Microwave Glycation of Bambara Protein-Rice Starch Composites

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ABSTRACT---- ***Glycation of food proteins has a great potential of improving the functionality of food systems which can lead to increased utilization of food as well as contribute to the elimination of food insecurity in developing countries. The objective of this paper was to evaluate the glycation potential of Bambara protein-rice starch composite using a microwave oven. The glycation of Bambara protein-rice starch composites was achieved by microwave heat treatment at varying composite ratios and microwave times. Conditions for glycation were optimized using the mixture composite design of Response Surface Methodology. The maximum glycation for the Bambara protein-rice starch composite was 8.99 (µg/10mg). The optimum conditions were found to be 0.6g protein, 0.4g rice starch and 6.0min of microwave heating time. Bambara proteins can be used in food industries especially in the formation of glycated food proteins to improve the functionalities of locally manufactured food systems with unique food functionalities.***

Keywords--- composite, functionality, glycation, response surface methodology

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1. INTRODUCTION

Despite the increase in production of agricultural foods, Africa's food and nutrition insecurity status is growing worse (Frimpong, 2013). One of the reasons is the underutilization of most staples crops with Bambara groundnut as a typical example. Africa's food insecurity situation if not addressed appropriately could lead to an increase in nutritional deficiencies viz., protein energy malnutrition, vitamin A deficiency, iron deficiency anaemiain addition to huge economic losses to growers and the nation as a whole.

There is also increasing demand by consumers for foods with improved functionality. Food scientists have therefore been searching for new and innovative methods of improving the functional properties of food systems. Furthermore, consumers are now becoming more conscious of their health and are therefore demanding for processed foods with little or no chemical additives.

It is known that Maillard reaction, carried out under dry state and well controlled processing conditions such as temperature, relative humidity and time, is an adequate method for improving functionality of proteins while optimizing structural components(Morgan *et al*., 1997).

The glycation of food through Maillard reaction is a complex reaction which involves the condensation of reducing sugars with amino groups of protein. Glycation endows food proteins with improved functional properties, such as solubility, water retention capacity, gelling capacity, and emulsifying properties. It occurs under mild and safe conditions and requires no extraneous chemicals (Liu *et al*., 2012a).

The physicochemical properties and structure of glycoconjugates are also greatly affected by glycation. Corzo*et al*. (2010) discovered that glycated sodium caseinate (with galactose/lactose/dextran) exhibited increased viscosity with increasing incubation time. Glycation also results in modification of secondary structure of food protein which brings about improved functional properties of food protein. The tertiary and quaternary structure of food protein also changes after glycation, resulting in the alteration of functional properties.

Despite the abundance of literature in this regards, the extent to which the tertiary or quaternary structure changes remains unclear (Liu *et al*., 2012b). Early Maillard reaction products and melanoidins are currently gaining a lot of attention due to their reported health-promoting properties and their potential use as functional food ingredients (Martin *et al*., 2009). Liu *et al*. (2012a) revealed that the emulsifying properties of the grafts of ovalbumin–dextran were much better than those of commercial emulsifiers. Recent studies have shown that functional properties of β-lactoglobulin, such as thermal stability and foaming capacity, are improved after modification by the Maillard reaction, depending on the sugar used during modification (Chevalier *et al* ., 2001). On the other hand, studies have shown that a diet rich in advanced glycation end products induces inflammatory mediators and decrease insulin sensitivity in both animals and humans (Lin *et al*., 2002).
The Maillard reaction, produced from an amino acid–sugar model system, has been known to be associated with the formation of compounds with pronounced antioxidant activity (Yoshimura *et al*., 1997). A study was carried out by Sun *et al*. (2006) revealed that α-lactalbuminglycated with a rare sugar (D- allose) possessed stronger antioxidant properties.
Maillard reaction is influenced by several factors whose regulation can control the glycation of food proteins. Silvan*et al*. (2011) were able to control the formation of early Maillard reaction products by the use of ferulic acid leading to the preparation of glycoproteins containing low amounts of advanced glycation end products with the potential to be used as functional food ingredients. The extent of glycation is affected by factors such as type of carbohydrate, protein content, reaction temperature, time, pH among others. It was observed by Olivier *et al.* (2006) that the sugar reactivity towards caseinate was faster with glucose, followed by fructose and lactose.

