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3 **Optimization of Processing of Fermented**

4 **Cassava Semolina (*attiéké*) Fortified with**

5 **Soybean Proteins**

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12 **ABSTRACT**

Aims: This study was carried out to optimize the processing of soybean proteins-fortified *attiéké*.

Methodology: Response surface methodology was used to describe the effects of ferment and soy contents, and fermentation time on the protein content, pH, and acceptability of the *attiéké* product. A central composite design consisted of twenty-three experiments was conceived using the Galiachi design.

Results: Results showed that the experimental data were adequately adjusted in the second-order polynomial model. Protein content and overall acceptability were significantly influenced by soy content. pH was affected by the three studied factors. The optimum conditions were 11.41% of ferment, 6.35% of soybean content and 18h 7min 48s of fermentation time. Under these conditions, the protein content (6.62%), the pH (4.57) and the overall acceptability (3.41) were within defined target range.

Conclusion: The obtained results could be used in the artisanal and modern industries for the processing of *attiéké* with high nutritional value.

13 *Keywords: attiéké, soybean, optimization, response surface methodology*

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15 **1. INTRODUCTION**

16

17 Cassava is the staple food of about 800 million people living in the Third World [1]. The use

18 of cassava as a food is limited because it is nutritionally deficient in protein, vitamins and

19 minerals [2]. In Côte d'Ivoire, it occupies with a yield of 6.7 tons / ha, the second rank in

20 terms of production volume (4.5 million tons in 2016) [3]. It is transformed into a dozen

21 dishes, which the best known are: *attiéké*, *placali*, *gari*, *attoukpou* and *tapioca* [4].

22 *Attiéké* is the main by-product of cassava in Côte d'Ivoire. The processing of cassava into

23 *attiéké* is as follow: crushed cassava fermentation, mash dewatering followed by sieving,

24 granulating, sun drying and steaming of granular product [5]. However, the problem with the

25 consumption of this dish is its low nutritional value. *Attiéké* is known for its high caloric value

26 and low protein content [6]. A local protein-rich product, such as soybeans, should be

27 introduced to improve its nutritional balance. Protein fortification is the incorporation of

28 protein-rich food resources into staple food which is widely used and consumed [7].

29 Soybean is an excellent source of protein (40%) and lipids (20%) [8]. The protein

30 composition of these seeds largely covers the essential amino acid requirements of the

31 organisms. The fat is low in saturated fatty acids that have a high atherogenic effect. It is one

32 of the oilseeds richest in polyunsaturated fatty acids, accounting for 54 to 72% of total lipids

33 [9]. Among them, linoleic acids (omega 6) and alpha-linolenic (omega 3) are the main fatty

34 acids essential to the organism because they cannot be synthesized [10]. Due to its  
 35 nutritional composition, it could validly replace animal proteins [11].

36 Moreover, fermentation plays an important role in food technology in developing countries,  
 37 particularly in the processing of *attiéké* [12]. In the traditional fermentation processing,  
 38 natural microorganisms are used in the production and preservation of foods. These  
 39 processing improve the nutritional value, flavour and other qualities of foods [13].

40 The ferment content commonly known as "mangnan", fermentation time and incorporation of  
 41 soy flour play important roles during the processing of soybean proteins-fortified *attiéké*.  
 42 Indeed, the soy incorporation to the cassava dough in order to increase the protein content  
 43 causes problems of acceptability, because it changes the organoleptic and functional  
 44 properties of produced semolina [14]. To date, no research has been reported on improving  
 45 the acceptability of soybean proteins-fortified *attiéké*. In this work, response surface  
 46 methodology was applied to study the effects of ferment content, soy content and  
 47 fermentation time on the protein content, pH and acceptability of fortified *attiéké* product.  
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## 49 2. MATERIAL AND METHODS

### 50 2.1 Raw Materials

52 The main raw materials were cassava roots and soybeans. Fresh cassava roots (*Manihot*  
 53 *esculenta* Crantz) and yellow soybeans (*Glycine max* L. Meril) were purchased from the  
 54 market of Bonoua (Côte d'Ivoire) and the National Centre of Agricultural Research (Côte  
 55 d'Ivoire), respectively.  
 56

### 57 2.2 Experimental Design

58 The central composite design [15], with three factors and five levels was used in this study.  
 59 Factors (independent variables) were ferment content, soy content and fermentation time.

