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The effect of some fruit juice extracts on Sickle Cell Erythrocytes

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Preparations of Haemoglobin from sickle cell (HbSS) erythrocytes were subjected to varying processes of deoxygenation using 2.0% sodium metabisulphite solution. Polymerization was induced by the 2.0% sodium metabisulphite. The polymerization of the HbSS led to subsequent sickling. Remarkable polymerization inhibitions of the haemoglobin by the fruit juice extracts were observed. The ascorbic acid content in mg/100mls of the fruit juice extracts are as follows:

Carica papaya (Pawpaw)	**=**	**33.0**
Magnifera indica (Mango)	**=**	**30.4**
Ananas comosus (Pineapple)	**=**	**43.2**
Citrus aurantifolia (Lime)	**=**	**39.5**
Citrus sinensis (Orange)	**=**	**37.3**
Anarcadium occidentale (Cashew)	**=**	**34.2**

The Vitamin C (ascorbic acid) contents in micromole (μm) following the polymerization inhibition potency experiments gave the results as: Pineapple (488 μm), Lime (443 μm), Orange (423 μm), Cashew (388 μm), Pawpaw (375 μm) and mango (340 μm).
The polymerization inhibition experiment left Ananas comosus (Pineapple) with the highest and Magnifera indica (Mango) with the least Vitamin C content, but there was no marked difference in the polymerization (hence, sickling) inhibition potency of the fruit juice extracts. This is indicative of the fact that all the juice extracts contain Vitamin C to an appreciable quantity. This research work aims at studying the effect of vitamin C on sickle cell erythrocyte.
Key words:Sickle cell, Erythrocytes, Fruit juice extracts, Serum, Vitamin C, Haemoglobin.

INTRODUCTION

The erythrocytes contain various components that perform different metabolic functions and prominent amongst these are the haemoglobins – that are responsible for the transport of oxygen from the lungs to other tissues. When deoxygenated, it is found that pure haemoglobin solution forms a gel (Hahn and Gilespie, 1927) as a result of the removal of oxygen, which of course reversible and enhanced by low pH and high temperature. It has been shown that there is the formation of small aggregates in the deoxy-sickle haemoglobin solution before gelation (Russin, *et al*., 1980). When cells containing HbS are suddenly deoxygenated, a short delay occurs before the cells begin to transform gradually into the typical sickly and holly-leaf shapes.

This cellular delay is probably related to a molecular delay which is due to condensation of molecules, prior to fibre formation. The magnitude of this delay time is highly dependent on the haemoglobin concentration and it is also sensitive to oxygen saturation and temperature. Since the polymerization of HbS occurs at low oxygen tension thereby depending on the presence of the deoxygenated conformation of haemoglobin, any factor that tend to shift the oxygenated to deoxygenated equilibrium should affect sickling. Hence when sodium metabisulphite is added to sickle cell erythrocyte (haemoglobin solution), being a strong reducing agent sodium metabisulphite converts HbO_2 to the Hb form and this deoxygenated form of Hb is very susceptible to polymerization. In sickle cell disease following the

Table 1: Shows the average change in O.D. value and standard deviation for n=6 number of juice extracts.

