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Use of response surface methodology to optimize the drying conditions of a bioactive ingredient derived from the African opaque sorghum beer

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The present work aims to optimize the heat drying conditions of a bioactive feed ingredient derived from the African opaque sorghum beer. The bioactive ingredient was dried at various temperatures 35 to 50°C and times 5 to 24 h. The effects of the drying conditions on the dry matter, water activity, pH, titratable acidity, bacteriocin, ethanol, lactic acid, yeasts, lactic acid bacteria contents and antimicrobial activity against indicator pathogens (*Staphylococcus aureus* ATCC 27844, methicillin resistant *S. aureus* (MRSA), *Salmonella typhi* R 30951401, *Klebsiella pneumoniae* ATCC 35657, *Escherichia coli* ATCC 25922, *E. coli* O157:H7 ATCC 700728 and *Candida albicans* MHMR) were studied. Results showed that the temperature was the main factor that affects the bioactivity indicators of the ingredient. The optimal conditions ensuring the best functionality of the ingredient were as follows: Temperature 42°C and drying duration, 24 h. At this optimum condition, water activity (0.49) was low enough to warrant adequate shelf life to the ingredient.

Key words: Bioactive ingredient, drying, optimization, response surface methodology.

INTRODUCTION

Thermal drying is a process of removing moisture from wet materials and is an important technique for the processing and preservation of some foodstuffs (Chen et al., 2013). It has a decisive effect on the quality of most

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Table 1. The central composite experimental design matrix (coded and real values for independent variable levels) for drying of antimicrobial ingredient.

Run	Levels of temperature		Levels of time	
	Coded value (X_1)	Real value (°C)	Coded value(X_2)	Real time (h)
1	0	42.5	0	14.5
2	0	42.5	0	14.5
3	0	42.5	0	14.5
4	0	42.5	0	14.5
5	0	42.5	0	14.5
6	0	42.5	0	14.5
7	+1	47	+1	20.2
8	-1	38	+1	20.2
9	-1	38	-1	8.79
10	+1	47	-1	8.79
11	+α	50	0	14.5
12	0	42.5	+α	24
13	-α	35	0	14.5
14	0	42.5	-α	5

commercial food products (Mujumdar, 2007). Sun drying has some significant disadvantages. It is time-consuming and labor-intensive, weather-dependent and may result in nutrients loss. Moreover, environmental contamination may occur during the sun drying (Maskan, 2001). Drying of biological materials such as bioactive ingredients containing living organisms is a complex process due to the simultaneous phenomenon of heat and mass transfer which occurs inside each particle (Torrez Irigoyen et al., 2014). In such conditions, the goal of drying is to preserve all functional properties of the ingredients after drying. To preserve the quality characteristics of such biological materials, a number of drying parameters could be optimized. These include drying temperature, flow-rate of drying air, pressure, power intensity, thickness of slices and drying time, which are dependent on the drying method (Singh et al., 2008; Sobukola et al., 2010; Manivannan and Rajasimman, 2011).

Response surface methodology (RSM) is a useful optimization tool, which has been applied in research to study the effect of individual variables and their interactions on response variables (Ahmed et al., 2014). It uses quantitative data from an appropriate experimental design to determine and simultaneously solve multivariate problems. Equations describe the effect of test variables on responses, determine interrelationships among test variables and represent the combined effect of all test variables in any response. This approach enables an experimenter to make efficient exploration of a process or system (Madamba, 2002). In the present study, we used the Response Surface Methodology to evaluate the simultaneous effects of the drying temperature and duration of a bioactive feed ingredient on its dry matter, water activity, pH, titratable acidity, bacteriocin, ethanol, lactic acid, yeasts, and lactic acid bacteria contents. Its antimicrobial activities against indicator pathogens such as *Staphylococcus aureus* ATCC 27844, methicillin resistant *S. aureus* (MRSA), *Salmonella typhi* R 30951401, *Klebsiella pneumoniae* ATCC 35657, *Escherichia coli* ATCC 25922*, E. coli* O157:H7 ATCC 700728 and *Candida albicans* MHMR were investigated and optimized.

MATERIALS AND METHODS

Inoculums and origin

The inoculum used is *kpete-kpete* which is the sediment of the Benin opaque sorghum beers known as tchoukoutou. *Kpete-kpete* consists of a mixture of lactic acid bacteria and yeasts (Kayodé et al., 2007) and is usually used as starter by women beer producers for a new beer production. The inoculum was supply by a woman producer of tchoukoutou in Abomey-Calavi.