60 The coded levels were: -1, - $\alpha$ , 0, + $\alpha$ , +1. The value of  $\alpha$  was given by:  $\alpha = \frac{\sqrt{k}}{k}$ , where  $k$  is the  
 61 number of factors.  $\alpha$  was equal to 0.577. The coded levels and their corresponding actual  
 62 values are listed in **Table 1**.  
 63  
 64

**Table 1. Factors and levels for the central composite design**

Factors	Symbol	Levels				
<b>Coded values</b>	$X_i$	-1	-0,577	0	+0,577	+1
<b>Ferment content (%)</b>	M	7	8,269	10	11,731	13
<b>Soy content (%)</b>	S	3	5,538	9	12,462	15
<b>Fermentation time (h)</b>	F	12	14,115	17	19,885	22

65  
 66 The number of necessary experiments (N) was determined by the following relation:  $N = 2^k +$   
 67  $2k + n_0$ , where  $k$  is the number of factors and  $n_0$  is the number of experimental points in the  
 68 central domain. For three factors and nine central points, twenty-three experiments were  
 69 needed. **Table 2** presents the experimental design.  
 70

**Table 2. Experimental design and observed value of response variables**

Samples	Coded values			Non-coded values			Observed values		
	$X_1$	$X_2$	$X_3$	M (%)	S (%)	F (h)	PC (%)	pH	OA

1	-0,577	-0,577	-0,577	8,269	5,538	14,115	3,56	4,93	3,97
2	0,577	-0,577	-0,577	11,731	5,538	14,115	6,13	4,74	3,45
3	-0,577	0,577	-0,577	8,269	12,462	14,115	8,03	5,61	2,72
4	0,577	0,577	-0,577	11,731	12,462	14,115	8,49	5,47	2,89
5	-0,577	-0,577	0,577	8,269	5,538	19,885	6,13	4,80	3,74
6	0,577	-0,577	0,577	11,731	5,538	19,885	6,57	4,61	3,33
7	-0,577	0,577	0,577	8,269	12,462	19,885	9,24	4,80	2,78
8	0,577	0,577	0,577	11,731	12,462	19,885	10,69	4,70	3,06
9	-1	0	0	7	9	17	7,83	5,07	2,81
10	1	0	0	13	9	17	8,04	4,62	3,00
11	0	-1	0	10	3	17	3,14	4,58	4,45
12	0	1	0	10	15	17	16,68	5,25	2,39
13	0	0	-1	10	9	12	7,86	4,83	3,19
14	0	0	1	10	9	22	9,10	4,64	3,30
15	0	0	0	10	9	17	8,32	4,71	3,23
16	0	0	0	10	9	17	7,86	4,64	3,11
17	0	0	0	10	9	17	7,42	4,69	3,05
18	0	0	0	10	9	17	8,69	4,80	3,11
19	0	0	0	10	9	17	8,96	4,58	3,11
20	0	0	0	10	9	17	7,29	4,75	3,08
21	0	0	0	10	9	17	8,97	4,72	3,28
22	0	0	0	10	9	17	8,33	4,75	3,28
23	0	0	0	10	9	17	7,69	4,77	3,33

71 Note. Xi: coded values; M: ferment content; S: soy content; F: fermentation time; PC: protein content; OA: overall  
72 acceptability; Hedonic scale for the determination of acceptability: 1=very bad, 2=bad, 3=acceptable, 4=good and 5=very good.  
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### 2.3 Preparation of soybean proteins-fortified attiéké

75 Soybean proteins-fortified *attiéké* was prepared according to method described by [14]. The  
76 tuberos roots of cassava were peeled, defibrated, cut, crushed and mixed with the ferment  
77 (7 to 13% of cassava pulp; cooked cassava roots for 10 min and fermented for 48 hours).  
78 The pre-fermented dough for 2 h was packed in the synthetic fibre bags and then wringed  
79 with a screw press until a mass dough was obtained. To this dough was added soy flour (3  
80 to 15% of cassava pulp). The mixture cassava-soy was fermented for 10 to 20 hours at room  
81 temperature to allow the development of aroma and taste as well as texture of soybean  
82 proteins-fortified *attiéké*. After the fermentation period, the dough was granulated, manually  
83 sieved and then partially dried. Two other sieves were carried out. The semolina was  
84 winnowed and steamed for 15 min in a couscous pot. The soybean proteins-fortified *attiéké*  
85 samples were dehydrated at 45°C for 48h.

