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Response Surface Methodology as an Approach for Optimization of Vinegar Fermentation Conditions Using Three Different Thermotolerant Acetic Acid Bacteria

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Abstract

This study aimed to investigate optimal fermentation conditions of biological acetic acid fermentation for vinegar production. Optimization was performed on 3 acetic acid bacteria strains namely VMA1, VMA7 and VMAO using Response Surface Methodology (RSM). A Box-Behnken-Design (BBD) was achieved with three different independent process parameters involving: fermentation temperature, original alcohol concentration and original acetic acid concentration and one dependent variable (acetic acid yield). The results showed that the mathematical models describe correctly the relationship between responses and factors (*F* values of the models ($p < 0.05$), R^2 (coefficient of correlation) respectively 0.96, 0.94, 0.98, and adjusted R^2 0.95, 0.92, 0.98). The maximum acidity was obtained respectively at fermentation temperatures, original alcohol concentrations and original acetic acid concentrations ranging from [37.5˚C - 45˚C], [16% - 20% (v/v)], [1.5% - 2% (w/v)] for VMA1, [40˚C - 45˚C], [14.5% - 20% (v/v)], [1.7% - 2% (w/v)] for VMA7 and [42˚C - 45˚C], [17% - 20% (v/v)], [1.5% - 2% (w/v)] for VMAO. The use of these acetic strains in the production of vinegar may seriously lead to a decrease or even an ablation of the costs related to the cooling of bioreactors especially in warm and hot countries, in the context of global warming.

Keywords

Vinegar, Response Surface Methodology, Box-Behnken-Design, Optimization

1. Introduction

Vinegar is one of the most valuable food products due to its adaptability and range of usages, and the global vinegar market has reached a value of US$ 2.27 Billion in 2021 and is expected to increase by 2.6% up to 2027 [1]. Vinegar is a well-known natural food product derived from alcoholic and subsequently acetous fermentation of carbohydrate-rich foods. Vinegar is widely used in the food industry; domestically for pickling vegetables and fruits, as an ingredient in condiments like ketchup, and mayonnaise; and traditionally as a food seasoning and preservative [2]. It is a two-step biochemical process, in which the first step involves the transformation of sugar into ethanol by the action of yeast usually Saccharomyces species, followed by the oxidation of ethanol into acetic acid under aerobic conditions by acetic acid bacteria (AAB) [3] [4] [5]. Fermentative oxidation of ethanol to acetic acid by AAB is dependent on two sequential reactions of membrane-bound pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) and aldehyde dehydrogenase (ALDH), both of which are localized on the periplasmic side of the inner membrane [6]. These strictly aerobic microorganisms, used in several biotechnological processes, have the particular ability to transform alcohols and sugar alcohols into the corresponding organic acids [7].

A major challenge in vinegar production is that AAB are extremely sensitive to environmental conditions [8]; these limiting factors affect their growth and production capacities through parameters such as temperature, pH, oxygen, ethanol concentration or acetic acid concentration in the culture medium [9] [10] [11]. These inhibitory parameters are difficult to avoid since they are often substrates, targeted products, or physicochemical conditions.

In general, optimization is the process of bringing together and applying the process by considering the interactions of independent variables, as well as the effects of the independent variables on the response in accordance with the determined objectives [12]. Response surface methodology (RSM) is an efficient experimental strategy to determine optimal conditions for a multivariate system rather than the conventional method of one factor at a time (OFAT) which is incapable of determining the true optimum [13]. It is a mathematical and statistical technique for designing experiments, model building, and assessing the effect produced by several factors [14].

In this study, the Response Surface Methodology was used to optimize the conditions of vinegar fermentation of 3 AAB strains (VMA1, VMA7 and VMAO). The aim of this study was to optimize the effect of 3 independent process parameters: fermentation temperature, original alcohol concentration and original acetic acid concentration using RSM coupled with BBD in order to obtain maximum acetic acid yield.

2. Materials and Method

2.1. Bacterial Strains

Three (3) AAB strains VMA1, VMA7, and VMAO previously isolated from fermented mango alcohol were tested in this study [15]. These strains were selected for their capacity to produce acetic acid, and their thermo-alcohol-acid tolerance capacities [16].

2.2. Statistical Experimental Design

Response surface methodology is an empirical modeling technique, and it is used to estimate the relationship between the set of controllable experimental factors and the observed results [17]. This study used RSM to determine the optimum conditions affecting the acetic acid fermentation process of three AAB strains VMA1, VMA7 and VMAO. The experiments are performed by using a Box-Behnken-Design. A BBD is a type of response surface design that do not have axial points, thus, we can be sure that all design points fall within your safe operating zone. BBD also ensure that all factors are not set at their high levels at the same time [18] [19]. The independent variables of acetic acid fermentation were X_1, X_2 and X_3 respectively fermentation temperature (FT), original alcohol concentration (OAC) and original acetic acid concentration (OAAC). Each of variables to be optimized was coded at 3 levels: −1, 0, and 1. **Table 1** shows the variables, their symbols and levels. This gives a range of these variables in the acetic acid fermentation process. Experimental design and statistical analysis of the data were performed using STATISTICA 10 for Windows™.

Variables were combined in 15 experiments repeated in triplicate. The experimental values were fitted in Equation (1) as a second order polynomial equation, including the linear and cross effect of the variables:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{j<1}^{n} \beta_{ij} X_i X_j + \sum_{j=1}^{n} \beta_{ij} X_j^2$$

where: Y represents the predicted response, i and j are linear and quadratic coefficients, respectively, β is the regression coefficient, and n is the number of variables studied in the experiments.

2.3. Preparation of Microorganisms and Inoculum

First, the strains were cultured in 100 mL of YPG broth (Yeast-extract, Peptone, Glucose), containing respectively 3% (w/v) yeast, 1% (w/v) Peptone, 3% (w/v) glucose. Then, the medium was incubated at 37˚C until the optical density (OD600 nm) of the suspension reaches 0.4.

Table 1. Levels of factors and variables chosen for Box–Behnken experimental design.

Variable	Symbol	Code-variable level		
		−1	0	1
Temperature (˚C)	X_1	30	37.5	45
Alcohol (v/v)	X_2	5	12.5	20
Acid acetic (w/v)	X_3	0	1	2

2.4. Fermentation Conditions

Acetic acid fermentations were carried out in YGKM fermentation medium containing 5% (w/v) Yeast, 5% (w/v) Glucose, 3.3% (w/v) K_2HPO_4, and 1.1% (w/v) $MgSO_4$. Sterile alcohol and acetic acid were added to the fermentation media supplemented at the specific concentrations listed in **Table 2**. The fermentation media were in each case inoculated with 10% (v/v) of pre-culture of the microorganism concerned. The flasks were then incubated at the temperature of each experiment (30˚C, 37.5˚C or 45˚C) with shaking at 150 rpm for a total of 21 days.

2.5. Titratable Acidity

In order to determine the amount of acid in the vinegar, the acetic acid will be titrated with a solution of 1N of sodium hydroxide (NaOH) using phenolphthalein as an indicator. All measurements were conducted in triplicate. The amount of acetic acid produced in g per 100 mL was calculated in using the following formula:

$$d^\circ = \left(10000 \times Vb \times Cb \times pm\left(\text{acetic acid}\right)\right) / \left(Va \times mv\left(\text{acetic acid}\right)\right)$$

Vb: average volume of sodium hydroxide needed to reach the end point;
Cb: concentration of NaOH solution;
pm: molar mass of acetic acid;
Va: Volume of vinegar sample;
mv: volumic mass of acetic acid.

