



Extraction of Rutin from Sweet Orange (*Citrus sinensis L*) Mesocarp using Soxhlet Extractor

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Abstract: Rutin is one of the flavonoids found in various plant species including sweet orange mesocarp. Crude extract of sweet orange mesocarp was obtained via soxhlet extraction method with 98% ethanol, and ethanol/water (60/40 % v/v) as solvents, for different extraction times viz 30, 60, 90, 120, 150, 180, and 210 mins at 75°C. The extracts were purified, concentrated, and characterized to obtain a total of 1,667 g of rutin at optimized conditions. The present study was intended for the optimization of extraction of rutin from sweet orange mesocarp. By the use of different process conditions such as extraction time, different solvents, and temperature the yield of rutin was investigated. Ethanol was found to be the best solvent for the extraction of rutin from sweet orange mesocarp. The result showed that optimum concentrations were obtained at 60 min with a yield of 247 g and 195 g with ethanol and ethanol/water solvents respectively. A model was proposed in this work and the data obtained was used to validate it. The final form of proposed models were $Y(x) = a_n x^n + a_{n-1} x^{n-1} + a_{n-2} x^{n-2} + \dots + a_1 x^1 + a_0$ Where $a_n, a_{n-1}, a_{n-2}, \dots, a_1$ are constants, $Y(x)$ = yield extract ($\mu\text{g/mL}$ of rutin) and x = extraction parameter being studied. The coefficient of the developed model were $R^2 = 0.9861$ and $R^2 = 0.9986$ for ethanol solvent extraction and ethanol/water solvent extraction respectively.

Keywords: Sweet orange mesocarp, rutin, soxhlet extractor, modeling equation, ethanol, ethanol/water mixture.

I. Introduction

Rutin, also called rutoside or sophorin, is one of the most popular phenolic compounds found in various plant species. It is a bioflavonoid found in a wide variety of plants, fruits and vegetables including orange, tomato, carrot, potato, apple peels and black tea, onion, (Fabjan et al., 2003). Rutin is a prominent flavonoid, it consists of a saccharide unit and an aglycone part (Spackova and Pazourek, 2013). It is a glycoside combining the quercetin flavonoid and phenolic compound. It is a natural compound belonging to the flavonol group and shows significant antioxidant property (Filipiak-Szok et al., 2012). Rutin has been utilized more in medical and pharmaceuticals where it has played an antioxidant role character. It has been used in inhibiting some cancers, reduction of capillary fragility, bruising, and swelling. It has also be used to cure venous insufficiency and improving micro-vascular blood flow (Roger, 2002). Rutin, as ferulic acid can reduce the cytotoxicity of oxidized low density lipoprotein (LDL) cholesterol and lower the risk of heart diseases (Del-Rio et al., 1997). Rutin supplements have also been promoted for the treatment of a wide spectrum of diseases (Anand and Surya, 2020). Rutin is also used as a food additive and flavouring agent for different drinks and food preparations, in cosmetics as well as for colouring purpose (Fathiazad et al., 2006).

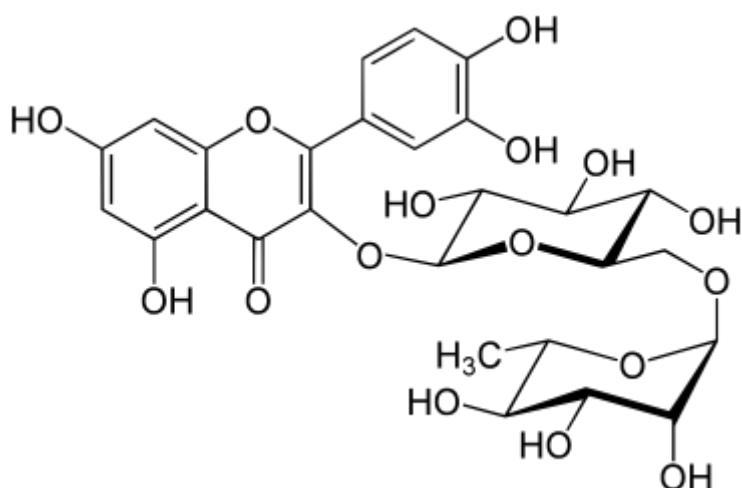


Figure 1. Chemical structure of rutin (quercetin 3-β-D-rutinoside)