Glycation of food through Maillard reaction is therefore an excellent option to improve the functionality of food systems thus increasing the utilization of food and ultimately reduce food insecurity in Africa. Since it does not involve the use of extraneous chemicals, glycation would also be readily acceptable to health conscious consumers. This study focused on contributing to finding a safer and simple technology that could convert most natural proteins into glycated proteins for commercial or industrial purposes. The objective for this project was to evaluate the glycation potential of Bambara protein and rice starch using a microwave oven.

2. METHODOLOGY

Sources of Material
Bambara groundnut *(Vignasubterranea)* and rice (*Oryzaglaberrima*)were obtained from a local market in the Kumasi metropolis of Ashanti Region of Ghana. Hexane was procured from Ghana Nut Limited, *Techiman*intheBrong- Ahafo Region of Ghana. All other chemicals used in this research were procured from Sigma Aldrich Company Limited, USA.

Sample Preparation
Bambara groundnuts were sorted and cleaned to remove dust and foreign materials and subsequently milled into flour with a hammer mill (F8, England). Rice samples were also sorted and cleaned. The samples were then coarse milled into flour with a hammer mill (F8, England). The dry Bambara groundnut flour was defatted using the semi-continuous Soxhlet (1879) in a solid-liquid extractor (E1VS, France). Proteins were subsequently extracted from the defatted Bambara groundnut flour using a protocol described by Gomez-Brenes*et al*. (1983). After this, the protein content was determined to verify the extraction process. This was done according to protocol described by Bradford (1976).Starch was extracted from rice according to a protocol described by Baah*et al.* (2005).

Experimental Design
Design Expert (2008) software was used to randomize the factors and the levels at which they were varied. A total of twenty-one runs for the Bambara protein- rice starch composites, were randomly generated by the Design Expert (2008) for the experiment. Table 1indicate the specified levels at which each factor were varied during the experiment.

Glycation of Protein-Starch Composites
Protein-starch glycation reaction was achieved by microwave heating according to a protocol described by Guan *et al*., (2006)which was carried out in a microwave oven (CMX20, U.K) of 800W power levels. For each run, the protein and starch were separately weighed according to the ratios generated by the Design Expert (2008) as shown in Table 2. For each run, the composites weremixed together in a tightly capped centrifuge tube and agitated in a vortex mixer (SA7, U.K) for 2min.

The lids of the centrifuge tubes were opened and placed in a covered plastic container for 2h alongside an opened beaker containing water. This was done to increase the moisture content of the samples. The samples were then heated by microwave irradiation at a temperature of 90^{o}C according to the time generated by the Design Expert for each run. The mixture was then cooled for 3min in an ice bath to stop the reaction. Each experimental run of reaction mixtures was heat-treated in duplicates.

Determination of Soluble Proteins in Glycated Samples

Ten milligrams (10mg) of each sample was weighed into a centrifuge tube and 1ml of Phosphate Basal Saline (PBS) added to the sample. The solution was mixed by shaking the content of the centrifuge tube for 2h in an orbital shaker (98001 Cat, USA) after which it was then centrifuged for 1h at 2500 rpm. Hundred micro liters (100 µl) of the supernatant which contained the soluble proteins was taken for protein determination.

The protein content was then determined for each run of sample according to the method of Bradford (1976) using Bovine Serum Albumin (BSA) as standard and Coomassie protein assay reagent. An aliquot of 100 µl of the supernatant for each run was measured into a test tube after which 2.5ml of Bradford reagent was added and kept for 5min for the reaction to come to completion. The absorbance of the each sample was measured at 595nm using a spectrophotometer (UV/VIS 1601, Japan). An aliquot of 20µl was injected into the spectrophotometer (UV/VIS 1601, Japan) and the absorbance was read in triplicates for each sample and the average taken. The absorbance was compared to that of the standard curve that was previously obtained. The protein content representing the total soluble glycated protein was then calculated from a standard curve generated using bovine serum albumin (BSA) as the standard.

