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Response Surface Methodology Models for Optimization of Traditional Fermentation of Cowpea Leaves

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Abstract

Cowpea leaves is one of the African leafy vegetables that has been promoted to mitigate food and nutrition insecurity in arid and semi-arid lands of western and eastern Africa. Spontaneous fermentation of this vegetable is one of the traditional processing techniques that have been heavily utilized to improve the keeping quality and sensory attributes while inadvertently imparting health benefits. However, the vegetable has been shown to have minimal fermentable sugars for optimal action of the bacteria thus the process tends to be slow and the product quality less optimal. The current study utilized the response surface methodology (RSM) models of the central composite design to optimize the spontaneous fermentation of cowpea leaves. The RSM model generated 20 runs with the independent variables being concentrations of sugar and salt and period of fermentation in days whereas the pH and titratable acidity of the fermented vegetable were the response variables. The models showed significant ($p<0.01$) changes in the pH and titratable acidity with R^2 of 0.89 and 0.60, respectively. Change in the concentration of sugar and period of fermentation significantly ($p<0.05$) affected the pH and titratable acidity of the fermented vegetables. Salt concentration and interaction between the independent variables did not significantly ($p>0.05$) influence the changes in the response variables. The RSM model generated the optimal fermentation conditions as 2% salt and 5% sugar concentration and 16 days fermentation period; optimal response variables were 3.8 and 1.23% for pH and titratable acidity, respectively with a desirability of proportion of 85.9%. Optimal concentrations of sugar and salt and period of fermentation improved the action of the natural culture in fermented cowpea leaves. To avert the challenges of poor product quality and slow fermentation process among the traditional communities, the addition of sugar and salt to the vegetables and optimization of fermentation period should be observed.

Introduction

The utilization of cowpea leaves in sub-Saharan Africa (SSA) for food is a strategy towards alleviating food insecurity and micronutrient deficiencies in the region. Traditional processing techniques including fermentation have been employed to enhance product diversification of this vegetable (Owade et al., 2019). The need to enhance vegetable processing through optimal techniques is emphasized by the regular scarcity of the vegetable during drought as a study done in Kenya among cowpea leaves producing households established that utilization of the vegetable decreases by three quarters in the off-season (Owade et al., 2020). Whereas communities adopt various low-cost processing techniques in resource-constrained settings, less optimal processing parameters often result in poor product quality that render the adoption and continued practice of the techniques unsustainable. For instance the fermentation techniques employed in the production of the traditional soured vegetables are spontaneous (Kasangi et al., 2010), a process which is very slow and the product quality less optimal. The continued practise of this otherwise less optimal processing technique has scientific justification for it inadvertently imparts health benefits while improving the bioavailability of some micronutrients. Fermentation of vegetables reduces the level of antinutrients such as oxalates, flavonoids and nitrites; which would otherwise inhibit the availability of minerals for absorption into the body (Wafula, 2017). With such great scientific evidence backing the continued practice of vegetable fermentation, improving the technology and process parameters while assuring cost-effectiveness provides avenues of commercialization and up-scaling utilization of cowpea leaves. Therefore the current study sought to optimize the process parameters for production of traditional soured cowpea leaves through utilization of low-cost processing techniques adoptable in resource-constrained settings that produce the vegetable.

Material and Methods

Cowpea leaves were harvested at optimal stage of maturity, 6 weeks after emergence, and washed. The residual water in the washed vegetables was not drained to allow its use in complete immersion of the vegetables. Experimental runs were generated through the response surface methodology (RSM) models of the Central Composite Design (CCD) of the Design Expert 11 software. Three different factors were evaluated for optimization and they included concentration of sugar and salt and the period of fermentation. The minimum and maximum entries of the factors as established by Muchoki (2007) were used in this study (Table 1). Six centre points and twenty experimental runs were generated in the study. Fermentation was done using low cost processing techniques illustrated in Figure 1. The response variables evaluated in the study were pH and titratable acidy. The data were analysed using the analysis of variance (ANOVA) for quadratic models illustrated in equation 1.

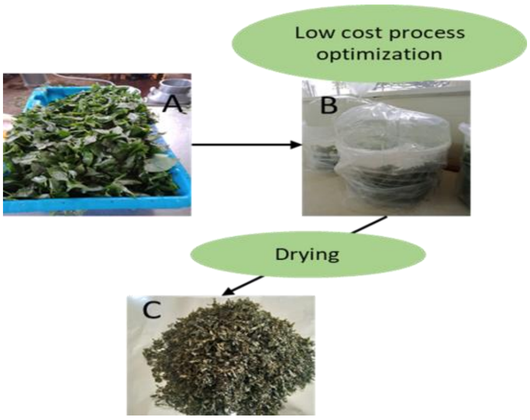

Figure 1: Traditional processing of dried soured cowpea leaves. A- fresh cowpea leaves, B-cowpea leaves under low cost fermentation and C-dried soured leaves.

Table 1: The minimum and maximum levels of factors in the central composite design

Factor	**Units**	**Minimum**	**Maximum**	**-α**	**+ α**
Concentration of salt	%	1	5	0.977311	6.02269
Concentration of sugar	%	1	5	0.977311	6.02269
Period of fermentation	Days	1	21	-4.11345	21.1134

$$y = a + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 BC + \beta_6 AC + \beta_7 A^2 + \beta_8 B^2 + \beta_9 C^2,$$ **Equation 1;**

whereby y is the response variable; a is a constant; A, B and C are fermentative parameters to be optimized; and β_1 *to* β_9 are coefficients.

