Application of response surface methodology for studying the influence of soaking, blanching and sodium hexametaphosphate salt concentration on some biochemical and physical characteristics of cowpeas (Vigna unguiculata) during canning

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Abstract

Response surface methodology and central composite design for $K = 3$ was used to study the combined effect of blanching time (0–12 min), soaking time (0–24 h) and sodium hexametaphosphate [(NaPO₃)₆] salt concentration (0–1%) on moisture, ash, leached solids, phytates, tannins and hardness (texture) of cowpeas during canning. Regression models were developed to predict the effects of variables on the studied indices. Blanching, soaking and salt concentration all had significant positive effects on moisture content, ash content, leached solids, phytates, tannins and hardness of the canned cowpeas with significant interaction between all the factors with high regression coefficients (72.0–91.4%). The use of blanching and soaking prior to canning led to increasing moisture content and leached solids while significant decreases were observed for phytates, tannins and hardness of the canned cowpeas. Addition of sodium hexametaphosphate salt during soaking also caused significant ($p < 0.05$) decreases in phytates, tannins and the hardness of the seeds. The combination of blanching, soaking and sodium hexametaphosphate salt can therefore be used to retain the mineral content of cowpeas during canning while reducing the anti-nutritional factors and the hardness of the canned cowpeas with acceptable product quality characteristics.

Keywords: Response surface methodology; Soaking, blanching; Sodium hexametaphosphate salt; Cowpeas; Nutritional and anti-nutritional factors

1. Introduction

Cowpea (Vigna unguiculata L. Walp) also known as black eye peas, is one of the most commonly utilised legumes in Africa. It is an important crop in some areas of the tropics where it provides more than half the plant protein in human diets (Afoakwa, 1996; Sefa-Dedeh, Frimpong, K, Afoakwa, & Sakyi-Dawson, 2000). Cowpeas constitute about 52% of the total world output of grain legumes. Major cowpea producing countries in the world include Niger, Nigeria, USA, Burkina Faso, Brazil, Ghana and Uganda (Sefa-Dedeh, Sakyi-Dawson, & Afoakwa, 2001). Cowpeas are consumed in various forms such as green pods, tender green leaves, green seeds and dry seeds. Usually the processing of cowpeas for consumption involves mainly traditional methods. These methods include soaking, dehulling, steaming and cooking by boiling in excess water. An important feature of cowpeas with respect to other legumes is their high protein content. Some varieties of cowpeas available in Ghana have a protein content ranging from 24% to 27% (Osei, Sefa-Dedeh, Frimpong, & Collison, 1996; Afoakwa, Sefa-Dedeh, & Cornelius, 2002).
To make cowpeas edible and to increase their shelf life, they are usually processed and preserved by cooking or sterilisation of the dry beans to develop acceptable flavour, texture, and inactivate anti-nutritional factors to make the bean protein nutritionally available to human life. This process usually involves the soaking of the cowpeas in water, draining and cooking or sterilising in fresh boiling water or brine. Factors such as storage conditions, soaking treatment and cooking method influence the cook ability or sterilisability and acceptability of the cowpeas (Phillips & Mcwatters, 1991; Afoakwa, Sefa-Dedeh, & Sakyi-Dawson, 2004). Some research efforts have led to the gradual development and adoption of various preservation methods to make cowpea available all year round. In most developed countries, cowpeas are packaged dry, either raw or precooked, canned in water, tomato sauce or molasses; and canned in combination with other vegetables and meat or as constituents of soups, salads and dips. Even though traditional drying method of cowpea is economically cheaper, canning is proven to give cowpea a longer shelf life (Bressani, 1993; Afoakwa, Sefa-Dedeh, Kluvitse, & Sakyi-Dawson, 2003). The canning industry is constantly improving processing methods, enhancing quality and product safety. Processing methods designed, heighten retention of nutrients and effective use of energy. 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).