The peel of sweet orange (*Citrus sinensis L*) fruit is one of the often overlooked part of the sweet orange plant. When sweet orange is consumed the peel, fruit pith residue, and seeds are regarded as waste. Direct disposal of these wastes to the environment causes serious problems as these contain bioactive compounds. Release of these bioactive compounds to the open landfills cause bad odor and spread disease, and disposal to water bodies or seepage to the underground water table deteriorates water quality and harms aquatic life. To this end, a number of research are being focused on the development of better reuse methods to obtain value-added phytochemicals as well for safe disposal. Sweet orange peel possess various bioactive properties and contains a variety of chemical compounds, including carotenoids, steroids, essential oils, terpenoids, ethyl esters, alkane groups and flavonoids (Okwi and Emenike 2006). The peel of sweet orange is divided into two parts: albedo or mesocarp (a white spongy tissue inner layer of the peel) and flavedo or the epicarp or exocarp (the outermost layer of the peel). Iman et al., 2020, determined the total phenolic content (TPC) and total flavonoid content (TFC) in different parts of sweet orange including, leaves, flowers, seeds, juice, exocarp, and mesocarp. The leaves were reported to contain the highest percentage of TPC while the mesocarp was reported to contain the highest percentage of TFC (Iman et al., 2020). However, the authors did not identify/separate the respective flavonoid compounds in the orange parts. Rutin obtained from orange mesocarp has been used to remove some metal ions. Ogali et al. (2008) reported the use of unmodified orange mesocarp residue after extraction of rutin to bind 56% of Mg, 81% of Zn, 71% of Cu, 73% of Pb and 85.05% of Cd, while the modified residue using 2,4-Dichloro-6-(Phenoxy-4'-sulphonic acid)-1,3,5-triazine bound 63.05% of Mg, 37% of Zn, 43.25% of Cu, 33.05% of Pb and 86.45% of Cd. Also Millicent et al., 2012, reported the use of modified orange mesocarp resin after extraction of rutin to removal of zinc, copper, nickel and cobalt ions from aqueous solution using modified orange mesocarp extract (Carboxylated-Toluene Di-isocyanate Orange Mesocarp Extract Resin (CTOMER) and Sulphonated-Toluene Di-isocyanate Orange Mesocarp Extract Resin (STOMER)).

Soxhlet extractor is a liquid- solvent extraction and the most common method for separating bioactive components from their natural resources (Ahmad, 2009). It is employed for the extraction of compounds with limited solubility in a solvent, and the impurity is insoluble in that solvent. The advantages of this method over other extraction methods (Nandan and Meena 2015), includes: (i) the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium, (ii) the temperature of the system remains relatively high due to the heat applied to the distillation flask, (iii) sample throughput can be increased by simultaneous extraction in parallel, (iv) it has the ability to extract more sample mass and it is non-matrix dependent. In addition to a comparable less solvent consumption, Soxhlet extractor is easy to maintain and of low cost. However, the high temperature and long extraction time in the Soxhlet extraction may increase the possibilities of thermal degradation of the chemical structure of the material and therefore reduces its bioactivity. Also, the presence of a solvent in the extract may affect the kinetics of crystallisation and the crystal morphology of the product. Meena et al., 2015, extracted rutin from pineapple peel using soxhlet

