# EFFECT OF VINEGAR ON THE STABILITY OF SUNFLOWER OIL-IN-WATER EMULSION STABILIZED BY GELATINIZED BAMBARA GROUNDNUT FLOUR

**[1,2*]Adeyi, O., [1]Ikhu-Omoregbe, D. and [3]Jideani, V. A.**

*[1]Department of Chemical Engineering, Cape Peninsula University of Technology, Cape Town 7530, South Africa*

*[2]Department of Chemical Engineering, Landmark University Omu-Aran Kwara State, Nigeria*

*[3]Department of Food Technology, Cape Peninsula University of Technology, Cape Town 7530, South Africa*

*Corresponding author: e-mail: adeyioladayo350@yahoo.com

## *ABSTRACT*

*The influence of vinegar 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. Vinegar significantly affected ($p < 0.05$) emulsion stability of BGNF-stabilized emulsion. Vinegar at all studied concentrations in the emulsion increased droplet size and physical instability of BGNF-stabilized emulsions. The results indicated that the stability of BGNF-stabilized emulsion can be controlled and manipulated using vinegar. The result provided the necessary information to understand the influence of vinegar on the stability of BGNF-stabilized emulsions for product and process development.*

***KEYWORDS: Bambara, emulsion, groundnut, stability***

## 1.0 INTRODUCTION

Emulsions are a class of dispersed systems that consist of two immiscible liquids, with one of the liquid dispersed as small droplets in the other called continuous phase (McClements, 1999). One of the important properties of emulsion is its stability. Emulsion stability is the ability to resist changes in the properties over time and it is a major parameter that determines the shelf-life of food emulsions. Food emulsions can be destabilized by phenomena like creaming, droplet flocculation, coalescence and Ostwald ripening or combination of two or more phenomena. Food emulsions can therefore be made kinetically stable by adding emulsifier (s) and/or stabilizer (s) which keep the dispersed phase suspended in a continuous phase. The unending demand for natural products by the consumers and increasing legislations for safe as well as healthy food by governments has however made synthetic emulsifiers and stabilizer in food systems increasingly unpopular.

The nutritional composition of bambara groundnut (BGN) indicates its potential as a natural food emulsifier/stabilizer and gelatinized bambara groundnut flour dispersion (BGNF) has been reported to stabilize sunflower oil-in-water emulsion (Adeyi *et al*., 2013). BGN is an underutilized African legume which belongs to the family fabaceae. 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 has also been reported to have great health significance (Murevanhema and Jideani, 2013). The high percentage of protein and carbohydrate contents as well as the probable protein-carbohydrate interactions during gelatinization and emulsion preparation made BGN to have high potential as main emulsifier/stabilizer in food emulsions.

Sunflower oil-in-water emulsion stabilized by gelatinized BGNF is an emulsion system that may find applications in food industries because of its numerous desirable properties (Adeyi *et al*., 2013). However, since most food emulsions contain vinegar (either as preservative or taste enhancer) as one of their major additives during formulation, it is therefore necessary to investigate the influence of vinegar on the properties of sunflower oil-in-water emulsion stabilized by gelatinized BGNF to get a better understanding for product and process development in food industries. Therefore the objective of this study was to investigate the effect of vinegar on the stability of sunflower-oil-in-water emulsion stabilized by gelatinized BGNF. This is necessary for the future adoption of BGN as stabilizer in food industry.

## 2.0 MATERIALS AND METHOD

### *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 vinegar was purchased from a local store in Bellville, South Africa.

### *Emulsion preparation*

Milli-Q water containing various vinegar concentrations (0.5 – 8% (w/w)) were used for continuous phase preparations of emulsions. Emulsions were prepared from a dispersed phase and a continuous phase according to Adeyi *et al.* 2013. The dispersed phase consisted of SFO and continuous phase was gelatinized BGNF dispersion containing various vinegar concentrations (0.5 – 8% (w/w)). Continuous phase was made by dispersing 7 g BGNF in 53 g of vinegar 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) were 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.

### 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. 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). 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 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 v1.36 b (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 (Microsoft™ 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 equation (1) and (2), respectively.