3. Results and Discussion

This study used RSM to determine the optimum conditions affecting the fermentation process of vinegar production by three AAB strains VMA1, VMA7 and VMAO. The three independent variables, fermentation temperature, original alcohol concentration and original acetic acid concentration and its influence on acetic acid percent as an important response, were studied using Response Surface Methodology coupled with a 3 factors 3 levels Box-Behnken experimental Design. RSM is widely used for experimental data analysis; it creates a link between responses and control variables and guesses the response values of the control variables within a specific range [20]. This model anticipates experimental changes such as changing operating conditions and various processing steps, and define not only the influences of independent variables on the responses, but also the interaction between parameters to achieve best system performance [21] [22]. RSM has been used worldwide for the optimization of numerous industrial processes [23]-[35].

In this study, 45 runs of vinegar production were carried out according to the experimental design provided, using three AAB strains VMA1, VMA7, and VMAO. Analysis of the experimental data produced the following mathematical models presented in Equations (1)-(3):

Table 2. Experimental design.

Run	Code level			Factors in real terms		
	X_1	X_2	X_3	Temperature	Alcohol	Ac. Acid
1	−1	−1	0	30	5	1
2	−1	−1	0	30	5	1
3	−1	−1	0	30	5	1
4	0	−1	−1	37.5	5	0
5	0	−1	−1	37.5	5	0
6	0	−1	−1	37.5	5	0
7	0	−1	1	37.5	5	2
8	0	−1	1	37.5	5	2
9	0	−1	1	37.5	5	2
10	1	−1	0	45	5	1
11	1	−1	0	45	5	1
12	1	−1	0	45	5	1
13	−1	0	−1	30	12.5	0
14	−1	0	−1	30	12.5	0
15	−1	0	−1	30	12.5	0
16	−1	0	1	30	12.5	2
17	−1	0	1	30	12.5	2
18	−1	0	1	30	12.5	2
19	0	0	0	37.5	12.5	1
20	0	0	0	37.5	12.5	1
21	0	0	0	37.5	12.5	1
22	0	0	0	37.5	12.5	1
23	0	0	0	37.5	12.5	1
24	0	0	0	37.5	12.5	1
25	0	0	0	37.5	12.5	1
26	0	0	0	37.5	12.5	1
27	0	0	0	37.5	12.5	1
28	1	0	−1	45	12.5	0
29	1	0	−1	45	12.5	0
30	1	0	−1	45	12.5	0
31	1	0	1	45	12.5	2
32	1	0	1	45	12.5	2
33	1	0	1	45	12.5	2
34	−1	1	0	30	20	1
35	−1	1	0	30	20	1

Continued

36	−1	1	0	30	20	1
37	0	1	−1	37.5	20	0
38	0	1	−1	37.5	20	0
39	0	1	−1	37.5	20	0
40	0	1	1	37.5	20	2
41	0	1	1	37.5	20	2
42	0	1	1	37.5	20	2
43	1	1	0	45	20	1
44	1	1	0	45	20	1
45	1	1	0	45	20	1

$$\begin{aligned} Y_1 = {} & 3.20000 + 0.776250X_1 - 0.528333X_1X_1 + 0.644167X_2 \\ & + 0.119167X_2X_2 + 0.481250X_3 - 0.20000X_3X_3 \\ & + 0.032500X_1X_2 + 0.053333X_1X_3 + 0.235833X_2X_3 \end{aligned} \tag{1}$$

$$\begin{aligned} Y_2 = {} & 4.00222 + 0.868333X_1 - 0.279194X_1X_1 + 0.693583X_2 \\ & - 0.301361X_2X_2 + 0.896917X_3 - 0.631361X_3X_3 \\ & + 0.161667X_1X_2 + 0.035000X_1X_3 + 0.576167X_2X_3 \end{aligned} \tag{2}$$

$$\begin{aligned} Y_3 = {} & 3.570000 + 0.763542X_1 - 0.031042X_1X_1 + 0.0896667X_2 \\ & - 0.038125X_2X_2 + 0.537708X_3 - 0.157708X_3X_3 \\ & + 0.187500X_1X_2 + 0.454583X_1X_3 + 0.120833X_2X_3 \end{aligned} \tag{3}$$

3.1. Parameters Effects and Parameters Interactions

Equations (1)-(3) represent the relationship between response and fermentation process variables on acetic acid production respectively by VMA1, VMA7 and VMAO. Positive regression of the models indicated a synergic effect whereas negative coefficients indicate an antagonist effect on the dependent response variable [13]. A positive effect of a factor indicates that the corresponding response increases when the factor changes from low to high level, whereas a negative effect indicates that the corresponding response decreases when the factor varies from low to high level [36].

3.1.1. For VMA1

The results showed that the linear FT, the linear and quadratic OAC and the linear OAAC have a positive effect on the production of acetic acid. The interaction FT-OAC, the interaction FT-OAAC and the interaction OAC-OAAC also have a positive effect on the acetic acid production. While the quadratic FT and the quadratic OAAC have a negative effect on acetic acid production (**Table 3**).

3.1.2. For VMA7 and VMAO

The results exhibited that the linear FT, the linear OAC, the linear OAAC, the interaction FT-OAC, the interaction FT-OAAC and the interaction between

Table 3. The data of parameter effects and parameter interactions on the production of vinegar by strain VMA1.

Fact.	Estimated effects; Var: Ac.Acid; $R^2 = 0.96621$; Adj $R^2 = 0.95753$									
	3 fact., 1 Blocs, 45 Ess.; MC Residus = 0.034364									
	VD: Ac. Acid									
	Effect	Err-Type	t(35)	p	+95% Lim.Conf	−95% Lim.Conf	(coeffs)	Err-Type (coeffs)	+95% Lim.Conf	−95% Lim.Conf
Moy/Ord.Orig	3.20000	0.061792	51.78680	0.000000	3.07456	3.325444	3.200000	0.061792	3.074556	3.325444
(1) Temperature (L)	1.55250	0.075679	20.51422	0.000000	1.39886	1.706137	0.776250	0.037840	0.699432	0.853068
Temperature (Q)	−1.05667	0.111397	−9.48561	0.000000	−1.28281	−0.830519	−0.528333	0.055698	−0.641407	−0.415260
(2) Alcohol (L)	1.28833	0.075679	17.02361	0.000000	1.13470	1.441970	0.644167	0.037840	0.567348	0.720985
Alcohol (Q)	0.23833	0.111397	2.13950	0.039447	0.01219	0.464481	0.119167	0.055698	0.006093	0.232240
(3) Ac. Acid (L)	0.96250	0.075679	12.71816	0.000000	0.80886	1.116137	0.481250	0.037840	0.404432	0.558068
Ac. Acid (Q)	−0.40000	0.111397	−3.59077	0.001001	−0.62615	−0.173853	−0.200000	0.055698	−0.313074	−0.086926
1L*2L	0.06500	0.107027	0.60733	0.547555	−0.15228	0.282275	0.032500	0.053513	−0.076138	0.141138
1L*3L	0.10667	0.107027	0.99664	0.325781	−0.11061	0.323942	0.053333	0.053513	−0.055304	0.161971
2L*3L	0.47167	0.107027	4.40701	0.000095	0.25439	0.688942	0.235833	0.053513	0.127196	0.344471

OAC-OAAC have a positive effect on the acetic acid yield. However, the quadratic FT, the quadratic OAC and the quadratic OAAC showed a negative effect on acetic acid production (Table 4 and Table 5).