extractor, different organic solvents such as methanol, ethanol, water and ethyl acetate were used. They investigated the effect of different solvents, soaking time, different solvent percentages, effect of pH, different volumes of hexane with methanol as solvent and extraction time on the yield of rutin. The highest rutin concentration for optimized condition was reported as 30 µg/mL. Methanol was found to be the best solvent for the extraction of rutin from pineapple peel by soxhlet extractor. Meena et al., extraction was carried out for 90 min and an optimum concentration obtained at 50 min with a concentration of 38 µg/mL. They reported that for the Effect of different solvents on extraction of rutin, methanol (Dixit, 2014) showed the best results and the concentration of rutin obtained was 12 µg/mL this was followed by ethanol. For the Effect of soaking time on extraction of rutin, they incubated the samples for different time periods viz., 1, 2, 3 and 4 d. and reported that soaking for 1 d gave the best result and the concentration of rutin was 21 µg/mL. The Effect of different solvent composition on the extraction of rutin, was also examined and they reported the optimum solvent percentage to be 80% and the concentration of rutin found to be 23 µg/mL. For the Effect of different pH on extraction of rutin, the optimum concentration of rutin was 26 µg/mL observed at pH 7. Finally the Effect of extraction time with hexane on extraction of rutin was investigated and they reported that, at the 1 h, the concentration of rutin was 30 µg/mL. This solvent extraction using soxhlet extractor was conducted to verify the mathematical model proposed in their work. Anand and surya 2020, extracted rutin from peel of *Allium cepa* (onion) using Soxhlet extractor. Various organic solvents such as ethanol, methanol, water, and chloroform were used for evaluating its effect on the extraction of rutin optimum yield from peel of *Allium cepa*. Among all the solvents, Methanol (Ravi, 2016) was reported to show the best result for the extraction of rutin and the concentration was 7.5 µg/ml. Also investigated are the effects of different pH, solvent percentage, and extracting time. Their reports shows the optimum values of pH = 5.0 and rutin concentration of 24.5 ng/ml, optimum solvent percentage 80% and rutin concentration of 10.0 ng/ml, and optimum extraction time 24 hours and rutin concentration of 30.5 ng/ml. Rutin extracted was purified using column chromatography. Jaya and Amit 2015, in their work "Isolation and identification of flavonoid rutin from *Rauwolfia serpentine*" extracted rutin using soxhlet extraction method. The dried powdered leaves were subjected to extraction using 500 ml 80% methanol in a soxhlet extractor for 24 hours. The extract was concentrated in a rotatory vacuum evaporator to 50 – 60 ml. The isolation and identification was done using a thin layer chromatography and the authors reported observed yellow bands with Rf value of 0.45 and 0.31.

Although, most of the studies have shown that methanol extraction resulted in a higher yield of citrus flavonoids (Khan et al., 2014, 2018; M'hiri et al., 2014, 2015; Garcia-Castello et al., 2015). However, methanol has been reported to be toxic especially in food industrial applications and also environmentally unfriendly. Ethanol is considered "Generally Recognized As Safe" (GRAS) solvent by the US Food and Drug Administration and is the most recommended solvent during the food Industrial extraction processes (Yaqoob et al., 2020), therefore it was tested in this study. In order to optimize the utilisation of solvent in the extraction of bioactive components (Bhuiyan and Begum, 2009) from natural resources, an estimation of the extract yield obtained is necessary. Some modelling equations have been developed by some researchers in this regard. Nandan and Meena 2015, developed a modelling equation describing the extraction process of Quercetin, Rutin and Kaempferol from peel powder of *Citrus Medica* Peel. To enable their modelling they made the following hypotheses: (i) every powder particle is symmetrical, (ii) the mass transfer coefficient is constant, (iii) the solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the flavonoids (Khoddami et al., 2013) concentration in the solvent depends only on time, (iv) the transfer of the flavonoids is a diffusion phenomenon and independent of time, (v) at the interface, the concentration of flavonoids in the solution between the internal liquid (in pores) and external to particles are equal. The final form of the equation obtained from this modelling is:

$$E_s = (t) + B \quad 1$$

(Where A & B are equation constants, Es = yield extract (µg/ml of flavonoids) and t = extraction time (min). The final form of proposed model equation for Quercetin was Es = 0.0849(t) + 7.0286, and for rutin was Es = 0.0912(t) + 25.971, and for Kaempferol was Es = 0.0267(t) + 7.3714.

