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Effect of Water Adulteration on the Rheology and Antibacterial Activities of Honey

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Abstract

Honey was diluted with different percentages of water and was analysed rheologically at room temperature of 27°C. The rheological profiles of pure and impure honey samples were measured at low shear rates (0.01-4.16s^{-1}). This work developed a structural kinetic model, which correlated well with the rheological data. The new model was used to categorise honey samples using their average molecular weights as one of the distinctive properties. Also, the kinetics order in the new model predicts the number of active components in the "honey" undergoing deformation. Honey produced third order kinetics to depict the monomers, oligomers and water content in honey. Pure honey exhibits peculiar non-Newtonian rheological behaviour. The behaviour of water is Newtonian. Dilution of honey with different percentages of water turns the resulting fluid Newtonian from 10% dilution with water. This study analysed the antibacterial activities of honey and serially adulterated samples against Staphylococcus *aureus* and Pseudomonas *aeruginosa*. The antibacterial analyses of honey were conducted using Kirby Bauer's well diffusion method. The results indicated that pure honey exhibited a zone of inhibition against both organisms. Also, the diameter of the zone of inhibition decreased with increasing dilution of honey, suggesting a correlation with the rheological method.

Keywords: Honey, Honey dilution with water, Antibacterial activities. Rheology of honey, Rheological Models.

Major classifications: Food Science, Health Science.

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1. Introduction

Honey is a sweet natural fluid produced by honey bees (*Apis Mellifera*) from the nectar of flowers or the sap of plants (White, 1992). It comprises carbohydrates such as monosaccharides consisting of glucose and fructose. Oligosaccharides such as sucrose, maltose, melezitose, and raffinose are contained in honey (Adebiyi et al., 2004). Honey contains not more than 20% water (Bogdanov, 2004). Its water content is responsible for its rheological properties. Honey is reputed to have various nutritional and medicinal benefits (Ajibola et al., 2012). This and honey's pleasant taste of universal appeal ensure sustained high demand for the product (Anidiobu, 2021). However, the honey supply is quite constrained and hardly matches the request (Hilmi, Bradbear, & Mejia, 2011).

Consequently, honey always commands a relatively high price and is exposed to adulteration and imitation (Anidiobu, 2014). Adulterated, diluted or fake honey would not be expected to have genuine product quality. They would contain very little or none of the constituents that impart nutritional and medicinal values to the latter (Nwalor et al., 2014). There is, thus, a strong imperative to regularly determine the content of what is sold as honey in the market (Everstine, Spink, & Kennedy, 2013). A cheap material, water, is an essential adulterant of honey in the Northern part of Nigeria (Anidiobu, 2014). The poverty level in some northern states of Nigeria is enormous. They cannot afford other adulterating materials; some add water to enhance the volume of what is sold as honey in the market.

Since honey is hygroscopic because of its low water content, it will readily absorb the added water, altering the product's natural composition (White, 1992). Honey, in its pure form, exhibits antibacterial properties (Ndip et al., 2007). It extracts the water and nutrient in the organism, thereby inhibiting its growth. At this point, it has not killed the organism but, by its activity, rendered the organism latent. This type of action is called the bacteriostatic effect (Saissy, et al., 1995). Complete inhibition of growth maintained over a long period is essential in controlling infections. Also, it is relevant to note that if bacteria are kept in a state of bacteriostasis for an extended period, their capacity to recover is lost. It suggests that honey without dilution or full strength exhibits its antibacterial properties mainly through this means.

This study explores the efficacy of molecular weight determination using rheological modelling. Rheological modelling is a tool to track adulteration in honey. Secondly, it is to extract structural and compositional information from the rheological data. Thirdly, this work seeks a correlation between the rheological method and the antibacterial activities of honey.

1.2 Theory

This section aims to develop a structural kinetic model that can discriminate between pure and impure honey using average molecular weight distribution as one of its major distinguishing parameters. The Carreau-Yasuda model is improved upon to correlate pure from adulterated samples.