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### 2.4 Determination of experimental responses

92 Experimental responses were protein content, pH, and general acceptability. Their desired  
93 values were 6 to 12% for protein content, pH 4 to 4.80 and a score of 3 to 5 for the

94 acceptability of formulations. Protein content and pH were determined according to methods  
95 [16]. Proteins were assayed according to Kjeldhal's method. The factor 6.25 was used to  
96 calculate the protein content of samples. The pH was determined with a pH-meter. A  
97 hedonic test was also made to evaluate the overall acceptability of samples. The panel  
98 consisted of 20 people recruited based on their availability. The coded samples were  
99 presented in random order to panellists. Each panellist, isolated from others, received  
100 samples of about 50 g of samples. The test consisted of recording each formulation on a  
101 hedonic five (5) point scale ranging from very bad (1) to very good (5). The studied  
102 parameters were colour, aroma, taste and overall acceptability.

## 103 2.5 Statistical analyzes of the data

105 The values of the experimental responses were reported in Table 2. A second-order  
106 polynomial regression model was used to express Y as a function of the independent  
107 variables as follows:

$$108 \quad Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{11}x_1^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 \quad (1)$$
$$109 \quad \quad \quad + \beta_{23}x_2x_3 + \beta_{22}x_2^2 + \beta_{33}x_3^2$$

112  $x_1$ ,  $x_2$  et  $x_3$  : Independent variables, respectively for the ferment content, protein content  
113 and fermentation time

114  $\beta_0$  : coefficients de régression pour le polynôme (terme constant)

115  $\beta_1$ ,  $\beta_2$  et  $\beta_3$  : linear coefficients

116  $\beta_{11}$ ,  $\beta_{22}$  et  $\beta_{33}$  : quadratic coefficients

117  $\beta_{12}$ ,  $\beta_{13}$  et  $\beta_{23}$ : interactive coefficients

118 Y: response variables coefficients, respectively.  $X_i$  and  $X_j$  are the levels of independent  
119 variables in coded value.

120 The STATISTICA software version 7.1 (Statsoft, 2005) was used for multiple regression  
121 analysis, analysis of variance (ANOVA), and canonical analysis in the response surface  
122 regression procedure. The analysis includes Fisher's test (overall significance of model), its  
123 associated probability  $P(F)$ , and determination coefficient of  $R^2$  which measure the fit  
124 goodness of regression model. It also includes the  $t$ -value  $t$  for the estimated coefficients and  
125 the associated probabilities. The statistical significance test was based on the total error  
126 criteria with a confidence level of 95.0%. The surface diagrams of the quadratic models were  
127 obtained by maintaining a constant variable at the central level and by varying the two others  
128 in their experimental limits.

## 129 3. RESULTS AND DISCUSSION

### 130 3.1 Statistical modelling of responses

133 For the three response variables, the second order polynomial model was highly significant  
134 ( $p < 0.01$ ) and gave a good fit of experimental results with  $R^2 > 0.75$  (Table 3).

#### 135 3.1.1 Effect of variables on the protein content of formulations

137 Protein contents ranged from 3.14 to 16.68% for the various combinations of soybean  
138 proteins-fortified *attiéké* with a mean of 8.04%. The maximum protein content was observed  
139 with 10% ferment, 15% soy and 17 h fermentation. Cassava is low in protein compared to  
140 soy that is a good source of protein [2,17]. During the processing, the incorporation of soy  
141 flour to the cassava dough resulted in an increase in protein content. The results are in

142 accordance those of [18]. [19] have also shown that the substitution of wheat flour for peanut  
 143 seed, which is a legume, improves the protein content of pasta.