Preparation of antimicrobial ingredient

Sorghum flour (75% of dehulled sorghum grains and 25% of sorghum malt) is mixed with distilled water to obtain a dough with a water content of 45% The dough is inoculated with 10% (w/w) of *kpete-kpete*, kneaded into dough and allowed to ferment in a plastic bucket with lid for 36 h to allow optimum microorganisms propagation. The fermented dough samples were dried in a ventilated oven drier (Venticell, Fisher, Bioblock Scientific, MMM, Medcenter) for an indicated time and temperature as specified in the experimental design (Table 1). At each time and temperature point, samples were aseptically taken and put in the flask for microbiological analysis and antimicrobial tests.

Experimental design

An orthogonal rotatable Central Composite Design (CCD) for k = 2 factors was used to investigate the simultaneous effects of two

process variables on the dry matter (DM), water activity (a_w), pH, lactic acid (LA), lactic acid bacteria count (LAB), yeasts and moulds count (YM), bacteriocin production (BE) and the antimicrobial activity against indicator pathogens (*S. aureus* ATCC 27844, methicillin resistant *S. aureus* (MRSA), *S. typhi* R 30951401, *K. pneumoniae* ATCC 35657, *E. coli* ATCC 25922*, E. coli* O157:H7 ATCC 700728 and *C. albicans* MHMR) in a quadratic function. The design generated fourteen observations including six central points, four kernel points and four axial points. The design matrix and variable combinations are shown in Table 1.

Microbiological analysis

Total counts of LAB, yeasts and moulds were performed according to the method described by Nout et al. (1987). At each sampling time, duplicate samples (10 g) were diluted in 90 ml sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl, and 1000 ml distilled water, pH = 7.0) and homogenized with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Viable counts of lactic acid bacteria were determined on de Man, Rogosa and Sharpe Agar (MRSA, CM 361, Oxoid, Hampshire, England) containing 0.1% (w/v) natamycin (Delvocid, DSM, The Netherlands) with incubation in anaerobic jar (Anaerocult A, Merck KGaA, Germany) for 72 h. Yeasts and moulds were enumerated using Malt Extract Agar (MEA, CM 59 Oxoid, Basingstoke, Hampshire, England). MEA plates were incubated at 25°C for 72 to 120 h. The colonies were then counted and expressed as logarithmic colony forming units per gram (log_{10} cfu/g) of sample.

Antimicrobial activity

The capacity of bioactive ingredient to inhibit the indicator pathogens was determined by modifying the disc diffusion method of NCCLS (2003). Twenty milliliters (20 mL) of molten Mueller-Hinton Agar (MHA, CM 337, Oxoid, Hampshire, UK) were poured into sterile Petri dishes and allow to solidify. Hundred microliters (100 µL) of the overnight Mueller-Hinton broth (Oxoid, CM 405, Hampshire, UK) culture of each pathogen strain, which have been adjusted to 0.5 McFarland-turbidity, was spread on the plates. Once the plates were dried aseptically, five blank discs papers (6 mm in diameter) were placed onto the surface of the agar. The moist or dried sample of bioactive ingredient was reconstituted with sterile distilled water to obtain a solution of 500 mg mL^{-1}. This solution was stirred vigorously using a magnetic stirrer for 30 min and then centrifuged at 3 500 g for 30 min. Forty microliters (40 µL) of each supernatant were delicately placed into the discs. The plates were left at room temperature for 1 h so that the absorbed supernatant become diffused into the agar, and then incubated at 37°C for 24 h.

Physicochemical analysis

Determination of dry matter, pH, titratable acidity and a_w

The dry matter content was determined by oven drying of 5 g of grinded at 105°C until a constant weight was reached (AACC, 44-15 A, 1984). Titratable acidity and pH were determined as described by Hounhouigan et al. (1993). The pH was measured using a digital pH-meter (JENWAY, Model 3505, UK) calibrated with buffers at pH 4.0 and 7.0 (WTW, Weilheim, Germany). The water activity (a_w) of the dried samples was measured using a water activity meter (Rotronic HygroLab 2, 8303 Bassersdorf).

HPLC determination of lactic acids and ethanol

Lactic acids and ethanol were extracted in 1.5 ml centrifuge tubes from 50 mg samples with 1 ml of sulphuric acid (H_2SO_4, 5 mM) under mild agitation for 30 min. After extraction, samples were centrifuged at 3500 x g for 30 min then filtered through a 0.45 µm sieve before separation by HPLC using an Aminex HPX-87H^+cation-exchange column (BioRad Hercules, USA) at a column temperature of 37°C with UV-(210 nm) and IR-detectors (Mestres and Rouau, 1997). The eluent was 5 mM sulphuric acid at a flow rate of 0.6 ml min^{-1}. The injection volume of the sample was 20 µl. Lactic acids and ethanol was expressed as mg/g dry matter.

