



EFFECT OF CITRIC ACID ON PHYSICAL STABILITY OF SUNFLOWER OIL-IN-WATER EMULSION STABILIZED BY GELATINIZED BAMBARA GROUNDNUT FLOUR

Oladayo Adeyi*

Department of Chemical Engineering, Landmark University, Omu Aran, Kwara State, Nigeria

Daniel IO Ikhu-Omoregbe

Department of Chemical Engineering, Cape Peninsula University of Technology, Cape Town 7530, South Africa

Victoria A Jideani

Department of Food Technology, Cape Peninsula University of Technology, Cape Town 7530, South Africa

ABSTRACT

The influence of citric acid concentrations on the physical stability of sunflower oil-in-water emulsions (40 w/w% sunflower oil) stabilized by 7 w/w% bambara groundnut flour (BGNF) was investigated. Oil droplet sizes and emulsion microstructure were measured microscopically. Physical stability was studied using a vertical analyzer, Turbiscan MA 2000, by observing changes in backscattering flux (%) at 20°C. Citric acid significantly ($p < 0.05$) affected emulsion stability of BGNF-stabilized emulsion. Increased citric acid in the emulsion however, produced insignificant difference in droplet size and physical instability of BGNF-stabilized emulsions at all tested concentrations. The results indicated that the stability of BGNF-stabilized emulsion can be controlled and manipulated using citric acid. The result provided the necessary information needed to understand the influence of citric acid on the stability of BGNF-stabilized emulsions for product and process development

Keywords: Bambara groundnut, Oil-in-water emulsion, Physical stability, Citric acid, Sunflower oil

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1. INTRODUCTION

Oil-in-water (O/W) emulsions occur in many industrial processes and are the basis of many food products (Sun *et al.*, 2007; Dickinson and Golding, 1997) and a few examples include products like ice cream, low-fat spreads, and cream liqueurs (Dickinson and Golding, 1997). Oil-in-water emulsion manufacture requires intense energy in order to disperse the organic phase (oil) in continuous phase (water). Emulsification process can be achieved using different machines such as rotor-stator systems (Batista *et al.* 2006; Schwarz *et al.*, 2000; Ax *et al.*, 2003) and high-pressure homogenizer (Sun *et al.*, 2007; Floury *et al.*, 2000; Chanamai and McClements, 2000). Emulsions are however, thermodynamically unstable and have tendency to breakdown overtime (Friberg and Larsson, 1997). Some of the destabilization mechanisms prevalent in food emulsions are creaming/sedimentation, flocculation, coalescence and Ostwald ripening. Food emulsions can therefore be made kinetically stable by adding an emulsifier which keeps the dispersed phase suspended in a continuous phase. However, consumer's demand for more natural food products has made synthetic emulsifiers in food systems increasingly unpopular. Researches in food emulsion technology have therefore been directed towards finding natural emulsifiers and stabilizers of comparable and better functionalities to replace the existing synthetic emulsifiers and stabilizers.

Among the class of additives frequently added to improve the organoleptic properties of oil-in-water food emulsion products are the acidulants / acid regulators. This class of food additives controls the acidity or alkalinity for safety and stability of oil-in-water food emulsion products. Acidulants gives sharp tastes to food and also act as preservatives. Commonly used food acidulants are acetic acid (Sarkar *et al.*, 2009; Klinkesorn *et al.*, 2005; Zivanovic *et al.*, 2004), citric acid, lactic acid, malic acid and tartaric acid (Igoe, 2011) to mention just a few. However, food acidulants / acid regulators have tremendous effects on the physical stability of oil-in-water emulsion (Demetriades *et al.*, 1997). Other factors having profound influence on the food emulsion stability are emulsifier and oil phase concentration (Sun and Gunasekaran, 2009; Ibrahim and Najwa, 2012), homogenizer type and processing variable (Huck-Iriart *et al.*, 2011; Tantayotai and Pongsawatmanit, 2005) and additives such as sodium chloride salt (Tantayotai and Pongsawatmanit, 2005; Demetriades, *et al.*, 1997).

Several methods are available for emulsion stability / instability characterization. These include zeta-potential measurement which is determined by measuring the electrophoretic mobility of the dispersed particles in a charged field (Roland *et al.*, 2003), particle size determination of emulsion which could be by laser diffraction method (Agboola *et al.*, 1998; Lorenzo *et al.*, 2008) or image analysis (Zúñiga *et al.*, 2012; Payet, and Terentjev, 2008) and optical characterization of emulsion by vertical scan analyzer (Camino and Pilosof, 2011; Huck-Iriart *et al.*, 2011; Lemarchand *et al.*, 2003). Optical characterization has been used to identify and quantify destabilization mechanisms prevalent in an emulsion system. One of the mostly used and reported vertical scanners is Turbiscan M.A 2000 (Lemarchand *et al.*, 2003; Cerimedo *et al.*, 2010).

