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Heat-induced gelation of whole egusi (*Colocynthis citrullus* L.) seeds

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Abstract

Despite the use of whole egusi (*Colocynthis citrullus* L.) seeds as a food ingredient for generations, there is no report on its gelling ability. This study evaluates the potentials of egusi seeds as a structure enhancer in food systems. Gelation and thermal characteristics of egusi seeds were examined by dynamic rheological testing and differential scanning calorimetry. Rheological data of gels, prepared at varied protein concentrations (3%, 6%, 10% and 20%, w/v) in 0.15 M NaCl, indicated that 6% (w/v) egusi protein was sufficiently high to produce properly-crosslinked networks ($G' = 8724$ Pa). Gels prepared at 10% (w/v) protein in 0.15 M NaCl, ($G' = 22,530$ Pa) were superior to gels treated with 0.5 M NaCl ($G' = 8385$ Pa). Structural stability of egusi meal increased as salt level increased. Elastic gels developed above the denaturation temperature ($T_d = 93.7$ °C) in the heating phase and continued during cooling, indicating that egusi seeds can be used as a gelling food ingredient.

Keywords: Egusi; Gelation; Dynamic rheology; Thermal denaturation

1. Introduction

Current world effort has been targetted at developing new oilseed crops which could be used for food, medicinal and industrial purposes (Yaniv, Schafferman, Elber, Ben-Moshe, & Zur, 1994). Melon seeds (*Cucurbita* spp., *Citrullus* spp.) are rich in oil and protein (Al-Khalifa, 1996). Although none of these oils has been used on an industrial scale, many are used as cooking oils in some African and Middle Eastern countries (El-Magoli, Morad, & El-Fara, 1979). Egusi (*Colocynthis citrullus* L.) belongs to the melon family of Cucurbitaceae but is often confused with other family members (Oyolu, 1977). It is cultivated in regions of West Africa, especially in Nigeria for the food in the seeds and as a crop inter-planted with maize, cassava and yam (Lagemann, Flinn, Okigbo, & Moormann, 1975). It produces bitter-flavoured melon-type fruits about the size of cantaloupe. Partially defatted egusi seed meal is made into patties that serve as a meat substitute. The undefatted meal is used in several dietary preparations that vary with the food habits of the people. For instance, egusi seeds are used as a major thickening and gelling ingredient in most southeastern Nigerian dishes. Whole egusi seeds are dry-roasted and consumed as a snack. Egusi kernels contain approximately 28.4% protein (60% in defatted flour), 52.0% oil, 3.6% ash, 2.7% fibre and 8.2% carbohydrate (Akobundu, Cherry, & Simmons, 1982). Its oil contains 63% linoleic acid, 16% oleic acid (Akobundu et al., 1982), and small amounts of linolenic acid (Akoh & Nwosu, 1992; Huang, Akoh, & Erickson, 1994; Udayasekhara-Rao, 1994). Such oil composition resembles that of safflower oil (Yaniv, Schafferman, Zur, & Shamir, 1996) and is very beneficial in human nutrition.

Oilseed proteins (e.g., egusi proteins) contribute useful functionality to food systems. According to Akobundu et al. (1982), protein isolates that differ in gel electrophoretic patterns and amino acid contents can be prepared from egusi flour in one- or two-step water and NaOH extractions. The water-holding (0.7 ml/g) and oil holding (2.6 ml/g) capacities of defatted egusi seed flour have been reported (Akobundu et al., 1982). This report further indicated that defatted egusi flour can form thick

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(mayonnaise-type) emulsions in the alkaline pH range, and stable foams at pH 5. Another functional property that can be of merit in the utilization of plant proteins is the ability to form gels. There is no published report on the gelation properties of egusi seeds, despite their use as a food ingredient for generations (Lagemann, 1977; Oyolu, 1977; Sanchez, Fuller, Yahiku, & Baldsin, 1972). An understanding of the thermal gelling properties of egusi seeds and their gelation mechanisms will enhance their utilization as a functional ingredient in food systems. The purpose of this study was to examine the rheological characteristics of egusi seeds and obtain some information on their potential as a structuring agent in food systems.

