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Effect of Germination Temperature on the Functional Properties of Grain Amaranthus

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Abstract Grain amaranthus in its ordinary state may be modified by germination to perform extraordinary role as emulsifier for our fast- growing food industry with a concomitant increase in food production to satisfy the ever-increasing population of the world with its associated food insecurity. Hence, this study aimed at determining the optimum germination temperature for the maximum improvement of the functional properties of grain amaranthus with the view of using this as an emulsifier in food processing. The dry grains were germinated at 30°C, 32°C, 34°C, 36°C, 38°C, 40°C and designated as T30, T32, T34, T36, T38, T40 respectively and T00 for the negative control. The water and oil absorption capacities (WAC and OAC) were determined by centrifuging the samples in water and then groundnut oil . The emulsifying capacity (EC) and emulsion stability (ES) were determined by homogenizing the samples in groundnut oil and later centrifuging. Result was expressed as mean and Analysis of Variance and Least Significant Difference were was used for comparison. The WAC increased from 107.58% in T00 to 118.97% in T30 with the peak value of 124.94% in T40. The OAC increased from 31.07% in the negative control to 33.13% in T30 with the peak value of 35.96% in T38. The emulsifying capacity was 2.0 1% in T00 and increased to 24.63% in T30. This property increased with increase in germination temperature to the maximum value of 31.17% in T40. The emulsion stability ranged between 1.20% in T00 to 2.31% in T36. Foam capacity in the negative control was zero and in T30 was 3.99% while the peak value was 8.45% in T32. The Hydrophile:Lipophile Balance (HLB) ranged between 3.28 in T38 to 4.16 in T40 which was higher than that of standard emulsifier, lecithin, with the value of 4.0. This shows that germinated grain amaranth may exhibit the same or even better emulsifying properties than lecithin which is a universal emulsifier even though it may not exert appreciable foaming properties where this is required.

Keywords: *germination, temperature, functional properties, grain amaranthus*

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1. Introduction

Grain amaranthus *(Amaranthus caudatus, Amaranthus cruentus, Amaranthus hypochondriachus*) which has been reported to originate from Peru (Birthe et al, 1987) is a hardy, fast-growing pseudo-cereal that has a promising potential as a nutritious food crop. The plant species are noted for high tolerance to arid conditions and poor soils where cereals cannot grow with ease (Omami et al., 2006 ; Brenner et al., 2000). They are easy to cultivate commercially and domestically making a good rotation crop responding well to fertilization. The seeds as well as the leaves of white amaranth are rich in good quality protein which is exceptionally high in lysine (Johnson and Helderson, 2011; Brenner et al., 2000), carbohydrate, fats, vitamins and minerals such as calcium, magnesium etc (Huerta-Ocampo and Barba de la Rosa, 2011 ; Akubugwo et al., 2007). Moreover, the seed proteins have some outstanding nutritional and functional properties (Mburu et al., 2012). In spite of these potentialities the present level of consumption of grain amaranth the all over the world is practically negligible but the development of processing technique to generate amaranth-based products with desirable functional and sensory properties could provide incentives to increase the production and consumption of the crop. This forms the basis for the germination of the seeds.

Amaranthus is a dual purpose plant which supplies tasty leafy vegetables as well as grains of high nutritional value. The vegetable amaranth has a protein content of 17.4% to 33.5%, total lipids of 10.6%, carbohydrate with appreciable amount of β-carotene, vitamin B 12, vitamin C, niacin, thiamine, riboflavin and minerals like magnesium, phosphorous, zinc, potassium, calcium and iron(Akubugwo et al., 2007). The seeds or grains of *Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus hypochondriachus* have been noted for the potential to increase world food production. The seeds are cream, golden or pink in colour and are comparable with cereals in composition but relatively small in size, barely bigger than mustard seed. The protein content of grain amaranth species varies from 12.5% to 17.6% which is