3.2. Suitability and Adequacy of Models

The suitability of model and data can be seen from the value of R^2. The coefficient of determination (R-Squared) represents the proportion of variability in the data explained or accounted by the model [37]. The determination coefficient (R^2) value indicates the extent to which the independent variables can explain the observed response values. R^2 values range from 0 to 1. The closer to the value of 1, the equation of the model approaches the experimental data [38] [39]. A good fitted model should have a minimum R^2 of 80% an R^2-value that is greater than 0.9 proves a very high degree of correlation [40]. The results of data analysis showed that the R^2 values of the models are 0.96, 0.94, and 0.98 respectively for VMA1, VMA7 and VMAO. These R^2 values indicate an excellent fit between predicted and experimental values. Meaning for VMA1, VMA7 and VMAO respectively 96%, 94% and 98% of the variability of the response can be explained by the model. The adjusted R^2 in this study were found to be respectively 0.95, 0.92 and 0.98 which are high and clarified the significance of the model, implying that the predicted and experimental values of the acetic acid production are quite similar for all three strains.

The adequacy of models is also supported by the F values of the models as shown in Tables 6-8. Values of "Prob F" < 0.0500 indicate models are significant

Table 4. The data of parameter effects and parameter interactions on the production of vinegar by strain VMA7.

Fact.	Estimated effects; Var: Ac.Acid; R^2 = 0.94042; Adj R^2 = 0.9251									
	3 fact., 1 Blocs, 45 Ess.; MC Residus = 0.1066117									
	VD: Ac. Acid									
	Effect	Err-Type	t(35)	p	−95% Lim.Conf	+95% Lim.Conf	(coeffs)	Err-Type (coeffs)	−95% Lim.Conf	+95% Lim.Conf
Moy/Ord.Orig	4.00222	0.108838	36.77224	0.000000	3.78127	4.223175	4.002222	0.108838	3.781269	4.223175
(1) Temperature (L)	1.77667	0.133299	13.02836	0.000000	1.46606	2.007278	0.868333	0.066649	0.733028	1.003639
Temperature (Q)	−0.55839	0.196211	−2.84586	0.007357	−0.95672	−0.160060	−0.279194	0.098105	−0.478359	−0.080030
(2) Alcohol (L)	1.38717	0.133299	10.40643	0.000000	1.11656	1.657778	0.693583	0.066649	0.558278	0.828889
Alcohol (Q)	−0.60272	0.196211	−3.07181	0.004101	−1.00105	−0.204393	−0.301361	0.098105	−0.500526	−0.102197
(3) Ac. Acid (L)	1.79383	0.133299	13.45722	0.000000	1.52322	2.064445	0.896917	0.066649	0.761611	1.032222
Ac. Acid (Q)	−1.26272	0.196211	−6.43554	0.000000	−1.66105	−0.864393	−0.631361	0.098105	−0.830526	−0.432197
1L*2L	0.32333	0.188513	1.71518	0.095155	−0.05937	0.706035	0.161667	0.094257	−0.029684	0.353018
1L*3L	0.07000	0.188513	0.37133	0.712633	−0.31270	0.452702	0.035000	0.094257	−0.156351	0.226531
2L*3L	1.15233	0.188513	6.11275	0.000001	0.76963	1.535035	0.576167	0.094257	0.384816	0.767518

Table 5. The data of parameter effects and parameter interactions on the production of vinegar by strain VMAO.

Fact.	Estimated effects; Var: Ac.Acid; R^2 = 0.98059; Adj R^2 = 0.97559									
	3 fact., 1 Blocs, 45 Ess.; MC Residus = 0.246589									
	VD: Ac. Acid									
	Effect	Err-Type	t(35)	p	−95% Lim.Conf	+95% Lim.Conf	(coeffs)	Err-Type (coeffs)	−95% Lim.Conf	+95% Lim.Conf
Moy/Ord.Orig	3.570000	0.052344	68.20291	0.000000	3.463736	3.676264	3.570000	0.052344	3.463736	3.676254
(1) Temperature (L)	1.527083	0.064108	23.82055	0.000000	1.396938	1.657229	0.763542	0.032054	0.698469	0.828615
Temperature (Q)	−0.062083	0.094364	−0.65791	0.514899	−0.253653	0.129486	−0.031042	0.047182	−0.126826	0.064743
(2) Alcohol (L)	1.793333	0.064108	27.97371	0.000000	1.663188	1.923479	0.896667	0.032054	0.831594	0.961740
Alcohol (Q)	−0.076250	0.094364	−0.80804	0.424526	−0.267819	0.115319	−0.038125	0.047182	−0.133910	0.057660
(3) Ac. Acid (L)	1.075417	0.064108	16.77513	0.000000	0.945271	1.205562	0.537708	0.032054	0.472635	0.602781
Ac. Acid (Q)	−0.315417	0.094364	−3.34255	0.001986	−0.506986	−0.123847	−0.157708	0.047182	−0.253493	−0.061924
1L*2L	0.375000	0.090662	4.13624	0.000210	0.190946	0.559054	0.187500	0.045331	0.095473	0.279527
1L*3L	0.909167	0.090662	10.02807	0.000000	0.725113	1.093221	0.454583	0.045331	0.362556	0.546610
2L*3L	0.241667	0.090662	2.66557	0.011551	0.057613	0.425721	0.120833	0.045331	0.028806	0.212860

[41]. The Models F-values of 22.34, 5.83 and 5.33 indicated the statistical significance of the experimental models and were statistically significant ($p < 0.05$).

3.3. Significance and Magnitude of the Factors

It is important to determine the most influential variable in the acetic acid

Table 6. Examination of F value model for VMA1.

Source	Sum of squares	df	Mean Square	F-value	p-value	
Model	11.64	9	1.29	22.34	0.0002	significant

Table 7. Examination of F value model for VMA7.

Source	Sum of squares	df	Mean Square	F-value	p-value	
Model	15.19	6	2.53	5.83	0.0076	significant

Table 8. Examination of F value model for VMAO.

Source	Sum of squares	df	Mean Square	F-value	p-value	
Model	10.10	6	1.68	5.33	0.0104	significant

production for each AAB strain. Pareto chart provide information about the most influential process variables. It reveals significance and magnitude of the factors that influence the process, depending on the significance level [42] [43].

Figures 1-3 present the Pareto chart of the acetic acid fermentation process using respectively strains VMA1, VMA7 and VMAO. It shows the order and significance of the factors.

For strain VMA1, fermentation temperature (L) is the most influential variable in the acetic acid production, and then followed by the original alcohol concentration (L) and the original acetic acid concentration (L).

For strain VMA7, original acetic acid concentration (L) is the most influential variable in the acetic acid production, and then followed by fermentation temperature (L) and the original alcohol concentration (L).

For strain VMAO, original alcohol concentration (L) is the most influential variable in the acetic acid production, and then followed by fermentation temperature (L) and the original acetic acid concentration (L).