144 The analysis of variance for the protein content response surface design shows that the  
 145 linear effect of soy content is significant ( $p\beta_2 < 0.001$ ). The other design terms are not  
 146 significant ( $p > 0.05$ ); which shows the absence of interaction effects and significant quadratic  
 147 effects (Table 3). The equation of the second order polynomial representing the response of  
 148 protein content is written in the following empirical design with 10 coefficients:

149 
$$\text{TP} = 8,17 + 0,65 \times M + 4,64 \times S + 1,06 \times F - 0,41 \times M \times S - 0,43 \times M \times F + 0,15 \times S \times F$$
  
 150 
$$- 1,05 \times M^2 + 0,93 \times S^2 - 0,50 \times F^2$$

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 152  
 153 The evolution of semolina protein content is illustrated in Figure 1. When the fermentation  
 154 time is maintained in centre of experimental domain, the semolina response surface  
 155 indications show that the protein content varies between 4 and 12% (Figure 1a). The  
 156 presence of linear effect for the soy content was observed and the effect of the ferment  
 157 content was not very important. The response observed in Figure 1b illustrates that the  
 158 protein content evolves in a bell shape with contents varying from 4.5 to 8.5%, when soy  
 159 content is maintained at the centre of experimental domain. According to the indications of  
 160 the response surface of Figure 1c, the protein content varies between 4 and 14%, when the  
 161 ferment content is maintained in centre of experimental domain. The fermentation time linear  
 162 effect does not seem important. This result disagrees with those of [20], where the protein  
 163 content of gari increases with fermentation time.

164 The results of optimization show that the protein content of semolina depends of the  
 165 incorporated soy content. Indeed, the increase of soy content causes an improvement of  
 166 protein content, because soy is considered a rich protein source. These results are  
 167 concordance with those of [21] that showed that incorporation of 10% soy increased the  
 168 protein content of sorghum-maize couscous from 10.47 to 15.66%. In addition, the variation  
 169 of ferment content and fermentation time did not have significant impacts on protein content.  
 170 The fermentation does not seem to influence of the protein content. The presence of soy in  
 171 the formulations would be at the origin of obtained results. This result disagrees with those of  
 172 [22], who in their studies claimed that fermentation causes a significant increase of the  
 173 protein content of gari and composite flour in proportionally to incorporation of the cashew  
 174 nut content. [23] stated that the fermentation time does not influence the protein content of  
 175 "Doklu" (food based on fermented maize). Contrariwise, the study results of [24] and [25]  
 176 indicate that the fermentation time has a significant positive effect on the protein content of  
 177 gari.

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**Table 3:** Analysis of variance for response surface design

Variables	df	Protein content				pH				Overall acceptability			
		SC	MC	F value	P value	SC	MC	F value	P value	SC	MC	F value	P value
<b>Terms</b>	9	112,50	12,50	5,61	0,003	1,43	0,16	10,03	<b>&lt;0,001</b>	3,69	0,41	17,42	<b>&lt;0,001</b>
<b>M (<math>\beta_1</math>)</b>	1	1,99	1,99	0,89	0,361	0,14	0,14	8,80	<b>0,011</b>	0,002	0,002	0,07	0,797
<b>S (<math>\beta_2</math>)</b>	1	100,53	100,53	45,14	<b>&lt;0,001</b>	0,50	0,50	31,81	<b>&lt;0,001</b>	3,12	3,12	132,44	<b>&lt;0,001</b>
<b>F (<math>\beta_3</math>)</b>	1	5,24	5,24	2,35	0,149	0,34	0,34	21,13	<b>&lt;0,001</b>	$3,6 \cdot 10^{-4}$	$3,6 \cdot 10^{-4}$	0,01	0,904
<b>M*S (<math>\beta_{12}</math>)</b>	1	0,15	0,15	0,07	0,798	0,002	0,002	0,15	0,701	0,24	0,24	10,11	<b>0,007</b>

