Effect of Germination Temperature on the Functional Properties of Grain Amaranthus

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Received February 26, 2014; Revised March 05, 2014; Accepted March 26, 2014

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 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 2.01% 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

Cite This Article: Paulina Oludoyin ADENIYI, and Veronica A. OBATOLU, “Effect of Germination Temperature on the Functional Properties of Grain Amaranthus.” American Journal of Food Science and Technology, vol. 2, no. 2 (2014): 76-79. doi: 10.12691/ajfst-2-2-5.

1. Introduction

Grain amaranthus (Amaranthus caudatus, Amaranthus cruentus, Amaranthus hypochondriacus) 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 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 hypochondriacus 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 defatting, 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 capacity of grain amaranth while thermal
treatments increased the water absorption capacity 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 hypochondriacus
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:

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

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. Emulsifying 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.

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

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:

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

\[
\text{OAC} = \frac{0.9281(\text{Initial oil volume} - \text{unabsorbed oil volume}) \times 100}{0.5g}
\]

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:

\[
\text{Foam Capacity} = \frac{\text{Volume after whipping} - \text{Volume before blending}}{\text{Volume before blending}} \times 100
\]

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

\[
\text{Foam Stability} = \frac{\text{Initial volume} - \text{Final Volume}}{\text{Initial volume}} \times 100
\]

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, 33.13% in T30 (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.

| 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| 3.99  | 0.53  |
| T34     | 124.33 | 31.07  | 24.58 | 2.31  | 4.00| 8.45  | 6.24  |
| 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 hydrophilic-lipophilic 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 hydrophilic-lipophilic 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% (Pacheco de Delahaye 1987). This signifies that for emulsion stability a composite flour and antinutritional factors in the germinated grain for its 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 desirable 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. Morestilt, 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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