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Response surface methodology for studying the effect of processing conditions on some nutritional and textural properties of bambara groundnuts (*Voandzeia subterranea*) during canning

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Abstract
The response surface methodology and central composite rotatable design for $K=3$ was used to study the combined effect of blanching, soaking and sodium hexametaphosphate salt concentration on moisture, ash, leached solids, phytates, tannins and hardness of bambara groundnut during canning. Regression models were developed to predict the effects of the processing parameters on the studied indices. Significant interactions were observed between all the factors with high regression coefficients ($64.4\%–82.6\%$). Blanching and soaking of the seeds prior to canning led to increases in moisture content and leached solids, while significant decreases were observed for phytates, tannins and hardness of the canned bambara groundnuts. Increasing the concentration of sodium salt added during soaking caused significant ($P \leq 0.05$) decreases in phytates, tannins and the hardness of the seeds, suggesting that pre-canning treatments of blanching, soaking and sodium hexametaphosphate salt addition can be used to effectively reduce the phytates, tannin levels with minimal mineral (ash) loss and enhanced textural integrity of the canned bambara groundnuts.

Keywords: Response surface methodology, soaking, blanching, sodium hexametaphosphate salt, bambara groundnuts, anti-nutritional factors, textural properties

Introduction
Bambara groundnut (*Voandzeia subterranea*) is an African crop, which produces an almost balanced food. It originated in the Sahelian region of present-day West Africa, and derived its name from the bambara tribe who now live mainly in Mali. Although considerably less popular throughout the world, cultivation of bambara groundnut has remained common in all of West Africa. It is a drought-tolerant and easy-to-cultivate crop, which makes very little demand, if at all, on the soil (Doku 1996). It serves as an important source of protein in the diets of a large percentage of the population in Africa, particularly to poorer people who cannot afford expensive animal protein, by being among the least expensive, most easily transported non-processed protein
sources for both rural and urban dwellers. It is ranked the third most important grain legume after groundnut (*Arachis hypogaea* L.) and cowpea (*Vigna unguiculata*) (Rachie and Silvester 1977; Baryeh 2001; Afoakwa et al. 2004).

Nutritionally, most researchers agree that lysine is high, while methionine and calcium are low (Doku 1996). Studies by Amarteifio et al. (1997) revealed that bambara groundnut contains 6–12% fat, 14–24% protein and 28–53% carbohydrates, and they make a well-balanced food with a caloric value equal to that of a high-quality cereal grain. Moderate amounts of B vitamins and small amounts of minerals and vitamins A are reported (FAO 1988). They can be eaten raw without ill effect, but cooking will destroy the inhibitor and allow the body to make maximum use of the seed protein (FAO 1988). To make bambara groundnut edible and to increase its shelf-life, the beans are usually dried, processed and preserved by cooking or sterilization to develop acceptable flavour, texture, and inactivate anti-nutritional factors to make the bean’s protein nutritionally available to human life. The processing method involves soaking the bambara groundnut in water, draining and cooking or sterilizing in fresh boiling water or brine. Factors such as storage conditions, soaking treatment and cooking method influence the cookability or sterilizability and acceptability of the bambara groundnuts. In spite of the wide utilization of bambara groundnuts as food in most developing countries, its availability is seasonal and therefore limited to its harvesting season, making it unavailable all year round. Canning of bambara groundnuts is therefore suggested to increase its availability all year round and to provide alternative food processing approaches to the commodity.