Results and Discussion

The RSM models generated significantly ($p<0.01$) predicted the variations both in the pH and titratable acidity. The R^2 for the models were 0.5995 and 0.8852 for the titratable acidity and pH, respectively; the constants were 0.7 and 4.1, respectively. The model for the prediction of titratable acidity of had an F-value of 7.98 with a minimal chance of 0.18% of noise occurring whereas that of pH had an F value of 8.56 with a 0.12% chance of noise occurring in the model. Sugar and period of fermentation and the quadratic terms of period of fermentation are significant factors with coefficients of -0.2, -0.2, and 0.5 that significantly ($p<0.01$) influenced the pH. Vatansever et al. (2017) reported similar findings in vegetables they evaluated as with increasing substrate, the pH declined. The pH reduced with increasing sugar concentrations, whereas salt concentration had no effect on the pH (Figure 2). The beneficial effect of reducing pH of the vegetables is it induces antimicrobial activity in the vegetables (Medina et al., 2016).

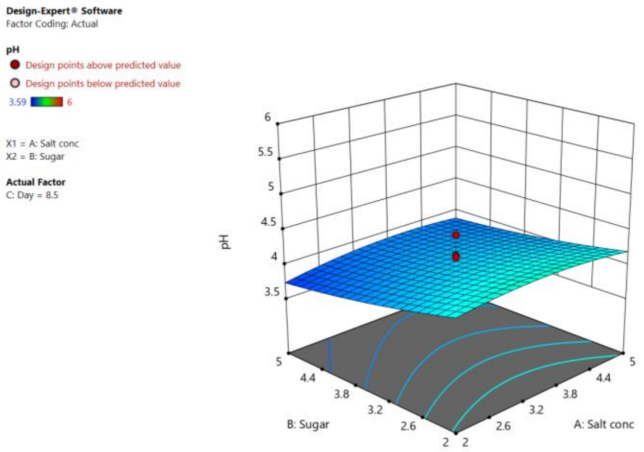

Figure 2: pH of spontaneously fermented cowpea leaves with different sugar and salt concentrations

Concentration of the sugar and period of fermentation with coefficients of 0.2 and 0.3 significantly ($p<0.05$) predicted titratable acidity contents of the fermented vegetables; salt concentration did not significantly ($p>0.05$) affect the titratable acidity. With increasing sugar concentration, the titratable acidity also increased (Figure 3). Under fermentation, the substrate that is sugar is converted to lactic acid by the lactic acid bacteria, LAB (Vatansever et al., 2017). The production of the acid indicates growth of the LAB, and this would continue to the point the sugars are depleted, thus no further conversion.

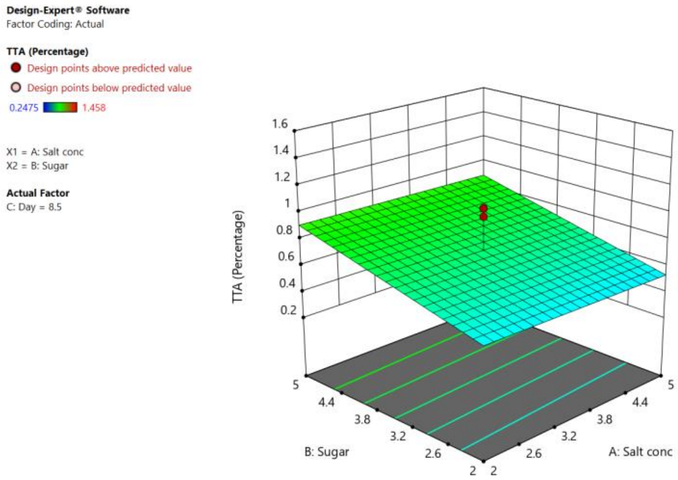

Figure 3: Titratable acidity of spontaneously fermented cowpea leaves with different sugar and salt concentrations

The optimal parameters for fermentation of cowpea leaves were established as 2% (w/w) salt concentration, 5% (w/w) sugar concentration and 16 days of fermentation period. The optimal point for the response variables were established as a pH of 3.8 and TTA of 1.23%, with a desirability of 85.9%. In as much as it is expected that with increasing concentration of the substrate and period of fermentation, the rate of process should increase, the addition of salt and the interactions of these factors adds the another perspective of setting in of osmotic stress that otherwise slows down the process (Jagannath et al., 2012). In their study, Muchoki et al. (2010) optimized the fermentation parameters at 3%(w/w) for both salt and sugar concentrations with 21 days of fermentation, however, the limitation in their study was the use of the one by one factor method of optimization. The method ignored the interactions between the fermentative parameters under study.

Conclusions and Outlook

The study established that the addition of sugar and salt in the vegetables improves the fermentative process of the vegetables. However, there is need to observe optimized period of fermentation, 16 days, in order to ensure that the pH and the titratable acidity are optimal. Whereas, this study successfully established the optimal fermentative parameters for traditionally soured product, there is further need to evaluate the nutritional quality, texture and acceptability of the optimally soured cowpea leaves. Further studies should also evaluate the health benefits derivative from the intake of this soured products inclusive of nutrient availability.

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