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Study of the *hibiscus esculentus* mucilage coagulation–flocculation activity

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ABSTRACT. The flocculent activity of *Hibiscus esculentus* (gombo) mucilage traditionally used for a local beer (Tchapalo) clarification in Côte d'Ivoire was studied using the method of the experimental designs. Of the three factors selected that are the volume of mucilage (X_1), the temperature (X_2) and the pH (X_3), sole X_1 and X_3 appeared influential. The lowering of turbidity observed is 23 % approximately. The general approach suggested in this work makes it possible to approach the operating conditions. The mucilage of gombo presents a better activity in alkaline water. A relatively low volume of gombo gives better results. Moreover, the heat treatment seems to be less determining during the process of coagulation-flocculation. @JASEM

One of the significant industrial problems in the preparation of drinks is the presence of disorders due to the existence of fine particles. However the consumers generally consider and sometimes wrongly that everything that is not limpid is impure. This explains the detailed attention that the industrialists carry to the clarifying of drinks (drinking water, fruit juice, etc). The methods used to solve this problem are decantation, filtration, centrifugation, coagulation-flocculation (Francesci et al, 2002; Gregor et al, 1997; Gregory, 1977). This last is especially used when the suspended particles are of too small size to settle or to be eliminated by traditional filtration. It is a question of adding an inert substance and without taste called coagulant whose role is to provoke the coagulation and the flocculation of awkward colloids. Substances generally used for the clarification of water are the aluminium sulphates, iron sulphates, aluminium chloride etc, while wine industrialists use gelatine, albumin, etc (Jules, 1977). In recent works (Faby et al, 1993; Nacoulima et al, 2000) it was shown that plants such as *Moringa oleifera*, *canavalia ensiformis*, *bombax costatum* presented an interesting flocculating activity. The aim of this paper is to study the flocculating activity of the fresh stems mucilage of Gombo (*Hibiscus esculentus*).

MATERIALS AND METHODS

Materials

The work concerned water of 35 NTU (Nephelometric Turbidity Unit) initial turbidity. For each run, measurements of turbidity were made using a portable turbidimeter ORBECO-HELLIGE model 966 and viscosity was performed with a viscometer SBIF NDJ-7. The tests of clarification were carried out with a flocculator BIOBLOCK model 11196 according to the method of Jar-test

Methods: For mucilage obtaining, approximately 300 g of gombo fresh stems were mixed in 150 mL water. The sticky liquid (mucilage) obtained was centrifuged at 600 turns per minute during
2 hours in order to eliminate the suspended particles. The viscosity of the solution obtained were about 2 mPa.S.
The samples of turbid water were taken in a purifying station and let at rest during 24 hours in order to eliminate the large particles by decantation. The turbidity of water was set at 35 NTU before use. The tests of clarification were performed according to the Jar test method (Knight, 1950). The study was led according to a strategy based on the experimental designs which are statistical tools for analysis, making it possible to highlight the relations between the parameters and their effects on a phenomenon studied (a turbidity for instance). These methods allow minimizing the numbers of runs to be carried out to make the study (Goupy, 1988; Feinberg, 1996). Being given that we did not have any information on the pace of the turbidity (Y) evolution according to the parameters (volume of mucilage X_1, temperature X_2 and pH of water X_3), it seemed judicious to consider the use of a composite matrix allowing to estimate coefficients of a polynomial mathematical model of second degree.

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2 + a_{12}X_1X_2 + a_{13}X_1X_3 + a_{23}X_2X_3$$

The coefficients were estimated by a multiple linear regression procedure of the software Excel 2000 (Microsoft Corporation, Louviers, France). The runs

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were carried out in a random order (Feinberg, 1996 and 1999)

RESULTS AND DISCUSSIONS

Table 1 and 2 represent respectively the experimental parameters variation and the results, while tables 3 illustrates the polynomial coefficients, the standard deviation related to each coefficient, the Student's t coefficients (Feinberg, 1996) and the probability associated to the criterion t. This probability represents the risk α to be mistaken by rejecting the assumption that the coefficient is equal to zero. When α is small then the coefficient is significant.

Table 1*: parameters and experimental domain*

Variables	Factor	Units	Low level (-)	High level (+)
X_1	Mucilage volume	mL	15	25
X_2	Temperature	°C	14	40
X_3	pH	-	5.6	10.4

Table 2 *: Experimental design and results*

run	**X_1**	**X_2**	**X_3**	**Y (NTU)**
1	15	14	5.6	25.2
2	25	14	5.6	26.6
3	15	40	5.6	24.4
4	25	40	5.6	24.6
5	15	14	10.4	22.4
6	25	14	10.4	23.7
7	15	40	10.4	22.1
8	25	40	10.4	23.2
9	10	27	8	25.2
10	30	27	8	28.8
11	20	15	8	26.5
12	20	65	8	24.8
13	20	27	4	27.3
14	20	27	12	26.8
15	20	27	8	25.1
16	20	27	8	24.4
17	20	27	8	26.7
18	20	27	8	27.2
19	20	27	8	26.8
20	20	27	8	24.9

Table 3 *: model coefficients and statistical analysis*

	Model Coefficient	Type error	Student's t coefficient	Probability
a_0	25.933	0.742	34.967	**0.000**
a_1	0.736	0.492	1.496	**0.165**
a_2	-0.473	0.492	-0.961	0.359
a_3	-0.750	0.492	-1.524	**0.159**
a_{11}	-0.175	0.643	-0.272	0.791
a_{22}	0.100	0.643	0.156	0.879
a_{33}	0.200	0.643	0.156	0.879
a_{12}	-0.139	0.679	-0.290	0.778
a_{13}	-0.619	0.679	-1.286	0.227
a_{23}	-0.619	0.679	-0.253	0.805