1.2.1 The Structural Kinetic Model (SKM) on the Nigerian Honey

From Anidiobu (2021), the structural kinetic model is given as follows:

$$\eta = \eta_\infty + (KM^A - \eta_\infty)\left[kt\ \ (n-1)+1\right]^{1/(1-n)} \quad (1)$$

When applied to a sample, the SKM (equation 1) would produce the fluid's average molecular weight of the sample. Analyses for different shear rates (γ) would yield k, which is directly proportional to the rate constant of sample deformation γ . Likewise, n (expected to take values in the range 1 - 4) is the order of the structure breakdown process. Where, η_o is the zero shear or zero time viscosity of the fluid. K and A = are Mark Houwink Constants, while M is the average molecular weight of the liquid. A is generally in 0.5 to 0.8 (Launay et al., 1986).

1.2.2. Carreau-Yasuda model (CYM) (Yasuda, 1979)

The Carreau-Yasuda Model (Equation 2) is an empirical model with five adjustable parameters, α, λ, n, η_0, and η_∞ .

$$\eta(\dot{\gamma}) = \eta_\infty + (\eta_o - \eta_\infty)\left[1+(\dot{\gamma}\lambda)^a\right]^{\frac{n-1}{a}} \quad (2)$$

This model describes a non-Newtonian time-dependent flow with apparent viscosities η_∞ at zero and infinite shear rates. The parameter λ is the viscous relaxation time. It defines the position of the change from shear-thickening to shear-thinning behaviour – (1/ λ) marks the critical rate of shear at which viscosity begins to decrease. The power-law slope is (n-1). The value of n changes with the content of the fluid. The parameter "α" is dimensionless and introduced by Yasuda to the Carreau equation. It is related to the breadth of the transition region between η_o and the power-law section.

Mark-Houwink's equation, given as $\eta = KM^a$ (3), is introduced in equation (2) by replacing the zero rate of shear viscosity with a term reflecting the average molecular weight of the fluid. Thus,

$$\eta(\dot{\gamma}) = \eta_\infty + \left(\mathrm{KM}^{\mathrm{A}} - \eta_\infty\right)\left[1 + (\dot{\gamma}\lambda)^a\right]^{\frac{n-1}{a}} \tag{4}$$

2. Methods

The experimental methods involve sample preparation, rheological characterisation, and antibacterial analysis of honey.

2.1 Sample Preparation

Sample A is pure honey, or a control sample was harvested and processed for this work. Samples AW1, AW2, AW3, AW4, AW5, and AW6 were produced by adulterating honey (A) with 5%, 10%, 15%,20%.25% and 30% of distilled water.

Table 1: Details of Samples Collection and Location Parameters

S/N	Sample	Location	Location Coordinates	Collection Date	Production Date
1.	A	Federal Poly Ado-Ekiti	7.6211°N, 5.2214°E	30-11-2021	15-01-2012
2.	AW1	5% Adulteration with Water			01-12-2021
3.	AW2	10% Adulteration with water			01-12-2021
4.	AW3	15% Adulteration with water			01-12-2021
5.	AW4	20% Adulteration with water			01-12-2021
6.	AW5	25% Adulteration with water			01-12-2021
7.	AW6	30% Adulteration with water			01-12-2021

2.2 Rheological Measurements

The rheograms of the samples were obtained with the aid of the Brookfield DV3T Programmable Rheometer. The principle of operating the DV3T Ultra is to drive a spindle (immersed in the test fluid) through a calibrated spring. The spring deflection measures the viscous drag of the fluid against the spindle. The spring deflection is measured with a rotary transducer. The viscosity measurement range of DV3T is determined by the rotational speed of the spindle size and the dimension of the spindle. The sample container in which the spindle is rotated and the full-scale torque of the calibrated spring also determine the viscosity measurement range. All measurements were carried out through a cone radius of 24 mm and a 0.8 cone angle; this gives the gap height of 0.1 mm at the cone's circumference. The effect of water adulteration on honey rheology was investigated at 27ºC. The samples were made to rest in the container for at least 30 minutes before the analysis. It allowed for the complete structural buildup from shear-induced flow into the container. Each experimental measurement was repeated after 24 hours to verify the reproducibility of the results.