Thermal processing is an important method of food preservation in the manufacture of shelf-stable canned foods, and has been the cornerstone of the food processing industry for more than a century. The quality of canned food is dependent on many factors such as the retort temperature, can size and shape, thermal properties and other processing parameters. To obtain the best quality product, each combination of processing conditions has to be carefully studied (Chen and Ramaswamy 2002; Simpson et al. 2006). Some work on cowpea seeds noted that pre-canned cowpea is usually soaked in cold soft water for 10–12 h, although an occasional lot may require somewhat less soaking depending on the moisture content of the peas and the hardness of the water (Lopez 1987; Afoakwa et al. 2005). Also, the addition of 0.2% sodium hexametaphosphate has been found to be satisfactory in water having 26–29 grains total hardness per gallon. Sodium hexametaphosphate has the general purpose of stabilizing or improving texture. It softens the texture of canned peas, beans, meat and poultry. The time of blanching depends on the texture and type of peas being blanched (Lopez 1987). Over blanching or blanching at too high temperature may cause the peas to split their skins. However, some canners have been successful in packing cowpeas by giving them a short soak (2–4 h), followed by a somewhat longer blanch (10–15 min). With some types of peas, this long blanch procedure seems to give firmer and less mushy peas, and this procedure requires very careful control of soaking and blanching times, temperatures and careful checking of filling weights. As bambara groundnuts can be suitable for canning, like cowpeas, it is important to study the processing conditions that would yield the best quality canned products from them and how these processing conditions affect the nutritional and textural properties of the canned beans.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for the modelling and analysis of problems in which a
response of interest is influenced by several variables with the objective of optimizing the response. RSM has important application in the design, development and formulation of new products, as well as in the improvement of existing product design. It defines the effect of the independent variables, alone or in combination, on the processes. In addition to analysing the effects of the independent variables, this experimental methodology generates a mathematical model that describes the chemical or biochemical processes (Myers and Montgomery 1995; Anjum et al. 1997; Afoakwa et al. 2002; Sefa-Dedeh et al. 2003). This work was therefore aimed at employing the techniques of response surface methodology to study the effects of soaking, blanching and sodium hexametaphosphate salt concentration [(NaPO3)6] on some biochemical and textural properties of bambara groundnut (V. subterranea) seeds during canning.

Materials and methods

Materials

Bambara groundnut seeds (V. subterranea) was obtained from the Crop Research Institute of the Council for Scientific and Industrial Research of Ghana and used for the study.

Experimental design for response surface methodology. A central composite rotatable design of the experiment was set up using Statgraphics software with experimental study variable number $K=3$, for independent variables including the blanching time ($X_1$), soaking time ($X_2$) and sodium hexametaphosphate concentrate ($X_3$). The process variables to be used in the central composite rotatable design for $K=3$ could be processed using the software. This will indicate the dependent variable limits and their values. The dependent variables studied included the following: moisture content of the canned bambara groundnuts, ash content, leached solids, phytates, tannin content of canned product and hardness (texture) of canned bambara groundnuts.

Twenty sample combinations were generated from the software in experimental design using the design matrix and variable combinations in experimental runs as shown on Tables I and II. The bambara groundnuts were canned using tin cans with dimensions of 44.0 mm $\times$ 83.7 mm. The pre-processing conditions as indicated in the various combinations generated in the experimental design were conducted on the bambara groundnuts and canned in a still vertical retort at 121°C (250°F) for 30 min. The data collated from the experiments conducted on the various combinations were then tabulated accordingly and analysed using stepwise regression analysis.

Table I. Process variables and their levels used in the central composite rotatable design for $K=3$.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Code</th>
<th>Variable level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$-1.682$</td>
</tr>
<tr>
<td>Blanching time (min)</td>
<td>$X_1$</td>
<td>0</td>
</tr>
<tr>
<td>Soaking time (h)</td>
<td>$X_2$</td>
<td>0</td>
</tr>
<tr>
<td>[(NaPO3)6] (%)</td>
<td>$X_3$</td>
<td>0</td>
</tr>
</tbody>
</table>
The optimization process. A stepwise multiple regression analyses was conducted on the data from the central composite rotatable design to relate the blanching time, soaking time and sodium hexametaphosphate (salt) concentration \([(\text{NaPO}_3)_6]\) to the moisture content, ash content, leached solids, phytates, tannin content and hardness of the canned bambara groundnuts. The response surface models were generated and presented as three-dimensional plots in the function of two factors (blanching time and soaking time) while the salt concentration \([(\text{NaPO}_3)_6]\) is kept constant. Adequacy of the model equation for predicting optimum response values was tested in the experiment using a blanching time of 0\(\text{--}12\) min, a soaking time of 0\(\text{--}24\) h and a salt concentration \([(\text{NaPO}_3)_6]\) of 0--1%. Three optimal processing conditions of the canning procedures of the Bambara groundnuts were determined from the mathematical models. In order to obtain these optimal values, the first partial derivatives of the regression equations were carried out according to \(X_1, X_2\) and \(X_3\) and sorted.

Analytical methods

Moisture content determination. The moisture content of the samples was determined using the AOAC (1990) method 950.40 of oven drying at 105°C for 6 h. The experiment was conducted in triplicate and the mean value determined.

Ash content determination. The ash content of the samples was determined using the procedure as outlined in the AOAC approved method 14.41 (AOAC, 1990). The crucibles were heat dried at 600°C in a muffle furnace for 30 min and cooled in desiccators. The crucibles were weighed and the initial weight noted. About 2 g sample were weighed and ashed at 600°C in the muffle furnace overnight. The sample was then cooled and weighed and the percentage ash determined.
Leached solids. The leached solids in the samples were determined according to the procedure as outlined in Lopez (1987). A 10 ml aliquot of the drained water from samples after canning was dried at 105°C in an air oven for 24 h. The weight of the residue was determined after drying. This was carried out in triplicate and the mean value reported as grams per gram of dry sample.