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

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

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

### Optical characterization of emulsion stability

The stability of oil-in-water emulsions stabilized with BGNF was monitored using Turbiscan MA 2000 (Formulaction, France). 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 ɸ of the particles according to the equations (3), (4) and (5), (6), respectively(Camino and Pilosof, 2011).

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

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

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

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

Where $T,\ T_o\ ,\ r_i, l^*,\ d,\ \phi,\ BS$ 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.

## 3.0 RESULT AND DISCUSSION

### Effect of Vinegar on droplet size distribution

Figure 1 shows the oil-droplet size distribution of the emulsion (7% (w/w) BGNF and 40% (w/w) SFO) containing various concentrations of vinegar (0 - 8% w/w). All the oil droplet distributions presented a similar Gaussian shape. The presence of vinegar at various concentrations affected the oil droplet distribution curves relative to emulsion without vinegar. Similar observation was reported by Romero *et al.* (2009) on the effect of pH on oil droplet distribution of highly concentrated oil-in-water crayfish flour-based emulsions. The influence of vinegar on the droplet size distribution of emulsions whose BGNF matrix contained vinegar in the range of 0.5 - 8% (w/w) were similar as there was no much difference in the oil-droplet distribution curves. When compared with the distribution of emulsion without vinegar, the oil droplet size distribution curves of all other emulsions with vinegar, irrespective of concentration, have shifted to the right and broader indicating an increase in oil-droplet size (Romero *et al.*, 2009).

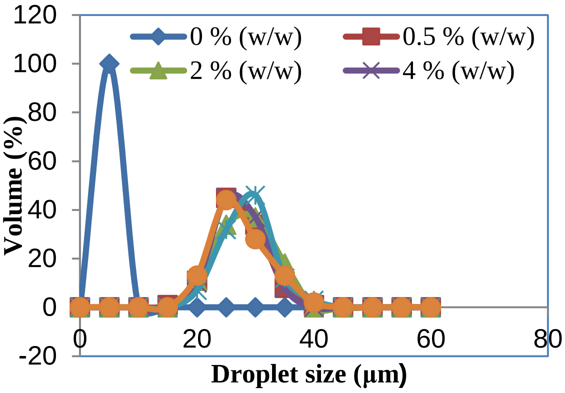

***Figure 1. Droplet size distribution of dispersed phase particles in emulsion formulated with 7% (w/w) BGNF and 40% (w/w) SFO containing vinegar at various concentrations***

Table 1 is the particle size volume surface mean diameter ($d_{3,2}$) and the equivalent volume mean diameter ($d_{4,3}$) of the emulsions with and without vinegar measured immediately after emulsion preparation. The $d_{3,2}$ gives information regarding size of the emulsion where most oil-droplet fall while $d_{4,3}$ is a measure of changes in oil-droplet size involving emulsion destabilization process.

*Table 1 Effect of vinegar on particle size*[1,2]

| Conc of Vinegar (% w/w) | $d_{3,2}$(µm) | $d_{4,3}$(µm) |
|---|---|---|
| 0 | 3.45 ± 0.10$^{a}$ | 3.66 ± 0.11$^{a}$ |
| 0.5 | 26.19 ± 0.86$^{bc}$ | 26.90 ± 0.42$^{bc}$ |
| 2.0 | 27.10 ± 0.49$^{bc}$ | 27.70 ± 0.54$^{cd}$ |
| 4.0 | 25.86 ± 0.23$^{b}$ | 26.39 ± 0.29$^{b}$ |
| 6.0 | 27.62 ± 0.91$^{c}$ | 28.35 ± 0.64$^{d}$ |
| 8.0 | 26.37 ± 0.46$^{bc}$ | 27.16 ± 0.52$^{bc}$ |