The soaking operation varies with the condition of the raw cowpeas and it has reported to cause a significant decrease in anti-nutrients, as the anti-nutrients are leached into the soak water (Lopez, 1987). Soaking for 18 h removed 65% of hemagglutinin activity in peas and soaking for 24 h at room temperature removed 66% of the trypsin (protease) inhibitor activity in mung beans, 93% in lentil, 59% in chickpea and 100% in broad bean. Other components lost are tannins, phytates and oligosaccharides. The loss of nutrients can be minimised through the soaking of whole seeds. The soaking solution employed has significant effect on the texture of the beans. Water with 4–9 grains hardness is considered ideal for soaking and blanching cowpeas and an addition of 0.2% Sodium hexametaphosphate has been found to be satisfactory in water having 26–29 grains total hardness per gallon (Lopez, 1987). Sodium hexametaphosphate has been used in the canning industry for decades as an acceptable food additive, and it is used generally for the purpose of stabilising or improving texture of dried peas or seeds. As new cowpea varieties are developed for human consumption by the use of canning techniques, it is important to study the influence of soaking, blanching and sodium hexametaphosphate [(NaPO3)6] salt concentration on the nutritional, anti-nutritional and physical characteristics of the newly developed cowpea (V. unguiculata) seeds.

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 and the objective is to optimise the response. The rationale for the use of RSM models is that it determines the optimum operating conditions for the system as well as the region of the factor space in which the operating specifications are satisfied. It also ensures simplicity of calculation of the model parameters (Sefa-Dedeh, Cornelius, Sakyi-Dawson, & Afoakwa, 2003). This work was therefore aimed at studying the influence of soaking, blanching and sodium hexametaphosphate [(NaPO3)6] salt concentration on some nutritional, anti-nutritional and physical characteristics of cowpea (V. unguiculata) seeds during canning operations using response surface methodology.

2. Materials and methods

2.1. Materials

Cowpea seeds (IT87D195Y) was obtained from the Crop Research Institute of the Council for Scientific and Industrial Research (CSIR) of Ghana and used for the study.

2.2. Experimental design for response surface methodology

A Central Composite Rotatable Design (CCRD) of the experiment was set up using the Statgraphics software with experimental study variable number $K = 3$, for independent variables including blanching time ($X_1$), soaking time ($X_2$) and sodium hexametaphosphate concentrate ($X_3$). The process variables to be used in the CCRD 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 cowpeas, ash content, leached solids, phytates, tannin content of canned product and hardness (texture) of canned cowpeas.

Twenty sample combinations were generated from the software in experimental design using the design matrix and variable combinations in experimental runs as shown in Tables 1 and 2. The cowpeas 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 cowpeas 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.

2.3. The optimisation process

A stepwise multiple regression analyses was conducted on the data from the Central Composite Rotatable Design to relate blanching time, soaking time and sodium hexametaphosphate (salt) concentration, \([\text{[(NaPO}_3\text{)]}_6]\) to moisture content, ash content, leached solids, phytates, tannin content and hardness of the canned cowpeas. The response surface models were generated and presented as 3-dimensional plots in the function of 2 factors (blanching time and soaking time) whilst the salt concentration \([\text{[(NaPO}_3\text{)]}_6]\) is kept constant. Adequacy of the model equation for predicting optimum response values was tested in the experiment using the blanching time of 0–12 min, soaking time of 0–24 h and salt concentration \([\text{[(NaPO}_3\text{)]}_6]\) of 0–1%. Three optimal processing conditions of the canning procedures of the cowpeas were determined from the mathematical models. In order to get these optimal values, the first partial derivatives of the regression equations were done according to \(X_1\), \(X_2\) and \(X_3\) and sorted.

2.4. Analytical methods

2.4.1. Moisture content determination

The moisture content of the samples were 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.

2.4.2. Ash content determination

The ash content of the samples was determined using the procedure as outlined in the Association of Official Analytical Chemists’ 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 of sample were weighed and ashed at 600 °C in the muffle furnace overnight. It was then cooled and weighed and the percentage ash determined.

2.5. 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 done in triplicate and the mean value reported as g/g dry sample.

Table 1

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<th>Independent variables</th>
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<td>Soaking time (h)</td>
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<td>([\text{[(NaPO}_3\text{)]}_6]) (%)</td>
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Table 2

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BT—blanching time, ST—soaking time and SHPC—sodium hexametaphosphate concentration.
2.6. Phytic acid and tannin determinations

The phytic acid and tannin levels in the samples were determined according to the procedure as outlined in Bainbridge, Tomlins, Wellings, and Westby (1996).

