an optimum solution for the valorization by alcoholic fermentation of the press residues obtained after processing the sea buckthorn fruit to obtain juice. By identifying the optimal fermentation conditions, it was obtained a novel low alcohol refreshing, energizing and healthy sea buckthorn beverage. The obtained product will have superior nutritional and sensory properties in comparison to other similar fruit beverages on the market due to the special raw material used in its production.

Several statistical approaches have been used to optimize the process factors maximizing yield of the product. Response Surface Methodology (RSM) is one such approach that has been extensively studied and applied in bioprocess technology. It minimizes the number of observations and thus provides more precise and accurate results [11]. The present research was conducted with the application of response surface methodology (RSM) for optimization, using a Box-Behnken design.

2. Matherials and methods

2.1. Yeast inoculum preparation

The yeast used for the experimental trials was a strain of *Saccharomyces cerevisiae* Killer (former *Bayanus*), (IOC 18 - 2007), purchased from Enzymes & Derivates SA company, Piatra Neamț, Romania. The yeast was rehydrated in water at 37°C (1: 10, w: v), in a clean container. The content was slowly stirred and then left to rest for 20 minutes. Then, must was added progressively so that the yeast starter can acclimatize to the specific must conditions (pH, sugars, temperature, etc.). In order to obtain an active starter culture sufficiently concentrated to achieve desired fermentation, Hydra PC was incorporated (1: 1, w: w) in the yeast stock solution. Hydra PC is an adjuvant naturally rich in magnesium, also bought from Enzymes & Derivates SA. It optimizes yeast rehydration by reinforcing its plasma membrane, which is essential to yeast growth and metabolism.

2.2. Fermentation of Hippophae rhamnoides press residues

Fruits of sea buckthorn (*H. rhamnoides* L.) were collected in Botoșani County, Romania. Fruits were cleaned and the juice was extracted by cold pressing. The press residues were collected and submitted to a maceration phase in water in 1:2 proportion (w:v), at 25˚C for 5 hours. Maceration step will contribute to all the specific characteristics of smell, appearance and taste of the final product. Since the fermentation medium obtained thereby lacked in the requisite sugar content (1.3˚Brix), sugar level was adjusted to 7˚Brix. Also, 100 ppm of SO_2 was added in the form of potassium bisulfite (Sigma Aldrich) to inhibit the growth of unfavorable microorganisms. Further, a mix of ammonium salts, inactivated yeast, thiamin and diammonium phosphate was added to ‘help’ the fermentation process. This ameliorated sea buckthorn marc juice was further inoculated with target concentration of yeast and the pH was set using sodium carbonate of absolute purity, from Sigma Aldrich. The fermentation processes were conducted for three days, according to the respective runs as suggested in a Box-Behnken Design (BBD) matrix (Table 1).

2.3. Experimental design and optimization

The fermentation process for the sea buckthorn marc was established by response surface methodology (RSM), using Design-Expert 12 software. RSM is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariable equations simultaneously [12]. This system was employed to determine the best combination of variables for desired ethanol concentration (3%). Box–Behnken design with three factors at three levels (-1, 0 and +1) was undertaken with six center points in a set of 18 experiments. For statistical calculations, the independent variables were coded as X_1 (coded temperature, range 18 ÷ 30 °C), X_2 (coded pH, range 2.85 ÷ 5) and X_3 (coded inoculum size, range 0.02 ÷ 2 %), according to Table 2. This design is an experimental design of response surface methodology that studies the effect of the chosen variables and their interactions.

Table 1.
Experimental design (Box-Behnken Design matrix)

Run order	Temperature (ºC)	pH	Inoculum size (% w/v)	Ethanol (% v/v)
1	24	2.85	0.2	2.08
2	24	3.925	0.11	2.21
3	24	3.925	0.11	2.21
4	24	3.925	0.11	2.11
5	24	3.925	0.11	2.15
6	18	3.925	0.02	1.32
7	30	3.925	0.02	2.96
8	24	5	0.2	3.29
9	24	3.925	0.11	2.32
10	18	5	0.11	1.84
11	30	5	0.11	3.19
12	30	3.925	0.2	3.5
13	18	2.85	0.11	0.91
14	24	2.85	0.02	2.05
15	24	3.925	0.11	2.33
16	18	3.925	0.2	1.52
17	24	5	0.02	2.3
18	30	2.85	0.11	3.53