Bambara groundnut (BGN), an indigenous African legume with many fascinating properties has been reported to stabilize oil-in-water emulsion (Adeyi *et al.*, 2014; Adeyi *et*

al., 2016) and thus a potential natural stabilizing composition. BGN contained carbohydrate contents of 49 - 63.5%, protein content of about 15 - 25%, fat contents of about 4.5 - 7.4%, fiber content of 5.2 - 6.4, ash of 3.2 - 4.4 % and 2% mineral (Murevanhema and Jideani, 2013). It was reported to have great health significance. Oil-in-water emulsion containing 40 w/w% sunflower oil stabilized by 7 w/w% bambara groundnut flour (BGNF) was reported as the optimum formulation having the highest physical stability. However, since most food emulsions contain citric acid as acidulant or acid regulator in their recipe during formulation, it is necessary to investigate its compatibility with the BGNF and its effect on the characteristics of oil-in-water emulsion. Therefore the objective of the study was to investigate the effect of citric acid concentrations on the physical stability of oil-in-water emulsion stabilized with BGNF. This is necessary for the future adoption of BGNF as a natural emulsifier/stabilizer in food industries and for process and product development.

2. MATERIALS AND METHOD

2.1. Materials

Dried BGN seeds of brown variety were purchased from Triotrade Gauteng CC, South Africa. The seeds were washed, and dried at 50°C for 48 hrs by using cabinet drier (Model: 1069616). The dried seeds were milled into flour using a hammer mill and screened through 90 µm sieve to give BGNF. A commercial brand (Ritebrand) of 100% sunflower oil (SFO) purchased from a local supermarket was used without purification as the hydrophobic dispersed phase in this work. Milli-Q water was used in the preparation of all the emulsions. Food grade citric acid was purchased from a local store in Bellville, South Africa.

2.2. Emulsion preparation

Citric acid solution of various concentrations (0.5 - 6% (w/w)) were prepared and used to prepare the continuous phase of the emulsions. Emulsions were prepared from a dispersed phase and a continuous phase according to the method of Adeyi *et al.* (2014). The dispersed phase consisted of SFO and continuous phase was gelatinized BGNF dispersion containing various citric concentrations (0.5 - 6% (w/w)). Continuous phase was made by dispersing 7 g BGNF in 53 g of citric acid solutions. The resulting dispersions were gelatinized at a temperature of 84°C for 10 minutes with constant stirring. The resulting gelatinized BGNF dispersions (GBGNFD) were weighted in order to ascertain the amount of water loss during gelatinization. Water loss during gelatinization was compensated for by adding Milli-Q water to the GBGNFD, stirred and allowed to cool down to 20°C. SFO of 40 % (w/w) was added into the gelatinized BGNF. Emulsions (100 g) were made by homogenizing SFO and gelatinized BGNF at 20°C using an Ultra Turrax T-25 homogenizer (IKA, Germany) for 10 minutes at the speed of 11000 r/min.

2.3. Quantification of droplet sizes and distributions of emulsion by image analysis

Microstructure of the emulsions immediately after emulsion preparation was analyzed in terms of droplet size and droplet size distribution according to the method of Adeyi *et al.* (2014). Each emulsion was diluted with Milli Q-water at a ratio of 1:5 (w/w) in order to avoid overlapping and agglomeration of oil droplets which can affect further image analysis and processing. Droplet sizes were determined from the images of the oil-in-water emulsion obtained with a light microscope (Ken-A-vision TU-19542C, Ken-a-Vision Mfg Co. Inc., USA). Emulsion samples were poured onto microscope slides and covered with glass cover

slips and visualized using X40 objective lens. The microscope focus and the light intensity were carefully controlled and optimized in order to obtain the sharpest possible boundaries between the oil-droplets and the surrounding GBGNFD. The images were captured with a digital camera mounted on the microscope. Image processing and further analysis was carried out using public domain software image J v1.36b (Caubet *et al.*, 2011; Perrechil and Cunha, 2010). The diameters of the oil droplets were measured one by one by an operator (Tcholakova *et al.*, 2004). A substantial number of droplets ($N = 1000$) were counted in order to obtain statistical estimate of the oil-droplet diameters and oil droplet size distribution in each sample. Droplet size distributions were generated by grouping the droplets into classes belonging to a common interval. Droplet size frequency distributions were computed using MS-Excel (MicrosoftTM Excel 2007) (Bellalta *et al.*, 2012). Oil-droplet sizes were obtained in terms of volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter ($d_{4,3}$). The volume-surface mean diameter ($d_{3,2}$) and equivalent volume-mean diameter, $d_{4,3}$ were calculated using Eq. (1) and (2) respectively.

$$d_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (2)$$

Where n_i is the number of droplets with diameter d_i (μm).