2. Materials and methods

2.1. Source of materials and sample preparation

Egusi seeds (dehulled) were obtained from a market in Umuahia, Nigeria and used without further purification. Whole seeds were ground with a Scovill food processor, model 702-4 (Scovill Inc., Hamilton Beach, USA) to obtain the full-fat seed meal. Samples were analyzed as is (ground without sieving) since the material is predominantly used in this form by West Africans (especially Nigerians). Tests were conducted on the low-hull (≈10% hulls) egusi seeds since, on a commercial scale, these will probably be most representative of practical industrial egusi seeds. Proximate analysis (AOAC, 1990) of whole egusi kernels indicated a protein content of 29.1%, and 52.9% oil, 4.3% ash, 5.4% moisture and 8.3% total carbohydrate (determined by difference). For rheological analysis, dispersions of egusi kernel meal at protein concentrations of 3%, 6%, 10% and 20% (w/v) were tested. The protein levels were varied to identify the concentration that will be sufficiently high to produce properly crosslinked heat-induced networks. Dispersions of whole egusi kernel meal were prepared in distilled water (control) and 0.05, 0.15 and 0.5 M NaCl solutions. A pH of 7.2 was determined for egusi seed meal at room temperature and all samples were analyzed at this pH.

2.2. Rheology: assessment of network properties

The method described by Arntfield, Murray, and Ismond (1990) was used with some modifications. An advanced Rheometer 2000 (AR2000, TA Instruments) operated in a small amplitude oscillatory mode, was used to monitor egusi seed meal network formation during heating and cooling, and to characterize the rheological properties of resulting gels. The rheometer was equipped with 40 mm parallel (flat) plate geometry and a built-in automated sensitivity. Input strain amplitude for dynamic analysis was 0.02, a value found to be in the linear viscoelastic region in a previous experiment (Arntfield et al., 1990). This strain was used for all rheological measurements. Approximately 1 ml egusi seed meal dispersion was placed between parallel plates in the rheometer and the gap between the plates was set at 1 mm when the upper plate was lowered. To prevent drying, samples were covered with a solvent trap, sealed with a thin layer of paraffin oil (Mallinckrodt, Paris, Kentucky, USA; Lot No. 6358 KJPC) during the procedure. Samples were heated and cooled over a temperature range of 25–102 °C at 2 °C/min using a frequency of 1 Hz to minimize stress on the samples during network formation. Sample temperature was controlled by an automated circulating tap water system. Rheological data were collected at 2 min intervals with a thermal equilibrium time of 10 s. At the end of each phase (heating and cooling phase), the final temperature was held for 2 min. Frequency sweeps of the final gel were measured over a range of 0.1–10 Hz at 25 °C. The storage modulus (G') and loss tangent ($\tan\delta = G''/G'$) for representative frequency sweep curves are reported. Values at 1 Hz were recorded for comparison. The use of a single frequency (1 Hz) for data comparison has been reported (Arntfield et al., 1990; Cai & Arntfield, 1997). All analyses were conducted in duplicate and average values were calculated.

2.3. Calorimetry

The thermal properties of egusi seed meal were assessed to identify its denaturation temperature (T_d) and enthalpy of denaturation (ΔH), as well as to evaluate the effect of chosen environment (i.e., NaCl) on protein or macromolecule conformation prior to thermal gelation. Differential scanning calorimetry (DSC) analysis was conducted using a Dupont 9900 Thermal Analyzer (TA Instruments, DE) with a 910 Cell Base (Leger & Arntfield, 1993). Thermal curves were obtained using 10–15 μl of sample and a heating rate of 10 °C/min with an empty pan as reference. The sample was heated over a temperature range of 25–130 °C in a standard DSC cell that had been calibrated with both indium and sapphire standards. Each sample was analyzed in triplicate. Both the T_d (measure at the point of maximum heat flow) and ΔH were calculated using the General Analysis utility software (version 2.2) available for the instrument.