[1] *Values are means ± standard deviations; Means 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 mean of oil-droplet size ranged from 3.45 to 27.62 µm and 3.66 to 28.35 µm for $d_{3\ 2}$ and $d_{4,3}$ respectively as affected by vinegar concentrations (0 to 8 % (w/w)). The influence of vinegar in the BGNF matrix was deleterious to emulsion as its presence at all studied concentrations increased the droplet sizes of the resulting oil-in-water emulsion relative to BGNF matrix without vinegar. Vinegar is a sour tasting liquid which contained acetic acid as its main component. Vinegar at various concentrations had been incorporated into the BGNF during continuous phase preparation by gelatinizing BGNF dispersions with vinegar prior to emulsification process. The presence of vinegar during the gelatinization of BGNF dispersion must have weakened the strength of the BGNF matrix responsible for emulsion formation. Ohishi *et al.* (2007) reported a decrease in viscosity of rice starch and flour pastes when gelatinized with acetic acid. The authors attributed decreased viscosity to loosening or destruction of the structures during gelatinization with acetic acid. Generally, there appeared to be no trend on the oil droplet sizes ($d_{3,2}$and $d_{4,3}$) as can be seen in Table 1. However, the presence of vinegar as low as 0.5 % (w/w) increased the oil droplet sizes more than seven time relative to the emulsion without vinegar. There was a significant difference ($p<0.05$) between the particle sizes of emulsions without and with vinegar. Some of the emulsions containing vinegar however showed significant differences in droplet sizes while others were not significantly different. The differences and similarities in particle sizes could be as a result of different levels at which vinegar at various concentrations caused BGNF structural loosening.

### Effect of vinegar concentration on microstructure

Figure 2 shows the microstructures of emulsions whose BGNF matrix contained vinegar at various concentrations. The images were obtained immediately after emulsion preparation and thus showed the influence of vinegar on the emulsion forming ability of BGNF matrix. The micrographs showed that vinegar has a negative effect on the ability of the BGNF matrix to form emulsions. The micrographs of emulsions presented relatively larger particles with some fewer smaller oil droplets which indicated a polydispersed system.

Creaming and oil droplet aggregation phenomena in an emulsion system have been explained to depend on the oil-droplet size and distribution of such systems. Emulsions of high polydispersity are more prone to creaming due to differential creaming speeds of individual large and small droplets (Nor Hayati, 2009). The presence of vinegar in the BGNF matrix at all studied concentrations had produced emulsions with bigger droplet sizes relative to the matrix without vinegar as can be visualized in the micrographic images. This probably was because of the weakened film strength of the BGNF matrix containing vinegar relative to the matrix without the vinegar. The weakening of the film strength during gelatinization might probably have been a result of the hindrance posed by vinegar to form polymer network of required strength necessary for emulsion formation and stabilization. The microstructures of the emulsions with vinegar were however difficult to differentiate micro graphically. This is an indication of comparable influences of vinegar at all studied concentrations on the emulsion forming ability of BGNF polymers

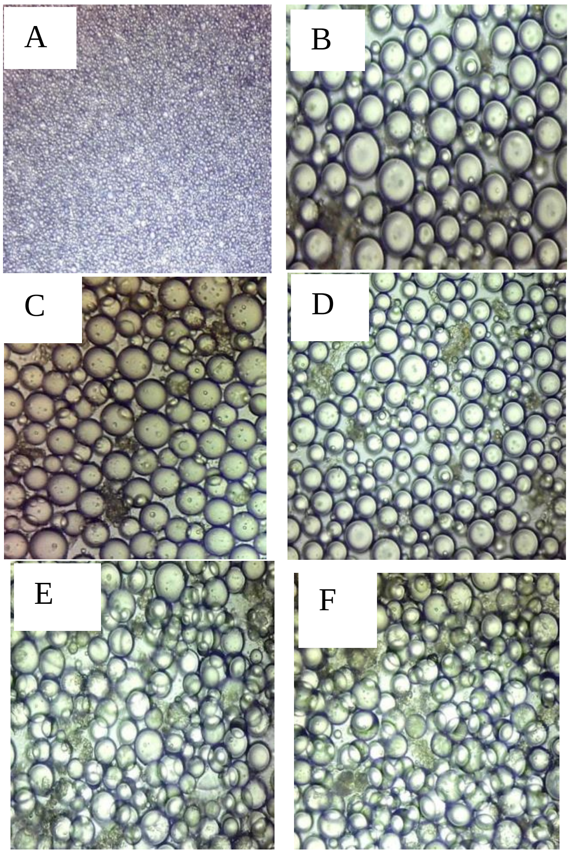

***Figure 2: Micrographs (X 40 magnifications) of emulsions containing vinegar at (A) 0 % (w/w) (B) 0.5 % (w/w) (C) 2 % (w/w) (D) 4 % (w/w) (E) 6 % (w/w) (F) 8 % (w/w)***