Meena et al., 2015 developed modeling equation for extraction of rutin from pineapple peel using soxhlet

extractor. To Model the extraction of rutin using soxhlet extractor in order to describe the rutin from the peel of pineapple to the bulk of the solvent, Meena et al., made the following hypothesis (i). The mass transfer coefficient is constant. (ii). the solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the rutin concentration in the solvent depends only on time, (iii). the transfer of the rutin was a diffusion phenomenon and independent of time. By this hypothesis, they developed an Equation of the form (Meena et al., 2015),

$$E(t) = At^2 + Bt + C$$

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Where A, B and C are constants, E_s =yield extract (mg/L of rutin) and t=extraction time (min). Their final form of proposed equation was given as: $E(t) = -0.011t^2 + 1.2591t$ with $R^2=0.962$

Anand and Surya 2020 developed modelling equation for extraction process of rutin extracted from the peel of *Allium cepa* by soxhlet extractor. To model the extraction process of rutin, the authors made some hypothesis: (i) The mass transfer coefficient remains constant (Manasa et al., 2014). (ii) The raffinate phase solvents and rutin concentration in the onion extract depending on time. (iii) The transfer of rutin is time-independent and a diffusion process. Applying the above hypothesis, the authors proposed that the rutin transfer from raffinate phase to extract phase can be described by the following linear equation: $E_s = A(t) + B$ for rutin, where A&B are constants, E_s = Extraction yield (rutin in $\mu\text{g}/\text{ml}$) and t = time per extraction in minutes. The determined equation at the end for rutin as reported by Anand and Surya was $E(t) = 0.134t + 9.766$ with $R^2 = 0.922$. E_s = Yield extract (rutin in $\mu\text{g}/\text{ml}$) and t = extraction time (minutes). To our knowledge no modelling equation has been developed for the extraction process of rutin from sweet orange mesocarp. Therefore, in this study sweet orange mesocarp which contains higher levels of flavonoids (Iman et al., 2020) and has a different sample matrix was used as a test material. Extraction parameters such as the solvent nature, temperature, solvent composition ratio, and extraction time, influence the yields of phenolic compounds (Li et al., 2006; Khan et al., 2014; M'hiri et al., 2014; Garcia-Castello et al., 2015; Yaqoob et al., 2020; Iglesias-Carres et al., 2019).

The present study was intended for the optimization of extraction of rutin from sweet orange mesocarp. By the use of different process conditions such as extraction time, different solvents, different solvent composition ratio and temperature the yield of rutin will be investigated. The extraction temperature was tested up to 80°C to determine the inflection point of the maximum yield of rutin, In addition, the number of extracts was also optimized by repeated experiments to overcome the equilibrium limit, which corresponds to the exhaustion of flavonoids in the sample matrix (M'hiri et al., 2014). The parameters were evaluated individually through single-factor experiments and developed an optimized extraction method for maximum recovery of rutin.

II. Materials and Methods

Chemicals and reagents: Ethanol, distilled water, hexane, aluminum chloride, sodium nitrite, sodium hydroxide of Analytical grade were used for the study.