Bacteriocin extraction

Bacteriocin was measured by the method of Burianek and Yousef (2000) modified as follows: 10 g of the tested ingredient were withdrawn in a Falcon tube to which one added 30 ml of distillated water stirred vigorously using a magnetic stirrer for 60 min. Blend was centrifuged at 3 500 g for 30 min. The supernatant fluid was harvested and chloroform was added (1:2, v/v) then stirred vigorously using a magnetic stirrer for 30 min. The blend was allowed to settle for 30 min and the aqueous layer was removed delicately, using a micropipette. Afterward, the precipitated phase (interface between aqueous layer and solvent) and solvent (chloroform) were removed and dried. The residual aqueous layer remaining in the flask was cooled and weighed. The solvent (chloroform) is evaporated using a rotary evaporator. After one hour of drying in the oven (130°C) the flask is cooled with the desiccators then weighed again. The weight (m) of bacteriocin expressed in mg/g is calculated as follows:

$$m = \frac{[(m_f - m_i) - m_0]}{m_e}$$

Where m_i is an initial mass of flask, m_f is final mass of flask; m_o is mass of peptide initially contained in sorghum flour used.

Statistical analysis

All statistical analyses were carried out by employing the statistical package Minitab 14 (Minitab Inc., USA). A second order polynomial model was fitted to the mean data values to obtain regression equations. The independent variables were the temperature (X_1) of drying (35 to 50°C) and the duration (X_2) of drying (5 to 24 h). The chosen model was a second degree polynomial regression with interaction for Y variables.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2.$$

Where Y is the response, b_0, b_1, b_2, b_3, b_4 and b_5 are model coefficients for intercept, linear, quadratic and interaction terms, respectively, and x_i are coded independent variables.

The fitted polynomial equations were expressed in 3D response surface graphs, in which the response is presented on the vertical axis and two factors at the two horizontal perpendicular axes. The analysis of variance (ANOVA) was used to determine significant differences between independent variables ($p < 0.05$).

Verification of the model

The criteria for evaluating the reliability of the simulations were the percentage error observed between experimental and predicted values. Thus, the experimental value was compared with the predicted one from the optimized model by calculating the percentage error to determine the adequacy of the drying process and response surface models. The percentage error, PE which is lower than 10 % indicates a good fit (McLaughlin and Magee, 1998; Kek et al., 2014).

Table 2. The measured data for response surface analysis of the effect of drying conditions on the quality of antimicrobial ingredient.

Code	D.M	a_w	pH	TA	Alcool	LA	BE	O157 :H7	E. coli	S. aureus	MRSA	C. albicans	S. typhi	K. pn	LAB	YM
1	67.64	0.86	4.15	1.95	0.85	10.15	19.55	13.12	12.75	12.25	12.50	14.62	13.25	12.12	7.93	7.71
2	66.58	0.88	3.86	3.64	0.34	12.28	12.81	12.62	13.25	13.00	12.00	13.12	12.00	12.00	7.83	7.17
3	69.84	0.85	3.92	3.33	0.11	17.23	11.92	14.12	13.62	12.00	13.50	13.00	16.00	12.12	7.92	7.09
4	68.25	0.86	3.88	3.45	0.89	12.46	13.09	12.00	12.00	13.75	13.00	15.12	14.12	11,75	7.93	7.14
5	69.87	0.85	3.91	3.43	0.60	21.11	12.11	12.12	11.75	12.25	12.87	12.75	13.50	10.75	7.44	7.08
6	69.61	0.85	3.93	3.35	0.67	20.54	15.95	11.25	13.12	14.00	11.12	13.25	14.00	13.25	8.10	7.25
7	92.59	0.26	4.61	2.07	0.08	15.19	13.82	13.00	12.62	12.50	12.12	13.00	12.12	10.25	5.16	0.00
8	74.80	0.84	3.95	3.16	0.57	19.83	16.36	12.62	13.50	12.25	13.00	14.87	13.25	11.00	8.06	7.10
9	62.62	0.90	3.94	3.56	1.19	13.12	15.97	12.00	12.75	13.00	13.50	15.00	14.50	12.12	8.12	7.22
10	77.76	0.84	4.15	3.4	0.20	14.95	12.47	12.87	12.50	13.62	11.00	14.12	15.00	13.00	7.14	6.24
11	90.72	0.31	4.43	2.15	0.01	13.44	13.23	12.25	12.12	12.00	11.87	12.00	13.12	10.00	5.03	0.00
12	90.74	0.40	4.27	2.47	0.02	10.18	13.67	11.87	12.00	11.87	13.00	13.62	12.00	10.87	7.35	6.19
13	65.02	0.89	3.91	3.34	0.99	17.34	13.58	13.12	12.25	13.00	12.25	14.87	15.00	12.12	8.22	7.13
14	59.78	0.88	3.89	3.81	1.54	17.99	16.73	14.00	13.62	12.00	12.62	14.25	15.50	14.12	8.43	7.95