2.4. Optical characterization of emulsion stability

The stability of oil-in-water emulsions stabilized with BGNF was monitored using Turbiscan MA 2000 (Formulation, France) according to the method of Adeyi *et al.* (2014). BGNF stabilized emulsion (6 mL) were introduced in a cylindrical glass cell and inserted into Turbiscan MA 2000. The optical reading head of the machine scanned the whole length of the sample and acquired both the transmission and backscattered data every 40 μm and 30 minutes for 6 hr. The transmission and backscattering curves generated provided transmission and backscattered light flux in percentage (%) relative to the internal standard of the machine as a function of sample height. Both the transmission and backscattering fluxes were dependent on the particle mean diameter, d , and volume fraction ϕ of the particles according to the Eq.s (3), (4) and (5), (6), respectively (Camino and Pilosof, 2011).

$$T = T_o e^{-\frac{2r_i}{l^*}} \quad (3)$$

$$l^* = \frac{2d}{3\phi Q_s} \quad (4)$$

$$BS = \frac{1}{\sqrt{l^*}} \quad (5)$$

$$l^* = \frac{2d}{3\phi(1-g)Q_s} \quad (6)$$

Where T , T_o , r_i , l^* , d , ϕ , BS are transmitted fluxes, transmittance of the continuous phase, measurement cell internal radius, photon mean free path, particle mean diameter, particle volume fraction, backscattered flux respectively Q_s and g are optical parameters given by Mie theory. The analysis of the emulsion stability was carried out as a variation of backscattering profiles over time because of the opaque nature of the emulsion nil transmission flux. The stability or instability of the dispersion was observed and evaluated by conducting repeated multiple scans overtime, each one providing a curve and all curves were overlaid on one graph to show stability or otherwise of the dispersion over time.

3. DATA ANALYSIS

IBM Statistical Package for the Social Science (IBM SPSS, version 22) was used for data analysis. The results were subjected to multivariate analysis of variance (MANOVA) to determine mean differences between treatments and Duncan's multiple range tests was conducted to separate mean differences where differences exist. Results were expressed as mean $\pm$ standard deviation.

4. RESULTS AND DISCUSSION

4.1. Effect of citric acid on droplet size distribution

Figure 1 presents the oil-droplet size distribution of optimum BGNF emulsion (7% (w/w) BGNF and 40% (w/w) SFO) as affected by various concentrations of citric acid. Citric acid had a notable effect on the oil droplet size distributions of the emulsions. Franco *et al.* (2000) also reported observable effect of pH and previous protein thermal treatments on the droplet size distribution of pea protein stabilized oil-in-water emulsion.

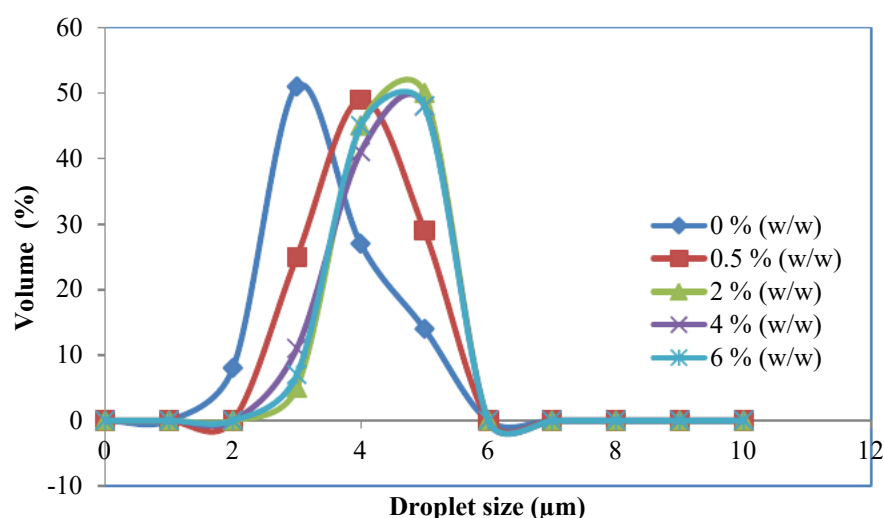


Figure 1 Particle size distribution of emulsions whose BGNF matrix contained various concentrations of citric acid

Although they all have closely comparable height, the oil droplet distribution curve widths of BGNF emulsions with citric acid shifted a bit to the right when compared with the BGNF emulsion without citric acid. All the curves of emulsions containing citric acid, irrespective of concentration were closely related. The presence of citric acid in the BGNF emulsions increased the oil-droplet size relative to the emulsion without citric acid. Table 1 compares the droplet size of the emulsions in terms of volume surface mean diameter ($d_{3,2}$) which provided information regarding where most oil particle fell (Adeyi *et al.*, 2014) and equivalent volume-mean diameter ($d_{4,3}$) which is related to changes in droplet size involving destabilization process (Camino and Pilosof, 2011). Both the $d_{3,2}$ and $d_{4,3}$ of the emulsions depended on the concentrations of citric acid.