Statistical Analysis

The response data for glycation obtained from the analysis was loaded and fitted to models using Design Expert (2008).The model that best fit the data was identified by evaluating regression parameters such as regression (R^2), adjusted regression (adj. R^2), predicted regression (pred. R^2), and adequate precision (adeq. precision). The variations between the factors and data obtained from the response were evaluated by determining the analysis of variance. The significance of the interaction among the factors and response were determined by the p-value and F-value. The response surface (40) methodology was used to optimize the glycation process using the response obtained from the glycation experiment.

3. RESULTS

Data Analysis

The following regression equations were generated for the model of Bambara protein-rice starch composites.

Y= 8.35A + 6.67B - 9.04AB -0.61AC + 2.32BC -10.75ABC

Where:
A=Bambara protein
B= Rice starch
C= Microwave time

The analysis of variance (ANOVA) for Bambara protein-rice starch composites is represented in Table 3. The Fisher's F-value recorded was5.49 and there is only 0.45 % chance that the model F-value is due to noise. The p-value for the model terms AB, BC, ABC were all significant model terms as their corresponding values of prob>F are each less than 0.05. The fit of a model was also expressed by the coefficient of regression (R-Squared). The predicted and adjusted R^2 squares were 0.65 and 0.53,respectively while the adequacy of prediction was 7.27 as shown in Table 4.

Effect of Different Bambara Protein–Rice Starch Combinations on the Extent of Glycation

Figure 1 shows the interaction among Bambara protein, rice starch and microwave time in the form of a three dimensional response plot. It can be observed from the response surface plot that an increase in protein content from 0.6g to 0.8g resulted in a corresponding increase in glycated protein content. On the other hand, a decrease in microwave time however resulted in an increase in glycated protein content.

Maximum glycation (represented by the red region) of 8.47(µg/10mg) was realized after glycation as shown in Figure 2. For that maximum glycation to occur, microwave time below 4.0 min, Bambara protein content above 0.75g and rice starch content below 0.25g is required. It can also be observed that high glycation of 7.89(µg/10mg) also occurred when Bambara protein-rice starch combination was 0.6g protein and 0.4g rice starch at microwave time of 6.0 min. The minimum glycation which is represented by the blue region was however recorded on the contour representing 4.3 (µg/10mg) which was a ratio of 0.7g protein and 0.3g starch. On that contour, the microwave time required ranged from 5.0 to 6.0min.

Optimization of Conditions for Glycation of Bambara Protein- Starch Composites

The response data for glycation obtained from the analysis was loaded and fitted to models to generate the optimum conditions for maximum glycation. After optimization, the optimum conditions for maximum glycation of were; 0.6 g of Bambara groundnut, 0.4 g of rice starch and microwave time of 6.0 min. The desirability of the optimization was 0.86 as shown in Table 5. On the other hand, glycation as high as 8.96(µg/10mg) could also be achieved at the optimum conditions of 0.8g protein, 0.2g starch and 2min. microwave time. These conditions however gave a slightly lower desirability of 0.85 as well as slightly lower glycated protein content.

A plot of the maximum glycation against Bambara protein and rice starch at the optimum microwave time of 6.0 min is shown in Figure 3. From that graph, it can be observed thatglycated protein content decreased gradually from a value of 9.0(μg/10mg) with a corresponding increase in the protein content of the composites. This gradual decrease was continued until it reached minimum glycation value of about 3.5 (μg/10mg) after which thereon it started increasing gradually upon further increase in protein content.

4. DISCUSSION

Data Analysis

The Fisher's F-value of 5.49for the model implies that the model is significant. There is only 0.45% chance that the model F-value is due to noise. Noise factors in an experiment refer to unexplained variations in the samples or factors that are impossible or too expensive to control (Steinberg and Burnsztyn, 1993). P-values were used to evaluate the significance of the coefficients, which where needed to understand the pattern of mutual interactions among the test variables. The smaller the magnitude of the P-value, the more significant it's corresponding coefficient. Model terms are significant when their P-vaues (prob>F) are less than 0.05. However, values greater than 0.10 indicate that the model terms are not significant. From the results, that the combine factor effect of AB, BC, ABC are all significant model terms as their corresponding values of prob>F are each less than 0.05.