S/N	*Control*	*Pawpaw*	*Mango*	*Pineapple*	*Orange*	*Lime*	*Cashew*
	X S.D ±	X S.D ±	X S.D ±	X S.D ±	X S.D ±	X S.D ±	X S.D ±
1	0.102± 0.10	0.038± 0.02	0.067± 0.10	0.042± 0.04	0.033± 0.03	0.032± 0.02	0.038± 0.03
2	0.163± 0.14	0.081± 0.05	0.099± 0.13	0.095± 0.07	0.081± 0.05	0.078± 0.05	0.093± 0.06
3	0.231± 0.18	0.123± 0.07	0.154± 0.13	0.158± 0.11	0.137± 0.09	0.132± 0.07	0.148± 0.09
4	0.322± 0.25	0.174± 0.07	0.196± 0.14	0.222± 0.15	0.215± 0.13	0.217± 0.13	0.251± 0.20
5	0.418± 0.28	0.233± 0.06	0.251± 0.15	0.301± 0.19	0.278± 0.17	0.300± 0.21	0.353± 0.22
6	0.500± 0.35	0.302± 0.11	0.328± 0.14	0.383± 0.25	0.354± 0.20	0.387± 0.26	0.451± 0.32
7	0.595± 0.38	0.378± 0.15	0.402± 0.23	0.461± 0.29	0.432± 0.20	0.458± 0.31	0.552± 0.40
8	0.665± 0.38	0.462± 0.20	0.473± 0.27	0.545± 0.33	0.511± 0.23	0.550± 0.34	0.632± 0.40
9	0.764± 0.43	0.545± 0.24	0.554± 0.31	0.609± 0.35	0.599± 0.29	0.616± 0.36	0.725± 0.43
10	0.838± 0.43	0.625± 0.27	0.655± 0.33	0.707± 0.36	0.677± 0.28	0.709± 0.39	0.811± 0.44

Data used for plotting of graph in Fig. 1

inadequacy of oxygen, the erythrocytes get deformed leading to sickle cell anaemia in the patients. Available literatures show that sickle cell anaemia usually occur when there are deficiencies in zinc, iron, folic acid, vitamin B complex, but majorly vitamin C. Sources of vitamin C include: pepper, tomato, tangerine, cherry, guava, pawpaw, pineapple, raspberry, grapes, watermelon, mango etc.

El-Hazmi (1994) in his experiment showed that plasma level of vitamin C were significantly lower in patients with severe sickle cell anaemia compared with the normal control group. He further asserted that this is so due to the continuous oxidative stress which put the patients in the deoxygenated position in the oxygenated - deoxygenated equilibrium of the haemoglobin.

Also preliminary research on dietary habits have shown that food and nutrient intake by patients of sickle cell in general meets or even exceeds recommendations and is not significantly different from healthy controls (Chiu, *et al*., 1990). Many studies have also shown that the polymerization of HbS, which occurs at low oxygen tension is associated, usually with low blood levels of antioxidants, including carotenoids, vitamins A and E and most importantly vitamin C (Jain, S.K and Williams, D.M., 1985).

FRUITS

Fruits are permanent crops and are eaten raw by man. Fruits and seeds provide the bulk of the foods eaten by man and under improved methods by livestock (Komlafe, 1979). Many industrial chemicals and drugs are also extracted from fruits. These fruits are rich in vitamins and the essential minerals for healthy human growth. The aim of this work hence, is to find out if the fruit juice extracts have antisickling potentials and if they do they are expected to prolong the delay time prior to polymerization of the deoxy-HbSS. It is only when this is achieved that one can now begin to suggest their capability as having therapeutic relevance in the disease of sickle cell.

These fruits juice extracts contain vitamins and minerals and there is every need to look for alternative, but nutritional, less expensive and non-toxic therapies for the management of sickle cell disease. For example, Cajanus cajan seeds (Ekeke and Shode, 1985) which has antisickling properties is used for SCD management. Accordingly, a pretreatment of HbSS cell suspensions using Carica papaya leaf extract has been shown to inhibit polymerization, hence, sickle cell formation (Imaga N.O.A, *et al*., 2009). Vitamin C as an antioxidant affects the kinetics of haemoglobin polymerization and inhibit the time course for the sickling of erythrocytes.

MATERIALS AND METHODS

Fruits: The fruits whose juices were extracted include: Cashew (Anarcadium occidentale), Mango (Magnifera indica), Pawpaw (Carica papaya), Sweet orange (Citrus sinensis), Lime (Citrus aurantifolia), Pineapple (Ananas comosus). The various fruits were weighed, juices extracted using hand presser. Filtration was done using filter paper and repeated using muslin cloth. The volumes of the extracts were recorded.