2.3 Rheological Curve Fitting

For the five-parameter structural kinetic model, the order of breakdown kinetics, *n*, was assigned numbers to ascertain which would give the least error. In this study, integers 1 to 4 were tested. The preset shear rates, experimental apparent viscosities and time of deformation from the experiment were utilised. It leaves the zero and infinite shear viscosities (η_0 and η_∞) which are not practicable to measure. The rate constant of deformation (k) is determined from the time versus apparent viscosity data as unknowns. Extrapolation of the data to the *t = 0* lines η_0 was estimated. With this latter determination and a guess of η_∞, *k* may be explicitly determined for each data point from (equation 5) which gives,

$$k = \frac{\theta^{(1-n)} - 1}{(n-1)t} \tag{5}$$

Since $\theta(\gamma, t) = \dfrac{\eta - \eta_\infty}{\eta_o - \eta_\infty}$

Also, Γ is defined by, $$\Gamma = \left(\frac{\eta - \eta_\infty}{\eta_o - \eta_\infty}\right)^{1-n} \quad (6)$$

Then $$\Gamma = tk(n-1) + 1 \quad (7)$$

Since *k* is projected to depend only on the shear rate, a scheme was developed by which an optimised value of *k* was determined for each shear rate. This scheme seeks a solution to yield the minimum coefficient of variation (CV) of *k*. The reasonable assumption justifies this decision that *k* should be a constant at the constant rate of the shear condition of the SKM data. The combination of η_∞ *n* and *k* that produces this optimum CV is utilised, along with the corresponding average molecular weight, *M,* as the parameters of the SKM of the sample used for the (experiment's) preset rate of shear.

2.4 Determination of Antibacterial Properties of Honey

This section is introduced to study the correlation or otherwise of antibacterial activities of honey with the rheological characteristics. The antibacterial activities of honey were tested using Kirby Bauer's well diffusion method (Cheesbrough, 2010).

2.4.2 Preparation of Test Organism

Stocked cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa* used in this study were obtained from the Microbiology Laboratory Unit, University Teaching Hospital Ado-Ekiti State. The isolates were identified based on standard microbiological techniques, and sub-cultured in nutrient agar slope at 37˚C for 24hrs (Chessbrough, 2010).

2.4.3 Antimicrobial Assay

The antibacterial activity of different honey samples against *Staphylococcus aureus* and *Pseudomonas aeruginosa* tested using ager diffusion technique. The test materials were prepared by diluting honey sample in sterilized, double distilled water at different dilution (concentration) 5%,10%,15%,20%, 25% and 30%. The plates were prepared using 20mils of sterile nutrient agar. The surfaces of the plates were inoculated using a 100µl of 0.5 Macfarland standardized inoculums suspension of bacterial and allowed to dry. Wells, 6.0mm in diameter were cut from the culture media using sterile metal cylinder and then filled with the test samples. The plates were incubated at 37oC and observed after 24 hours for clear, cellular inhibition zones around the wells were measured using venial caliper (CLSI, 2020).

3. Results and Discussion of Results

3.1 Results

Figure1 shows the effect of water adulteration on honey rheology. Also, figure 2 shows the ACYM curve-fit of sample AH1 at 27˚C. Figure 3 is the plot Γ against time at a shear rate of $0.01s^{-1.}$ Figure 4 is the optimisation of k and generation of infinite shear viscosity of Sample A. Figure 5 is the plot of k against the shear rate of Sample A. Figure 6 is the correlation of rheological and antibacterial activities of honey upon water adulteration. Table 1 shows details of sample collection and location Parameters. Table 2 summarises Rheological Curve-fit using the new Structural Kinetic Model (SKM). Table 3 summarises the rheological ACYM curve fit of samples at 27˚C. Table 4 shows honey's antibacterial effect on *Staphylococcus aureus*, while table 5 shows honey's antibacterial effect on *Pseudomonas aeruginosa*.

3.2 Discussion of Results

This study focused on the solution to ongoing honey adulteration in Nigeria. The rheological method of solving this problem was analysed. A correlation between rheological characterisation and antibacterial activities of honey was sought to validate the rheological characterisation. During the rheological characterisation of honey, SKM and ACYM were utilised to study the effect of water dilution or adulteration on the rheological behaviour of honey. The impact of adulteration on honey's antibacterial activities was carried out using *Staphylococcus aureus and Pseudomonas aeruginosa*.