2.7. Seed hardness (texture)

The hardness of seeds after canning was determined using a TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, England). The test cell used was the Warner-Bratzler Blade. 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: test speed of 1.5 mm/s and distance of 11 mm. The test was replicated five times and the average peak force recorded.

2.8. Statistical analysis

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

3. Results and discussion

3.1. Effect of process variables on the moisture content of canned cowpeas

The model obtained for moisture content when the IT87D195Y cowpea was used for the canning was:

$$
Z = 5.447071 - 0.37596X_1 - 0.16487X_2 + 0.10423X_3 + 0.02312X_4X_5 + 0.41888X_1X_3 - 0.01523X_1X_2X_3 + 0.00274X_2^2 - 1.13888X_2^3
$$

with an $R^2$ of 90.0%. There was a strong and significant influence of the linear factors of blanching time and salt concentration on the moisture content. Statistical analysis conducted on the data showed that salt concentration and blanching time both had significant ($p \leq 0.05$) quadratic and linear effects on the model, with soaking time having only quadratic effect. The model could explain 90.0% of the variations in moisture content, meaning only 10.0% of the variation was due to other factors not included in the model. The response plots (Fig. 1a–c) show that blanching time, soaking time and the salt concentration, all had significant effects on the moisture content of the canned cowpea with significant interaction between all the factors.

The response surface plots generated showed a curvilinear plots with both blanching time and soaking time (Fig. 1a–c). This implies that the moisture content of the cowpea seeds reduced slightly during the first few hours of soaking and then increased again till the end of the soaking time. Similar trends were observed for the blanching time with all the different salt concentrations used. Decreased moisture in canned food products have been reported to lowers the enzymatic deterioration as well as microbial and chemical activities in the product hence increases the shelf life of the product (Lopez, 1987). From the study, the optimal pre-processing conditions required to achieve an optimal moisture content suitable for the new cowpea were identified as blanching time of 5 min, soaking time of 12 h and salt concentration of 0.5%.

3.2. Effect of the process variables on ash content of the canned cowpeas

The model obtained for ash content when IT87D195Y variety was:

$$
Z = 5.447071 - 0.37596X_1 - 0.16487X_2 + 0.10423X_3 + 0.02312X_4X_5 + 0.41888X_1X_3 - 0.01523X_1X_2X_3 + 0.00274X_2^2 - 1.13888X_2^3
$$

with an $R^2$ of 87.2%. There was a significant ($p \leq 0.05$) influence of the linear factors of blanching and soaking time for the cowpeas. It was observed from the statistical analysis that soaking time and blanching time had significant ($p \leq 0.05$) linear effect on the model, but no quadratic effect. The model could explain about 87.0% of the variations in the ash levels.

The response surface plots generated showed slight increases in ash content with increasing soaking time, with consequential decrease with increasing blanching time of cowpeas soaked with no salt addition (Fig. 2a). However, no observable influences were noted with increasing soaking and blanching times for the seeds soaked with 0.5% and 1% salt concentrations (Fig. 2b and c). This means that at these concentrations, blanching and soaking did not have any influence on the ash content of the cowpea seeds and therefore the mineral content of the cowpeas are retained.

The ash content of the cowpea soaked in no salt addition was also found to decrease with the increasing blanching time (Fig. 2a). This observation might be due to the fact that during blanching of cowpeas soaked without the sodium salt, some amount of minerals are leached into the blanching medium in the form of leachates thereby decreasing the ash content of the cowpeas. Lopez (1987) 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). He further explained that 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. However, the addition of the sodium salt during the soaking caused no reduction in the ash content during blanching (Fig. 2b and c), imply-
ing that the ash (mineral) content of the cowpeas are retained when the soaking is done with the addition of sodium hexametaphosphate salt.

3.3. Effect of process variables on leached solids of canned cowpea

The regression model obtained for leached solids when the IT87D195Y cowpea was used for the canning was:

\[ Z = 1.322319 - 0.24665X_1 - 0.12834X_2 - 2.53147X_3 + 0.01121X_1X_2 + 0.28909X_1X_3 + 0.12619X_2X_3 - 0.01726X_1X_2X_3 + 0.00567X_1^2 + 0.00181X_2^2 + 0.45173X_3^2, \]

with an \( R^2 \) of 91.4%. There was a significant (\( p \leq 0.05 \)) influence of the quadratic factor of soaking time. It was observed that soaking time had significant (\( p \leq 0.05 \)) quadratic effect on the model, but no linear effect. The model could explain about 91.0% of the variations in the leached solids.