Collection and Processing of Plant Material

Freshly sweet Orange (*Citrus sinensis*) fruits were sourced from mile 1 market in Port Harcourt, Rivers State, Nigeria. The oranges were thoroughly washed with clean water and air dried for 1 days at room temperature to remove moistures and impurities. They were then peeled with kitchen knife to remove the epicarp or flavedo. After which the endocarp was removed to obtain the mesocarp or albedo. The mesocarp were washed with deionized water, and air dried for 3 days at room temperature to remove moisture and impurities. The dried Orange peel (mesocarp) were crushed into a fine powder with a commercial grinding machine, sieved to 150 μm particles sized and stored in an airtight bottle at room temperature for extraction. The crushing was done to increase the surface area of the sample in order to achieve proper interaction and penetration between the extracting solvent and shell materials during the extraction.

Soxhlet extraction

Extraction was done at Biochemistry research laboratory, University of Port Harcourt. The study adopted a solvent extraction mechanism (Akaho et al., 2019; Nandan and Meena, 2015). Extraction process was carried out in batches and in triplets for each batch. Samples of dried powder Orange mesocarp peel were measured in

300g and wrapped with filter paper. The wrapped samples were introduced into the main chamber (thimble) of the Soxhlet extractor, while 350ml of ethanol was poured into 500ml flat bottom flask. The chamber was fitted into the flask containing the ethanol and was connected to the condenser, pipe and the heating mantle. At the end of the set-up, the heating mantle (a source of heat) was turned on. The system was allowed to achieve a temperature range between 70°C and 80°C, which is the boiling point temperature range of the ethanol and was heated to reflux for 1-2hrs. The process was repeated until there was a clear ethanol in the thimble, and the extract collected as mixture in the glass reagent bottle. The crude extract then was filtered and ethanol was evaporated on vacuum rotary evaporator to obtain a concentrated crude extract. The concentrated extract was further air/sun dried for 24 hours to ensure solvent has completely evaporated and the product is in powder form before storage in an air tight bottles.

Purification of the soxhlet crude extract

Extract obtained from the soxhlet extractor contain many other components along with rutin. So the impurities are to be removed to get purified rutin. Crude extract obtained after the soxhlet extraction, can be purified by two different methods: solvent- solvent extraction and column chromatography or crystallization.

Solvent – Solvent Extraction:

Solvent – Solvent extraction was done with extract (i.e., obtained from soxhlet extractor) and hexane of different proportions by varying extract: hexane proportion in the ratio of 1:0.5 to 1:2.0 used as solvent (Nandan and Meena, 2015). Extraction was done in separating funnel for 1hr to 2hrs and then the two phases: raffinate and extract phases were separated. The sample is collected from both the phases for the estimation of rutin concentration. The raffinate was later filtered and the filtrate discarded to remove fats, waxes and pigments.

Column Chromatography

In this method, 200 micron particle size silica gel was used as stationary phase. Before starting the experiment a piece of cotton was first inserted into the column towards outlet and the column was fixed to the clamp tightly. A sea sand of 1cm bed was poured in the column. Silica gel powder was added in the column up to 10cm length from the neck of the column. The solvent ethanol was then run through the column up to the bed was entirely wet. Excess solvent was added on the top of the silica gel bed and the column gently tapped with hand or soft materials. After tapping gentle pressure can be applied. Before loading the sample in the column, little silica gel was added to the sample. To load the sample into the column, 20ml of sample was poured along the side walls of the column, and sand added on the top of the sample. Samples were collected for every 5 minutes from the column and 1ml of sample was taken from each test tube and quantitatively determine the rutin content by using spectrophotometer (Pawar and Salunkhe 2013). The rutin obtained was concentrated again using a rotary evaporator and recrystallized from ethanol to obtain pure rutin. The pure rutin obtained was characterized and verified using infrared spectroscopy and yield and purity of the isolated rutin were determined using GCMS.

Crystallization

A saturated solution of rutin was created by dissolving the concentrated rutin extract in ethanol (solvent). The saturated solution was cooled slowly to a temperature of 4 °C. A small amount of pure rutin crystals (seed crystals) were added to the solution to initiate crystallization. The solution was allowed to stand for 24 hrs. At end of 24 hrs, the solution was filtered and crystals of rutin formed were collected. The crystals were washed with a small amount of cold ethanol to remove impurities. The pure crystals of rutin were dried in a desiccator to remove any remaining solvent. During the process of crystallization, the pH of solution was maintained at pH of 5 to ensure rutin solubility for effective crystallization. Also cooling rate was controlled to prevent rapid crystallization, which can lead to impure crystals.