Phytic acid and tannin determinations. The phytic acid and tannin levels in the samples were determined according to the procedure as outlined in Bainbridge et al. (1996).

Seed hardness (texture). The hardness of seeds after canning was determined using a TA-XT2 Texture Analyser (Stable Micro Systems, Haslemere, Surrey, UK). The test cell used was the Warner-Bratzler Blade (Stable Micro Systems). The peak force required to cut through five seeds was determined. The seeds were placed longitudinally across the groove in the sample holder and cut perpendicularly across the axis of the seeds. The test conditions used were a test speed of 1.5 mm/sec and a distance of 11 mm. The test was replicated five times and the average peak recorded.

Statistical analysis

All the statistical analysis and graphical presentations were carried out using Statgraphics 5.1 (Graphics Software Systems, STCC, Inc., Rockville, MD, USA). The significant probability was set at $P \leq 0.05$.

Results and discussion

Effect of process variables on the moisture content of canned bambara groundnuts

The regression model obtained for the moisture content of the canned bambara groundnuts was:

$$Z = 37.7995 - 0.2492X_1 - 0.7345X_2 - 15.7905X_3 + 0.0086X_1X_1 - 0.0916X_2X_2 + 13.2493X_3X_3 - 0.01189X_1X_2X_3,$$

with $R^2 = 74.41\%$. There was significant ($P \leq 0.05$) influence of the quadratic factors of soaking time, blanching time and salt concentrations on the moisture content of the canned bambara groundnuts. The model could explain about 74.4% of the variations in the moisture content observed. Thus, 25.6% of the variation was attributed to factors not included in the model.

The response surface plots generated for the canned bambara groundnut showed a curvilinear plot with both soaking and blanching times (Figure 1). The moisture content of the bambara groundnut was initially low during the first 8 h of soaking and then increased gradually until the end of the 24 h of soaking. Observed trend for the blanching time differed slightly in the sense there was slight and insignificant reduction in the moisture content within the first 4 min of blanching, after which the moisture increased consistently until the end of the 10 min of blanching. The effects of these two processing parameters on the moisture content of the bambara groundnut showed similar trends in all the different salt concentrations (Figure 1). This indicates that soaking bambara groundnuts in salt concentrations of 0.5% and 1% results in increased moisture content of the canned product. Further blanching of the seeds for 4–10 min results in a 2–4% increase in the moisture of the canned product, suggesting that the processes of soaking, blanching and sodium salt addition
employed during canning of bambara groundnuts do not greatly affect the moisture content of the canned product.

Effect of the process variables on the mineral (ash) content of the canned bambara groundnuts

The model obtained for ash content of the bambara groundnuts was:

\[ Z = 2.3948 + 0.009X_1 + 0.2986X_2 - 0.3497X_3 - 0.00228X_1X_1 - 0.0258X_2X_2 + 0.6008X_3X_3 - 0.0022X_1X_2X_3, \]

with \( R^2 = 66.5\% \). There was a significant \( (P \leq 0.05) \) influence of the quadratic factor of blanching time and the linear of the soaking time for the canned bambara groundnut. The model could explain about 66.5\% of the variation in the ash levels.

The response surface plot generated for the bambara groundnut showed general decreasing trends in the ash content with increasing soaking time (Figure 2). The blanching effect on the moisture, however, was different. Blanching caused the ash content to increase gradually during the first 8 min of blanching and then decreased again upon increasing blanching to 10 min. The effect of the salt concentration on the

Figure 1. Response surface plot for the moisture content of the canned bambara groundnut.

Figure 2. Response surface plot for the ash content of the canned bambara groundnut.
ash content of the bambara groundnuts was not significant enough to cause any change.

The decreasing trend noted in the ash content during soaking could be due to leaching of minerals from the seeds into the soaking medium with the subsequent uptake of moisture into the seeds cells. The combined effect of soaking and blanching, however, triggered sharp and consistent decreases in the ash content of the product. This might be due to leaching of minerals in the form of leached solids as a result of the susceptibility of minerals to destruction by temperature of the blanching medium as well as the duration of the blanching period. This analogy is supported by Lopez (1987), who reported that loss of vitamins and minerals during blanching can be significant and is a function of surface area per mass of the product, degree of maturity of the product, type of blanching (hot water or steam), blanching time and method of cooling after blanching (water or air). Also nutrient losses that occur during blanching are caused by leaching, oxidation of water-soluble nutrients and thermal destruction, and that water-soluble vitamins are the most affected. Even though there were no variations in the different salt concentrations, it would be economical for industrial purposes to use the sodium salt at 0.5% concentration. From the model, the optimal conditions required for soaking and blanching of the seeds without much mineral losses to the products were a soaking time of 12 h, a blanching time of 6 min and a salt concentration of 0.5%.