### Effect of vinegar concentration on the storage stability of emulsions

Figures 3 and 4 are the Turbiscan profile (physical stability graph) of the BGNF emulsions with vinegar concentration of 0, 0.5 and 2% (w/w) and 4, 6 and 8 % (w/w) respectively scanned at 30 mins interval for a period of 360 minutes at $20^{o}$C. The emulsion stability curves are presented in both the normal and reference Turbiscan mode with backscattering flux (%) at ordinate and tube length at abscissa respectively. The reference modes (positioned right to the normal mode in Figures 3 and 4 were the alternative representation of the stability curves where the firstly scanned profile was used as the reference profile relative to the other scans and was assigned a value of 0%. The first scans in all of the emulsion stability curves gave the intrinsic information regarding the emulsions and its backscattering flux (%) gave the representation of the microstructure of the recently prepared emulsions.

The initial backscattering value, BS $_{AVo}$ (%) is a parameter that is highly dependent on the number of oil droplets in an emulsion system (Cerimedo *et al.,* 2010). The higher the number of the oil droplets in the emulsion system, the higher the BS $_{AVo}$ (%). Table 2 shows the effect of vinegar concentrations on BS $_{AVo}$ (%). Vinegar had observable influence on BS $_{AVo}$ (%). Sodium caseinate and trehalose also showed observable effects on the BS $_{AVo}$ (%) of oil-in-water emulsion (Cerimedo *et al.,* 2010). The mean of BS $_{AVo}$ (%) ranged between 95.21 and 70.48 % for vinegar concentration between 0 and 8 % (w/w). There was no general trend of increase or decrease of BS $_{AVo}$ (%) in relation to increase in concentration of vinegar. However, there was a general decrease in BS $_{AVo}$ (%) of the emulsions containing vinegar relative to emulsion without vinegar. Emulsions containing 0.5, 2, 4, 6, and 8 % (w/w) vinegar showed similar BS $_{AVo}$ (%).

***Table 2: Effect of vinegar concentration on Initial backscattering value[1,2]***

| Concentration of vinegar | Initial backscattering [$BS_{AVo}$] value (%) |
|---|---|
| 0 | 95.21 ± $0.01^{a}$ |
| 0.5 | 71.17 ± $0.87^{b}$ |
| 2.0 | 72.01 ± $1.75^{b}$ |
| 4.0 | 70.48 ± $0.29^{b}$ |
| 6.0 | 71.27 ± $1.63^{b}$ |

| | |
|---|---|
| 8.0 | $70.77 \pm 0.23^b$ |

[1] *Values are means ± standard deviations;* [2] *Means with different letters within the same column are significantly different from each other ($p < 0.05$).*

The reduction in the initial backscattering values ($BS_{AVo}$(%)) of emulsions with vinegar relative to emulsion without vinegar is a result of less numerous oil-droplets formed by the BGNF matrix containing vinegar. Although there was no significant difference in the $BS_{AVo}$(%) of all the emulsions containing vinegar, emulsion without vinegar showed a significant difference ($p < 0.05$) from other emulsions with vinegar and recorded the highest $BS_{AVo}$ (%).The result of the reference mode of Turbiscan profile in Figures 3 and 4 (right hand side) showed relative changes with respect to time in all emulsions. Backscattering flux (%) decreased along the entire tube length in all the emulsion with and without vinegar. Decreased backscattering flux (%) was as a result of oil droplet aggregation which could be as a result of oil-droplet flocculation or coalescence. Emulsion stabilized with sodium caseinate was reported to exhibit similar behaviour (Dickinson, 1997; Huck-Iriart *et al*., 2011). The observed decrease in the backscattering flux overtime was because of increased oil-droplet size which caused the mean path of photon (l*) to increase because of an increase in the average distance between the oil-droplets. The presence of vinegar at all concentrations induced similar destabilization mechanism in BGNF emulsion.