REAGENTS AND SPECIMEN

Blood Samples: The blood samples used were obtained from the chemical pathology laboratory of the University of Port Harcourt Teaching Hospital. In all cases the blood samples from different individuals were genotyped electrophoretically at pH 8.4 on cellulose acetate and the HbSS determined. Other reagents used

Table 2: Shows the average change in O.D. and S.D. for n=6 number of extracts (when fruit juice extracts were boiled in water bath for 10 minutes). Data used for plotting of graph in Fig. 1

S/N	Control	Pawpaw	Mango	Pineapple	Orange	Lime	Cashew
	X ± S.D	X ± S.D	X ± S.D	X ± S.D	X ± S.D	X ± S.D	X ± S.D
1	0.136± 0.002	0.132± 0.001	0.134± 0.004	0.125± 0.001	0.111± 0.002	0.133± 0.001	0.112± 0.000
2	0.209± 0.004	0.198± 0.002	0.195± 0.009	0.200± 0.001	0.191± 0.002	0.199± 0.001	0.189± 0.000
3	0.295± 0.006	0.270± 0.002	0.271± 0.000	0.281± 0.001	0.287± 0.002	0.287± 0.001	0.278± 0.000
4	0.408± 0.006	0.385± 0.004	0.389± 0.000	0.387± 0.001	0.389± 0.002	0.395± 0.001	0.382± 0.000
5	0.517± 0.008	0.488± 0.006	0.489± 0.000	0.499± 0.001	0.501± 0.002	0.503± 0.001	0.448± 0.900
6	0.612± 0.011	0.588± 0.007	0.601± 0.000	0.595± 0.001	0.592± 0.002	0.601± 0.001	0.599± 0.001
7	0.726± 0.010	0.692± 0.009	0.700± 0.000	0.700± 0.001	0.707± 0.002	0.711± 0.001	0.700± 0.001
8	0.797± 0.004	0.770± 0.013	0.777± 0.000	0.779± 0.001	0.777± 0.002	0.779± 0.001	0.776± 0.000
9	0.913± 0.014	0.880± 0.017	0.879± 0.000	0.888± 0.001	0.889± 0.002	0.888± 0.001	0.890± 0.000
10	0.989± 0.016	0.984± 0.019	0.970± 0.000	0.971± 0.001	0.976± 0.002	0.977± 0.001	0.973± 0.000

included sodium metabisulphite (2.0% w/v), control (0.1ml distilled H_2O for polymerization), distilled and deionized H_2O, fruit juice extracts, glacial acetic acid (5%), ascorbic acid standard, ascorbic acid 2,6–Dichlorophenol indophenol (0.025%).

METHODS

Principles: Sickle cell erythrocytes sickle or become crescent-shaped when deprived of oxygen. Deoxygenation was carried out using sodium metabisulphite solution. The rapidity with which the reaction occurs varies with the amount of HbS present in the cell. The reducing agent was 2% sodium metabisulphite solution. 0.1ml of distilled H_2O was used as control throughout the experiment since it maintains the osmolarity of the blood.

a) POLYMERIZATION OF HAEMOGLOBIN (HbS)
PROCEDURE:

i. For Control: Into a test tube was added 4.8ml of 2.0% sodium metabisulphite ($Na_2S_2O_5$), 0.1ml of haemolysate, and 0.1ml of distilled H_2O. The mixture was properly shaken and O.D measured (taken) using spectronic 20 spectrophotometer at 700nm.

Absorbance (O.D) values were taken at one (1) minute interval for 10 minutes at 700nm to determine turbidity. The values were observed increasing with time probably as a result of increased polymerization with time.

ii. For Juice Extracts and Vitamin C: Into a test tube was added 4.8ml of 2.0% sodium metabisulphite ($Na_2S_2O_5$), 0.1ml of haemolysate and 0.1ml of extract/or vitamin C. The mixture was properly shaken and O.D measured using spectronic 20 spectrophotometer at 700nm. Also, the absorbance values were taken at one (1) minute interval for 10 minutes at 700nm to determine turbidity.

Graphs of Optical density (O.D) were plotted against time, tangents applied to the curves and rate of sickling calculated.

b) ESTIMATION OF VITAMIN C USING VISUAL TITRATION METHOD (Varley, 1969)
PROCEDURE:

10ml aliquot solution from the vitamin C standard was mixed with 10ml of glacial acetic acid. This 20ml solution was titrated against 0.025% 2,6–dichlorophenol indophenol. The volume of the vitamin extract that decolourized the blue colour of the reagent to pink was noted. Titration was done in triplicate. The titrations as described above were repeated with the extracts from each sample and their end point (volume of extracts that decolourized the reagent to pink of the same intensity) was noted.