3.4. Three-Dimensional and Two-Dimensional Response Surface Plots

Figures 4-6 illustrate the 3D and 2D response surface plots of the acetic acid fermentation using respectively strains VMA1, VMA7 and VMAO. The surface plots were generated to determine the best levels and interactions between the parameters in order to determine the optimal conditions for maximum acetic acid production. Surface plot shows a functional relationship between a designated dependent variable (Y), and two independent variables (X and Z) [44] [45]. The plots showed the pair-wise combinations of the three process variables, (FT-OAC, OAC-OAAC, FT-OAAC); the interaction of two independent variables and their effect in the acetic acid production was assessed while fixing the

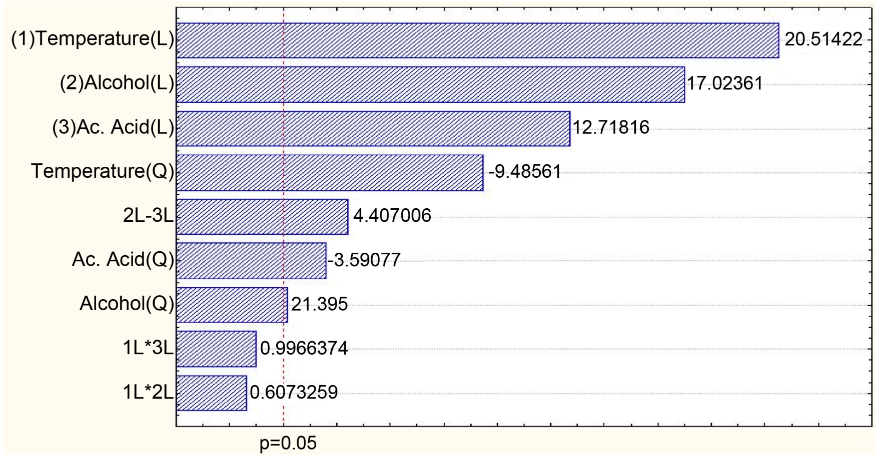

Figure 1. Pareto chart of the standardized effect estimate (Absolute Value) using strain VMA1.

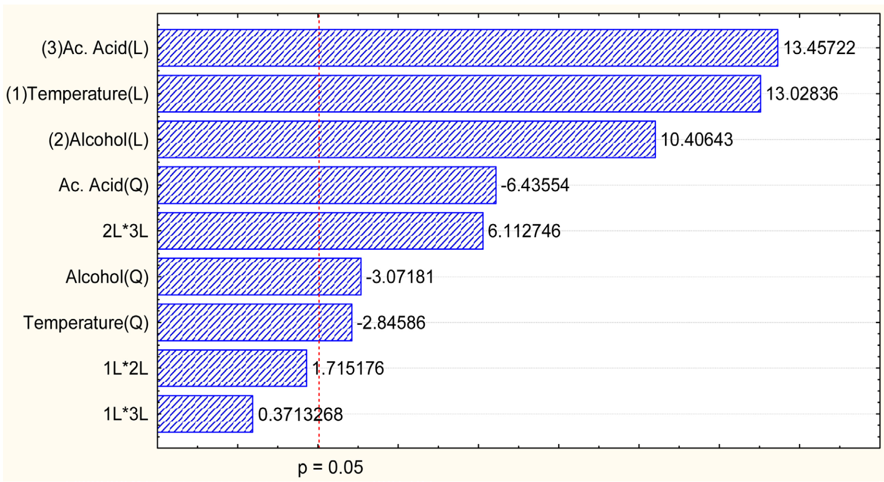

Figure 2. Pareto chart of the standardized effect estimate (Absolute Value) using strain VMA7.

value of the third variable.

3.4.1. For VMA1

Figures 4(a)-(c) show the combined effect of FT-OAC, OAC-OAAC and FT-OAAC on acetic acid production by AAB strain VMA1 while OAAC, FT and OAC values were respectively kept constant at 1% (w/v), 37.5˚C and 12.5% (v/v). It was observed that acetic acid yield increased as the two independent parameters were increasing. Low values of FT (≤34˚C) associated with low values of OAC (≤6% (v/v)) led to decrease of acetic acid production. In the same way, whatever the OAC value, lower FT (≤30˚C) and low OAAC values (≤0.2) have a negative effect on acetic acid yield. Likewise, low OACs (≤10% (v/v)) have a negative effect on acetic acid yield whatever the OAAC values.

3.4.2. For VMA7

Figures 5(a)-(c) present the combined effect of FT-OAC, OAC-OAAC and

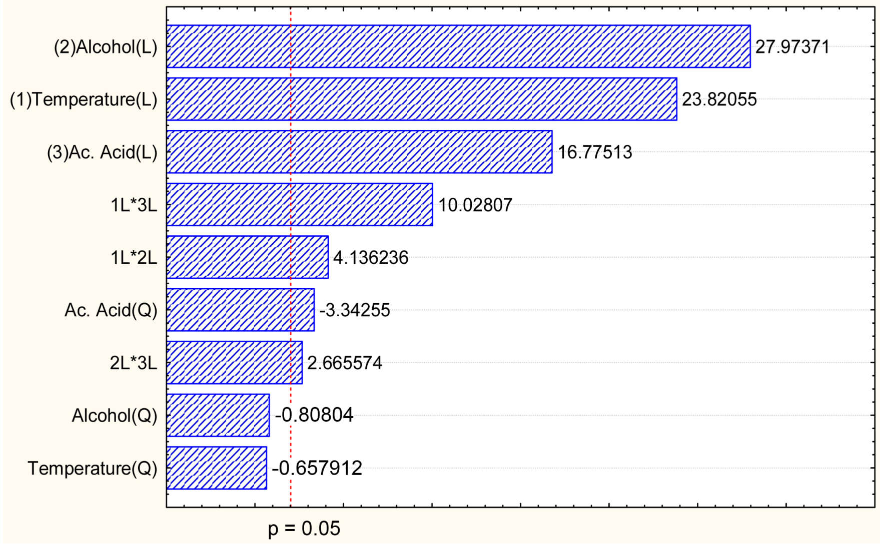

Figure 3. Pareto chart of the standardized effect estimate (absolute value) using strain VMAO.

FT-OAAC on acetic acid production by AAB strain VMA7 while OAA concentration, FT and OAC values were respectively fixed at 1% (w/v), 37.5˚C and 12.5% (v/v). Low FT [30˚C - 35˚C] decreased the medium yield when associated with OAC values between [6% - 13.5% (v/v)] and OAAC ≤ 0.4% (w/v). Low values of OAAC (≤0.3% (w/v)) have a negative effect in the AA production whatever the OAC. In the contrary, high OAAC ranging [1.7 - 2] associated with fermentation temperatures between [33˚C; 39˚C] give good responses.

3.4.3. For VMAO

Figures 6(a)-(c) present the combined effect of FT-OAC, OAC-OAAC and FT-OAAC on acetic acid production by AAB strain VMAO while OAA concentration, FT and OAC values were respectively fixed at 1% (w/v), 37.5˚C and 12.5% (v/v).

It was observed that low values of OAC and FT ≤ 34.5˚C led to a decrease of the fermentation yield whatever the OAAC. Likewise, low FT even associated with high OAC ([18% - 20% (v/v)], as well as OAAC ≤ 0.6% (w/v) associated with OAC ≤ 13.5% (w/v) decreases the production of AA. Low OAC (≤10% (v/v)) and low values of OAAC (≤0.4% (w/v)) have a negative effect on AA production whatever the fermentation temperature value.

These results allowed us to define the following optimization ranges for each of the strains used in this study.