O157,H7, E. coli O157,H7 ATCC 700728; E. coli*, E. coli* ATCC 25922; S. aureus*, Staphylococcus aureus* ATCC 27844; MRSA, methicillin resistant *Staphylococcus aureus; C. albicans, Candida albicans* MHMR*; S. typhi, Salmonella typhi* R 30951401 and *K. pn, Klebsiella pneumoniae* ATCC 35657.

$$PE(\%) = \left(\left| \frac{m_{exp} - m_{pre}}{m_{exp}} \right| \right) * 100$$

Where m_{exp} is experimental value and m_{pre} is predicted value.

RESULTS AND DISCUSSION

Effects on physic-chemical properties

Long shelf life of dried product is closely related to their low moisture content (Atalar and Dervisoglu, 2015). For all treatments (Table 2) the dry matter varied from 59.78 to 92.59%. The highest dry matter was obtained for the treatment 7 and the lowest for the treatment 14. The linear and quadratic regression model for dry matter was given in Table 3. As could be expected, it clearly appears that the drying temperature as well as the drying duration significantly influenced the dry matter of the bioactive ingredient. Only 3% of the total variations are not explained by the model. Additionally, the significant p-value of F, particularly for linear and quadratic regression model indicates that dry matter from bioactive ingredient had a good model fit due to the high value of R^2 and F. Figure 1a shows the surface plots for the effect of the independent variables on the moisture content. From this figure., it was observed that water content decreases continuously with drying time and increasing drying temperature. Such increase in the product dry matter content, due to water loss, is desirable since it could improve shelf life of the product (Kayodé et al., 2012).

Besides, the main objective of drying is to decrease the a_w of various perishable materials to values <0.5, in order to enable their storage at ambient temperature (Bonazzi and Dumoulin, 2011). Water activity of wet bioactive ingredient was 0.93. During the drying process, it decreased significantly to reach the value of 0.26 for treatment 7 (Table 2). Table 3 shows that the linear, quadratic and interaction terms of all independent variables (drying time and drying temperature) was highly significant and negatively affected the water activity of the bioactive ingredient. This is confirmed by its surface plot (Figure 1b). The same trend was reported by Kek et al. (2014) for drying of guava.

For all the treatments applied, the pH values of dried bioactive ingredient range from 3.86 to 4.61. Figure 1d shows the trend in the pH as function of the two independent variables. As shown in Table 3, pH was found to be a function of the linear and quadratic effects of drying temperature and drying

Table 3. Regression coefficients, coefficient of determination (R^2), and p-values for the second order polynomial equations.

Coef.	D.M	a_w	pH	TA	Alcool	L.A	Bac.	*E. coli* O157:H7	*E. coli*	*S. aureus*	MRSA	*C. albicans*	*S. typhi*	*K. pn*	LAB	YM
Constant																
b_0	68.936	0.861	3.952	3.195[a]	0.5634	15.7211	14.282	12.5251	12.7808	12.5251	12.4927	13.711	13.7926	11.9693	7.796	7.1558
Linear																
b_1	7.935[a]	-0.168[a]	0.1819[a]	-0.3386	-0.3261[b]	-0.9749	-0.6942	-0.0208	-0.1411	-0.0833	-0.4206	-0.7889[b]	-0.3940	-0.3562	-1.0585[a]	-2.0918[a]
b_2	8.232[a]	-0.151[a]	0.1155[b]	-0.4151[b]	-0.3427[b]	-0.6340	-0.3515	-0.2930	-0.1914	-0.2187	-0.1313	-0.2409	-1.0435[b]	-0.9726[a]	-0.3068[c]	-0.9726[b]
Quadratic																
b_3	3.653[a]	-0.090[a]	0.0933[c]	-0.1568	-0.0415	-0.0109	-0.2552	-0.0392	-0.1695	-0.0809	-0.1641	-0.0045	0.0697	-0.3691	-0.5107[a]	-1.4146[a]
b_4	2.710[b]	-0.076[a]	0.0608	-0.0142	0.0595	-0.4601	0.3928	-0.1294	-0.0562	-0.2849	0.1066	-0.176	-0.0422	0.1489	-0.0541	-0.1494
Interaction																
b_5	0.662	-0.130[a]	0.038	-0.2325	0.1250	-1.6175	0.24	-0.1225	-0.1575	-0.0925	0.4050	-0.2475	-0.4075	-0.4075	-0.2525	-1.5275[b]
R^2	**0.972**	**0.995**	**0.881**	**0.629**	**0.772**	**0.149**	**0.148**	**0.129**	**0.207**	**0.234**	**0.392**	**0.555**	**0.582**	**0.750**	**0.932**	**0.953**