3. Results and discussion

3.1. Thermal characteristics of egusi seed meals

The denaturation of egusi seed meal was assessed by observing the effects of three NaCl concentrations on the thermal properties (T_d, ΔH). The influence of NaCl

concentrations on the thermal stability could be described as either stabilizing (causing T_d values to increase with increasing concentration) or destabilizing (causing T_d values to decrease). NaCl concentration significantly affected the thermal denaturation properties of egusi seed meals (Table 1). Samples prepared in the presence of NaCl had higher thermal denaturation temperatures than the control containing no salt ($T_d = 93.7$ °C). A trend was observed among the four samples; increased NaCl concentration led to higher T_d values. The increase in T_d with the inclusion of low NaCl concentration (0.05 M) can be attributed to non-specific ion effects on electrostatic interactions between charged groups on the protein and other macromolecules. This resulted in stabilization of the protein molecules against thermal denaturation. Differences in T_d values, in all four treatments, indicated that NaCl exerts ion-specific stabilizing or destabilizing effects at varied concentrations. The ΔH specifically describes the actual heat flow into the macromolecule during the thermal denaturation process. The greater the heat flow, the greater is the state of nativity known to exist in the egusi seeds before the heat treatment. Despite the increase in thermal T_d, the ΔH value for the egusi meal without NaCl ($\Delta H = 8.03$ J/g) was similar to those at 0.05 and 0.15 M NaCl series (Table 1), indicating no change in protein conformation at these salt levels. However, the ΔH value for egusi meal at the 0.5 M NaCl ($\Delta H = 3.69$ J/g) level was markedly lower than the sample prepared without salt (Table 1). Damodaran (1988) offered two possible explanations for this phenomenon. Although higher T_d values reflect increased resistance to thermal denaturation, the tertiary and quaternary structures of the stabilized protein may not be the same as that of the native molecule. Consequently, a lower ΔH can be obtained. On the other hand, the apparent decrease in ΔH values may be due to aggregation of denatured protein. Hence, the exothermic heat effect of such an aggregation process may partly offset the endothermic heat flow measured by DSC. Despite the fact that a high level of Cl^- is seen as destabilizing in this study, the T_d value was quite high ($T_d = 108.2$ °C). Similar results have been reported for soy globulin (Damodaran, 1988) where, in the presence of destabilizing anions, T_d values rose until the anion concentration reached 0.5 M and the destabilizing effect became more pronounced as concentrations were increased to 2–3 M.

3.2. Gelation properties of egusi seed meal

3.2.1. Influence of NaCl concentration

The rheological properties (G' and $\tan\delta$ values) of the final gels are presented in Table 1. The G' relates to the number of interactions or crosslinks within a gel system, while $\tan\delta$ gives an indication of the type of network where a lower value is more elastic. We observed differences in gel characteristics in all four treatments. The extent to which structure developed among the NaCl series was much more than that of the gel prepared without NaCl ($G' = 3252$ Pa). It appears the ability of egusi proteins to form gels in the presence of NaCl is related to the thermal denaturation temperature of the proteins when exposed to each salt concentration. As the T_d increases, structure development is enhanced (high G' values), and the resulting gel networks were superior in overall strength. This was observed when the NaCl concentration was increased from 0.05 M ($G' = 16,605$ Pa) to 0.15 M ($G' = 22,530$ Pa). At 0.5 M NaCl concentration, an increase in T_d resulted in a decrease in storage modulus ($G' = 8385$ Pa). According to Léger et al. (1993), as the T_d increases, structure development is delayed, and the resulting gel networks are inferior in overall strength and structure. Alternatively, the decrease in G' value at 0.5 M NaCl concentration may be due to excessive aggregation of denatured protein, resulting in lower gel strength. Tan δ values were similar in all four treatments, suggesting that the actual crosslinking pattern was similar in all the samples.