Table 2.
Factors involved in Box-Behnken design in terms of actual values

Factor	Name	Low value	High value
X_1	Temperature	18	30
X_2	pH	2.85	5
X_3	Inoculum size	0.02	0.2

2.4. Fermentation monitoring and ethanol determination

BlueSens sensors were used to monitor the fermentation trials. The collected data were logged with BacVIS Software. To this extent, spectral sensors in infrared domain were mounted on the fermentation recipients. This modern gas analysis allowed the control of fermentation processes in real-time and allowed the overseeing of the ethanol content during the fermentation processes of the sea buckthorn press residues.

3. Results and discussion

The data of ethanol yield according to Box-Behnken design shown in table 1 indicated that there was a variation in each ethanol production in response to the eighteen trials employed (ethanol concentration: 0.91 – 3.53%). These variations reflected the importance of medium optimization in obtaining the desired ethanol concentration. Also, the experiment successfully indicated that all the three independent variables, i.e., temperature, pH and inoculum size had a profound effect on ethanol yield, 3D response surface plots at intermediate level of temperature, pH and inoculum size and interaction between different variables as depicted in Fig. 1a-c.

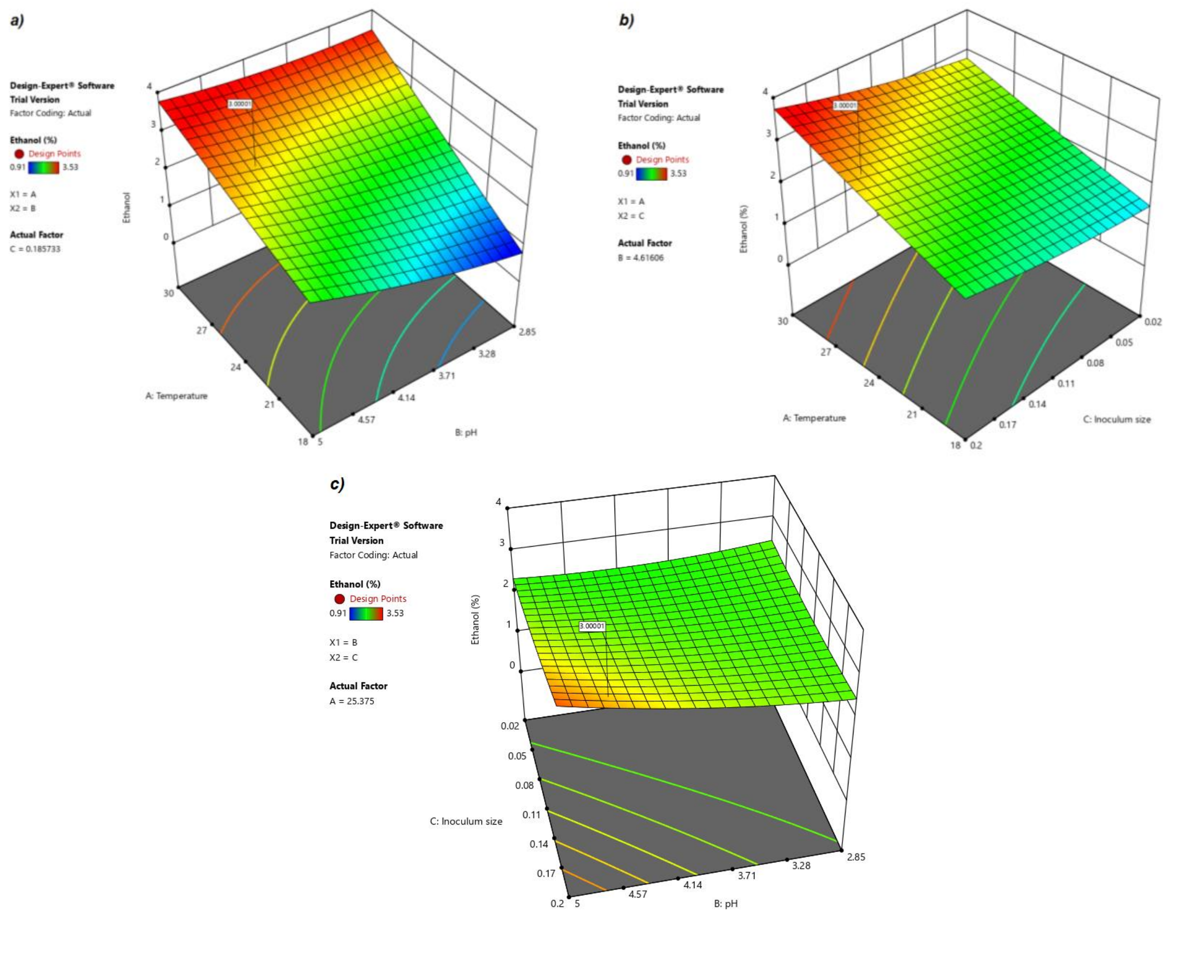

Fig. 1. 3D surface plots of temperature vs. pH (a), temperature vs. inoculum size (b) and pH vs. inoculum size (c) on ethanol content

On the entire domain of pH, high temperatures lead to high concentrations of ethanol (Fig. 1a), while the gradual increase of both temperature and inoculum size (Fig. 1b) and pH and inoculum size (Fig. 1c) resulted in higher values of ethanol (Fig. 1b). These trends of variations of ethanol yield considering those three independent variables were also observed in earlier reports of Kumar *et al.* (2009) for mango fermentation [13] and of Sevda & Rodrigues (2011), too, for guava fermentation [14]. Several previous studies suggested that these three primary factors have an important influence on the fermentation kinetics [15, 16]. Thus, the present study indicated that *Hippophae rhamnoides* press residues could be successfully capitalized in the production of a refreshing drink with target ethanol content of 3% vol. in 3 days, with the following optimized conditions: temperature of 25.38 °C, pH of 4.62 and inoculum size of 0.18 %.