Fig. 1. Response surface plot for moisture content of the canned cowpea at (a) 0%, (b) 0.5% and (c) 1% salt concentration.
The response plots (Fig. 3a–c) show that soaking and blanching times had significant effects on the leaching of solids from the cowpea seeds into the soaking medium. The leached solids were found to increase with increasing soaking time and blanching time in all the different salt concentrations. Phillips (1993) reported that soaking cowpea seeds for 12 h removes 65% of anti-nutrients, which are leached into the soaking water. It is therefore suspected that some dissolved solids were leached from the cowpea seeds when the plant cell comes into contact with a solution of lower water potential for at least 12 h and also when blanched for about 10 min. From the plots the optimal conditions required to achieve the optimal leaching were blanching time of 5 min, soaking time of 12 h and salt concentration of 0.5%. Even though the leaching might remove some anti-nutrients from the cowpea seeds prior to canning, too high amount of leached solids can bring about low quality
3.4. Effect of process variables on phytate content of canned cowpeas

Phytic acid (myoinositol hexaphosphate) is the principal source of phosphorus in dry beans. The interaction
of phytate with proteins, vitamins and several minerals is considered to be one of the factors that limit the nutritive value of dry beans (Bressani, 1993). The regression model generated for phytate content when the IT87D195Y cowpea was used for the canning gave a high regression coefficient of 79.5% with a significant lack of fit. The equation generated from the model was: 

\[ Z = 12.531266 + 131.2220X_3 + 0.06626X_1X_2 - 9.04057X_1X_3 - 7.89521X_2X_3 + 0.69867X_1X_2X_3 - 0.03099X_1^2, \]

with an \( R^2 \) of 79.5%. There was a strong

![Response surface plot for phytate content of the canned cowpea at (a) 0%, (b) 0.5% and (c) 1% salt concentration.](image)

Fig. 4. Response surface plot for phytate content of the canned cowpea at (a) 0%, (b) 0.5% and (c) 1% salt concentration.
and significant ($p \leq 0.05$) influence of the linear factors of soaking time, salt concentration $[[\text{NaPO}_3]_6]$ and blanching time. Statistical analysis on the data revealed that soaking time, blanching time and salt concentration $[[\text{NaPO}_3]_6]$ all had significant ($p \leq 0.05$) linear effect on the model. The model could explain about 80.0% of the variations in the phytate level.

The response surface plots generated (Fig. 4a–c) showed that the salt concentration and soaking time all had significant effects on the phytate levels of the cowpeas. Increased salt concentration and soaking time resulted in consistent decreases in the phytate contents. Increasing soaking time caused significant decreases in the phytate content, as the anti-nutrients were suspected

![Response surface plots](image)

Fig. 5. Response surface plot for tannin content of the canned cowpea at (a) 0%, (b) 0.5% and (c) 1% salt concentration.
to be leached into the soaking medium at all the salt concentrations. Pearson (1976) reported that soaking cowpea seeds for 18 h removed 65% of hemagglutinin activity, and soaking mung bean for 24 h at room temperature removed 66% of the trypsin (protease) inhibitor activity, 93% in lentil, 59% in Chickpea and 100% in broad bean. In addition, it has been reported that blanching for long time removes most or all of the anti-nutrients in cowpeas seeds (Phillips, 1993). Nutrient losses that occur during blanching are caused by leach-

Fig. 6. Response surface plot for heed hardness of the canned cowpea at (a) 0%, (b) 0.5% and (c) 1% salt concentration.
ing, oxidation of water-soluble nutrients and thermal destruction (Lopez, 1987). The results showed that the soaking cowpea seeds for 12 h in 0.5 salt concentration and blanching for 5 min would remove all the phytates in the cowpeas.