comparatively higher than that of maize and most other grains (Mburu et al., 2012) with a methionine and lysine content of 0.6 to 1.7 and 3.4 to 6.4g/ 16g N respectively (Mburu et al., 2012). The amino acid profile of the protein is close to the optimum composition suggested by FAO/WHO hence it is superior to some other grains (Mburu et al., 2012). The protein efficiency ratio (PER) comparable to that of casein with total digestibility of 90% (Venskutonis and Kraujalis, 2013) and was found to be altered by defattening, extrusion, roasting and popping (Muyonga et al., 2014). It has a lipid content of 10 to 17 % with a relatively high degree of unsaturated fatty acids and also a good source of vitamins and minerals especially calcium and magnesium (Mburu et al., 2014). The carbohydrate content is which is mainly starch with very small granules and it is two to five times more hydrolyzed than maize starch, hence, it is applicable in food, chemical and other industries (Srichuwong et al.,2012).The starch was also found to withstand four freezing and thawing cycles before marked syneresis occurs (Srichuwong et al., 2012) hence, it could be suitable for frozen desserts.

Limited research studies have been carried out on the functional properties of this grain and the effect of germination. Pachelo de Delahaye in 1987 reported an oil absorption capacity, water absorption capacity, emulsifying activity and emulsion stability of 150%, 420%, 40% and 42% respectively when the grains were subjected to 60°C for 4 hours. The emulsion formed was found to be stable after 24 hours while increase in the temperature of treatment did not affect the oil and water absorption capacity. Germination increased the oil absorption capacityof grain amaranth while thermal treatments increased the water absorption capacity to 5.1 and 6.3 g/g in *A. caudatus* and *A. cruentus* respectively (Gamel et al., 2006).Germination reduced the foam stability and the level of phenolic compounds, phytate and enzyme inhibitors but increased the flour dispersibility (Gamel et al., 2006). The Water Absorption Index of this grain increased from 3.45 to 3.82 when germinated at 32°C for 16 hours but reduced at 24 hours and lesser hours of germination periods (Chauhan and Singh, 2013). There is need for more research study on grain amaranth in order to maximally utilize it for consumption, hence, this study determines the effect of germinating grain amaranth at different temperatures on the functional properties of the grain.

2. Materials and Method

Collection of seeds: Seeds of hybrid between *Amaranthus cruentus* and *Amaranthus hypochondriachus* of ascension number NH 84/452 were collected from National Institute of Horticultural Research and Training (NIHORT) Idishin, Ibadan, Nigeria. The seeds were cleaned manually by aspiration and the moisture content was determined.

Moisture Content determination: This was carried out using A.O.A.C. (1980) method. Into clean, dry, cooled and weighed moisture cans 5g of the sample was weighed and oven-dried at 105°C for 18 hours after which it was cooled in a dessicator and then weighed. The moisture content was calculated thus:

$$\%\ \text{Moisture content} = \frac{\left(\begin{matrix}\text{final weight of the can} \\ +\ \text{sample} - \text{weight of can}\end{matrix}\right)\times 100}{\text{Weight of sample}}$$

Germination of seeds: This was done using the method of Paredes -Lopez and Mora- Escobedo, (1989) with slight modification. The seeds were surface-sterilized by soaking in 0.1% sodium hypochlorite for 10 minutes after which it was washed with distilled water and soaked again in distilled water for another 10 minutes. After draining the water the seeds were spread evenly in a single layer on a dampened 4 layered muslin cloth placed in plastic trays. These were then incubated at temperatures 30°C, 32°C, 34°C, 36°C, 38°C and 40°C for 24 hours in Ikemoto Brand Incubator, Germany and were designated T30, T32, T34, T36, T38, and T40 respectively while T00 was the negative control. The germinated grains were oven-dried in Cole Parmer oven,U.S.A at 45°C for 18 hours, cooled, milled and sifted after which the following functional properties were determined: emulsifying capacity, emulsion stability, water absorption capacity, oil absorption capacity, foam capacity and stability.