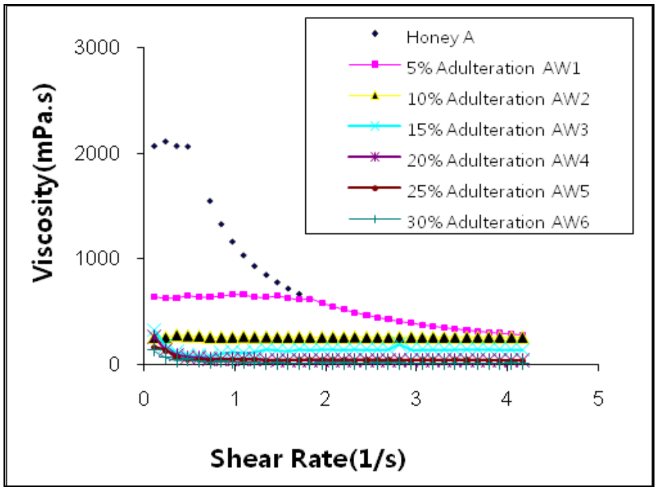

Figure1: Effect of Water Adulteration on Honey Rheology

Figure 1 shows the effect of water adulteration on the rheology of honey. Sample A is pure honey. It indicates that pure honey first exhibited shear thickening behaviour at the inception of flow and low shear rates but later assumed an essentially shear thinning behaviour at higher shear rates. Kurzberck, Oster, Mu¨nstedt, Nguyen, & Gensler (1999) suggested that the presence of chain branches gave rise to strain hardening, which they said was a necessary stability property for polymers undergoing deformation. The clusters and aggregates of particles initially present as colloids or suspensions in the fluid were reversibly destroyed by shear. Triantafillopoulos (1988) explained this shear thinning behaviour as a result of isothermal and reversible disorientation of the fluid structures.

The rheograms show that the non-Newtonian features of apparent viscosities of honey decrease significantly with the addition of water. Just 10% adulteration with water turns the resulting fluid towards Newtonian behaviour. The higher the water concentration extra, the less viscous the resulting fluid becomes.

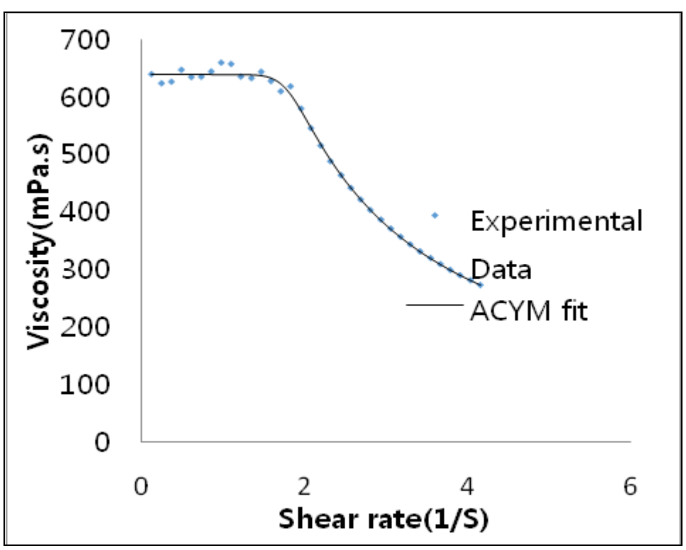

Figure 2: ACYM Curvefit of Sample AH1 at 27˚C

Figure 2 is the rheological curve fit using the Amended Carreau-Yasuda model on sample AW1. It shows that the model followed well the experimental result. The zero shear viscosity dropped from about 2084.06mPa.s for honey to 642.25mPa.s for 5% adulteration with water.