RESULTS

Polymerization of Haemoglobin S by Turbidity Measured At 700nm.

The experiment was carried out on different samples of haemoglobin SS (HbSS) from different patients.

From table 1 above, it shows that in comparison with the control, all the fruit juices were found to exhibit measurable inhibition of HbS polymerization in the order; C. papaya > M. indica > C. sinensis > C. aurantifolia > A. comosus > A. occidentale, as represented in fig. 1 while fig. 2 is as in fig. 1, except that the anti sickling factor seems to be denatured or inactivated or inactivated slightly by heat (see Appendix A). The absorbance for each extract, mostly mean values were presented graphically (see Appendix B).

Those for ascorbic acid are also shown graphically (Appendix C).

From these graphs the initial rate of change of polymerization were obtained by drawing tangents on each graph at zero time. No marked differences were observed in the polymerization inhibition ability of the various fruit juice extracts, although there were little variations.

Appendix A

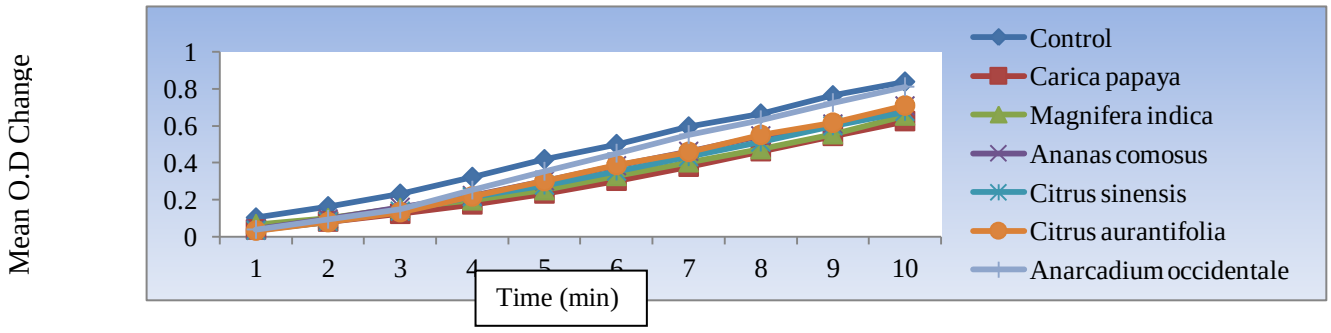


Fig. 1: Effect of fruit juice extracts on HbSS polymerization unheated.

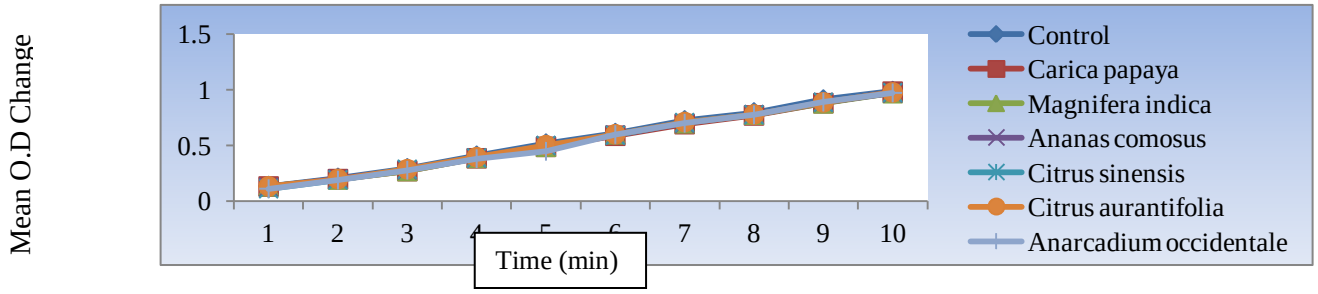


Fig. 2: Effect of fruit juice extract on HbSS polymerization (Heated).