3.2.2. Effect of protein concentration

In rheological tests, the egusi protein concentration ranged from 3% to 20% (w/v). There is no information on the concentration at which egusi seed meal (a major thickening and gelling ingredient in most Nigerian dishes) can form gels. When the egusi seed meal dispersion was heat-treated (at 0.15 M NaCl and pH 7.2), a white slurry material was obtained at 3% (w/v) egusi protein, whereas a white opaque gel resulted at higher

Table 1
Effects of NaCl concentration on the thermal parameters (T_d, ΔH) and rheological characteristics (G', $\tan\delta$) of egusi seed meal gels (at 10%, w/v protein and pH 7.2)

NaCl level (M)	T_d[a] (°C)	ΔH[a] (J/g protein)	G'[a] (Pa)	Tan δ[a]
0.00	93.66 ± 0.57	8.03 ± 0.46	3252 ± 195	0.24 ± 0.009
0.05	97.25 ± 0.30	8.02 ± 0.31	16,605 ± 205	0.20 ± 0.001
0.15	100.49 ± 0.68	8.64 ± 0.61	22,530 ± 198	0.22 ± 0.001
0.50	108.15 ± 0.88	3.69 ± 0.15	8385 ± 185	0.20 ± 0.00

Rheological measurements were taken during frequency sweep (after the cooling cycle) at 1 Hz.
[a] Mean ± SD.

Table 2
Effect of protein concentration on the rheological properties of egusi seed meal gels (at 0.15 M NaCl and pH 7.2)

Protein (%, w/v)	G' (Pa)[a]	Tan δ[a]
3	36 ± 3	0.24 ± 0.021
6	8724 ± 535	0.28 ± 0.009
10	$22{,}530 \pm 198$	0.22 ± 0.001
20	$86{,}745 \pm 2355$	0.21 ± 0.004

Measurements were taken during frequency sweep (after the cooling cycle) at 1 Hz.
[a] Mean $\pm$ SD. For the plot of G' as a function of protein concentration; $R^2 = 0.975$.

protein concentrations (6–20%, w/v). The rheological characteristics of whole egusi seed meal, at varied protein concentration, are shown in Table 2. Increase in egusi protein concentration resulted in high G' values for all samples. This indicates increased crosslinking in egusi gels as the protein concentration increased. Very strong and elastic gel networks (high G' values) were produced at 10% and 20% (w/v) egusi protein levels (Table 2). The formation of elastic networks may be due to the electrostatic interactions between NaCl and the macromolecules (protein, lipid, polysaccharides) present in whole egusi seed meal. The rheological data showed that whole egusi seed meal can produce properly crosslinked ($G' = 8724$ Pa) networks at 6% protein concentration. Overall, tan δ values for egusi seed meal gels were unaffected by egusi protein concentration for all samples (Table 2), suggesting similar structural patterns for the four treatments.

3.3. Mechanisms for network formation

Temperature sweep curves in rheological tests for whole egusi seed meal helped to show the course of network formation (G' and tan δ) as a function of temperature. The gel network developed above the T_d in the heating phase, and continued in the cooling phase. Representative of temperature sweep curves were the effects of NaCl concentrations on the G' values of egusi seed meal gel at pH 7.2 and 10% (w/v) protein (Fig. 1(a)). Network formation (deviation from the baseline) started at $\approx$85 °C for all four treatments and G' values increased as temperature increased. The shapes of the curves were similar for all four treatments, indicating similar mechanisms for network development during heating. At the end of the heating phase, the sample treated with 0.5 M NaCl gave the lowest G' values, whereas the 0.15 and 0.05 M NaCl samples gave higher and highest values, respectively. Low G' values for the 0.5 M NaCl treatment may be attributed to a salt concentration effect. At 0.5 M NaCl, conformational changes may have impeded structure development during the early stages of gelation, resulting in fewer crosslinks and thus, low G' values. Alternatively, the