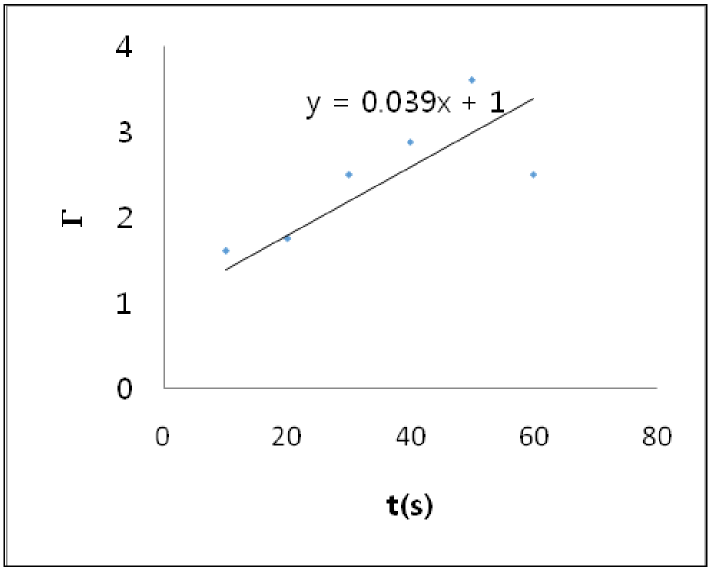

Figure 3: Plot of Γ against t at shear rate of $0.01s^{-1}$

Figure 3 shows the Γ plot against t in equation 7 above, where k (deformation rate) is obtained in the structural kinetic model. The sequence above was repeated for all the samples used in this study.

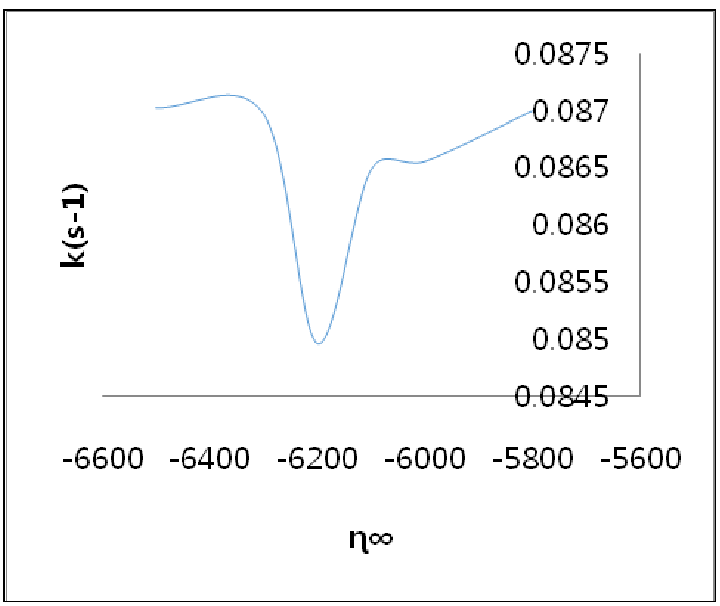

Figure 4: Optimisation of k for Sample A

Fig.4 is the plot for optimisation of infinite time viscosity at a shear rate of $0.01s^{-1}$. The figure obtained the minimum (best value) of infinite time viscosity. Fig 5 shows the dependence of the deformation rate for sample A on the shear rate.

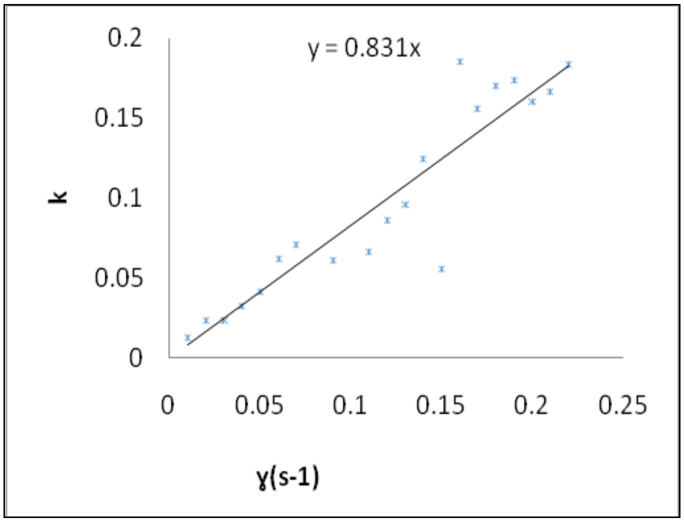

Figure 5: Plot of k against shear rate of Sample A

Table 2 summarises rheological curve fitting results using the Structural Kinetic Model. It was observed that pure sample A produced 202 g/mol as its molecular weight distribution. There are sequential decreases in adulterated honey's average molecular weight distribution with increases in adulteration. It is expected since the molecular weight distribution of the adulterating material is far lower than that of honey. The high molecular weight values (>180 g/mol) exhibited by honey in Table 2 could result from high melezitose, colloidal materials, and polymerised and crystalised monosaccharides present in honey. The addition of water, which has a molecular weight of 18 g/mol, led to a sequential reduction in the molecular weight of the resulting adulterated samples.