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Table 1 Effect of citric acid concentration on the particle size ^{1, 2}

Citric acid concentration (%) (w/w))	d _{3,2} (μm)	d _{4,3} (μm)
0	3.45 ± 0.10 ^a	3.66 ± 0.11 ^a
0.5	3.85 ± 0.98 ^b	3.94 ± 0.59 ^b
2.0	4.03 ± 0.84 ^{bc}	4.06 ± 0.56 ^{bc}
4.0	4.02 ± 0.42 ^{bc}	4.06 ± 0.42 ^{bc}
6.0	4.19 ± 0.28 ^c	4.23 ± 0.14 ^c

¹ Mean values with different letters within the same column are significantly different from each other ($p < 0.05$).

² d_{3,2} refers to the volume surface mean diameter of the emulsions; d_{4,3} is the equivalent volume-mean diameter of the emulsions.

The d_{3,2} and d_{4,3} ranged between 3.45 – 4.19 μm and 3.66 – 4.23 μm, respectively. The oil droplet sizes of emulsion without citric acid were significantly different from emulsions with citric acid. The smallest and largest d_{3,2} and d_{4,3} were found in emulsions without citric acid and 6% (w/w) respectively. Citric acid is an acidulant and has been earlier reported as an agent for adjusting the pH of various systems including emulsions (Solowiej, 2007; Miquelim *et al.*, 2010; Taherian *et al.*, 2007). Contrary to the observations above, Franco *et al.* (2000) reported significant decrease in oil-droplet size with increase in pH up to emulsion pH close to protein isoelectric point for pea protein stabilized emulsion. Chanamai and McClements (2002) also reported that the droplet sizes of gum Arabic and modified starch stabilized emulsions were insensitive to acid within pH range of 3 - 9. The difference in our results with other researchers on the influence of pH on particle size may probably be connected to the method used for the incorporation of citric acid into the emulsion system and some physicochemical properties of BGNF. Like other organic acids, citric acid might have hydrolysed and changed the molecular conformation of the BGNF (Majzoobi and Beparva, 2014) during continuous phase preparation and this could have decreased the matrix strength necessary for emulsion formation.

4.2. Effect of citric acid on the microstructure

Figure 2 presents the photomicrographs of freshly prepared emulsions formed with BGNF matrix containing various concentrations of citric acid.