Emulsifying capacity and stability: Emulsifying capacity of a substance is its ability to mix with both hydrophilic (water-loving) and hydrophobic (lipophilic) substances to form homogeneous mix. This property is a resultant effect of the hydrophilic and lipophilic groups in the substance such as proteins, phospholipids while complex carbohydrates like gums, pectin and starch stabilize the emulsion formed. Emusifying capacity was determined using the method of Yasumatsu et al., 1972 with minor modification. 1g of the sample was suspended in 50ml of distilled water and 50ml of refined groundnut oil was added to it. The mixture was homogenized with Ace Homogenizer, U.S.A at 10,000 rpm for 1 minute. The emulsion obtained was divided evenly into two 50ml centrifuge tubes and centrifuged in Hettich Universal Centrifuge, Germany at 4,100 rpm for 5 minutes. The emulsifying capacity was calculated thus:

$$\text{Emulsifying capacity} = \frac{\text{Height of emulsified layer}\times 100}{\text{Height of whole layer}}$$

Emulsion stability: The centrifuge tubes were heated for 30 minutes at 80°C and then cooled under tap water for 15 minutes before re-centrifuging at 4,100 rpm for 5 minutes.

$$\textbf{Emulsion stability} = \frac{\text{Height of remaining emulsified layer}\times 100}{\text{Height of whole layer}}$$

Water Absorption Capacity (WAC): This was determined using the method of Ige et al.,1984. 10ml distilled water was mixed vigorously with 1.5g of the sample and agitated 4 times with a glass rod, allowing 10 minutes resting periods between each mixing. The suspension was then centrifuged at 3,250 rpm for 25 minutes. The supernatant was decanted and the tubes were air-dried and the WAC was calculated thus:

$$\text{WAC} = \frac{\left(\text{final weight} - \text{Initial weight}\right)\times 100}{\text{Sample weight}}$$

Oil Absorption Capacity (OAC): The method of Ige et al.,1984 was used to determine this. Refined groundnut oil (3ml) of density 0.9281g/ml was added to 0.5g of the sample in a 15ml conical graduated centrifuge tubes and stirred with a glass rod for 1 minute. After 30 minutes at room temperature the tubes were centrifuged at 3,200 rpm for 25 minutes. The volume of the unabsorbed oil was determined and OAC was calculated thus:

$$\frac{0.9281(\text{Initial oil volume} - \text{unabsorbed oil volume}) \times 100}{0.5\text{g}}$$

Foam Capacity and Stability: These were determined using the method described by Giam et al.,1994 with minor modification. A measured quantity (2g) of sample was blended with 100ml of distilled water in a Moulinex blender, France and whipped at 1,500 rpm for 5 minutes. This was then poured into a 250ml measuring cylinder and the foam capacity was calculated thus:

$$\text{Foam Capacity} = \frac{\left(\begin{array}{l}\text{Volume after whipping} \\ -\text{Volume before blending}\end{array}\right) \times 100}{\text{Volume before blending}}$$

Foam stability was determined by measuring the volume of the foam after 120 minutes.

$$\text{Foam Stability} = \frac{(\text{Initial volume} - \text{Final Volume}) \times 100}{\text{Initial volume}}$$

Statistical Analysis: Analyses were done in triplicate and result expressed in mean. Analysis of variance was used to compare the mean between groups while LSD ($p<0.05$) was used in determining the significant differences between one group and the other.

3. Result and Discussion

3.1. Water Absorption Capacity (WAC)

There was a significant increase ($p< 0.05$) in the WAC from 107.58% in T00 to 118.97% in T30 to the peak value of 124.94% in T40 (Table 1).This shows that germination at 40°C produced samples with highest amount of water-loving, hydrophilic compounds which may be in form of proteins, carbohydrates such as sugars, gums, starch as well as water-soluble vitamins. The values of WAC for T00 differed from the report of Pachelo de Delahaye (1987) which was 420% after subjecting the grains to 60°C for 4 hours. The range of the WAC values was 117 to 125%. Pink colouration was observed in all the germinated samples after addition of water but was mostly intense in T30. This shows that the pigment amaranthine is a polar compound and can be extracted using polar solvents.

3.2. Oil Absorption Capacity (OAC)

The OAC ranged from the least, 31.07% in T00 to the highest. 35.96% in T38 (Table 1). There exists significant difference ($p<0.05$) between these values. Comparing these with the results of Pachelo de Delahaye (1987) which was 150%, these values are very low but higher than that of soy protein which was 0.5% (Chamba et al., 2013). This could be as a result of the different treatments given to the grains and the difference in climatic conditions where the experiments were carried out.