It appears that whatever the experimental conditions, the turbidity of the samples varies from 22.1 to 28.8 NTU. Assuming that a coefficient is significant (statistically different from 0), if the associated probability is 0.200 (20 %) (Feinberg, 1999; Dagneli, 1975), the significant coefficients are a_0, a_1 and a_3. It appears that the influential factors on the turbidity are the gombo extract volume and the pH. On the other hand, the temperature has no proven effect. Moreover, it should be noted that the square terms (quadratic) and the interactions do not influence the turbidity of water. It results that a simple experimental design of first degree (a complete factorial design for instance) would have been enough to undertake this study and consequently to reduce the number of run to 8. The mathematical model connecting the response Y to the factors is brought back finally to a simpler model, as follows:

$$Y = 25.933 + 0.736X_1 – 0.750X_3$$

To reduce water turbidity, it is necessary to decrease the volume of coagulant and increase the pH. Moreover, these two factors were separately studied, since there are not interactions between them. For each study, we vary the factor concerned and let us maintain the other ones constant. Figures 1 and 2 indicate the results obtained.

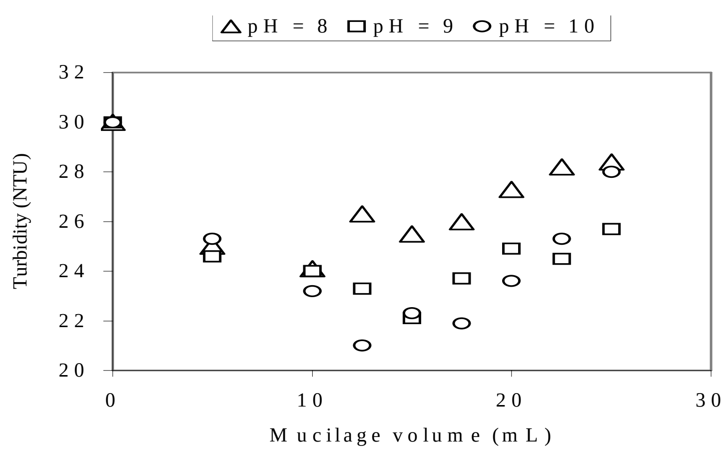

Fig.1: Turbidity as a function of the mucilage volume

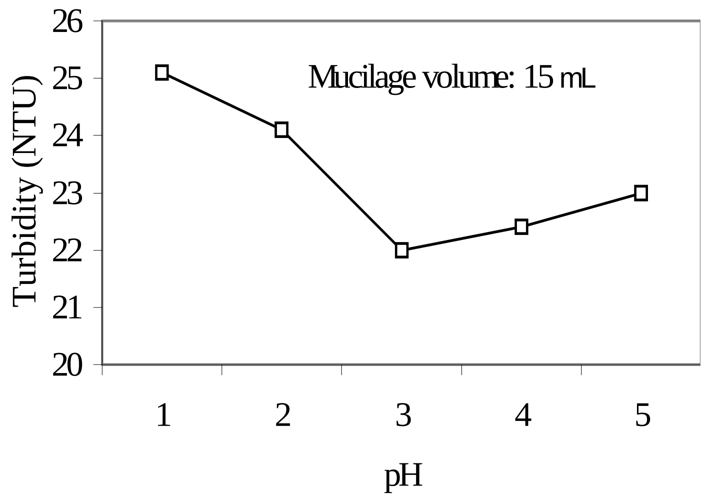

Fig.2: Turbidity as a function of pH

It appears at figure 1 that whatever the pH, the turbidity of the samples presents the same space (a decrease then a growth starting from 15 mL of gombo extract). The reduction in turbidity is more significant when the pH increases, so when the alkalinity of the mixture is high. However, the evolution according to volume seems to follow a nonlinear curve, whereas the experimental design shows the opposite. This apparent contradiction is not one. Indeed, with the experimental design, the minimal value used was 15 mL. However, in this last study (evolution according to volume), this minimal value is set at 0 mL, so the experimental field is not the same in both cases. The increase in the turbidity observed with the volume of gombo extract is explained by the saturation of the medium in flocculating agent. Indeed, beyond a threshold (here between 10 and 15 mL), the flocculating activity of the extract becomes negligible in front of the muddy due to its excess.

In the other hand, the extract volume to be added being fixed, the pH of water is varied from 4 to 11. The results obtained with three different volumes are illustrated by figure 4. In the range of studied, it is observed that whatever the volume of gombo mucilage, the turbidity decreases when the pH increases. The reduction in turbidity is significant when the volume of mucilage used is 15 ml, confirming the preceding results.

Although the gombo mucilage coagulation mechanism is unknown in the actually state of the study, it is possible to make two assumptions to explain the phenomenon observed. The mucilage, from its sticky nature, contains polymer molecules (Nacoulima et al, 2000). The flocculating activity can be either due to a chemical reaction, or a complex formation. The first one supposed that these polymers have functions likely to react with the colloidal particles. That is possible if the polymers in question are protein as already shown by former work (Folkard et al, 1992). In the case of the second assumption, it will then be necessary to admit the presence of interactions by the formation of complex with the colloids particles. This would contribute to increase their weight and to involve their flocculation.

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