<b>M*F (<math>\beta_{13}</math>)</b>	1	0,16	0,16	0,07	0,791	$2,10^{-4}$	$2,10^{-4}$	0,01	0,912	0,006	0,006	0,26	0,621
<b>S*F (<math>\beta_{23}</math>)</b>	1	0,02	0,02	0,009	0,926	0,22	0,22	13,70	<b>0,003</b>	0,04	0,04	1,78	0,204
<b>M<sup>2</sup> (<math>\beta_{11}</math>)</b>	1	2,12	2,12	0,95	0,347	0,08	0,08	5,11	<b>0,042</b>	0,11	0,11	4,76	<b>0,048</b>
<b>S<sup>2</sup> (<math>\beta_{22}</math>)</b>	1	1,67	1,67	0,75	0,402	0,15	0,15	9,20	<b>0,009</b>	0,14	0,14	6,19	<b>0,027</b>
<b>F<sup>2</sup> (<math>\beta_{33}</math>)</b>	1	0,48	0,48	0,22	0,648	0,02	0,02	1,09	0,315	0,02	0,02	0,81	0,383
<b>Residues</b>	13	28,954	2,227	-	-	0,207	0,016	-	-	0,306	0,023	-	-

184 M : ferment content (%) ; S : soy content (%) ; F : fermentation time (h) ; SC : sum of squares ; MC : mean of squares ;  
185 Fisher's F test ( $p \leq 0,05$ ).

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### 3.1.2 Effect of variables on the pH of formulations

188 The pH of formulations ranged from 4.58 to 5.61 with a mean of 4.83. The lowest pH was  
189 observed with 10% ferment, 3% soy and 17 h fermentation. pH is an acidity indicator of a  
190 food. The incorporation of the soy flour resulted an increase of pH value. Adding soy would  
191 tend to make *attiéké* less acidic. This result is in accordance with those of [17] who reported  
192 in their study on the effect of soybean treatments on gari-soy quality parameters that soy  
193 fortification tends to make gari less acidic. In addition, pH is a determining parameter in the  
194 development of characteristic food flavours [26]. The results of [12] highlight the decrease in  
195 the pH values of *attiéké* with ferment content and fermentation time while those of [27] and  
196 [25], the decrease of pH with the fermentation time.

197 The analysis of variance of pH response surfaces showed that the linear effects of ferment  
198 and soy contents and fermentation time are significant. Indeed, the regression coefficients  
199  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  have  $p$ -values inferior to 0.05 (Table 3). This result shows that each of three  
200 parameters has a specific effect on the pH of food. However, there is firstly the absence of  
201 interaction effects of the ferment content and soy content, secondly of the ferment content  
202 and the fermentation time, because the coefficients each of these effects has  $p$ -values that  
203 are superior to 0.05. Contrariwise, there are synergistic effects between the variables "soy  
204 content and fermentation time" ( $p < 0.05$ ). The equation of second order polynomial  
205 representing the pH response is written in the following empirical design with 10 coefficients:

206

$$207 \text{ pH} = 4,71 - 0,17 \times M + 0,33 \times S - 0,27 \times F + 0,05 \times M \times S + 0,02 \times M \times F - 0,50 \times S \times F \\ 208 \quad + 0,20 \times M^2 + 0,27 \times S^2 + 0,09 \times F^2$$

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210 pH response surfaces showed three important linear effects of ferment content, soy content  
211 and fermentation time. In addition, there is an interaction effect with the combination of soy  
212 content and fermentation time. In Figure 1d, the fermentation time is maintained in centre of  
213 experimental domain and causes a pH variation of 4.6 to 5.6. As for Figure 1e where the  
214 variable "soy content" has been maintained, the pH varies between 4.6 and 5.4. pH values  
215 ranged from 4.6 to 6, when the ferment content is maintained at centre of the experimental  
216 domain (Figure 1f). The surface graphs in Figure 1 show that the pH is influenced by the  
217 three variables and the combination of soy content and fermentation time affects this  
218 parameter. [28] showed that co-fermentation of cassava dough and soy caused significant  
219 changes in pH, while fortification with soy caused minimal pH effects.

220 The surface curves show the presence of linear and synergistic effects of three variables on  
221 the pH. In addition, they show that increasing the protein content increase the pH value.  
222 Conversely, increasing the ferment content and fermentation time decreases the pH value.  
223 The activity of microorganisms, particularly lactic acid bacteria, would be responsible for this  
224 pH drop. They produce organic acids (lactic acid, acetic acid, propionic acid and butyric acid)  
225 during their growth [23]. The areas with the most interesting responses, characterized by pH  
226 values are close to control value (classic *attiéké*) of 4.72, areas are between 4.60 and 4.80.