The fit of a model was also expressed by the coefficient of regression (R-Squared). The R^2 value is always between 0 and 1 and the closer the R^2 is to 1.0, the stronger the model and the better it predicts the response (Haaland, 1989).The predicted and adjusted R^2 squares were 0.65 and 0.53 respectively, indicating that the model was fairly good in predicting variation of responses obtained. According to Montgomery and Myers (2002) adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. The ratio 7.27 therefore confirms that there was adequate model discrimination.

Effect of Bambara Protein- Rice starch Composites and Microwave Time on the Formation of Soluble Glycated Proteins

From the response surface plot in Figure 1, it can be seen that generally an increase in protein content from 0.6g to 0.8g was followed by a corresponding increase in glycated protein content obtained from the experiment. A similar trend was observed by Zhen-Chun *et al*., (2013)in a study conducted on the effects of protein to glucose ratio, substrate concentration, pH, reaction temperature, ultrasonication time and ultrasonic power on degree of grafting and degree of browning. It was revealed from their investigation that an increase in protein concentration led to an increase in degree of grafting in Mung bean protein-glucose substrate.

It can also be seen from the Figure 1 that, a decrease in microwave time unlike the protein content resulted in an increase in glycation. This observation is however contrary to observations made by Zhen-Chun *et al*., (2013) that an increase in ultrasonic time and temperature both led to an increase in degree of grafting. Observations made by Bourais*et al.*, (2006)also revealed that an increase in incubation time led to an increase in the amount of glycated proteins which is similar to the findings of Zhen- Chun *et al.*, (2013). Similar observations were also made by Achouri*et al.*, (2005) when they researched into the functional properties of glycated soy 11S-rich glycinin with glucose at varying incubation periods. In their research, a gradual increase in glycation from 34.8% to 46.5% was observed as incubation time increased from 6 to 48 h. According to Kato *et al*., (1993), glycation proceeds faster during the early stages compared with other stages in the reaction because, more e-amino groups of lysine are accessible for glucose interaction.

Figure 2 is a contour plot showing the effect of Bambara protein–rice starch combinations on glycation. Each contour curve represents an infinite number of combinations of two least variables with the other maintained at zero level (Ghodke*et al*., 2009).It can be seen from the plot that for each contour, different microwave times and protein- starch combinations is required to achieve that particular glycated protein content. The yellow region represents the area with maximum glycation whiles the blue represents the region with minimum glycation.

In Figure 2, it can be seen that for maximum glycation (represented by the red region) of 8.47 (μg/10mg) to occur, microwave time below 4.0 min, Bambara protein content above 0.75 g and rice starch content below 0.25 g are required. It can also be observed that high glycation of 7.89 also occurred when Bambara protein–rice starch combination was 0.6 g protein and 0.4 g rice starch at microwave time of 6 min. This implies that high glycation can be obtained by increasing the content of Bambara protein in the composites while reducing the time required for microwave heating. On the other, high glycation can equally be obtained by decreasing the Bambara protein content in the composite while rather increasing the time of heating in the microwave. A choice therefore has to be made by the manufacturer on which option to take which obviously will depend on cost of the production of Bambara protein as compared to the cost of operating the microwave oven for longer duration.

The minimum glycation is represented by the blue region was found on the contour representing 4.3 (µg/10mg). On that contour the microwave time required ranged from 5.0 to 6.0 min with a combination of 0.7g protein and 0.3g starch.

Dry glycation has a lot of industrial benefits compared to wet glycation as according to Oliver *et al*. (2006).Dry reactions are more desirable from an industrial perspective, based on the premise that they require less space and time to achieve the desired result than wet reaction conditions. Some dryMaillard reactions, however, particularly those involving formation of protein-polysaccharide conjugates derived from globular proteins, are performed over 2 to 3 weeks (Song *et al*., 2002). This effect can be reduced by using microwave heating as according to Guan *et al.*, (2006) compared to the classical heating; the microwave heating speeded up the graft reactions of soy protein isolate with sugars.