Analysis of the variance (ANOVA) showed that models are significant with respect to ethanol concentration (Table 3).

Table 3.
Analysis of variance (ANOVA) for the selected quadratic model of Box-Behnken design

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8.9	9	0.1	49.7	< 0.0001	significant
X_1 -Temperature	7.2	1	7.2	362.3	< 0.0001	
X_2 - pH	0.5	1	0.5	26.4	0.0009	
X_3 - Inoculum size	0.4	1	0.4	19.5	0.0022	
X_1X_2	0.4	1	0.4	20.3	0.0020	
X_1X_3	0.03	1	0.03	1.4	0.2623	
X_2X_3	0.2	1	0.2	11.6	0.0093	
X_1^2	0.002	1	0.002	0.09	0.7700	
X_2^2	0.07	1	0.07	3.4	0.1002	
X_3^2	0.03	1	0.03	1.5	0.2541	
Residual	0.2	8	0.02			
Lack of Fit	0.1	3	0.04	5.0	0.0561	not significant
Pure Error	0.04	5	0.008			
Cor Total	9.0	17				

The quadratic model thus generated could successfully explain that all three variables have a significant effect on the ethanol content, especially temperature, with $p < 0.0001$. In addition, the interactions between temperature and pH ($p < 0.01$) and pH and inoculum size ($p < 0.01$) were also having a significant effect on the ethanol content. The Model F-value of 49.70 implies the model is significant. Coefficient of determination R-Square value was 0.9824 indicated model's goodness of fit. The predicted R-Square value of 0.7821 was in reasonable agreement with the Adjusted R-Squared value of 0.9627. Thus, the final equation of process variables in terms of coded values on developed model for the production of *Hippopae rhamnoides* low alcoholic refreshing drink is as follows (equation 1):

Ethanol concentration = $2.22 + 0.9487\ X_1 + 0.2562\ X_2 + 0.22\ X_3 - 0.3175\ X_1X_2 +$

$0.0850\ X_1\ X_3 + 0.24\ X_2X_3 + 0.0204\ X_1^2 + 0.1254\ X_2^2 + 0.0829\ X_3^2$ **(1)**

To validate the predicted response, another fermentation process as a confirmatory run with the optimized conditions was conducted, in triplicate. The result found was in accordance with the predicted response and the ethanol content observed was 3 ± 0.2%.