- **VMA1**

  FT: [37.5˚C - 45˚C]

  OAC: [16% - 20% (v/v)]

  OAAC: [1.5% - 2% (w/v)]

- **VMA7**

  FT: [40˚C - 45˚C]

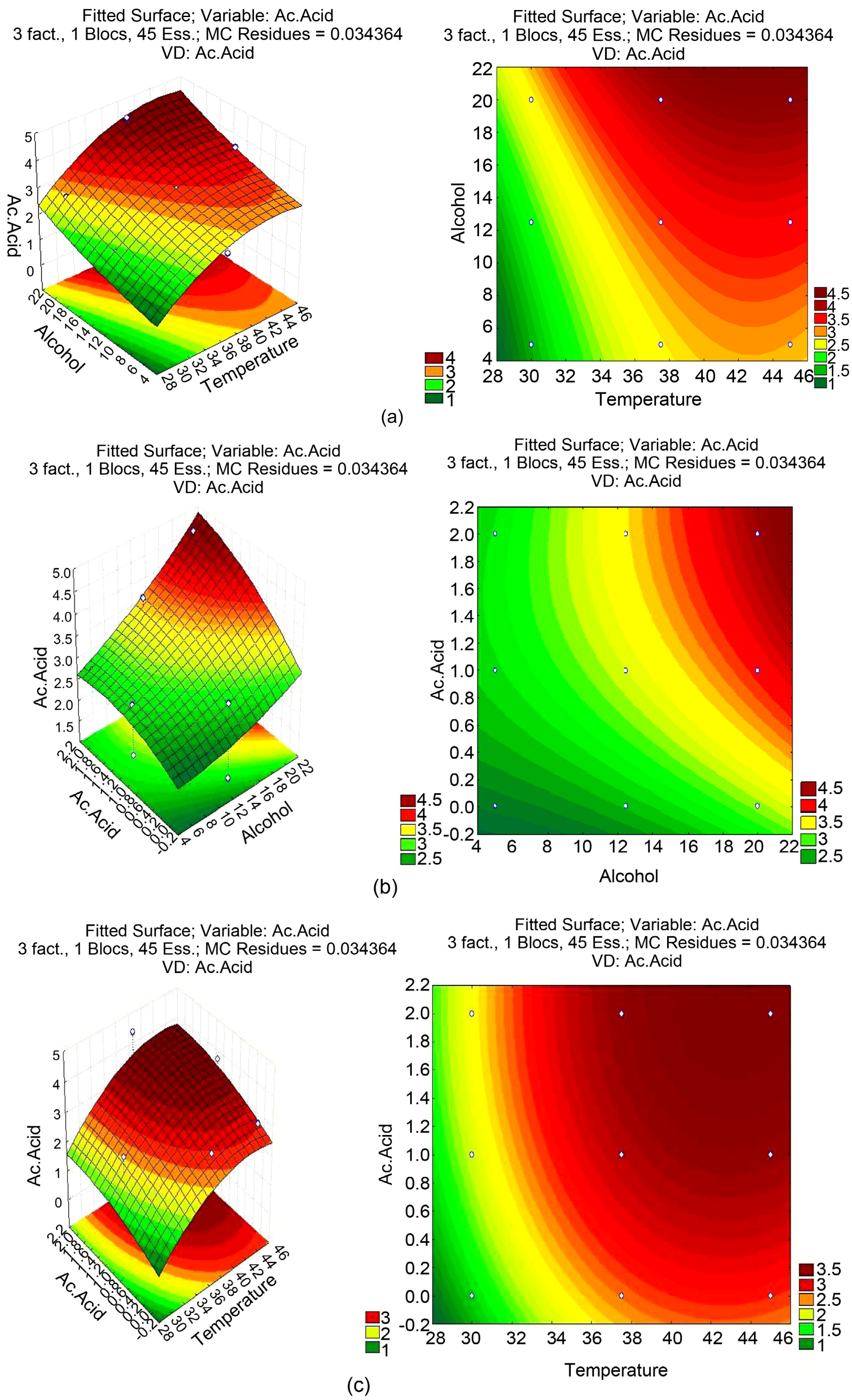

Figure 4. 3D and 2D surface plot for acetic acid yield using AAB strain VMA1. (a): Interaction Fermentation Temperature-Original alcohol concentration; (b): Interaction original alcohol concentration-Original acetic acid concentration; (c) Interaction Fermentation temperature-Original acetic acid concentration.

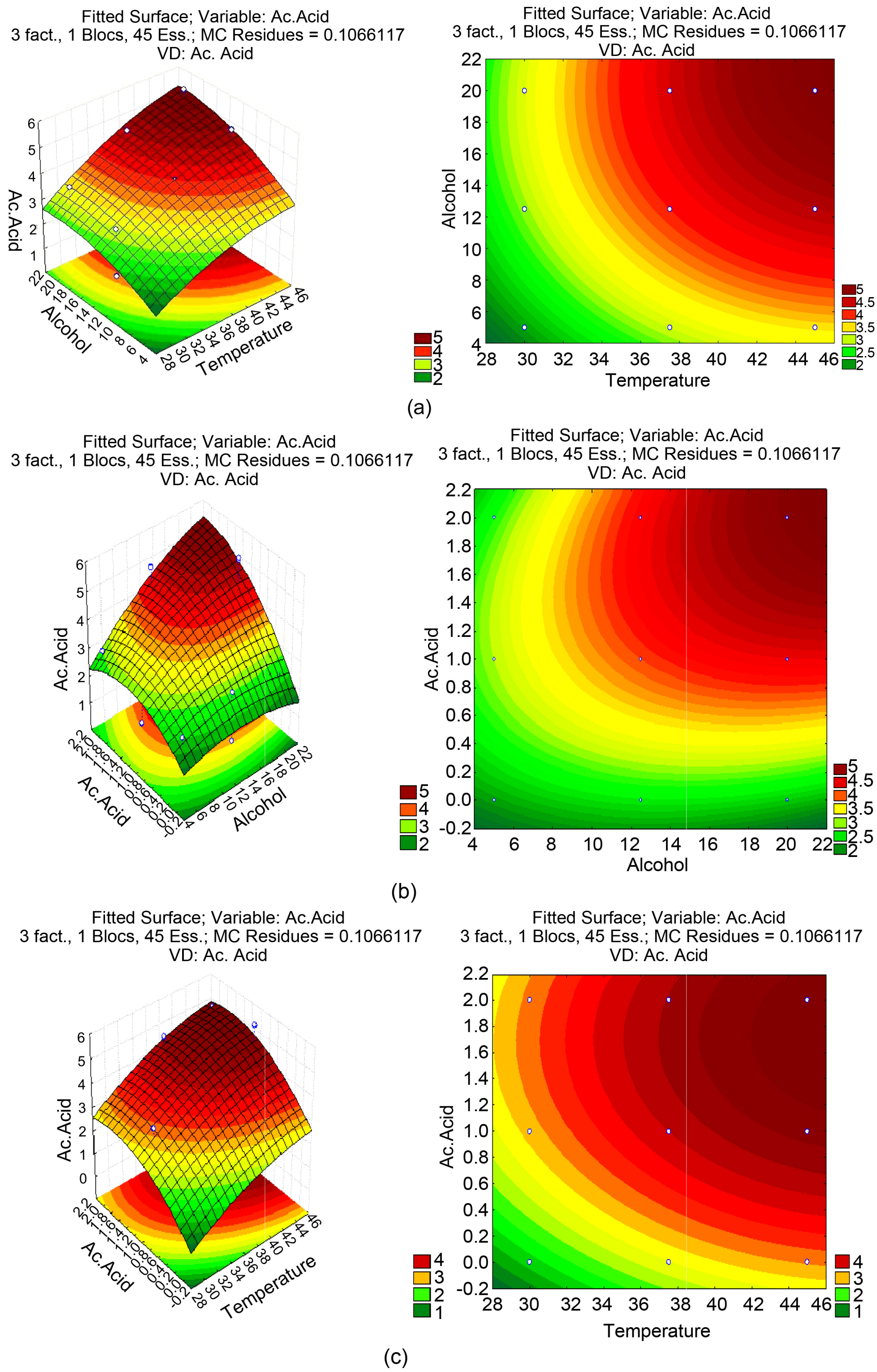

Figure 5. 3D and 2D surface plot for acetic acid yield using AAB strain VMA7. (a) Interaction Fermentation Temperature-Original alcohol concentration; (b) Interaction original alcohol concentration-Original acetic acid concentration; (c) Interaction Fermentation temperature -Original acetic acid concentration.