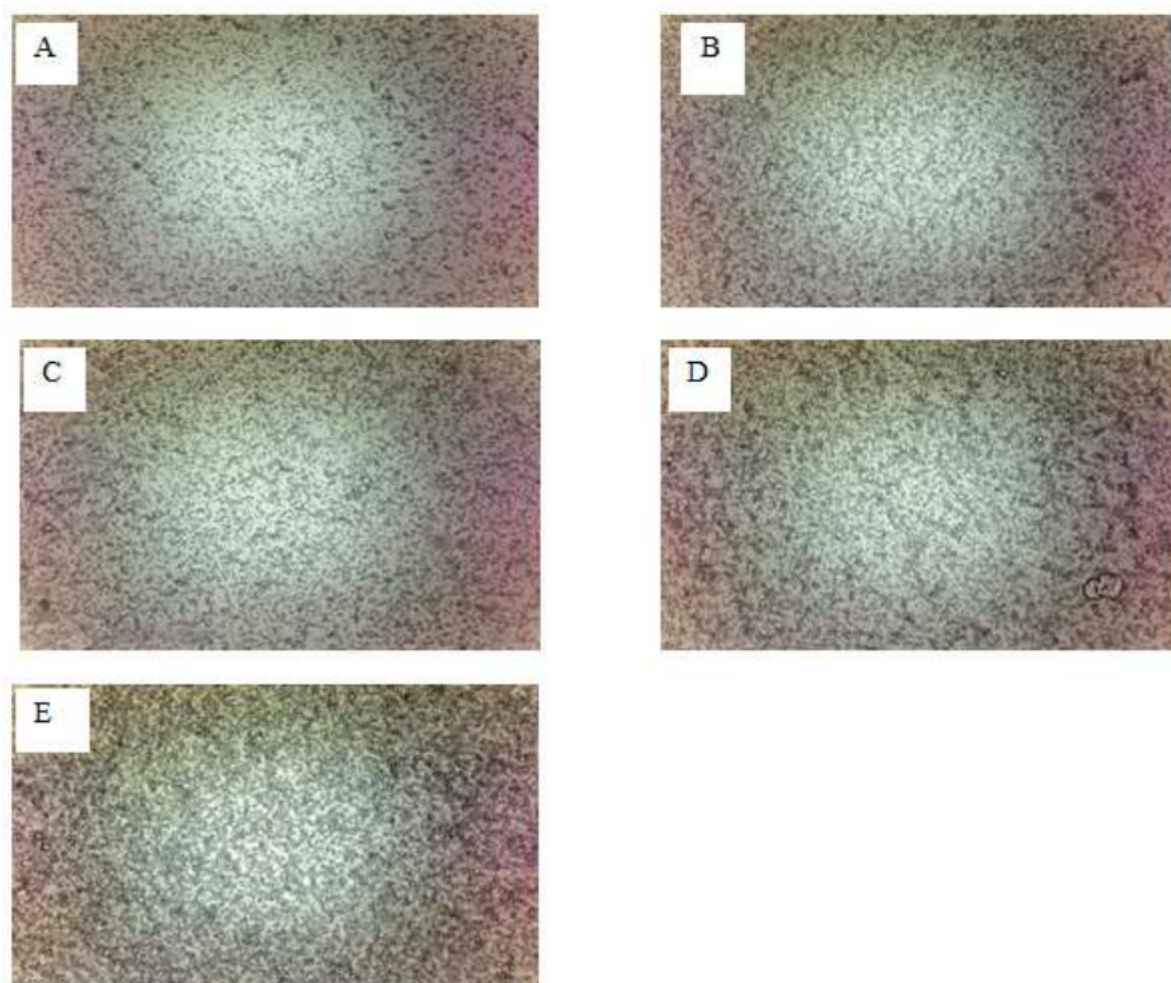


Figure 2 Photo micrographs of emulsions formulated with 7% (w/w) BGNF and 40% (w/w) SFO containing citric acid at concentrations of (A) 0%(w/w) (B) 0.5% (w/w) (C) 2% (w/w) (D) 4% (w/w) (E) 6% (w/w)

The figure compares the emulsion forming characteristics of BGNF matrix containing various concentrations of citric acid as well as also the influence of citric acid on the matrix-droplet and droplet-droplet interactions. The oil-droplet sizes of the emulsion with citric acid however showed some similarities and differences which could therefore be indicative of levels of the changes caused by citric acid concentrations in the molecular structure of BGNF. Figure 2 shows that all the emulsions were made up of evenly dispersed small spherical oil droplets surrounded by continuous phase BGNF matrix, irrespective of the concentrations of citric acid. The presence of citric acid at all concentrations in the BGNF dispersion during continuous phase gelatinization did not seem to greatly affect the emulsion forming properties of the resulting BGNF matrix. When compared with the BGNF matrix without citric acid, matrix with citric acid had comparable emulsion forming ability. As can be seen in the figure, there was no serious flocculation of the oil-droplets and the phenomenon of oil-droplet aggregations were not different in both the emulsion with and without citric acid.

4.3. Effect of citric acid on the storage stability of emulsion

Figures 3 and 4 present the stability of BGNF emulsions without and with citric acid at various concentrations (0 – 6% (w/w)). The graphs are the normal and reference modes of

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Turbiscan profiles of emulsions scanned at a regular interval of 30 minutes for 360 minutes at 20 °C. The reference modes of the Turbiscan graphs were placed right of the normal modes in Figs 3 and 4 and were constructed relative to the initial or the first scan. The initial scans of all the emulsions were assigned a value of 0% when constructing the reference mode and can be visualized at the ordinate of the normal turbiscan mode. The initial backscattering flux (BS_{AV0} (%)) provided the information regarding the structure of the freshly prepared emulsions and it is dependent on the oil droplet numbers. The more numerous the oil droplets in an emulsion the greater the backscattered light and hence the higher the backscattering flux. Since all the emulsions contained fixed amounts of SFO and BGNF, the information regarding the effect of various concentrations of citric acid in the BGNF matrix on their respective emulsion forming ability can be obtained from BS_{AV0} (%).

Table 2 presents BS_{AV0} (%) of the emulsions formed by BGNF matrix containing citric acid in the range of 0 – 6% (w/w). The mean of BS_{AV0} (%) was between 95.21 and 90.08 % with the highest and lowest values belonging to emulsion without citric acid and emulsion whose BGNF matrix contained 6% (w/w) citric acid respectively. All the emulsions with citric acid showed closely related BS_{AV0} (%) which is an indication that the presence of citric acid at all studied concentrations in the BGNF matrix affected the emulsion forming abilities in a similar way. Although the result indicated a significant difference between the emulsion without citric acid and emulsions containing citric acid, there seemed not to be much observable difference in their photomicrographs. The presence of citric acid in the BGNF dispersions during gelatinization caused little impediment to polymer network formation even at high concentration of 6% (w/w) and has consequently affected the matrix strength mildly.

The result of the Turbiscan reference mode showed the various destabilization mechanisms which characterized emulsions with and without citric acid. There were no observable differences between the graphs of emulsion with and without citric acid. There were peaks between 0 - 10 mm region and notable increases and decreases in the backscattering flux (%) along the entire tube length of all the Turbiscan profiles which was indicative of possible creaming and particle aggregation phenomenon, respectively. Particle aggregation phenomenon showed as decrease and increase in backscattering flux depending on the oil droplet sizes in an emulsion system.