Effect of process variables on the leached solids of canned cowpea

The model obtained for the leached solids of the canned bambara groundnut was:

$$Z = 0.6771 + 0.011X_1 - 0.00793X_2 - 0.5091X_3 + 0.0005X_1X_2 + 0.0092X_1X_3 + 0.4206X_2X_3 + 0.001X_1X_2X_3,$$

with $R^2 = 82.6\%$. There was a strong significant ($P \leq 0.05$) influence of the quadratic factors of soaking time, blanching time and salt concentration.

Statistical analysis indicated that the soaking time, blanching time and salt concentration had a significant ($P \leq 0.05$) quadratic effect on the model, but no linear effect. The model could explain about 82.6% of the variation in the leached solids. Thus about 17.4% of the variation was due to other factors not included in the model.

The response surface plot (Figure 3) showed that all three factors (soaking time, blanching time and salt concentration) influenced the leached solids of the bambara groundnut. It was noted that the level of leached solids of the bambara groundnut increased consistently during the 24 h of soaking. No much variation was observed with blanching within the first 4 h. However, the model showed slight increases with blanching after 6 min (Figure 3). This confirms the observation made with decreases in ash content during soaking and blanching, suggesting that some minerals and vitamins are leached into the soaking medium prior to canning. From the response plots, the optimal conditions required to achieve the level of leached solids in the canned bambara groundnut were identified as a soaking time of 12 h, a blanching time of 6 min and a salt concentration of 0.5%.

Effect of process variables on the phytate content of canned bambara groundnuts

Phytates are the principal source of phosphorus in dry beans. The interaction of phytates with proteins, vitamins and several minerals is considered one of the factors
that limit the nutritive value of dry beans (Afoakwa et al. 2004). Phytates have been reported to interfere with protein metabolism and decrease the utilization of proteins subjected to proteolytic digestion (O’Dell and Boland 1986). The model obtained for the phytate levels of the bambara groundnut was:

\[ Z = 4.91355 + 0.59634X_1 + 5.08527X_2 + 25.78484X_3 + 0.00791X_1X_2 - 0.64431X_1X_3 - 9.95468X_2X_3 + 0.017042X_1X_2X_3, \]

with \( R^2 = 66.1\% \). There was a significant \( (P \leq 0.05) \) influence of the linear factors of soaking time and salt concentration and the quadratic factor of blanching time. The model could explain about 66.1\% of the variations in phytate levels of the canned bambara groundnut. The response surface plot (Figure 4) indicates that all the three factors (soaking time, blanching time) had significant effects on the phytate level of the canned bambara groundnut.

There was a sharp and consistent decrease in the phytate levels of the canned product from the beginning to the end of the soaking period. For the blanching period, there was an initial increase in the phytate level, and this was further followed...
by a rapid decline in the phytate level of the canned bambara groundnuts on completion of the blanching time. The salt concentration did not have much influence on the phytate levels.

Phytates are affected by heating. Also, soaking under optimal conditions (55°C, pH 4.5–5.0) can reduce or eliminate phytates (Sandberg and Svarberg 1991; Hurrell 1997). This confirms the observation that the combined effects of soaking and blanching after 6 min greatly reduced the level of phytates present in the canned bambara groundnuts to insignificant levels. However, that effect is not pronounced if the soaked seeds are blanched for less than 6 min. The influence of increasing salt concentrations resulted in a slight and insignificant \( (P \leq 0.05) \) increase in the phytate levels of the canned bambara groundnuts. An important factor in the precipitation of phytate as its salts is the synergistic effect of two or more cations, which, when present simultaneously, may act together to increase the quantity of metallic phytate precipitated. The amount of these cations and the type present can determine this synergistic effect (National Academy of Sciences 1979).