b_1 and b_3, Coefficients for temperature; b_2 and b_4, coefficients for time; b_5, coefficient for interaction (temperature × time). [a] Significant at $p<0.001$, [b] significant at $p<0.01$, [c] significant at $p<0.05$; R^2 coefficient of determination.

time. The linear and quadratic effects ($p<0.01$) were positive. The interaction effect between time and temperature was found to be not significant (Table 3). Titratable acidity (Figure 1g) was mainly a function of the drying time with a negative linear effect ($p<0.05$) and insignificant linear effect of the drying temperature. Besides, the quadratic and interaction effects with temperature and time were found to be not significant (Table 3).

Effects on bacteriocin production, organic acid and ethanol contents

Bacteriocin, organic acids (such as lactic acid, acetic acid), ethanol, hydrogen peroxide are part of components capable of inhibiting pathogenic microorganisms. Statistical analysis showed no significant effect of the drying parameters on bacteriocin and lactic acid as reflected by the low values of the respective coefficients of determination (Table 3). Ahmad et al. (2014) reported that activity of bacteriocin is heat stable at temperature up to 80°C. The drying duration and temperature had not significant effect on bacteriocin activity of bioactive ingredient.

Yeasts are mainly responsible for ethanol production. The yeasts involved in the bioactive ingredient under study were previously reported to belong essentially to *Saccharomyces cerevisiae* (Kayodé et al., 2007). The ethanol content of the product decreases significantly with increasing temperatures. This could be expected since ethanol is highly volatile even at ambient temperature. Among the different treatments applied, the highest ethanol rate (1.54 mg/g) of bioactive ingredient was obtained with the treatment 14 and the lowest (0.01 mg/g) with the treatment 11 (Table 2). As shown in Table 3 and Figure 1c, only the linear effect of the drying parameters was significant on the ethanol content of the ingredient. The quadratic and interaction effects were not significant.

Effects on functional microorganisms

Surface plots and the model coefficients for viable microorganisms are presented in Figure 1e and f and Table 3, respectively. The total count of viable microorganisms is relatively constant at drying temperature between 35 and 43°C. Beyond this temperature, the viable microorganisms decreased sharply to reach a minimum level at 50°C. Thus, viable yeasts decreased from 6.24 to 0.0 log cfu/g whereas LAB decreased from 7.14 to 5.03 log cfu/g. In similar study, Kayodé et al. (2012) reported that the yeasts were much more sensitive to temperature compared to LAB which are able to survive at temperatures as high as 45°C. Higher temperatures and longer drying durations affect negatively the total count of viable microorganisms in the bioactive ingredient. Specifically, the linear and quadratic effects of the parameters are significant. It is well established that the loss of probiotics viability during convective thermal processing is related to cell injuries resulting from the combined effects of heat and mechanical stress (Behboudi-Jobbehdar et al., 2013). The interactive effects of the duration and temperature were not significant for LAB,