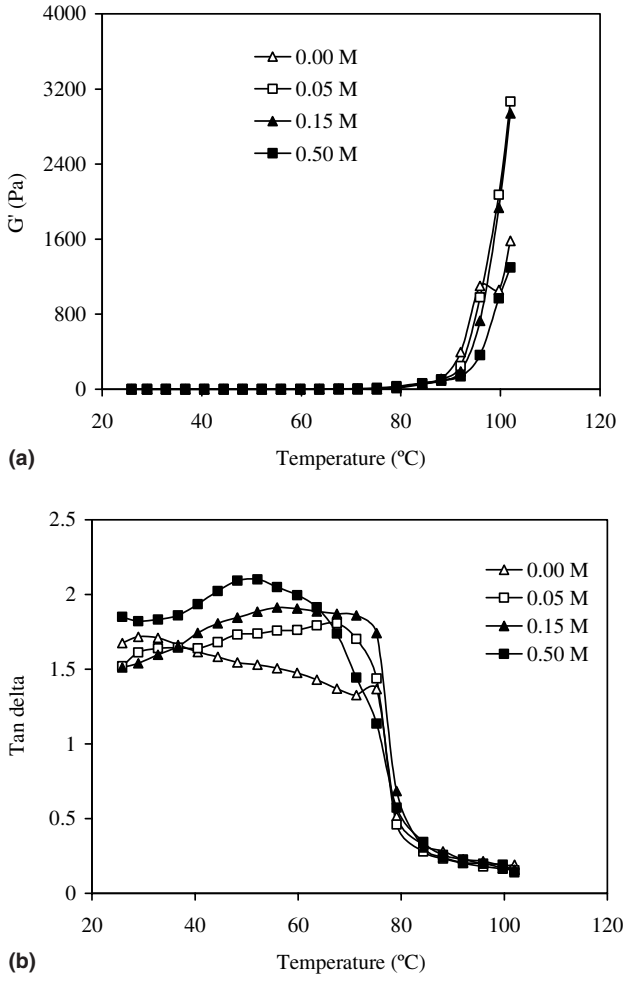

Fig. 1. Influence of NaCl concentration on the rheological characteristics, G' (a) and tan δ (b), of egusi seed meal gels (at 10%, w/v protein and pH 7.2) as a function of temperature during the heating regime.

decrease in G' value at high NaCl concentration may be due to excessive aggregation of denatured protein, resulting in inferior gel strength. The effects of NaCl concentrations (0.05, 0.15 and 0.5 M) on tanδ during the heating phase were compared (Fig. 1(b)). Tan δ values tended to be highly erratic before gel formation and the network appeared to be less elastic below 80 °C. The four treatments had lower tan δ values at higher temperature, indicating development of superior and more elastic gel network at higher temperature. It has been reported that hydrophobic interactions are favoured at high temperature (Leger & Arntfield, 1993). Therefore, the strong and elastic network obtained at high temperature may be due to this intermolecular force.

Data collected during the cooling phase are shown in Fig. 2. The NaCl series gave higher G' values than the control (0.0 M NaCl) sample. The 0.15 M NaCl sample produced the greatest increase in G' values, whereas the 0.0 M NaCl sample had the smallest increase. In the NaCl series, the 0.5 M NaCl sample had the lowest G'

