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# **Optimization of Processing of Fermented Cassava Semolina (***attiéké***) Fortified with Soybean Proteins**

**K. C. M. Kouakou** $^{1,2^*}$ **, D. B. C. Ebah<sup>2</sup>, S. S. Guédé** $^3$  **and G. A. Gbogouri** $^1$ 

*1 Department of Food Science and Technology, Nangui Abrogoua University, Abidjan, Côte d'Ivoire. <sup>2</sup> National Centre of Agricultural Research, Bingerville, Côte d'Ivoire. <sup>3</sup> Agropastoral Management Institute, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire.*

## *Authors' contributions*

*This work was carried out in collaboration among all authors. Author GAG designed the study. Author KCMK wrote the protocol and wrote the first draft of the manuscript. Author SSG performed the statistical analysis. Author DBCE facilitated the preparation of soybean proteins-fortified attiéké. All authors read and approved the final manuscript.*

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# **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 18 h 7 min 48 s 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.

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*Keywords: Attiéké; soybean; optimization; response surface methodology.*

## **1. INTRODUCTION**

Cassava is the staple food of about 800 million people living in the Third World [1]. The use of cassava as a food is limited because it is nutritionally deficient in protein, vitamins and minerals [2]. In Côte d'Ivoire, it occupies with a yield of 6.7 tons / ha, the second rank in terms of production volume (4.5 million tons in 2016) [3]. It is transformed into a dozen dishes, which the best known are: *attiéké, placali, gari, attoukpou* and *tapioca* [4].

*Attiéké* is the main by-product of cassava in Côte d'Ivoire. The processing of cassava into *attiéké* is as follow: crushed cassava fermentation, mash dewatering followed by sieving, granulating, sun drying and steaming of granular product [5]. However, the problem with the consumption of this dish is its low nutritional value. *Attiéké* is known for its high caloric value and low protein content [6]. A local protein-rich product, such as soybeans, should be introduced to improve its nutritional balance. Fortification is the incorporation of nutrient essential-rich food resources into staple food which is widely used and consumed [7].

Soybean is an excellent source of protein (40%) and lipids (20%) [8]. The protein composition of these seeds largely covers the essential amino acid requirements of the organisms. The fat is low in saturated fatty acids that have a high atherogenic effect. It is one of the oilseeds richest in polyunsaturated fatty acids, accounting for 54 to 72% of total lipids [9]. Among them, linoleic acids (omega 6) and alpha-linolenic (omega 3) are the main fatty acids essential to the organism because they cannot be synthesized [10]. Due to its nutritional composition, soybean could validly replace animal proteins [11].

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

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

## **2. MATERIALS AND METHODS**

## **2.1 Raw Materials**

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

## **2.2 Experimental Design**

The central composite design [15], with three factors and five levels was used in this study. Factors (independent variables) were ferment content, soy content and fermentation time. The coded levels were: -1, - α, 0, + α, +1. The value of α was given by:  $\alpha = \frac{\sqrt{k}}{k}$ , where is the number of factors. α was equal to 0.577. The coded levels and their corresponding actual values are listed in Table 1.

The number of necessary experiments (N) was determined by the following relation:  $N = 2<sup>k</sup>$ + 2k +  $n_0$ , where k is the number of factors and  $n_0$  is the number of experimental points in the central domain. For three factors and nine central points, twenty-three experiments were needed. Table 2 presents the experimental design.

## **2.3 Preparation of Soybean Proteinsfortified** *attiéké*

Soybean proteins-fortified a*ttiéké* was prepared according to method described by Kouakou et al. [14]. The tuberous roots of cassava were peeled, defibrated, cut, crushed and mixed with the ferment (7 to 13% of cassava pulp; cooked





<b>Samples</b>	<b>Coded values</b>			<b>Non-coded values</b>			<b>Observed values</b>		
	$\mathsf{X}_1$	$X_{2}$	$X_3$	M(%)	S(%)	F(h)	PC (%)	pH	ОA
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		$\mathbf{0}$	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		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

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

*Note. Xi: coded values; M: ferment content; S: soy content; F: fermentation time; PC: protein content; OA: overall acceptability; Hedonic scale for the determination of acceptability: 1 = very bad, 2 = bad, 3 = acceptable, 4 = good and 5 = very good*

cassava roots for 10 min and fermented for 48 hours). The pre-fermented dough for 2 h was packed in the synthetic fibre bags and then wringed with a screw press until a mass dough was obtained. To this dough was added soy flour (3 to 15% of cassava pulp). The mixture cassava-soy was fermented for 10 to 20 hours at room temperature to allow the development of aroma and taste as well as texture of soybean proteins-fortified *attiéké*. After the fermentation period, the dough was granulated, manually sieved and then partially dried. Two other sieves were carried out. The semolina was winnowed and steamed for 15 min in a couscous pot. The soybean proteins-fortified *attiéké* samples were dehydrated at 45°C for 48 h.