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### 3.1.3 Effect of variables on the overall acceptability of formulations

229 Acceptability ranged from 2.39 to 4.45 with a mean of 3.20. Maximum acceptability (the most  
230 accepted formulation) was noted with 10% ferment, 3% soy and 17 h fermentation time. The  
231 addition of soy flour has reduced the acceptability of *attiéké*. The main factors affecting the  
232 acceptability of a food are colour, aroma and flavour [29]. They determine a person's energy  
233 and nutrient intake. A person consumes less food if the food has characteristics lower than  
234 desired. Desirable sensory characteristics of foods determine acceptability and consumption  
235 [30]. The obtained results showed that the appreciation of soybean proteins-fortified *attiéké*  
236 varied with the formulation. The substitution of cassava with soy flour would have  
237 deteriorated the colour and taste of formulations compared to classic *attiéké*. According to  
238 [31], the fat content of soy is responsible for the flavor of a food. Colour is a determinant of  
239 the quality of any food and is a characteristic that the consumer immediately notices as it  
240 influences subjective sensory impression [29].

241 The study of [32] on the ability to process gluten-free couscous showed that the colour of  
242 couscous is an important criterion for consumers. The observed colours of the formulations  
243 would be related to the carotenoid content of semolina, which varies according to the soy  
244 content used as well as enzymatic and non-enzymatic browning reactions. In addition, the  
245 yellow index is correlated with protein contents. Aroma modifications of soybean proteins-  
246 fortified *attiéké* formulations have been observed compared to classic *attiéké*. Volatile flavour  
247 components are produced by heat, oxidation, and non-enzymatic activity on proteins, fat and  
248 carbohydrates.

249 The analysis of variance of the "general acceptability" response summarized in Table 3  
250 shows that the linear effect of the soy content is significant ( $p < 0.001$ ). In addition, the  
251 regression coefficient corresponding to the interaction effects of parameters "ferment and  
252 soy contents" is inferior to 0.05. These results show the presence of synergistic effect  
253 between these two parameters. The equation of second order polynomial representing the  
254 response of general acceptability is written in the following empirical design with 10  
255 coefficients:

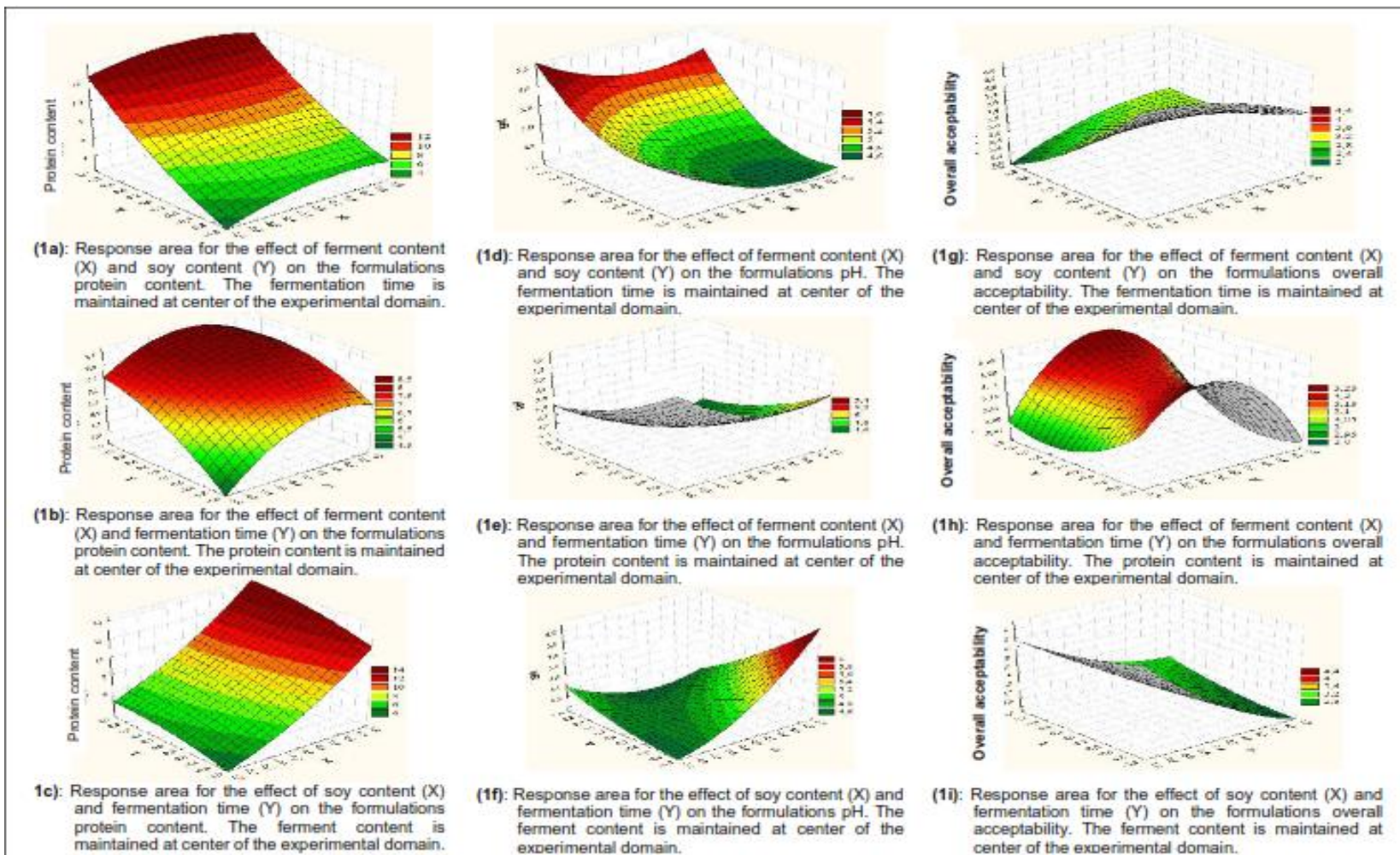
256  
257 
$$AG = 3,18 - 0,02 \times M - 0,82 \times S + 0,01 \times F + 0,52 \times M \times S + 0,08 \times M \times F + 0,22 \times S \times F$$
  
258 
$$- 0,24 \times M^2 + 0,27 \times S^2 + 0,10 \times F^2$$
  
259

260 The graphs confirm that there are two significant effects: a linear effect of soy content and an  
261 interaction effect between ferment content and soy content. The acceptability of formulated  
262 foods varies between 2 and 4.4 (Figure 1g); 2.9 to 3.25 (Figure 1h) and from 2.8 to 4.4  
263 (figure 1i) respectively, when the fermentation time, the soy content and the ferment content  
264 are maintained at centre of experimental domain. The results of [12] suggested that the  
265 fermentation time does not affect the general acceptability of classic *attiéké*, whereas the  
266 ferment content affects this acceptability. The sensory quality of formulated foods was  
267 significantly and mainly affected by the soy content. The addition of soy from 3 to 9%  
268 resulted in an acceptable food. Contrariwise, beyond 9% to 15%, acceptability is negatively  
269 affected. The ferment would also have an impact on the acceptability of formulations. The  
270 study of [14] corroborates this argument. In their study on improving the nutritional value of  
271 *attiéké*, the authors have shown that the addition of soy associated with the use of ferment  
272 and an adequate fermentation time improves not only the nutritional value, but also the  
273 sensory properties of soybean proteins-fortified *attiéké*.

274 The graph of main effects of acceptability confirms the results observed on the surface  
275 diagram of responses. The couple "ferment / fermentation time" has no influence on the

276 acceptability of formulations. We are interested in maintaining the acceptability of  
277 formulations so that it approaches of control who is classic *attiéké*. Indeed, more the higher  
278 acceptability values, more the formulation will have the characteristics of control. The  
279 acceptability responses we are interested in are the biggest answers. According to Figure 1,  
280 these responses are located in the brown colored zones. We note that these areas are at the  
281 upper limit of design. That is, to increase the acceptability values, the soy content must be  
282 decreased.





285 **3.2 Optimal parameters**

286 For response "protein content and overall acceptability", the stationary distance is greater  
 287 than 1, which means that their stationary points are outside of experimental area. They  
 288 cannot therefore be used to determine optimal parameters. Contrariwise, for the response  
 289 "pH", the stationary distance is less than 1; its stationary point is inside the experimental  
 290 domain. It can therefore be used to determine optimal conditions (Table 4).