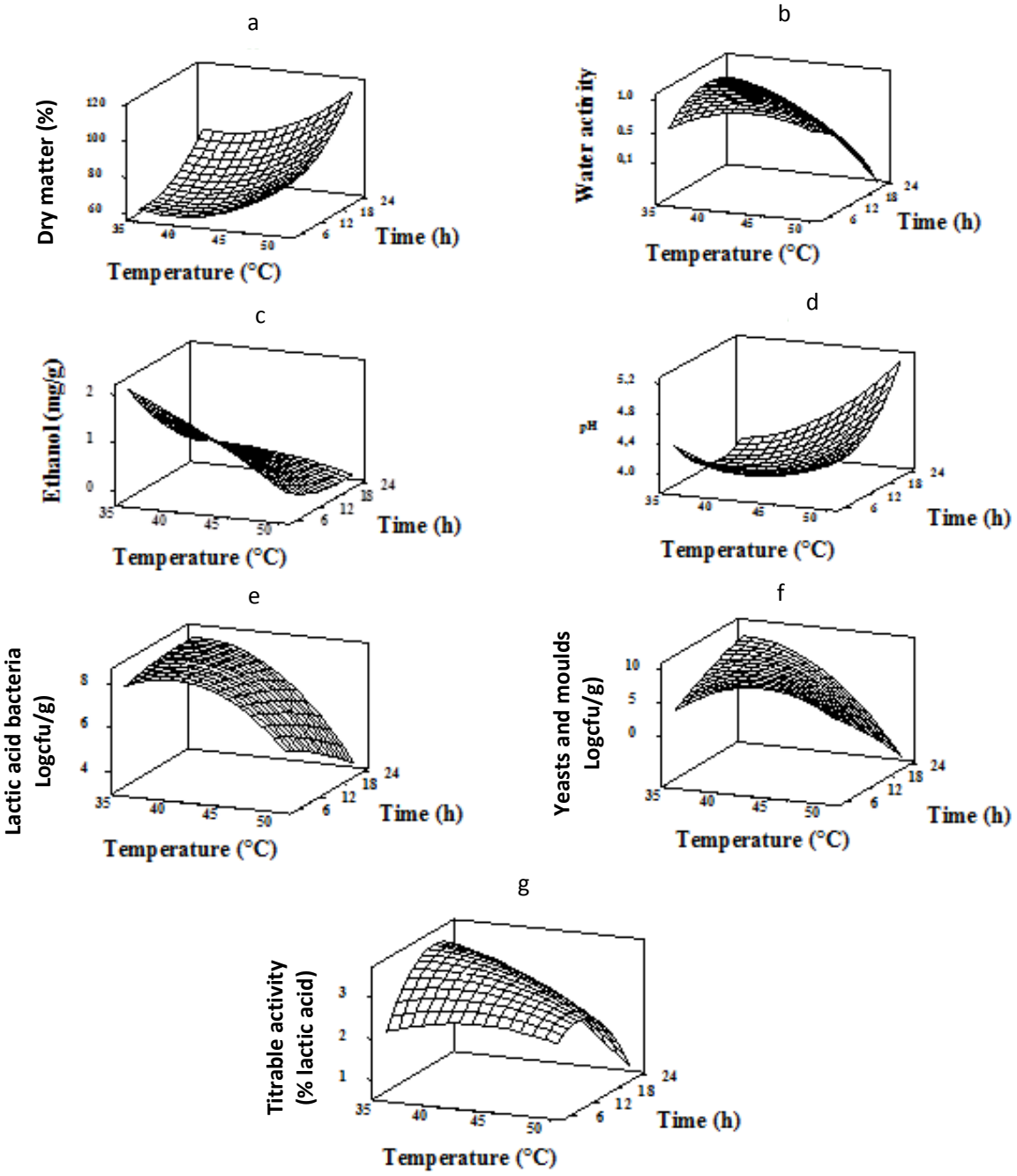

Figure 1. Response surface curves (3D) depicting the effects of drying temperature and time on (a) dry matter, (b) water activity, (c) ethanol, (d) pH, (e) lactic acid bacteria, (f) Yeasts and moulds and (g) titratable acidity with respect to significant process parameters.

whereas it significantly affect the yeasts and moulds contents of the product (Table 3).

Effects on antimicrobial properties

The inhibition diameter was used as an index for the antimicrobial activity of the bioactive ingredient investigated. The inhibition diameter of all indicator pathogens tested ranged from 10 mm (on *K. pneumoniae*) to 15.50 mm (on *S. typhi*). Clearly, the bioactive ingredient dried at different temperatures (35-50°C), preserved its antimicrobial properties. However, the analysis of variance (Table 3) indicated that the