3.5. Effect of process variables on tannin content of canned cowpeas

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 macro molecules. As components of food or feed, tannins reduce the biological value of dietary proteins (Bressani, 1993). The regression model obtained for the tannin content was: 

\[
Z = 5.529647 + 0.03476 X_2 - 0.01062 X_1 X_2 - 0.04044 X_2 X_3 + 0.01333 X_1 X_2 X_3, \text{ with an } R^2 \text{ of 72.0}%. 
\]

There was a significant (p ≤ 0.05) influence on the linear factors of soaking time. Statistical analysis conducted on the data showed that soaking time and blanching time had significant (p ≤ 0.05) linear effect on the model, but no quadratic effect. The model could explain about 72.0% of the variations in the tannin content.

The response plots (Fig. 5a–c) showed that increasing soaking time led to drastic decreases in the tannin content of the cowpeas with all the different salt concentrations used. This means that soaking cowpea seeds for about 24 h prior to canning can effectively reduce their tannin levels to insignificant amounts, independent on the salt concentration used in the soaking medium. The variation in the salt concentration did not bring any noticeable change in the patterns of the plots. Increasing blanching time however caused no effect on the tannin content of the cowpeas indicating that blanching and salt concentration do not influence the tannin levels during canning of cowpeas.

3.6. Effect of process variables on hardness of canned cowpeas

The model obtained for hardness of the cowpea was:

\[
Z = 215.618596 + 13.5476 X_2 - 0.01062 X_1 X_2 - 0.04044 X_2 X_3 + 0.01333 X_1 X_2 X_3, \text{ with an } R^2 \text{ of 75.8}%. 
\]

There was a strong and significant (p ≤ 0.05) linear effect in the quadratic factor of blanching and linear factor of soaking time, salt concentration [(NaPO$_3$)$_6$] and blanching time. It was observed from the statistical analysis that soaking time and salt concentration [(NaPO$_3$)$_6$] had significant (p ≤ 0.05) linear effect on the model, whilst blanching time has both quadratic and linear effect. The model could explain about 76.0% of the variations in the hardness level.

As shown in the response plots (Fig. 6a–c), all the factors had significant effects on the texture (hardness) of the canned cowpeas. The variations in the salt concentration brought about slight changes in the pattern of the response plots, which is an indication of the fact that increasing the salt concentration influences the hardness of the cowpeas (Fig. 6a–c). The plots revealed that increasing soaking time resulted in consistent decreases in the hardness of the canned cowpeas. According to Lopez (1987), cowpeas soaked for longer periods of time tend to become soft. On the other hand, the decrease in hardness as a result of the increasing soaking and blanching times were due to the fact that, there is a disruption of cell integrity as a result of an ion exchange reaction between sodium ions and the divalent ions in the intracellular cement during soaking (Pearson, 1976). Again, if cowpeas remain in water for a long time they become soft (Lopez, 1987). Earlier work by Sefa-Dedeh et al. (2001) reported that for acceptable cowpea product formulation, a hardness level of 500 g is ideal. From the study, it was observed that soaking time of 12 h and [(NaPO$_3$)$_6$] salt concentration of 0.5% with 5 min blanching gave a hardness value of 500 g and therefore would be optimal for achieving an acceptable product texture, which would not make the canned cowpeas too soft or hard after processing.

4. Conclusions

Blanching, soaking and sodium hexametaphosphate salt concentration all had significant effects on the moisture content, ash content, leached solids, phytates, tannins and the hardness of the canned cowpeas with significant interaction between all the factors. Blanching and soaking prior to canning led to increasing moisture content and leached solids while significant decreases were observed for the phytates, tannins and hardness of the canned cowpeas. The addition of sodium hexametaphosphate salt during the soaking operation also caused significant improvement (reduction) in the hardness of the seeds. The combination of blanching, soaking and sodium hexametaphosphate [(NaPO$_3$)$_6$] salt can therefore be used to retain the mineral content of cowpeas during canning while reducing the anti-nutritional factors and the hardness of the canned cowpeas with acceptable product quality characteristics. From the study, it was observed that the optimal pre-processing conditions required to achieve the optimum quality canned product from the cowpea cultivar were blanching time of 5 min, soaking time of 12 h and [(NaPO$_3$)$_6$] salt concentration of 0.5%. These conditions would give the best quality canned product from the IT87D195Y cowpea with improved nutritional quality and acceptable product quality characteristics.
References