In order to understand the effect of different concentrations of vinegar on emulsion stability, the mean value kinetics for oil droplet aggregation in the middle of the tube (20 - 40 mm) from backscattering flux (%) of the samples was analysed (Cerimedo *et al.,* 2010). Figure 5 showed the effect of vinegar concentrations on the backscattering flux (%) mean value kinetics. Vinegar concentrations had dissimilar effects on emulsion stability over time. Similar behaviour was reported by Cerimedo *et al.* (2010) for emulsions stabilized with concentrated sodium caseinate and trehalose. The farther the graph from the origin the less stable the emulsion becomes. Although the effect of vinegar on emulsion has induced destabilization at all studied concentrations, the destabilization kinetics did not however follow any particular trend. The graph showed that there were no clear differences between the effects of various concentrations of vinegar on emulsion stability within the period of study. However the destabilization kinetic graph of emulsion containing 8 % (w/w) vinegar was the closest to the origin among emulsions containing vinegar. In order to analyze emulsion stability at equilibrium time, the backscattering flux (%) at equilibrium was plotted against vinegar concentration. The equilibrium backscattering flux (%) is the final backscattering flux attained at the equilibrium time. Figures 3 and 4 showed that all of the studied emulsions have reached equilibrium at about 360 minutes. Therefore, the backscattering flux (%) values at the 360th minute were plotted against the respective vinegar concentrations. Figure 6 is the graph of the equilibrium backscattering flux (%) against concentration of vinegar and it showed clearly that vinegar concentrations affected the average equilibrium backscattering flux (%). Although there was no definite trend on the effect of vinegar concentrations on the equilibrium backscattering flux (%), emulsion containing, 8 % (w/w) of vinegar had the least average backscattering flux (%) at the end of studied time of 360 mins.

## 4.0 CONCLUSION

The results showed that comparable strength was possessed by BGNF matrix at all studied vinegar concentrations and this affected their emulsion forming ability similarly. Related structures of emulsions containing vinegar had influenced the particle aggregation and hence destabilization in a comparable similar and complicated manner. All emulsions containing vinegar possessed very large oil-droplets compared to emulsion whose BGNF matrix did not contain vinegar. Vinegar therefore caused changes in the properties of the BGNF polymer network during continuous phase preparations.