291 The coded coordinates of the stationary point are (0.47, -0.44 and 0.23) for the pH response.  
 292 They were converted to non-coded values (11.41%, 6.35%, 18.13 hours). At this stationary  
 293 point, the predicted values of protein content, pH and overall acceptability are 6.62%; 4.57  
 294 and 3.41. These values are in the interval of previously defined objectives, which are 6 to  
 295 12% for the protein content, 4 to 4.80 for the pH and 3 to 5 for the overall acceptability. We  
 296 could say that this stationary point corresponds to optimal point. The optimal parameters are  
 297 therefore: 11.41% ferment, 6.35% soy and 18.13 hours fermentation time. They allowed the  
 298 following optimal responses: 6.62% protein, pH 4.57 and 3.41 for acceptability. The  
 299 formulations respecting the desired responses are reported in Table 4.

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**Table 4:** Optimal parameters

Stationary point Coordinates s	Correspondent values			Variables			
	PC	pH	OA	PC	pH	AG	
Xs <sub>1</sub>	0,70	0,47	1,01	M (%)	12,10	11,41	13,04
Xs <sub>2</sub>	-2,37	-0,44	1,27	S (%)	-5,25	6,35	16,63
Xs <sub>3</sub>	0,40	0,23	-1,86	F (h)	19,02	18,13	7,72
Ds	2,51	0,68	2,47	Responses	3,10	4,57	2,64

302 Ds : distance from stationary point to centre of area; Xs<sub>1</sub>, Xs<sub>2</sub> et Xs<sub>3</sub> are the coordinates of stationary point ; M :  
 303 ferment content ; S : soy content ; F : fermentation time ; PC : protein content (%) ; OA : overall acceptability.

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**Table 5:** Optimal formulations after optimization by the response surface methodology

Formulations	Coded variables			Non-coded variables			Responses		
	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>
FO <sub>1</sub>	0,577	-0,577	-0,577	11,731	5,538	14,115	6,13	4,74	3,45
FO <sub>2</sub>	-0,577	-0,577	0,577	8,269	5,538	19,885	6,13	4,80	3,74
FO <sub>3</sub>	0,577	-0,577	0,577	11,731	5,538	19,885	6,57	4,61	3,33
FO <sub>4</sub>	0,577	0,577	0,577	11,731	12,462	19,885	10,69	4,70	3,06
FO <sub>5</sub>	1	0	0	13	9	17	8,04	4,62	3,00
FO <sub>6</sub>	0	0	1	10	9	22	9,10	4,64	3,30
FO <sub>7</sub>	0	0	0	10	9	17	8,32	4,71	3,23

306 FO: Optimal formulation, Ci: coded values, X<sub>1</sub> (ferment content), X<sub>2</sub> (soy content), X<sub>3</sub> (fermentation time), Y<sub>1</sub>  
 307 (protein content), Y<sub>2</sub> (pH), Y<sub>3</sub> (overall acceptability).

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**4. CONCLUSION**

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311 Results showed that the second-order polynomial model is sufficient to describe and predict  
 312 the response variables of the protein content, pH, and acceptability of soybean proteins-  
 313 fortified *attiéké*, using the ferment, soy content and fermentation time as independent  
 314 variables. Protein content and overall acceptability are affected only by the linear term of the  
 315 soy content. However, the pH is affected by the linear terms of three studied independent  
 316 factors. The adopted optimization method to find the best condition for the production of  
 317 soybean proteins-fortified *attiéké*, predicts that the optimum parameters in the experimental

318 domain are: 11.41% for the ferment content; 6.35% for the soy content and 18h7min48s for  
319 the fermentation time. Under such conditions, the formulated fortified *attiéké* has good  
320 acceptability with a protein content of 6.62% and a pH of 4.57. These results open the  
321 prospect of using soybean proteins-fortified *attiéké* to combat protein-energy malnutrition.  
322 However, further studies are needed to evaluate the impact of its consumption on human  
323 health.

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## COMPETING INTERESTS

327 Authors have declared that no competing interests exist.

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