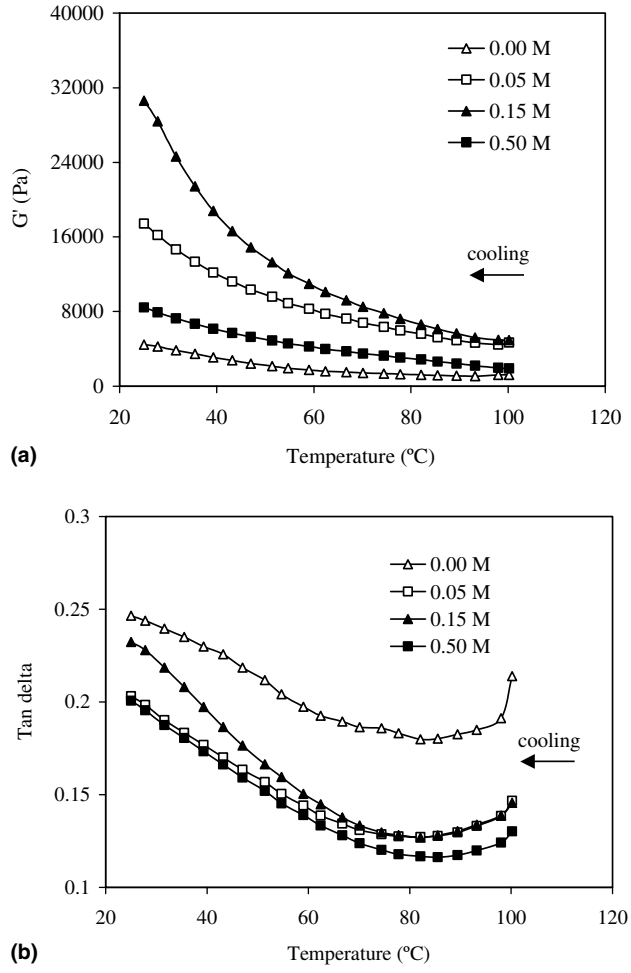

Fig. 2. Influence of NaCl concentration on the rheological parameters, G' (a) and tan δ (b), of egusi seed meal gels (at 10%, w/v protein and pH 7.2) as a function of temperature during the cooling phase.

values while the 0.15 M NaCl sample produced the highest G' values. Of note was the temperature at which the rate of change in G' increased. For the 0.5 M NaCl samples, the rate of change in G' increase occurred at ≈52 °C while that of the 0.05 and 0.15 M NaCl treatments occurred at ≈85 °C. Based on these results, it is evident that a higher NaCl concentration (0.5 M) extended the initial stage of structure formation. At higher concentrations, neutral salts (e.g., NaCl) are known to have ion-specific effects on hydrophobic interactions in addition to non-specific charge neutralization effects. These ion-specific effects on hydrophobic interactions are believed to arise from perturbations in bulk water structure, which affect protein–protein and protein–solvent interactions (von Hippel & Schleich, 1969). This phenomenon was observed at 0.5 M NaCl concentration in this study. In this respect, 0.5 M NaCl acted as a destabilizer of hydrophobic interactions, which resulted in binding of Cl^- ion to the protein molecule and created a net charge repulsion. Overall, 0.5 M NaCl may have prevented the formation of hydrophobic interactions, which in turn, prolonged the initial stage of gel formation and limited the extent of structure formation. The shapes of the cooling curves were similar (Fig. 2(a)) for all treatments, suggesting that all samples underwent similar gelation mechanisms. Similar shapes of curves suggest that the mechanism by which gelation occurred was not influenced by varied NaCl concentrations. Given that hydrogen bonding is favoured at low temperatures, the gradual increase in G' values during the final stage of gelation is attributed to this molecular force (Leger & Arntfield, 1993). Once the three-dimensional gel network was established, there was a solidification and/or stabilization of the network structure. This was reflected by the change in the slope of the cooling curves between 80 and 25 °C for 0.05 and 0.15 M NaCl-treated samples. Tan δ values showed similar behaviour (Fig. 2(b)). During the initial cooling phase (from 100 to 80 °C), tan δ values decreased, indicating a properly established elastic structure. Between 80° and 25 °C, tan δ values increased progressively, suggesting that the