APPENDIX B

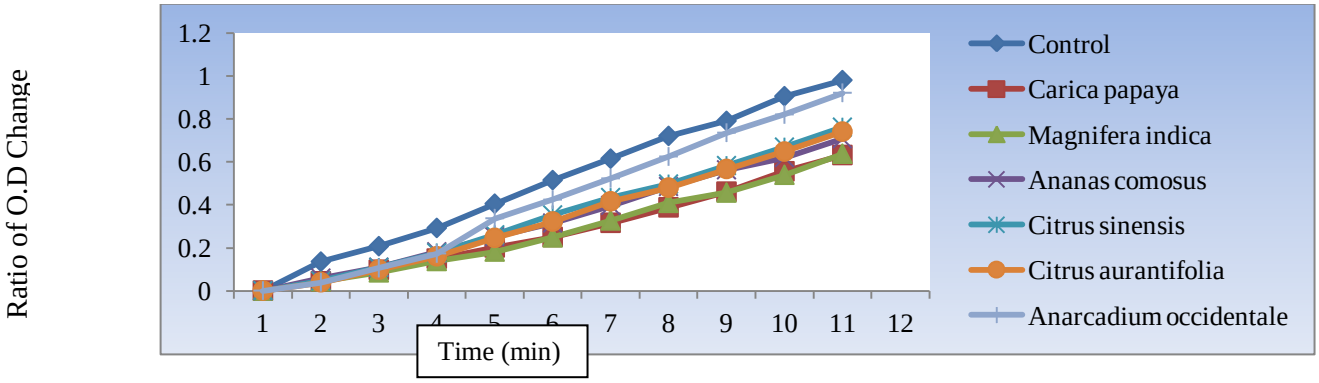


Fig. 3: Fruit juice extracts (Experiment 1).

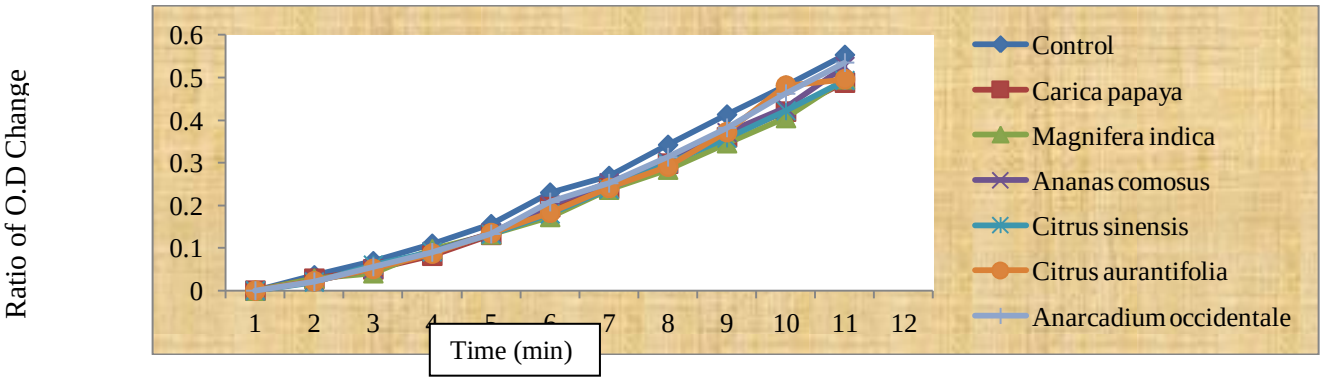


Fig. 4: Fruit juice extracts (Experiment 2).