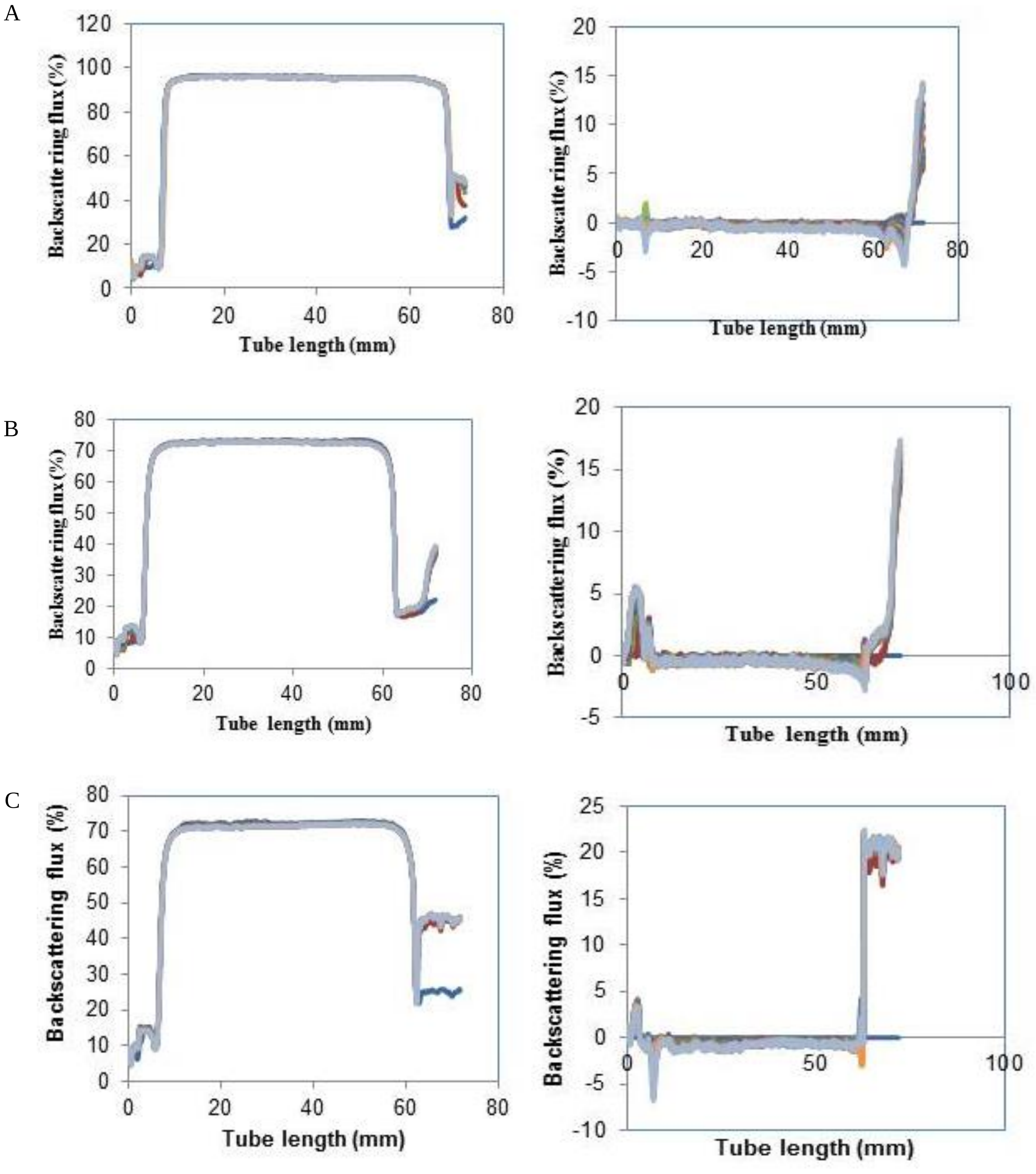

***Figure 3: Changes in the backscattering profile (BS%) as a function of sample height with storage time of BGNF (7% (w/w) stabilized emulsion containing vinegar at (A) 0 % (w/w) (B) 0.5 % (w/w) and (C) 2 % (w/w)***