4. Conclusion

In conclusion, the fermentation conditions were successfully optimized using Response Surface Methodology and Box-Behnken Design by analyzing the individual and interactive effects of fermentation temperature, inoculum size and pH on fermentation kinetics. The determined optimum conditions for a 3 days fermentation were: a temperature of 25.38 °C, a pH of 4.62 and an inoculum size of 0.18 % for obtaining a 3% vol. alc. beverage. Hence, sea buckthorn press residues can be capitalized by means of alcoholic fermentation in order to obtain a novel refreshing drink. Therefore, it can be stated that this study provides useful information that can help beverage processing industries achieve optimum yield in low alcoholic beverage production using sea buckthorn press residues as sole substrate.

6. References

[1]. MISIHAIRABGWI J., CHEIKHYOUSSEF A., Traditional fermented foods and beverages of Namibia, *Journal of Ethnic Foods*, 4(3): 145-153, (2017).

[2]. COTEA V. D., POMOHACI N., GHEORGHIŢĂ M. ŞT., Oenologie, *Editura Tipo Moldova*, Iaşi, 315 p., (2010).

[3]. ROOM R., BABOR T., REHM J., Alcohol and public health, *Lancet*, 365(9458): 519-530, (2005).

[4]. ARROYO-LÓPEZ F. N., ORLIĆ S., QUEROL A., BARRIO E., Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae, S. kudriavzevii* and their interspecific hybrid, *International Journal of Food Microbiology*, 131(2-3): 120-127, (2009).

[5]. LI T., MCLOUGHLIN C., Sea buckthorn production guide, *Pacific Agri-Food Research Centre, Agriculture & Agri-Food Canada Summerland*, 21 p., (1997).

[6]. ZEB A., Chemical and Nutritional Constituents of Sea Buckthorn Juice, *Pakistan Journal of Nutrition*, 3(2): 99-106, (2004).

[7]. BEVERIDGE T., LI T.S., OOMAH B.D., SMITH A., Sea buckthorn products: manufacture and composition, *Journal of Agricultural and Food Chemistry*, 47(9): 3480-3488, (1999).

[8]. RAȚI I.V., RAȚI L., Cătina albă în exploatații agricole, *Ministerul Agriculturii, Pădurilor, Apelor și Mediului, Agenția Națională de Consultanță Agricolă*, 127 p., (2003).

[9]. SMALL E., CATLING P. M., LI T., Blossoming treasures of Biodiversity: Sea Buckthorn (*Hippophae rhamnoides*) - an ancient crop with modern virtues, *Biodiversity*, 3(2): 25-27, (2002).

[10]. MANEA Ș., Cătina și uleiul de cătină. Aliment, miracol, sanătate, echilibru, *Hofigal*, 113 p., (2004).

[11]. MUNDARAGI A., THANGADURAI D., Process optimization, physicochemical characterization and antioxidant potential of novel wine from an underutilized fruit *Carissa spinarum* L. (*Apocynaceae*), *Ciência e Tecnologia de Alimentos*, 38(3): 1-6, (2017).

[12]. SEVDA S., RODRIGUES L., Extraction and Optimization of Guava Juice by Using Response Surface Methodology, *American Journal of Food Technology*, 7: 326-339, (2012).

[13]. KUMAR Y. S., PRAKASAM R. S., REDDY O. V. S., Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology, *International Journal of Food Science & Technology*, 44(11): 2320-2327, (2009).

[14]. SEVDA S., RODRIGUES L., Fermentative behavior of *Saccharomyces* strains during Guava (*Psidium guajava* L.) must fermentation and optimization of Guava wine production, *Journal of Food Processing & Technology*, 2: 2-9, (2011).

[15]. REDDY L. V. A., REDDY O. V. S., Effect of fermentation conditions on yeast growth and volatile composition of wine produced from mango (*Mangifera indica* L.) fruit juice, *Food and Bioproducts Processing*, 89(4): 487-491, (2011).

[16]. BLEOANCA I., BAHRIM G., Overview on brewing yeast stress factors, *Romanian Biotechnological Letters*, 18(5): 8559-8572, (2013).