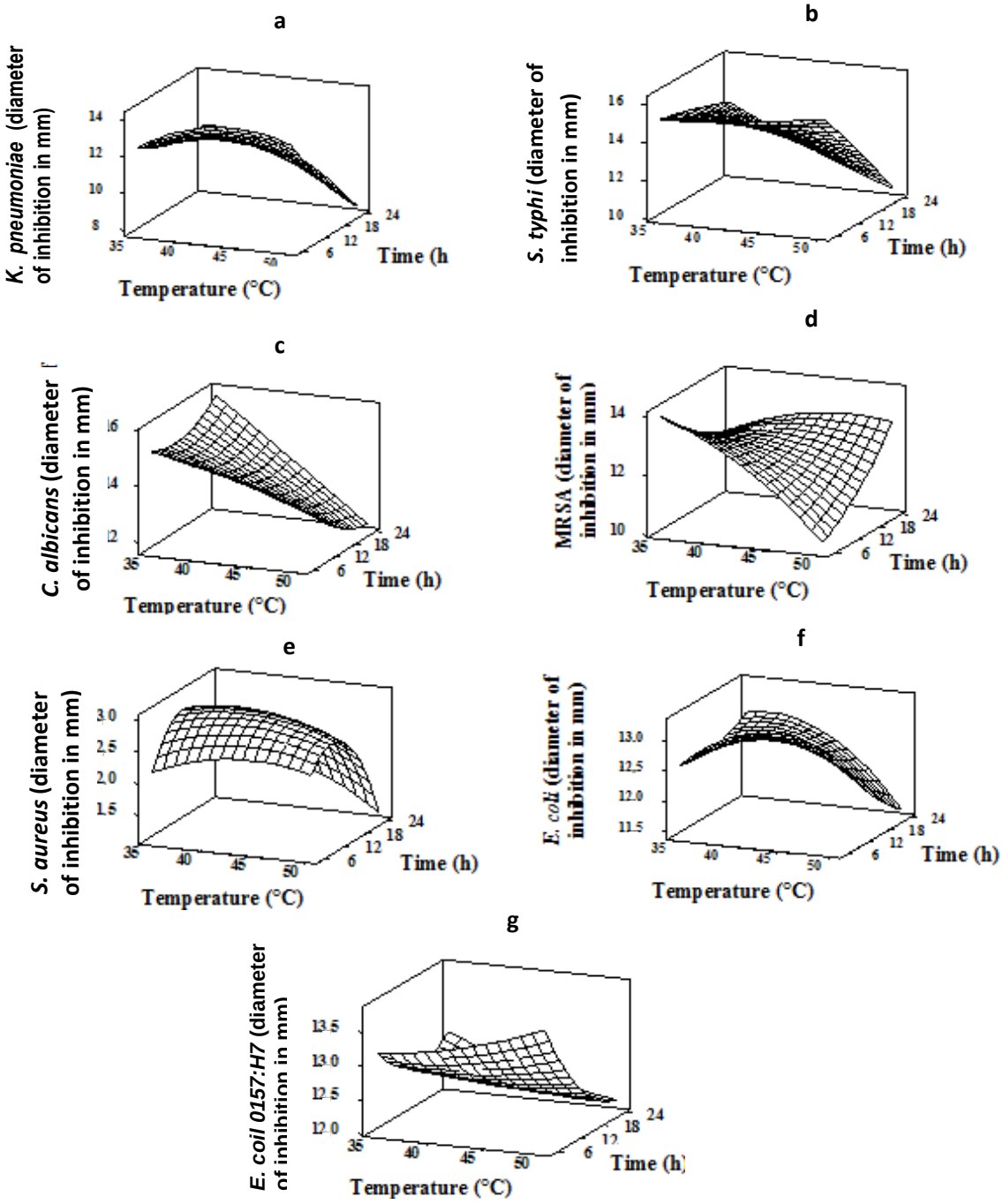

Figure 2. Response surface curves (3D) showing the effects of dried antimicrobial ingredient on (a) *K. pneumoniae*, (b) *S. typhi*, (c) *C. albicans*, (d) MRSA, (e) *S. aureus*, (f) *E. coli*, (g) and *E. coli* .O157:H7.

antimicrobial activity on three indicator pathogens (*K. pneumoniae* ATCC 35657, *S. typhi* R 30951401 and *C. albicans* MHMR) was closely related to the linear effects of temperature and time with significant values of the relative coefficients of determination values (R^2) which ranged from 0.56 to 0.75. As shown in Table 3 and Figure 2a, b and c, inhibitions of *C. albicans* MHMR, *S. typhi* R 30951401 and *K. pneumoniae* ATCC 35657 mainly depend on drying temperature. The quadratic and interactive effects of time and temperature were not significant. The coefficient of determination (R^2) obtained for the four (Table 3 and Figure 2d, e, f and g) remaining pathogens (methicillin resistant *S. aureus* (MRSA), *S. aureus* ATCC 27844, *E. coli* ATCC 25922 and *E. coli*

Table 4. Pearson's correlation coefficients between microbial inhibitions and some physicochemical and microbiological parameters.