Optimization of Conditions for Glycation of Bambara Protein- Starch Composites

The model constraints were used to generate the optimum conditions for maximum glycation of Bambara protein-rice starch composites. After optimization, the optimum conditions for maximum glycation of were; 0.6 g of Bambara groundnut, 0.4 g of rice starch and microwave time of 6.0 min as shown in Table 6. The desirability of the optimization was 0.86 which is good as it is closer to 1. The closer the desirability is to 1, the better the optimization. A plot of the maximum glycation against Bambara protein and rice starch at the optimum microwave time of 6.0 min is shown in Figure 3. It can be seen from the graph that the glycated protein content decreased gradually as protein content increased until it reached minimum glycation value of about 3.5 (µg/10mg) after which it started increasing gradually upon further increase in protein content. This is contrary to the trend to observed by Zhen-Chun *et al*., (2013) that glycation increased steadily with increasing protein concentration to a peak of 52.28 %and later followed by a decline in glycation upon further increase in protein concentration.

5. CONCLUSION

The glycation between protein from Bambara groundnut and starch from rice was optimized using a composite design of the response surface methodology. The output of the glycation was determined, revealing a maximum glycation of 8.99 (µg/10mg) at the optimum conditions of 0.6 g protein, 0.4 g rice starch and microwave time of 6.0 min. However high glycation of 7.89(µg/10mg) was also observed at the conditions of 0.8g Bambara protein, 0.2g rice starch and 2min. of microwave time.

Since glycation of protein is known to improve the functional properties of food systems, the functional properties of the glycated protein which yielded maximum glycation should be studied in a model food system. This will help to improve the functionality of a particular food product and can subsequently be adopted for industrial purposes.

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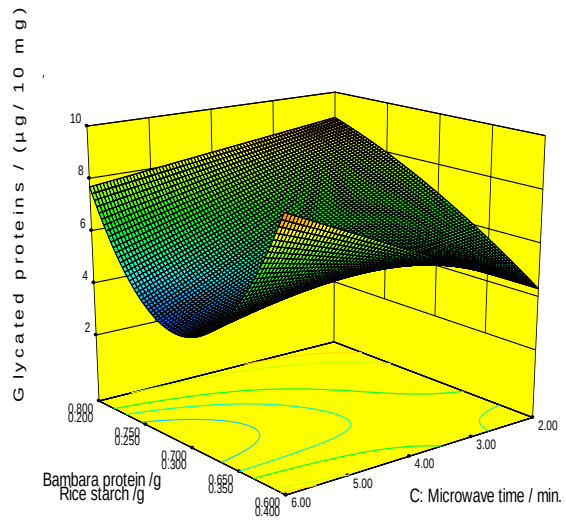

Figure 1: Response surface plot showing the variation of glycated protein content with Bambara protein-rice starch ratio and microwave time

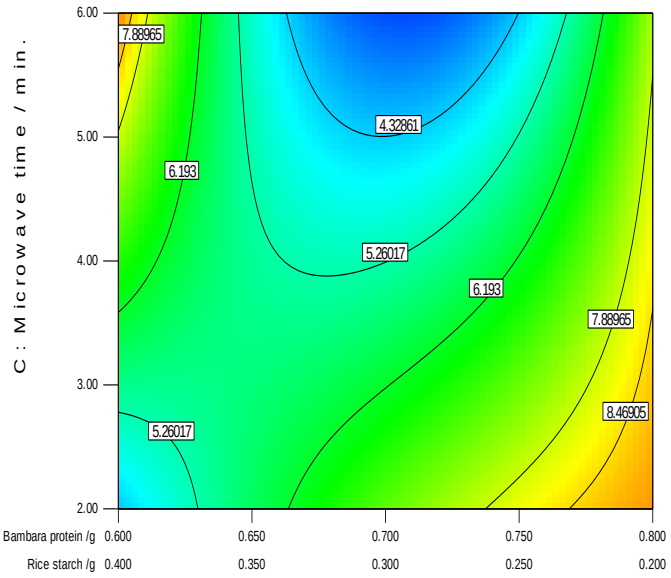

Figure 2: Response surface plot on the effect of Bambara protein-rice starch combinations and microwave time on glycation