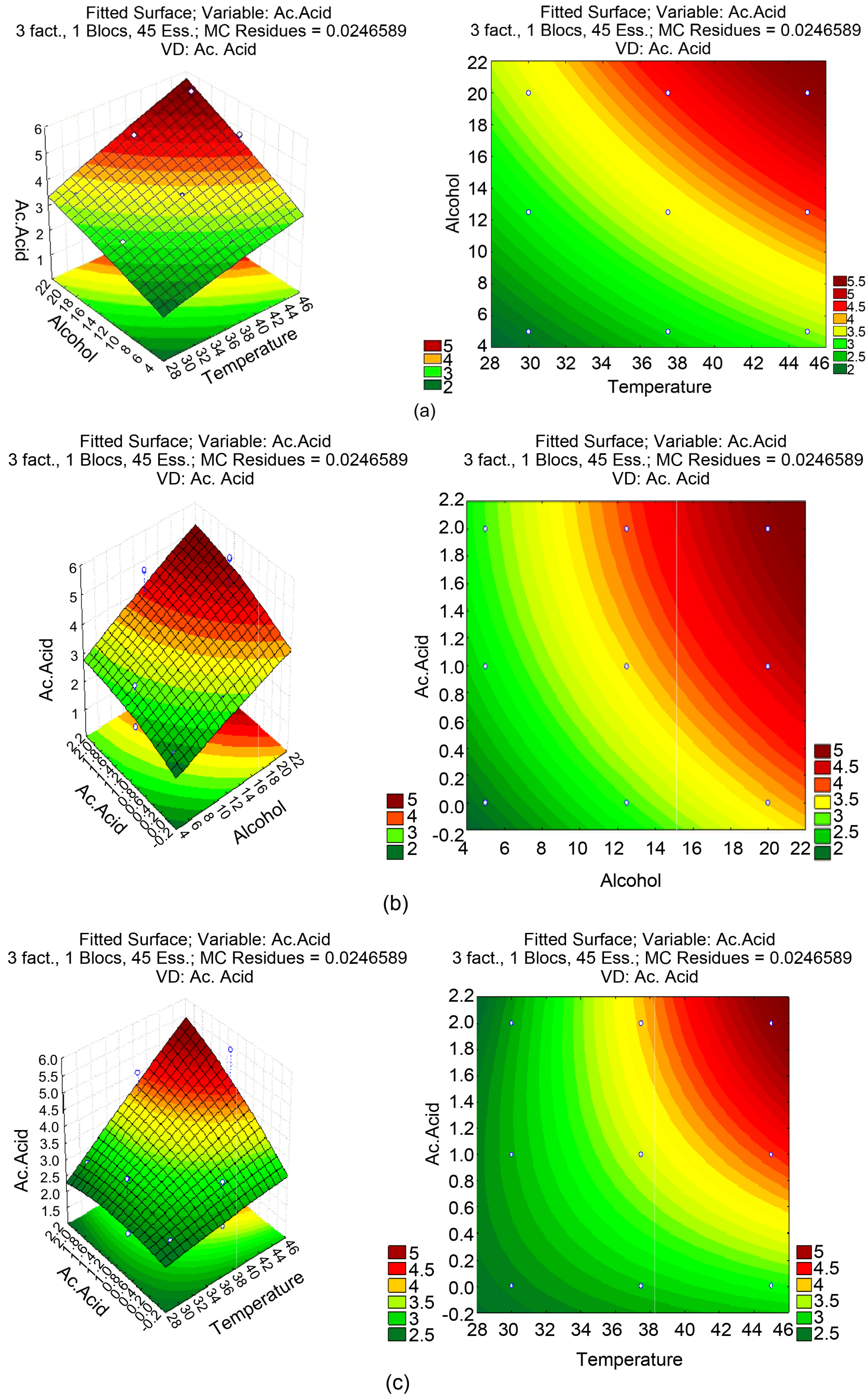

Figure 6. 3D and 2D surface plot for acetic acid yield using AAB strain VMAO. (a): Interaction Fermentation Temperature-Original alcohol concentration; (b) Interaction original alcohol concentration-Original acetic acid concentration; (c) Interaction Fermentation temperature-Original acetic acid concentration.

OAC: [14.5% - 20% (v/v)
OAAC: [1.7% - 2% (w/v)]

- **VMAO**

FT: [42˚C - 45˚C]
OAC: [17% - 20% (v/v)]
OAAC: [1.5% - 2% (w/v)]

These results confirm the resistant thermo-ethanol-acido character of the strains used in this study and previously described by Kourouma [16]. The use of these acetic strains in the production of vinegar could seriously lead to a decrease or even an ablation of the costs related to the cooling of bioreactors especially in warm and hot countries where the ambient temperature can easily exceed 37˚C, in a context of global warming. This would probably lead to a reduction in the total production cost, thus impacting the final price of the vinegar marketed, in addition to having a bank of acetic strains able to withstand such drastic conditions.

4. Conclusion

The oxidation of ethanol to acetic acid in order to produce vinegar is one of the best well-known uses of AAB in industries. However, during the acetification process, AAB are affected by many variables. This study used RSM coupled with BBD using 3 process parameters: fermentation temperature, original alcohol concentration and original acetic acid concentration to enhance the acetic acid production of 3 AAB strains, namely VMA1, VMA7 and VMAO. RSM optimization was successfully achieved. Results indicate an excellent fit between predicted and experimental values; the R^2 values are respectively 0.96, 0.94, and 0.98 which means 96%, 94% and 98% of the variability of the response can be explained by the model; the Model F-values of 22.34, 5.83 and 5.33 indicated that experimental models were statistically significant, with $p < 0.05$. The most influencing variables for AA production using VMA1, VMA7 and VMAO were respectively fermentation temperature, original acetic acid concentration and original alcohol concentration. The maximum acidity was obtained respectively at fermentation temperatures, original alcohol concentrations, original acetic acid concentrations ranging [37.5˚C - 45˚C], [16% - 20% (v/v)], [1.5% - 2% (w/v)] for VMA1; [40˚C - 45˚C], [14.5% - 20% (v/v)], [1.7% - 2% (w/v)] for VMA7; and [42˚C - 45˚C], [17% - 20% (v/v)], [1.5% - 2% (w/v)] for VMAO.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] IMARC (2023) Vinegar Market Size, Global Industry Overview, Key Players, Trends, Analysis, Latest Insights and Business Opportunities 2027. *Digital Journal*. https://www.digitaljournal.com/pr/vinegar-market-size-global-industry-overview-key-players-trends-analysis-latest-insights-and-business-opportunities-by-2027