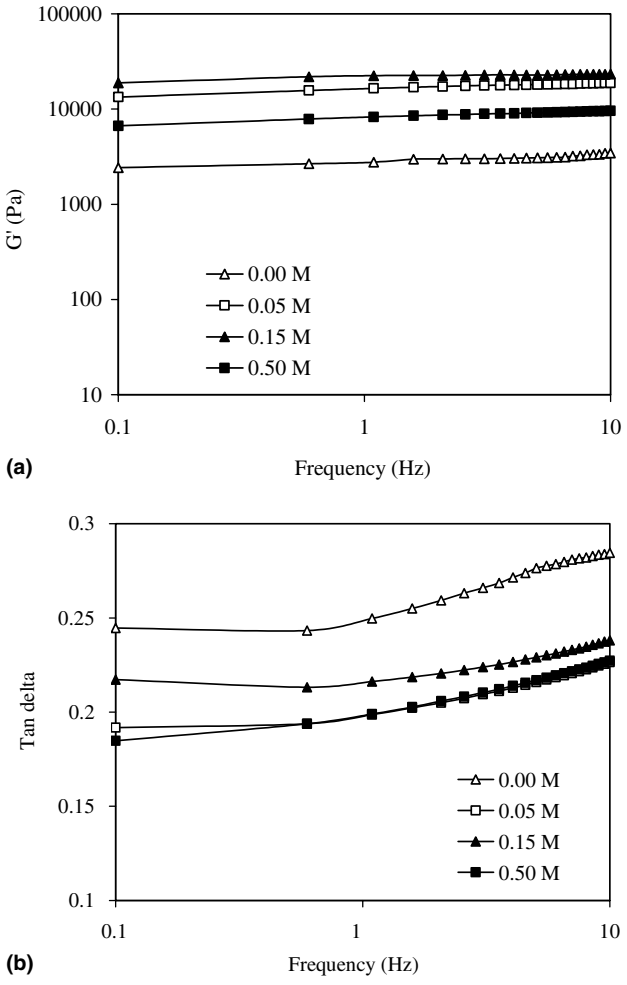

Fig. 3. The effect of NaCl concentration on the rheological properties, G' (a) and tan δ (b), of egusi seed meal gels (at 10%, w/v protein and pH 7.2) under a frequency sweep (after cooling).

samples appeared to lose their structural integrity. These results suggest that the forces responsible for stabilizing and/or strengthening the gel matrix in the mid to final cooling phase were disrupted. As a result, the structure of these samples disintegrated and produced gels with more fluid than elastic character. The 0.5 M NaCl sample had the lowest G' and tanδ values. Overall, the NaCl-treated samples had higher G' and lower tan δ values than the control (0.0 M NaCl) sample, indicating improved gel strength and elastic network structure in the presence of NaCl.

3.4. Network development after the cooling cycle

The frequency sweeps, in the rheological tests, examined final gel characteristics (gel produced after the cooling phase) of egusi seed meal networks. The frequency sweep data gave an overview of rheological behaviour as a function of oscillatory frequency. Representative frequency sweep curves for final gels of egusi seed meal (10%, w/v protein) treated with varied NaCl concentrations (0.05, 0.15, 0.5 M) at pH 7.2 are shown in Fig. 3. An almost linear relationship was observed between G' and the log of frequency (Fig. 3). Treating egusi seed meal with NaCl, produced gels with high G' and low tan δ values when compared to the gel without NaCl, which indicates that egusi seed meal gels had stronger and more elastic networks in the presence of NaCl.

4. Conclusions

Results of this study have demonstrated that whole egusi seeds can be used as a structuring ingredient in food systems, and this functionality can be modified by interactions with ingredients such as NaCl which is a typical food component. Egusi seed proteins may be the most important intrinsic factor that determines the gelation pattern of the meal; therefore, isolation of egusi seed proteins is currently under investigation.

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