Table 2 Effect of citric acid concentration on Initial backscattering value¹

Citric acid concentration (%) (w/w))	Initial backscattering flux (%)
0	95.21 ± 0.01 ^a
0.5	90.94 ± 1.19 ^b
2.0	90.80 ± 0.69 ^{bc}
4.0	90.21 ± 0.11 ^{bc}
6.0	90.08 ± 0.07 ^c

¹Mean values with different letters within the same column are significantly different from each other ($p < 0.05$)

Decrease in the backscattering flux was as a result of an increase in the oil-droplet size which correspondingly caused the mean path of photon (l^*) to increase because of an increase in the average distance between the oil-droplets (Celia *et al.*, 2009). This relationship between the backscattering flux and the oil-droplet size variation is presented in Eqs (5) and

(6) according to Mie theory. However, if the oil-droplet size is smaller than the wavelength of the light source and is increasing by flocculation or coalescence, then the backscattering flux can identify an increase and this is called Raleigh diffusion (Park *et al.*, 2010). Therefore, the more the oil droplets sizes increased the higher the backscattering flux in the Raleigh diffusion zone (Park *et al.*, 2010). No substantial information was obtained for creaming phenomenon as the migration rates obtained for all the emulsions were zeros. This is an indication that the droplet movement was very minimal within the time frame of study. In addition, no physical separation was observed with the naked eye.

Figure 5 showed the oil-droplet aggregation kinetics obtained in the middle of the Turbiscan tube (zone 20 - 40 mm) and was reported in the Turbiscan MA 2000 reference mode. It was expected that the graphs in Fig. 5 should fall below the zero line if the subsequently scanned profiles decreased with time of scanning relative to the first scan (Mie diffusion zone). However, some of the graphs were higher than the zero line which is an indication that successive scans increased relative to the first scan (Raleigh diffusion zone). The graphs compared the influence of citric acid at various concentrations on the oil droplet aggregation phenomenon. The farther the graphs from the origin, the less stable the emulsion. Figure 5 showed that citric acid concentrations had different effects on the droplet aggregation kinetics. No meaningful conclusion can however, be drawn on the trend of increase or decrease with citric acid concentrations. The oil-droplet aggregation kinetics was marked with high standard deviations which did not allow any valid conclusions within the time frame of study. The destabilization velocity of oil-in-water emulsion was strongly dependent on the droplet size and concentration (Perrechil and Cunha, 2010). The unresolved stability behaviour of all the emulsions containing citric acid may be as a result of the similar manner it has affected the microstructure of the emulsion. The results of the oil -droplet distribution, image analysis and initial backscattering flux showed that citric acid had produced similar characteristics at all concentrations.

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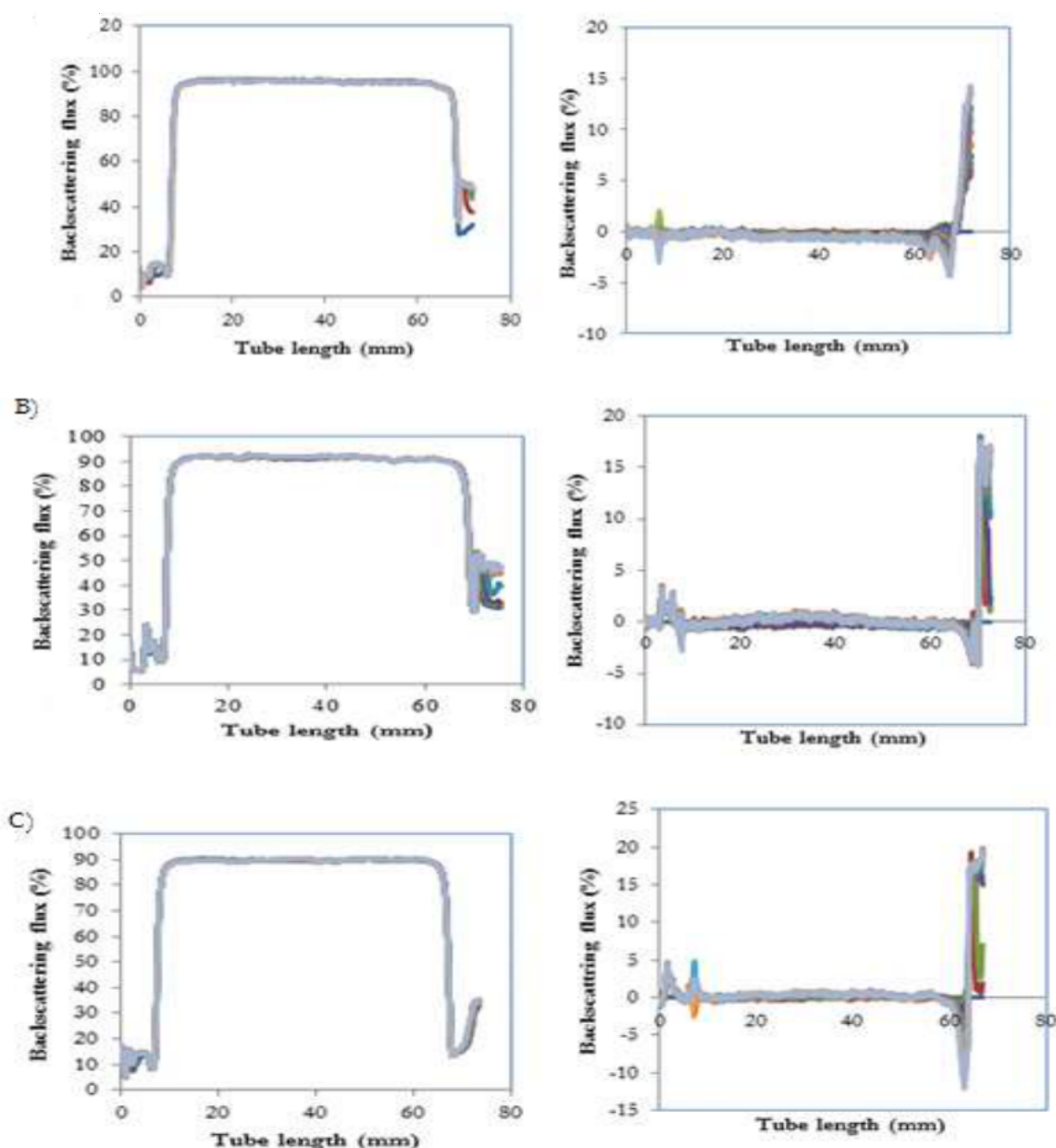