[2] Perumpuli, P.A.B.N. and Dilrukshi, D.M.N. (2022) Vinegar: A Functional Ingredient for Human Health. *International Food Research Journal*, **29**, 959-974. https://doi.org/10.47836/ifrj.29.5.01

[3] Hutchinson, U.F., Jolly, N.P, Chidi, B.S., Ngongang, M.M. and Ntwampe, S.K.O. (2019) Vinegar Engineering: A Bioprocess Perspective. *Food Engineering Reviews*, **11**, 290-305. https://doi.org/10.1007/s12393-019-09196-x

[4] Pazuch, C.M., Kalschne, D.L., Siepmann, F.B., Marx, I.M.G., Oliveira de, T.C.G., Spinosa, W.A., *et al*. (2019) Optimization and Characterization of Vinegar Produced from Rice Bran. *Food Science and Technology*, **40**, 608-613. https://doi.org/10.1590/fst.13919

[5] Vavřiník, A., Štůsková, K., Baron, M. and Sochor, J. (2022) The Production of Wine Vinegar Using Different Types of Acetic Acid Bacteria. *Potravinarstvo Slovak Journal of Food Sciences*, **16**, 556-567. https://doi.org/10.5219/1723

[6] Saha, D. and Das, P.K. (2023) Bioconversion of Agricultural and Food Wastes to Vinegar. In: Sharma, D.H. and Rao, D.P.S., Eds., *Updates on Fermentation*, IntechOpen, Rijeka. https://doi.org/10.5772/intechopen.109546

[7] Román-Camacho, J.J., Santos-Dueñas, I.M., García-García, I., Moreno-García, J., García-Martínez, T. and Mauricio, J.C. (2020) Metaproteomics of Microbiota Involved in Submerged Culture Production of Alcohol Wine Vinegar: A First Approach. *International Journal of Food Microbiology*, **333**, Article ID: 108797. https://doi.org/10.1016/j.ijfoodmicro.2020.108797

[8] Es-sbata, I., Lakhlifi, T., Yatim, M., El-Abid, H., Belhaj, A., Hafidi, M. and Zouhair, R. (2021) Screening and Molecular Characterization of New Thermo- and Ethanol-Tolerant *Acetobacter malorum* Strains Isolated from Two Biomes Moroccan Cactus Fruits. *Biotechnology and Applied Biochemistry*, **68**, 476-485. https://doi.org/10.1002/bab.1941

[9] El-Askri, T., Yatim, M., Sehli, Y., Rahou, A., Belhaj, A., Castro, R., *et al*. (2022) Screening and Characterization of New *Acetobacter fabarum* and *Acetobacter pasteurianus* Strains with High Ethanol-Thermo Tolerance and the Optimization of Acetic Acid Production. *Microorganisms*, **10**, Article No. 1741. https://doi.org/10.3390/microorganisms10091741

[10] Matsushita, K.., Azuma, Y.., Kosaka, T.., Yakushi, T.., Hoshida, H.., Akada, R. and Yamada, M. (2016) Genomic Analyses of Thermotolerant Microorganisms Used for High-Temperature Fermentations. *Bioscience*, *Biotechnology*, *and Biochemistry*, **80**, 655-668. https://doi.org/10.1080/09168451.2015.1104235

[11] Yuan, Y., Feng, F., Chen, L., Yao, Q. and Chen, K. (2013) Directional Isolation of Ethanol-Tolerant Acetic Acid Bacteria from Industrial Fermented Vinegar. *European Food Research and Technology*, **236**, 573-578. https://doi.org/10.1007/s00217-012-1885-6

[12] Yıkmış, S. (2019) Optimization of Uruset Apple Vinegar Production Using Response Surface Methodology for the Enhanced Extraction of Bioactive Substances. *Foods*, **8**, Article No. 107. https://doi.org/10.3390/foods8030107

[13] Chakraborty, K., Saha, S., Raychaudhuri, U. and Chakraborty, R. (2017) Vinegar Production From Vegetable Waste: Optimization of Physical Condition and Kinetic Modeling of Fermentation Process. *Indian Journal of Chemical Technology*, **24**, 508-516.

[14] Kahlouche, F.Z., Zerrouki, S., Bouhelassa, M. and Rihani, R. (2022) Experimental Optimization of Enzymatic and Thermochemical Pretreatments of Bread Waste by Central Composite Design Study for Bioethanol Production. *Water Science and*

Technology, **85**, 3436-3450. https://doi.org/10.2166/wst.2022.190

[15] Cire Kourouma, M., Mbengue, M., Sarr, K. and Toure Kane, C. (2021) Isolement, identification et caracterisation de souches de bacteries acetiques a partir dun alcool de mangue fermente. *International Journal of Advanced Research*, **9**, 271-281. https://doi.org/10.21474/IJAR01/12580

[16] Kourouma, M.C., Mbengue, M., Sarr, N.C.D., Sarr, K. and Kane, C.T. (2022) Thermoresistant, Ethanol-Resistant and Acid-Resistant Properties of Acetic Acid Bacteria Isolated from Fermented Mango Alcohol. *Advances in Microbiology*, **12**, 177-191. https://doi.org/10.4236/aim.2022.124014

[17] Ghosh, S., Chakraborty, R., Chatterjee, A. and Raychaudhuri, U. (2014) Optimization of Media Components for the Production of Palm Vinegar Using Response Surface Methodology. *Journal of the Institute of Brewing*, **120**, 550-558. https://doi.org/10.1002/jib.153

[18] What Are Response Surface Designs, Central Composite Designs, and Box-Behnken Designs? https://support.minitab.com/en-us/minitab/20/help-and-how-to/statistical-modeling/doe/supporting-topics/response-surface-designs/response-surface-central-composite-and-box-behnken-designs/

[19] Taheri, M. (2022) Techno-Economical Aspects of Electrocoagulation Optimization in Three Acid Azo Dyes' Removal Comparison. *Cleaner Chemical Engineering*, **2**, Article ID: 100007. https://doi.org/10.1016/j.clce.2022.100007

[20] Patil, P.S., Deshannavar, U.B., Ramasamy, M. and Emani, S. (2021) Production, Optimization, and Characterization of Sugarcane (*Saccharum officinarum*)-Papaya (*Carica papaya*) Wine Using *Saccharomyces cerevisiae*. *Environmental Technology & Innovation*, **21**, Article ID: 101290. https://doi.org/10.1016/j.eti.2020.101290

[21] Bekele Bayu, A., Abdissa Akuma, D. and Beyecha Hundie, K. (2022) An Integrated Approach to Optimization of Fermentation Conditions for Bioethanol Production From Local Leftover Injera Waste Using Central Composite Design. *Environmental Health Engineering and Management Journal*, **9**, 281-293. https://doi.org/10.34172/EHEM.2022.29

[22] Feng, J., Zhang, J., Zhang, J., He, Y., Zhang, R., Chen, C., *et al.* (2017) Enhanced Methane Production of Vinegar Residue by Response Surface Methodology (RSM). *AMB Express*, **7**, Article No. 89. https://doi.org/10.1186/s13568-017-0392-3

[23] Hasan, M.M., Rasul, M.G., Jahirul, M.I. and Khan, M.M.K. (2023) Fast Pyrolysis of Macadamia Nutshell in an Auger Reactor: Process Optimization Using Response Surface Methodology (RSM) and Oil Characterization. *Fuel*, **333**, Article ID: 126490. https://doi.org/10.1016/j.fuel.2022.126490