## **2.4 Determination of Experimental Responses**

Experimental responses were protein content, pH, and general acceptability. Their desired values were 6 to 12% for protein content, pH 4 to 4.80 and a score of 3 to 5 for the acceptability of formulations. Protein content and pH were determined according to methods [16]. Proteins were assayed according to Kjeldhal's method. The factor 6.25 was used to calculate the protein content of samples. The pH was determined with a pH-meter. A hedonic test was also made to evaluate the overall acceptability of samples. The panel consisted of 20 people recruited based on their availability. The coded samples were

presented in random order to panellists. Each panellist, isolated from others, received samples of about 50 g of samples. The test consisted of recording each formulation on a hedonic five (5) point scale ranging from very bad (1) to very good (5). The studied parameters were colour, aroma, taste and overall acceptability.

## **2.5 Statistical Analyzes of the Data**

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

$$
Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_1 x_1 + \beta_1 z_1 x_2 + \n\beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_3 x_3^2
$$
\n(1)

x1, x2 et x3 : Independent variables, respectively for the ferment content, protein content and fermentation time

β0: coefficients de régression pour le polynôme (terme constant)

 $β_1$ ,  $β_2$  et  $β_3$ : linear coefficients

 $β<sub>11</sub>, β<sub>22</sub>$  et  $β<sub>33</sub>$ : quadratic coefficients

 $β<sub>12</sub>, β<sub>13</sub>$  et  $β<sub>23</sub>$ : interactive coefficients

Y: response variables coefficients, respectively. Xi and Xj are the levels of independent variables in coded value.

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

## **3. RESULTS AND DISCUSSION**

## **3.1 Statistical Modelling of Responses**

For the three response variables, the second order polynomial model was highly significant (*p*<0.01) and gave a good fit of experimental results with  $R^2$ > 0.75 (Table 4).

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

Protein contents ranged from 3.14 to 16.68% for the various combinations of soybean proteinsfortified *attiéké* with a mean of 8.04%. The maximum protein content was observed with 10% ferment, 15% soy and 17 h fermentation. Cassava is low in protein compared to soy that is a good source of protein [2,17]. During the processing, the incorporation of soy flour to the cassava dough resulted in an increase in protein content. The results are in accordance those of [18]. Mridula et al. [19] have also shown that the substitution of wheat flour for peanut seed, which is a legume, improves the protein content of pasta.

The analysis of variance for the protein content response surface design shows that the linear effect of soy content is significant (*p*β2 <0.001). The other design terms are not significant (*p*> 0.05); which shows the absence of interaction effects and significant quadratic effects (Table 3). The equation of the second order polynomial representing the response of protein content is written in the following empirical design with 10 coefficients:

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

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

The results of optimization show that the protein content of semolina depends of the incorporated soy content. Indeed, the increase of soy content causes an improvement of protein content, because soy is considered a rich protein source. These results are concordance with those of [21] that showed that incorporation of 10% soy increased the protein content of sorghum-maize couscous from 10.47 to 15.66%. The variation of ferment content from 7 to 13% and fermentation time de 12 to 22 h did not have significant impact on protein content. The fermentation parameters does not seem to influence of the protein content. The presence of soy in the formulations would be at the origin of obtained results. In addition, the fermentation time may be insufficient to cause the modification of the protein content. Assohoun et al. [22] stated that the fermentation time from 24 to 72 h does not influence the protein content of "Doklu" (food based on fermented maize). This result disagrees with those of Fofana et al. [23], who in their studies claimed that fermentation causes a significant increase of the protein content of gari and composite flour in proportionally to incorporation of the cashew nut content. However, fermentation could increase protein digestibility by improving the uptake of essential amino acids or by eliminating undesirable antinutritional compounds such as phytates. Indeed, fermentation increased lysine, methionine and tryptophan contents of soybeans contained in the formulations. Contrariwise, the study results of Irtwange and Achimba [24] and Oduah et al. [25] indicate that the fermentation time has a significant positive effect on the protein content of gari.