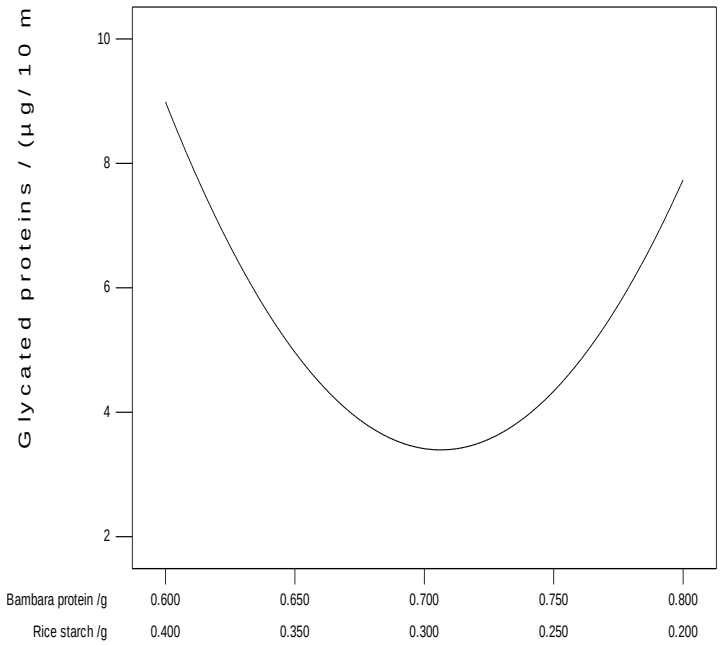

Figure 3: Response surface plot of maximum glycation of Bambara protein-rice starch composites at optimum time of 6.0 min.

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Table 1: Factors and level of variation

Factors	Level of variable
Bambara protein	**0.6 – 0.8g**
Rice starch	**0.2 – 0.4g**
Microwave time	**2.0 – 6.0min.**

Table 2: Factors and ratios of experimental runs for the glycation of Bambara protein – rice composites

Runs	A: Bambara protein/g	B: rice starch/g	C: Microwave time/min
1	0.70	0.30	4.0
2	0.75	0.25	5.0
3	0.70	0.30	4.0
4	0.75	0.25	3.0
5	0.60	0.40	4.0
6	0.70	0.30	4.0
7	0.65	0.35	3.0
8	0.60	0.40	6.0
9	0.80	0.20	6.0
10	0.70	0.30	6.0
11	0.60	0.40	2.0
12	0.70	0.30	2.0
13	0.70	0.30	4.0
14	0.80	0.20	4.0
15	0.80	0.20	2.0
16	0.60	0.40	2.0
17	0.80	0.20	6.0
18	0.60	0.40	6.0
19	0.70	0.30	4.0
20	0.70	0.30	4.0
21	0.70	0.30	4.0

Table 3: ANOVA for glycation of Bambara protein-rice starch composites

Source	Sum of Squares	DF	Mean Square	F-value	prob>F	Significance
Model	56.47	5	11.29	5.49	0.0045	significant
Linear mixture	3.67	1	3.67	1.79	0.2014	
AB	22.98	1	22.98	11.17	0.0045	
AC	1.07	1	1.07	0.52	0.4825	
BC	21.67	1	21.67	10.53	0.0054	
ABC	12.35	1	12.35	6.00	0.0271	
Residual	30.86	15	2.057			
Lack of fit	11.79	6	1.96	0.93	0.52	Not significant
Pure Error	19.07	9	2.12			
Cor Total	87.33	20				

Table 4: Summary of the statistics of the analysis of variance for the extent of glycation of Bambara protein-rice starch composites

Summary statistics	**Std. Dev.**	**Mean**	**C.V. %**	**PRESS**	**R-Squared**	**Adj. R-Squared**	**Pred. R-Squared**	**Adeq. Precision**
	1.43	6.26	22.91	69.74	0.65	0.53	0.20	7.27

Table 5: Optimum conditions for glycation of Bambara protein-rice starch composites

Optimum conditions					
Bambara protein /g	**Rice starch /g**	**Microwave time /min**	**Max. glycation response /µg/10mg**	**Desirability**	**Rank of selection**
0.6	0.40	6.00	8.99	0.86	1st
0.8	0.20	2.00	8.96	0.85	2nd