Table 2: Summary of Rheological Curve Fitting using the new Structural Kinetic Model (SKM)

Sample Code	Sample Identity	Molecular Weight(g/mol)	n	$k(S^{-1})$
A	Pure honey	202	3.0	3.26
AW1	5% Adulteration with water	190	2.5	1.05
AW2	10% Adulteration with water	181	2.0	0.94
AW3	15% Adulteration with water	174	1.5	0.75
AW4	20% Adulteration with water	165	1.5	0.69
AW5	25% Adulteration with water	156	1.5	0.63
AW6	30% Adulteration with water	146	1.5	0.61

The best-correlated results were obtained for the pure honey samples at third-order deformation kinetics. It is because three components of the honey constituents were active during deformation. It suggests that melezitose, other oligosaccharides and polymerised materials serve as the first, water as the second, and monosaccharides as the third active component.

The results of the deformation rate of samples in Table 2 suggest that it decreases with the adulteration of samples. In the preceding subsection, the rheograms were employed for the qualitative assessment of the samples. This assessment shows that honey's purity changes are reflected in its rheograms. In this subsection, however, quantitative analyses of samples by using models are sought. The ACYM was used to extract compositional information and assess the purity level of honey from the rheograms. Figure 2 is the ACYM curve fit of pure honey sample A at 27˚C. The ACYM was used to independently assess the molecular weight distribution of honey using Morrison's method (1999). ACYM gave an excellent curve fit of the rheogram of pure honey, A. The behaviour index value of 0.23, suggesting shear thinning behaviour, was obtained for pure Sample A at 27°C. The average molecular weight distribution of 205 g/mol was correlated. The high molecular weight calculated suggests a minor presence of higher oligosaccharides in the pure honey sample (Nguyen et al., 1998). High molecular weight oligomers such as melezitose and raffinose are found in honey (Nguyen et al., 1998).

Table 3: Summary of Rheological ACYM Correlation of Samples at 27°C

S/N	Samples	Sample Identity	n	Molecular Weight (g/mol)	λ(s)	η_o	a
1	A	Pure honey	0.23	205	2.09	2084.06	131.60
2	AW1	5% Adulteration with water	0.51	194	2.42	642.25	59.14
3	AW2	10% Adulteration with water	0.81	186	1.87	320.45	54.02
4	AW3	15% Adulteration with water	0.93	176	1.42	256.08	58.65
5	AW4	20% Adulteration with water	0.99	168	1.03	256.00	57.41
6	AW5	25% Adulteration with water	1.01	156	0.89	164.09	28.17
7.	AW6	30% Adulteration with water	1.05	146	0.80	132.12	26.03

Table 3 summarises rheological curve fitting results using ACYM at 27°C. The results show that the behaviour index in the ACYM increases with the increasing adulteration of honey (Lazaridou et al., 2004). Also, the molecular weight of samples sequentially decreased with its increasing dilution with water. The curve fit parameters reflected the varying degrees of impurity, as shown in Table 3.

A possible correlation between honey's rheological behaviour and its medicinal value, visible in its antibacterial activities, can be observed. Tables 4 and 5 summarise experimental results on the effect of water adulteration on thl.e antibacterial properties of honey.