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

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

The analysis of variance of pH response surfaces showed that the linear effects of ferment and soy contents and fermentation time are significant. Indeed, the regression coefficients β1, β2 and β3 have *p*-values inferior to 0.05 (Table 4). This result shows that each of three parameters has a specific effect on the pH of food. However, there is firstly the absence of interaction effects of the ferment content and soy content, secondly of the ferment content and the fermentation time, because the coefficients each of these effects has *p*-values that are superior to 0.05. Contrariwise, there are synergistic effects between the variables "soy content and fermentation time"  $(p < 0.05)$ . The equation of second order polynomial representing the pH response is written in the following empirical design with 10 coefficients:

$$
pH = 4,71 - 0,17 \times M + 0,33 \times S - 0,27 \times F
$$
  
+ 0,05 \times M \times S + 0,02 \times M \times F  
- 0,50 \times S \times F + 0,20 \times M<sup>2</sup>  
+ 0,27 \times S<sup>2</sup> + 0,09 \times F<sup>2</sup>

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

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

to control value (classic *attiéké*) of 4.72, areas are between 4.60 and 4.80.

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

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

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

The analysis of variance of the "general acceptability" response summarized in Table 4 shows that the linear effect of the soy content is significant (*p*<0.001). In addition, the regression coefficient corresponding to the interaction effects of parameters "ferment and soy contents" is inferior to 0.05. These results show the presence of synergistic effect between these two parameters. The equation of second order polynomial representing the response of general acceptability is written in the following empirical design with 10 coefficients:

 $AG = 3,18 - 0,02 \times M - 0,82 \times S + 0,01 \times F + 0,52 \times M$  $\times$  S + 0.08  $\times$  M  $\times$  F + 0.22  $\times$  S  $\times$  F  $-0.24 \times M^2 + 0.27 \times S^2 + 0.10 \times F^2$ 

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

The graph of main effects of acceptability confirms the results observed on the surface diagram of responses. The couple "ferment / fermentation time" has no influence on the acceptability of formulations. We are interested in maintaining the acceptability of formulations so that it approaches of control who is classic *attiéké*. Indeed, more the higher acceptability values, more the formulation will have the characteristics of control. The acceptability responses we are interested in are the biggest answers. According to Fig. 1, these responses are located in the brown colored zones. We note that these areas are at the upper limit of design. That is, to increase the acceptability values, the soy content must be decreased.

## **3.2 Optimal Parameters**

For response "protein content and overall acceptability", the stationary distance is greater than 1, which means that their

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## **Table 3. Analysis of variance for response surface design**

*M: Ferment content (%); S: Soy content (%); F: Fermentation time (h); SC: Sum of squares; MC: Mean of squares; Fisher's F test (p ≤ 0,05)*

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**Fig. 1. Effect of independent variables and response areas on the protein content, pH and overall acceptability of formulations**



#### **Table 4. Optimal parameters**

*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: ferment content; S: soy content; F: fermentation time; PC: protein content (%); OA: overall acceptability*





*FO: Optimal formulation, Ci: Coded values, X1 (ferment content), X2 (Soy content), X3 (fermentation time), Y1 (Protein content), Y2 (pH), Y3 (Overall acceptability)*

stationary points are outside of experimental area. They cannot therefore be used to determine optimal parameters. Contrariwise, for the response "pH", the stationary distance is less than 1; its stationary point is inside the experimental domain. It can therefore be used to determine optimal conditions (Table 4).

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

## **4. CONCLUSION**

Results showed that the second-order polynomial model is sufficient to describe and predict the response variables of the protein content, pH, and acceptability of soybean proteins-fortified *attiéké*, using the ferment, soy content and fermentation time as independent variables. Protein content and overall acceptability are affected only by the linear term of the soy content. However, the pH is affected by the linear terms of three studied independent factors. The adopted optimization method to find the best condition for the production of soybean proteins-fortified *attiéké*, predicts that the optimum parameters in the experimental domain are: 11.41% for the ferment content; 6.35% for the soy content and 18h7min48s for the fermentation time. Under such conditions, the formulated fortified *attiéké* has good acceptability with a protein content of 6.62% and a pH of 4.57. These results open the prospect of using soybean proteins-fortified *attiéké* to combat protein-energy malnutrition. However, further studies are needed to evaluate the impact of its consumption on human health.

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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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