**Effect of process variables on the tannin content of canned bambara groundnuts**

Tannins are naturally occurring compounds in legume seeds that contain sufficiently large number of phenolic hydroxyl or other suitable groups to enable it form effective cross-links proteins and other macromolecules. As components of food, tannins reduce the biological value of dietary proteins (National Academy of Sciences 1979). The model obtained for the tannin content of the bambara groundnut was:

\[
Z = 6.31355 - 0.100784X_1 + 0.506214X_2 - 0.10987X_3 + 0.00243X_1X_2 - 0.07601X_1X_3 + 0.02156X_2X_3 + 0.00489X_1X_2X_3,
\]

with \( R^2 = 65.2\% \). There was a significant \( (P \leq 0.05) \) influence of the quadratic factor of blanching time and linear factors of soaking time and salt concentration. The model could explain about 65.2\% of the variations in tannin content of the canned bambara groundnuts. The response surface plots developed (Figure 5) indicated that the soaking time and blanching time had significant effects on the tannin content of the canned bambara groundnut.

There was a slight reduction in the tannin content of the canned product from the beginning to the end of the soaking time. Blanching, however, caused a drastic decline
in the tannin levels of the canned bambara groundnuts. The combined effects of soaking the bambara groundnut in 0.5% salt sodium solution and blanching for between 8 and 10 min reduces the tannins to insignificant levels. The peak values for these two parameters (soaking time and blanching time) did not vary much at different salt concentrations. This trend could be explained by the fact that a soaking time of 12 h and a blanching time of between 8 and 10 min prior to canning of bambara groundnuts can effectively be employed to reduce their tannin levels to insignificant amounts, independent of the salt concentration used in the soaking medium.

Effect of process variables on the hardness of canned bambara groundnuts

Texture is one of the most important parameters used to assess the quality and acceptability of processed foods. The regression model obtained for hardness of the bambara groundnuts was:

\[ Z = 1398.3056 - 97.0545X_1 - 13.6317X_2 - 495.2921X_3 + 3.2443X_1X_1 + 1.2076X_2X_2 + 289.1576X_3X_3 + 0.0196X_1X_2X_3, \]

with \( R^2 = 79.2\% \). There was a strong significant (\( P \leq 0.05 \)) influence of the quadratic factor of soaking time and the linear factors of blanching time and salt concentration on the seed hardness of the canned bambara groundnuts. The model could explain about 79.2% of the variation in the hardness level of the seeds.

The response plots revealed that increasing soaking time resulted in a decrease in the hardness of the canned seeds (Figure 6a–c). However, increasing the blanching time did not influence the hardness of the seeds. Variation in the salt concentration from 0 to 1% brought about significant changes in the levels of hardness in the response surface plots, which is an indication of the fact that increasing the salt concentration influences the hardness of canned bambara groundnuts (Figure 6a–c).

The plots revealed that increasing the salt concentration led to significant decreases in hardness of the canned seeds (Figure 6a–c). This is suggested to be due to the fact that there were ion exchanges between the sodium ions and the divalent ions in the intracellular cement of the seeds during soaking with the salt solution leading to reduction in the hardness of the canned seeds (Pearson 1979). However, the hardness levels of the seeds soaked in 0.5% and 1% salt concentrations were comparable. This means, for economic reasons, a salt concentration of 0.5% would be ideal for the production of canned bambara groundnuts with an optimal softness. From the observed trend it can be deduced that the optimal conditions required to achieve the least hardness of canned bambara groundnuts were a soaking time of 12 h, a blanching time of 5 min and a salt concentration of 0.5%.

Conclusions

Soaking, blanching, and the sodium hexametaphosphate salt concentration all had varied effects on the moisture and mineral (ash) content, leached solids, phytates, tannins and the hardness of the canned bambara groundnuts, with significant interaction between all the factors. Soaking and blanching prior to canning led to increasing moisture content while significant decreases were observed for the phytates, tannins and hardness of the canned bambara groundnuts, with only minimal losses to the mineral content and leached solids when the seeds are soaked for 12 h. Increasing the levels of sodium hexametaphosphate salt during the soaking operation
Figure 6. Response surface plot for the heed hardness of the canned bambara groundnut at (a) 0%, (b) 0.5% and (c) 1% salt concentration.
caused a slight reduction in the texture (hardness) of the canned product. It was observed that soaking the bambara groundnuts for 12 h in a 0.5\% $[(\text{NaPO}_3)_6]$ salt concentration and blanching for 5 min gave the best quality product with only minimal loss to the mineral content. However, to reduce the anti-nutritional factors including phytates and tannins to insignificant levels with acceptable textural properties, the blanching time could be increased to 12 min. The combined effect of blanching, soaking and sodium hexametaphosphate $[(\text{NaPO}_3)_6]$ salt can therefore be used to effectively produce canned bambara groundnuts with enhanced nutritional and textural properties.

References