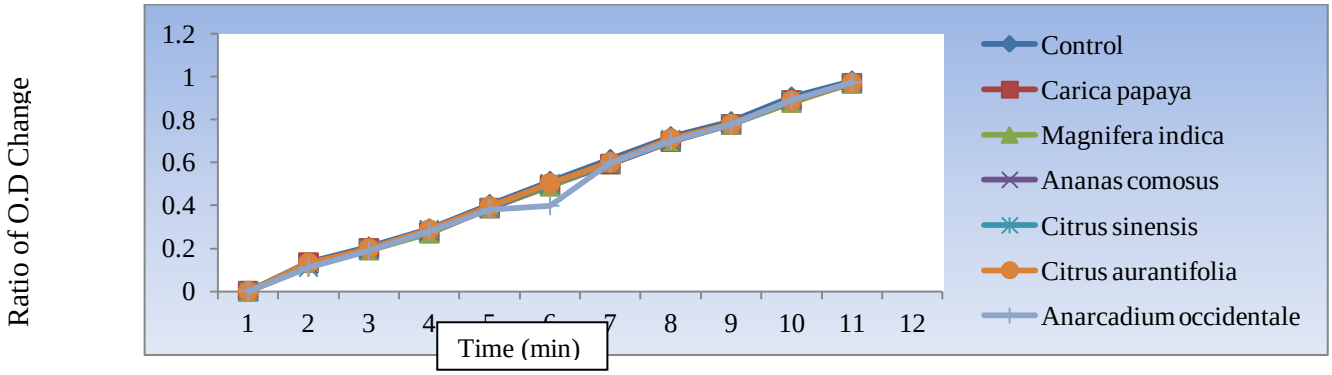


Fig. 5: Fruit juice extracts (Experiment 3).

APPENDIX C

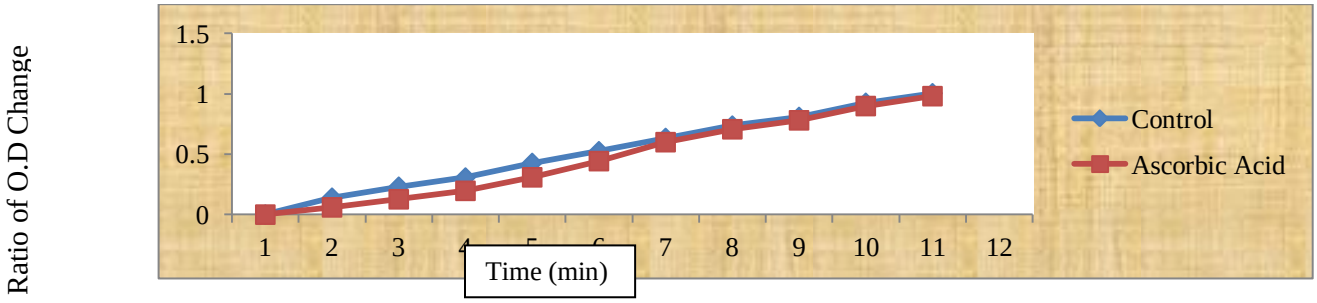


Fig. 6: Effect of Ascorbic acid on polymerization.

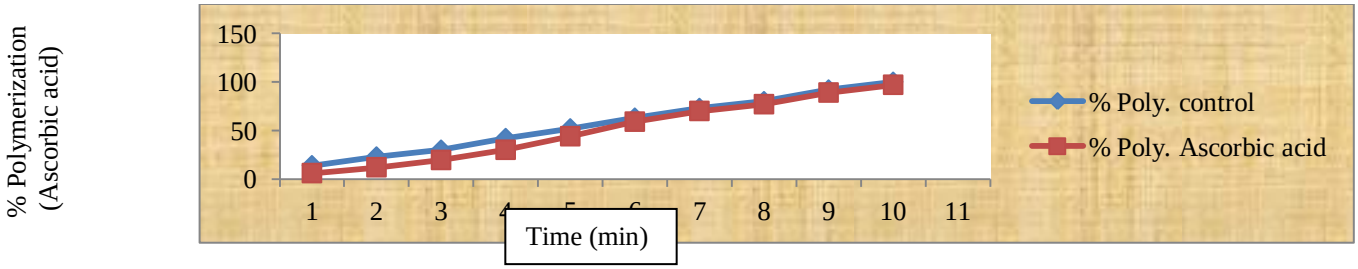


Fig. 7: % Effect of Ascorbic acid on HbSS (Polymerization).