Figure 3 Changes in the backscattering profile (BS%) as a function of sample height with storage time of BGNF (7% (w/w)) stabilized emulsions containing citric acid at (A) 0% (w/w) (B) 0.5% (w/w) (C) 2% (w/w)

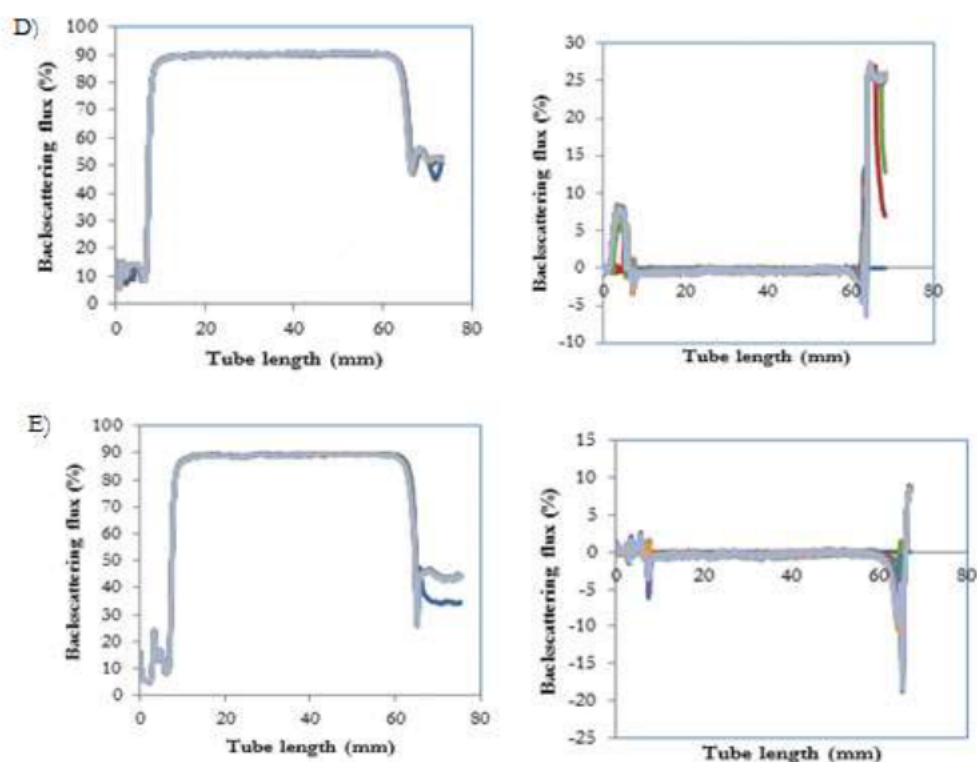


Figure 4 Changes in the backscattering profile (BS%) as a function of sample height with storage time of BGNF (7% (w/w)) stabilized emulsions containing citric acid at (D) 4% (w/w) (E) 6% (w/w)