[24] Özkan, A., Zannou, O., Pashazadeh, H. and Koca, I. (2023) Application of Biosolvents for the Extraction of Anthocyanins from Gülfatma Flowers (*Alcea apterocarpa* (Fenzl) Boiss): Optimization and Stability Approaches. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-022-03730-4

[25] Yang, S., Piao, Y., Li, X., Mu, D., Ji, S., Wu, R., *et al.* (2023) A New Decontamination Method for *Bacillus subtilisin* Pasteurized Milk: Thermosonication Treatment. *Food Research International*, **163**, Article ID: 112291. https://doi.org/10.1016/j.foodres.2022.112291

[26] Yıkmış, S., Erdal, B., Bozgeyik, E., Levent, O. and Yinanç, A. (2022) Evaluation of Purple Onion Waste from the Perspective of Sustainability in Gastronomy: Ultrasound-Treated Vinegar. *International Journal of Gastronomy and Food Science*, **29**, Article ID: 100574. https://doi.org/10.1016/j.ijgfs.2022.100574

[27] Yıkmış, S., Altıner, D.D., Ozer, H., Levent, O., Celik, G. and Çöl, B.G. (2022) Modeling and Optimization of Bioactive Compounds from Jujube (*Ziziphus jujuba mill.*) Vinegar Using Response Surface Methodology and Artificial Neural Network: Comparison of Ultrasound Processing and Thermal Pasteurization. *Journal of Food Processing and Preservation*, **46**, e17102. https://doi.org/10.1111/jfpp.17102

[28] Lohan, V., Pawar, K., Kumari, A. and Gehlot, R. (2022) Optimization of Fermentation Factors for Vinegar Preparation from Sugarcane *Jaggery* Using Response Surface Methodology. *Asian Journal of Dairy and Food Research*. https://doi.org/10.18805/ajdfr.DR-1730

[29] Wang, F., Song, Y., Vidyarthi, S.K. and Zhang, R. (2022) Physicochemical Properties, and Volatile Compounds of Blackened Jujube Vinegar as Prepared by Optimized Fermentation Process. *International Journal of Food Properties*, **25**, 288-304. https://doi.org/10.1080/10942912.2022.2032735

[30] Ndiaye, B., Sakho, M., Ayessou, N.C., Cisse, O.I.K., Cisse, M. and Diop, C.M. (2019) Optimization of a Tiger Nut-Based Yoghurt Formulation by Response Surface Methodology. *Food and Nutrition Sciences*, **10**, 1400-1418. https://doi.org/10.4236/fns.2019.1012100

[31] Oramahi, H.A., Rusmiyanto, E. and Kustiati (2021) Optimization of Wood Vinegar from Pyrolysis of Jelutung Wood (*Dyera lowii* Hook) by Using Response Surface Methodology. *Journal of Physics*: *Conference Series*, **1940**, Article ID: 012062. https://doi.org/10.1088/1742-6596/1940/1/012062

[32] Chen, C. and Chen, F. (2009) Study on the Conditions to Brew Rice Vinegar with High Content of γ-Amino Butyric Acid by Response Surface Methodology. *Food and Bioproducts Processing*, **87**, 334-340. https://doi.org/10.1016/j.fbp.2009.03.003

[33] Rajha, H.N., Darra, N.E., Hobaika, Z., Boussetta, N., Vorobiev, E., Maroun, R.G., *et al*. (2014) Extraction of Total Phenolic Compounds, Flavonoids, Anthocyanins and Tannins from Grape Byproducts by Response Surface Methodology. Influence of Solid-Liquid Ratio, Particle Size, Time, Temperature and Solvent Mixtures on the Optimization Process. *Food and Nutrition Sciences*, **5**, 397-409. https://doi.org/10.4236/fns.2014.54048

[34] Singh, S., Sharma, S., Sarma, S.J. and Brar, S.K. (2023) Roles of Process Parameters on the Ricinoleic Acid Production from Castor Oil by *Aspergillus flavus* BU22S. *Fermentation*, **9**, Article No. 318. https://doi.org/10.3390/fermentation9040318

[35] Li, M., Tang, H., Li, Z., Song, Y., Chen, L., Ran, C., *et al*. (2023) Optimization of the Production and Characterization of an Antifungal Protein by *Bacillus velezensis* Strain NT35 and Its Antifungal Activity against *Ilyonectria robusta* Causing Ginseng Rusty Root Rot. *Fermentation*, **9**, Article No. 358. https://doi.org/10.3390/fermentation9040358

[36] Halladj, F., Boukhiar, A., Amellal, H. and Benamara, S. (2016) Optimization of Traditional Date Vinegar Preparation Using Full Factorial Design. *Journal of the American Society of Brewing Chemists*, **74**, 137-144. https://doi.org/10.1094/ASBCJ-2016-2188-01

[37] Chicco, D., Warrens, M.J. and Jurman, G. (2021) The Coefficient of Determination R-Squared Is More Informative than SMAPE, MAE, MAPE, MSE and RMSE in Regression Analysis Evaluation. *PeerJ Computer Science*, **7**, e623. https://doi.org/10.7717/peerj-cs.623

[38] Germec, M. and Turhan, I. (2021) Predicting the Experimental Data of the Substrate Specificity of *Aspergillus niger* Inulinase Using Mathematical Models, Estimating Kinetic Constants in the Michaelis-Menten Equation, and Sensitivity Analysis. *Biomass Conversion and Biorefinery*.

https://doi.org/10.1007/s13399-021-01830-1

[39] Saha, P. and Banerjee, S. (2013) Optimization of Process Parameters for Vinegar Production Using Banana Fermentation. *International Journal of Research in Engineering and Technology*, **2**, 501-514. https://doi.org/10.15623/ijret.2013.0209076

[40] Tazulazhar, N., Loh, S.H., Ariffin, M. and Khalik, W. (2021) Optimization of Effervescent Tablet-Assisted Dispersive Liquid-Liquid Microextraction with Central Composite Design for Preconcentration of Stimulant Drug. *Sains Malaysiana*, **50**, 109-121. https://doi.org/10.17576/jsm-2021-5001-12

[41] Mohd Sharif, N.S.A., Thor, E.S., Zainol, N. and Jamaluddin, M.F. (2017) Optimization of Ferulic Acid Production from Banana Stem Waste Using Central Composite Design. *Environmental Progress & Sustainable Energy*, **36**, 1217-1223. https://doi.org/10.1002/ep.12560

[42] El-Naggar, N.E.-A., Mohammed, A.B.A. and El-Malkey, S.E. (2023) Bacterial Nanocellulose Production Using Cantaloupe Juice, Statistical Optimization and Characterization. *Scientific Reports*, **13**, Article No. 51. https://doi.org/10.1038/s41598-022-26642-9

[43] Jannah, S.N., Handayani, D., Purwantisari, S., Yulianto, M.E., Amalia, R. and Hartati, I. (2020) Application of Response Surface Methodology to the Optimization of Coco Vinegar Production as Antioxidant and Antidiabetes through Bubble Biofermentation Process. *AIP Conference Proceedings*, **2197**, Article ID: 060004. https://doi.org/10.1063/1.5140931

[44] Response Surface Methodology. https://jaeronline.com/wp-content/uploads/2020/04/3.-Response-Surface-Methodology.pdf

[45] Kaladhar, M., Kambagowni, V., Rao, C.S. and Rao, K.N. (2010) Optimization of Process Parameters in Turning of AISI202 Austenitic Stainless Steel. *Journal of Engineering and Applied Sciences*, **5**, 79-87.