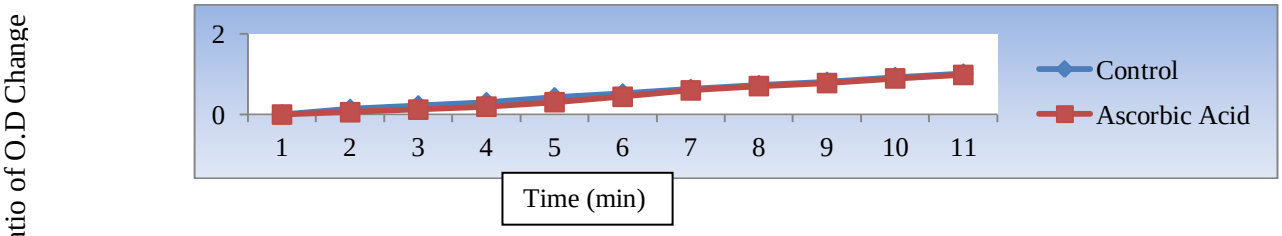


Fig. 8: Effect of Vitamin C on polymerization using two different tests.

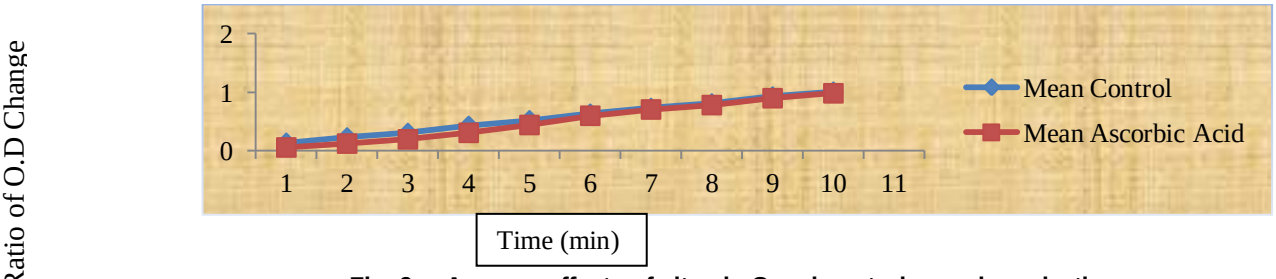


Fig. 9: Average effects of vitamin C and control on polymerization.

DISCUSSION AND CONCLUSION

Gelation of haemoglobin SS were observed to occur rapidly in the control experiments (i.e. with sodium metabisulphite and distilled water). This rapidity is due to sodium metabisulphite which as a result of its strong reducing power tends to remove oxygen from the immediate environment of the haemoglobin thereby eliciting gelation of the haemoglobin and the consequent sickling of erythrocytes. However, in the presence of the fruit juice extracts, the haemoglobin gelation rate was considerably reduced, showing that the fruit juice extracts contain factors that have antisickling potentials. But when the fruit juice extracts were subjected to boiling (by heating 0.1ml of each in a water bath for 10 minutes) their antisickling activity decreased (Table 2). However, major factors were later found to be vitamin C (Ascorbic acid). This was confirmed by using dry white crystal form of vitamin C on polymerization (Appendix C) and result found to be similar with those of the fruit juice extracts. This experiment was carried out by the 2,6–dichlorophenol indophenol visual titration method.

As a reducing agent, vitamin C reverses the deoxygenation activity of sodium metabisulphite and restores oxygen back to haemoglobin. It was observed that this inhabitation is concentration-dependent.

There were no significant differences in the antisickling effectiveness of the fruit juice extracts. This would be due to the closeness in the vitamin C content of the fruit juice extracts in micromoles as demonstrated. Carica papaya exhibited the best antisickling activity, although it does not contain the highest vitamin C content. It thus means that the extract contain other factors which act(s) as antisickling agent(s).

The vitamin C content of the citrus fruits C. sinensis and C. aurantifolia are very close.

From the results shown with dry white crystal form of vitamin C on polymerization and the estimation of vitamin C content in HbS polymerization, it could then be concluded that vitamin C is an antisickling agent.

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