Table 1. Effect of germination temperature on functional properties of grain amaranth

Samples	WAC(%)	OAC(%)	EC(%)	ES(%)	HLB	FC(%)	FS(%)
T00	107.58	31.07	2.01	1.20	3.46	0.00	0.00
T30	118.97	33.13	24.63	1.71	3.59	3.99	0.42
T32	119.13	33.13	24.14	1.62	3.60	8.45	6.24
T34	124.33	31.07	24.58	2.10	4.00	7.84	1.40
T36	117.84	34.46	27.80	2.31	3.42	3.92	0.53
T38	117.90	35.96	28.00	1.8	3.28	0.98	0.02
T40	124.94	30.06	31.17	1.23	4.16	0.97	0.00

WAC-Water Absorption Capacity
OAC-Oil Absorption Capacity
EC - Emulsifying Capacity
ES- Emulsion Stability
FC- Foam Capacity
FS- Foam Stability
HLB- Hydrophile:Lipophile Balance

3.3. Hydrophile: Lipophile Balance

Lecithin, a universal emulsifier exhibits a hydrophile: lipophile balance of 4.0 at which it attains maximum emulsifying capacity (Baseeth and Sebree 2011). Germinated grain amaranth also presented a similar characteristic. The least hydrophile: lipophile balance, 3.26 was observed in T36 while the peak, 4.16 (which is higher than that of lecithin) in T40 (Table 1).The value for the negative control is significantly different ($p<0.05$) from those of the germinated sample except T36 and T38.

3.4. Emulsifying Capacity (EC) and Emulsion Stability (ES)

The increase in the EC from 2.01% in T00 to 24.63% in T30 confirms the fact that germination effects improved functional properties of grains in addition to the improved nutritional value as reported by Paredes-Lopez and Mora Escobedo, 1989. The value for the negative control is significantly different ($p<0.05$) from those of the germinated samples. The EC for the germinated samples ranged from the least, 24.14% to the highest, 31.17% in T40 (Table 1). Soy flour, a renown natural emulsifier exhibited an EC of 48% (Paredes Lopez and Mora-

Escobedo, 1987) for it contains an appreciable amount of lecithin. Hence, germinated amaranth grain flour at 40°C for 24 hours may be a close substitute for soyflour as an emulsifier.

Even though germination improved the EC of grain amaranth the emulsion was not noticeably stable as shown in Table 1. The least value in the germinated samples was 1.23% in T40 while the peak was 2.31% in T38. These were not significantly different ($p < 0.05$) from that of the negative control (T00) which was 1.20%.

These values are very low compared to that of soy flour which was found to be 48% (Pachelo de Delahaye 1987). This signifies that for emulsion stability a composite flour of soy flour and germinated amaranth grain flour could be used. This will have an added advantage of increased nutritive value.

3.5. Foam Capacity and Stability

The values of foam capacity (Table 1) shows that grain amaranth flour did not foam at all but on germination it increased from 3.99% in T30 to the peak value of 8.45% in T32 and then to the least value, 0.97% in T40. There exists a significant difference ($p<0.05$) between the negative control and the germinated samples. This could be attributed to increased protein content and quality, and probably formation of some alginates (Balasubramanian and Sadasivam, 1987, Paredes- Lopez and Mora-Escobedo, 1989). The foam was not stable at all because the foam stability of most of the samples was close to zero except in T32 which is significantly different from other values as shown in Table 1. Hence we can deduce that germinated and ungerminated amaranth flours will not produce an acceptable result where foam production is desirable as in cakes, sponges, ice cream etc.

4. Conclusion and Recommendation

The optimum germination temperature that imparted the most desireable emulsifying properties comparable to the standard emulsifier, monoglyceride,in grain amaranthus was at 40°C while the highest foam capacity was in the sample germinated at 32°C. Hence germinated grain amaranth may be a suitable emulsifier in the food industry and if possible an egg replacer where reduced cost of production and cholesterol-free alternative is preferred, nevertheless more research study is needed in this area. Morestill, there is need to know the dispersibility and antinutritional factors in the germinated grain for its appropriate application as an emulsifier in the food industry.

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