Table 4: The Antibacterial Effect of Honey on *Staphylococcus aureus*

S/N	Sample Code	Description	Zone of Inhibition(mm)
1.	A	Ijebu Mushin	24
2.	AW1	5% Adulteration with water	25
3.	AW2	10% Adulteration with water	22
4.	AW3	15% Adulteration with water	-
5.	AW4	20% Adulteration with water	-
6.	AW5	25% Adulteration with water	-
7.	AW6	30% Adulteration with water	-

The results in Table 4 show that pure honey sample A was effective against *Staphylococcus aureus*, typified by the large zone of inhibition observed (24mm). The implication is that *Staphylococcus aureus* infected wounds not treated appropriately with antibiotics can be treated by topical application of honey (Chute et al., 2010). Upon 5% dilution with water, the zone of inhibition widens to 25mm. It was, however, reduced to 22mm on 10% dilution. At higher degrees of adulteration, the antibacterial activity disappeared.

Table 5: The Antibacterial Effect of Honey on *Pseudomonas aeruginosa*.

S/N	Sample Code	Samples	Zone of Inhibition(mm)
1.	A	Ijebu Mushin	25
2.	AW1	5% Adulteration with water	26.1
3.	AW2	10% Adulteration with water	22
4.	AW3	15% Adulteration with water	-
5.	AW4	20% Adulteration with water	-
6.	AW6	25% Adulteration with water	-
7.	AW7	30% Adulteration with water	-

Table 5 summarises the antibacterial Effect of Honey against *Pseudomonas aeruginosa*. Pure sample A exhibited suitable antibacterial activities against *Pseudomonas aeruginosa.* A clear zone of inhibition of 25mm was observed at full-strength honey. Upon dilution with varying degrees of water, a slight increase in the diameter of zone inhibition to 26.1 mm was observed at 5% dilution. The diameter of the zone of inhibition decreased to 22mm at 10% dilution. Beyond 10% dilution, no activity was observed.

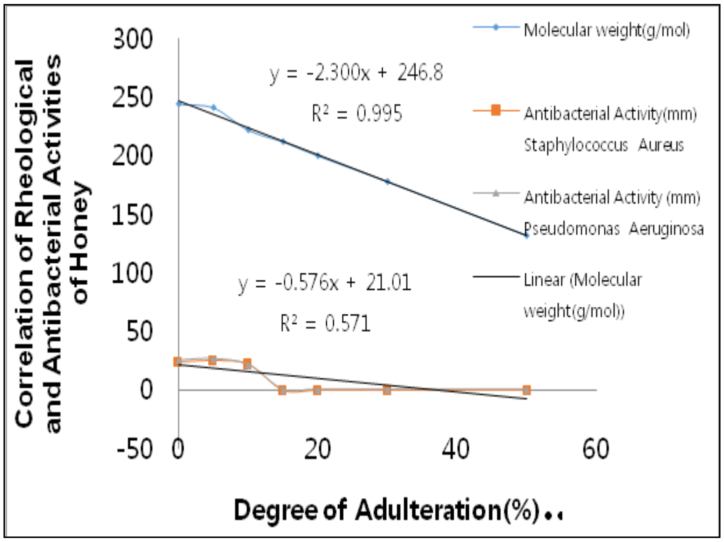

Figure 6: Correlation of Rheological and Antibacterial Properties of Honey upon Water Adulteration

Figure 6 shows the correlation of rheological and antibacterial activities of honey adulterated with water. It can be seen from the plots that the molecular weight of honey decreases with increasing dilution with water, the same way the antibacterial activities fall with increasing adulteration of honey.

Comparing the outcome of antibacterial activities (where the antibacterial activity of honey decreases with increasing dilution with water) with the rheological characterisation of honey shows that the antibacterial activities of samples behaved similarly to the rheological characterisation of honey. Thus, this study's rheological assessment of the quality of honey samples correlates with another independent honey quality index – the antibacterial activity.

4. Conclusion

Honey exhibited shear thickening behaviour at the inception of flow and predominantly shear-thinning behaviour as the flow progressed. The rheological behaviour of water is Newtonian behaviour. The dilution of honey with water pushes the viscosities of the resulting fluids to Newtonian behaviour. The Structural Kinetic Model followed the experimental results well and predicted the molecular weight of different samples. It discriminates pure honey from adulterated honey and determines the degree of adulteration. Pure Nigerian honey was effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Adulterations of honey with water beyond 10% lead to loss of antibacterial activities of the resulting samples, suggesting a correlation between the antibacterial and the rheological behaviour of honey.

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