	a_w	pH	*S. typhi* ATCC	*K. pneumoniae*	*E. coli O157 :H7*	*E. coli* ATCC	*S. aureus*	*MRSA*	*C. albicans*	LAB
a_w										
pH	-0.916**									
S. typhi	0.077	0.099								
K. pneumoniae	0.306	-0.268	0.649*							
E. coli O157 :H7	0.205	-0.053	0.478	0.282						
E. coli ATCC	0.170	-0.021	0.310	0.519	0.510					
S. aureus ATCC	-0.047	0.041	0.161	0.403	-0.457	-0.095				
MRSA	-0.338	0.424	0.121	-0.221	0.204	0.060	-0.504			
C. albicans	-0.248	0.327	0.312	0.402	0.062	0.096	0.311	0.296		
LAB	0.820**	-0.881**	-0.248	0.103	-0.112	-0.133	-0.085	-0.393	-0.352	
YM	0.889**	-0.878**	-0.018	0.160	0.129	-0.065	-0.168	-0.325	-0.423	0.931**

*Significant at 0.05; **Significant at 0.01.

Table 5. Predicted and experimental values of the response variables at optimum processing parameters by desirability function of RSM.

Variable	Optimum product				
	Limit	Desirability	Predicted values	Experimental values	PE(%)
pH	3.90-4.50	0.587	4.26	4.11±0.21	3.65
Water activity	0.4-0.5	0.999	0.45	0.49±0.01	8.16
Titratable acidity (%)	2.0-3.50	0.747	2.56	2.63±0.08	2.66
LAB (Log cfu/g)	7.0-8.0	0.649	7.33	7.63±0.27	3.93
Antimicrobial activity **					
K. pneumoniae	11.95-13.73	0.827	12.41	12.67±0.63	2.05
S. typhi	11.95-13.73	0.827	12.41	12.63±0.53	1.74
C. albicans	11.95-13.73	0.827	12.41	13.81±0.44	10.13

**Inhibition diameter (mm) of antimicrobial ingredient against some indicator pathogens.

O157:H7 ATCC 700728) were rather weak. There were no significant effects of the drying time and temperature on the antimicrobial activities of the ingredient vis-à-vis of these pathogens.

Relationship between some measured parameters

The Pearson's correlation coefficients between some measured parameters are presented in Table 4. High negative correlation exists between the a_w and the pH ($r = -0.916$; $p<0.01$). Except the correlation between microbial inhibition on *K. pneumoniae* and *S. typhi* ($r = 0.649$; $p<0.05$), no other significant correlation could be observed between microbial inhibition on indicator pathogens. Lactic acid bacteria, yeasts and moulds were positively correlated with a_w ($r = 0.820$; $r = 0.889$; $p<0.01$ respectively) and negatively with pH ($r = -0.881$; $r = -0.878$; $p<0.01$ respectively). It was clear that the lower the a_w the lower the viable cells count during the drying process. Interestingly, a low a_w is an indicator of a good viability of such microorganisms during storage (Vesterlund et al., 2012).

Optimization of process parameters and verification of models

To achieve optimum drying conditions for the bioactive ingredient in terms of optimal functionality, the desirability function was used to optimize the drying time and temperature. The desired goals for each response are summarized in Table 5. The limits of all responses at operating conditions were converted to a desirability function. The optimal conditions were found to be: temperature = 41.893°C and drying duration = 24.0 h. The composite desirability value of the optimum solution was 0.749 for the optimized temperature and time. Once the optimum conditions have been determined, we conducted additional independent experiments at these values. The experimental values were compared with the predicted from the optimized model and the PE was

calculated to determine the adequacy of the drying and response surface models. After verification, the experimental values were very close to the predicted responses (Table 5) and the percentage error indicated that the observed values were the same as the predicted values. Thus, all optimized models gave good fits to experimental data over the range of limit employed (PE<10%).

Conclusion

In the present research, we successfully used response surface methodology with CCD to optimize the drying process of bioactive ingredient containing viable thermo sensitive microorganisms. Statistical models show that the independent variables mostly marked the responses. Dry matter, water activity and viable microorganisms showed pronounced dependence on drying durations and temperatures. The optimal conditions for drying ensuring the best functionality of bioactive ingredient were found to be 42°C and 24 h. Under these conditions, experimental and predicted responses were not significantly different. At these water activity and water content values, the bioactive ingredient can be preserved and stored at ambient temperature with good shelf life.

Conflict of Interests

The authors have not declared any conflict of interests.

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