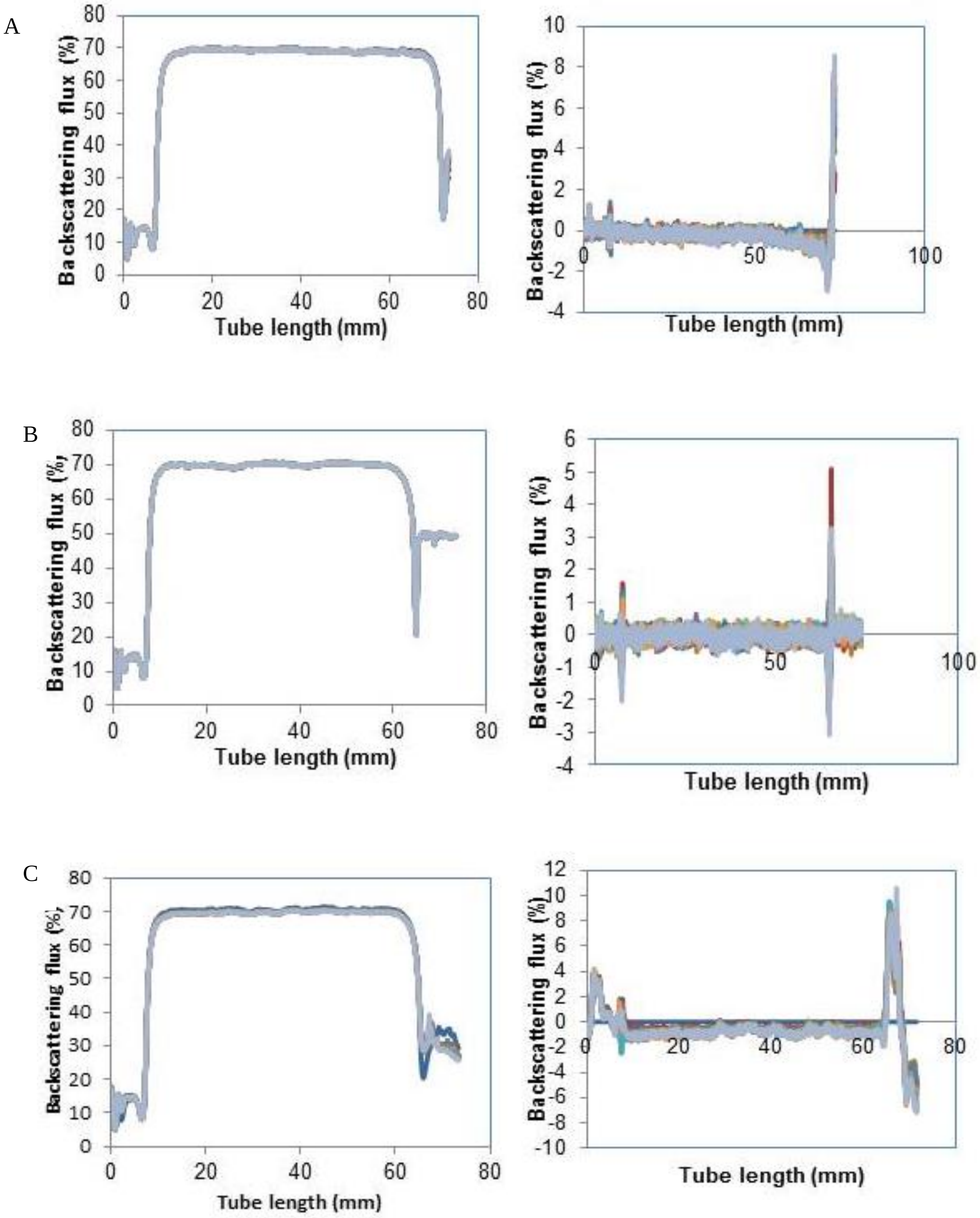

*Figure 4. Changes in the backscattering profile (BS%) as a function of sample height with storage time of BGNF (7% (w/w) stabilized emulsion containing vinegar at (A) 4 % (w/w) (B) 6 % (w/w) and (C) 8 % (w/w)*

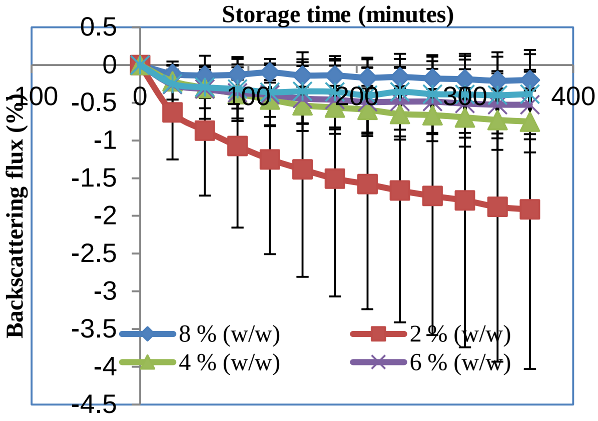

***Figure 5. Variation in backscattering in the 20 – 40 mm zone monitored over 360 minutes for sample stored in quiescent condition at 20°C for emulsion containing different concentrations of vinegar***

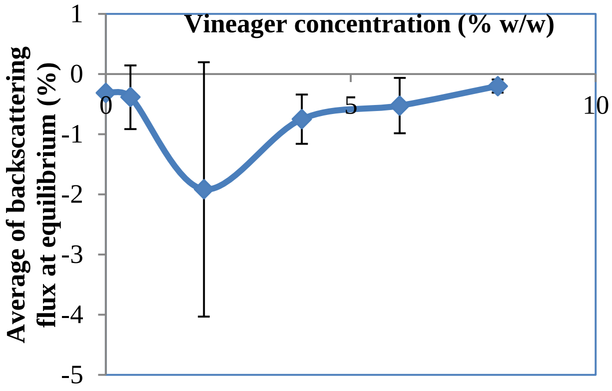

***Figure 6. Effect of addition of vinegaron emulsion stability (Average backscattering flux at equilibrium state)***

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