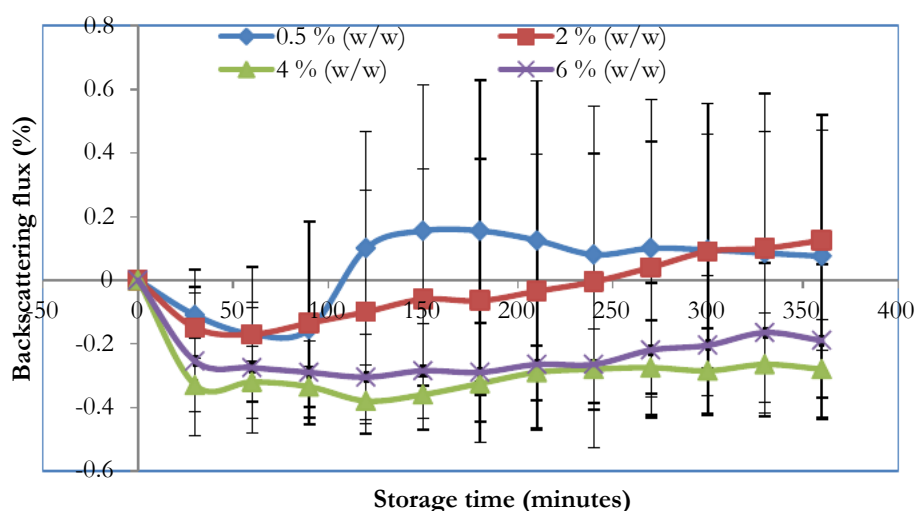


Figure 5 Effect of citric acid on backscattering in the 20-40 mm zone at 20°C

The equilibrium backscattering flux is detailed in Fig. 6 and it provides information regarding the influence of citric acid on the emulsion stability at the equilibrium time. The graph was generated by plotting the backscattering flux attained at the equilibrium studied time (360th minute) against the citric acid concentrations. It was expected that a stable formulation will be very close to the origin at the 360th minute. A third order polynomial was found to describe the effect of citric acid concentrations on emulsion stability with high coefficient of determination. Although the graph was marked with high standard deviations,

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the mean of the backscattering flux at the 360th minute showed that emulsion containing 0.5% (w/w) citric acid was marginally better.

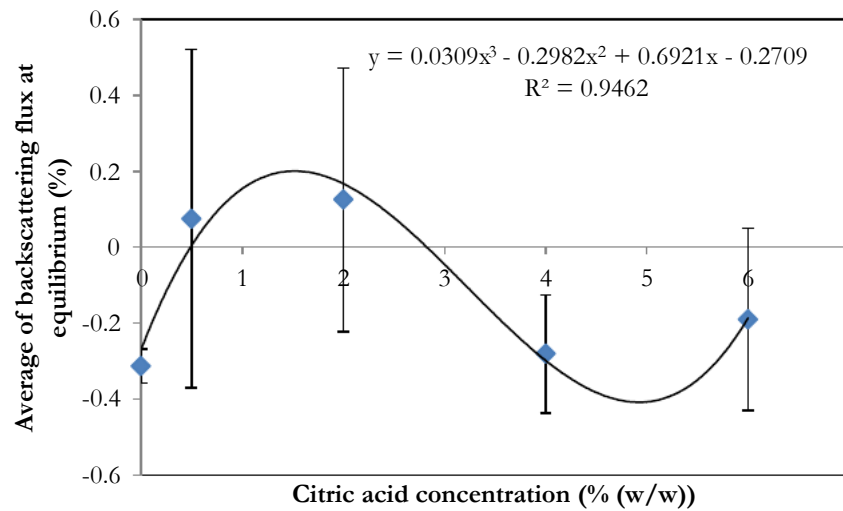


Figure 6 Effect of citric acid on emulsion stability (Average backscattering flux at equilibrium state)

5. CONCLUSION

Citric acid affected the stability of oil-in-water emulsion stabilized with BGNF. The effect at all concentrations had produced very similar effects on the emulsion forming ability of gelatinized BGNF dispersion. The initial backscattering showed that comparable concentrations of the droplets were formed by all the gelatinized BGNF dispersions containing citric acid. And this was strongly supported by the results of oil droplet size and microscopic analysis of the emulsions formed by citric acid containing gelatinized BGNF dispersions. Citric acid had affected the matrix strength and droplet-droplet interaction of emulsions comparably. Citric acid weakened the BGNF matrix and reduced the strength.

5.1. Nomenclature

T	transmitted fluxes (%)
BS	backscattered flux (%)
T_0	transmittance of the continuous phase
r_i	internal radius of the measurement cell
l^*	photon mean free path
d	particle mean diameter
Q_s and g	optical parameters given by Mie theory.
Φ	particle volume fraction,
d_i	diameter of oil droplets (μm).
n	number of oil droplets with diameter d_i
$d_{3,2}$	volume-surface mean diameter (μm)
$d_{4,3}$	equivalent volume-mean diameter (μm)
BS_{AVO}	Initial backscattering flux (%)
BGNF	bambara groundnut flour

GBGNF gelatinized bambara groundnut flour
SFO Sunflower oil

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