Modelling of Extraction Using Soxhlet Extractor

The transfer of Rutin from the peel powder to the bulk of the solvent, was modelled by adopting the same hypotheses of Nandan and Meena 2015: (i) every powder particle is symmetrical, (ii) the mass transfer coefficient is constant, (iii) the solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the flavonoids (Khoddami *et al.*, 2013) concentration in the solvent depends only on time, (iv) the transfer of the flavonoids is a diffusion phenomenon and independent of time, (v) at the

interface, the concentration of flavonoids in the solution between the internal liquid (in pores) and external to particles are equal. The final form of the equation obtained from this modelling is:

$Y(x) = a_n x^n + a_{n-1} x^{n-1} + a_{n-2} x^{n-2} + \dots + a_1 x^1 + a_0$ Where $a_n, a_{n-1}, a_{n-2}, \dots, a_1$ are equation constants, $Y(x)$ = yield extract ($\mu\text{g}/\text{mL}$ of rutin) and x = extraction parameter being studied.

III. Results and Discussion

Effect of different solvent composition on the extraction of rutin:

Different ethanol/water solvent percentage ratios – 50:50, 60:40; 80:20; and 100:0 were used for extraction of rutin. The optimum solvent percentage ratio was found to be 100:0 and with rutin yield to be 70.42%. (Fig.1)

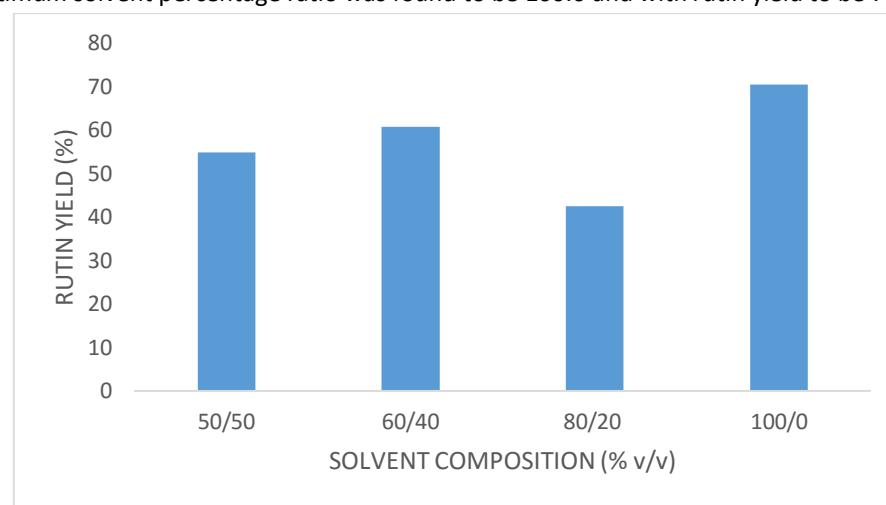


Figure 1. Effect of different solvent composition on extraction of rutin (ethanol/water ratio, %v/v)

Effect of extraction time with ethanol and ethanol/water on extraction of rutin:

Effect of extraction time on extraction of rutin was investigated with ethanol and ethanol/water solvents. To investigate the influence of the two solvents on extraction of rutin, different time intervals were taken: 30, 60, 90, 120, 150, 180, 210 minutes. It was observed that, the maximum yield of rutin with ethanol solvent was 247 g at 60 mins. (Fig.2) while the maximum yield of rutin with ethanol/water (60/40 % v/v) mixture solvent was 195 g at 60 mins. (Fig. 3)

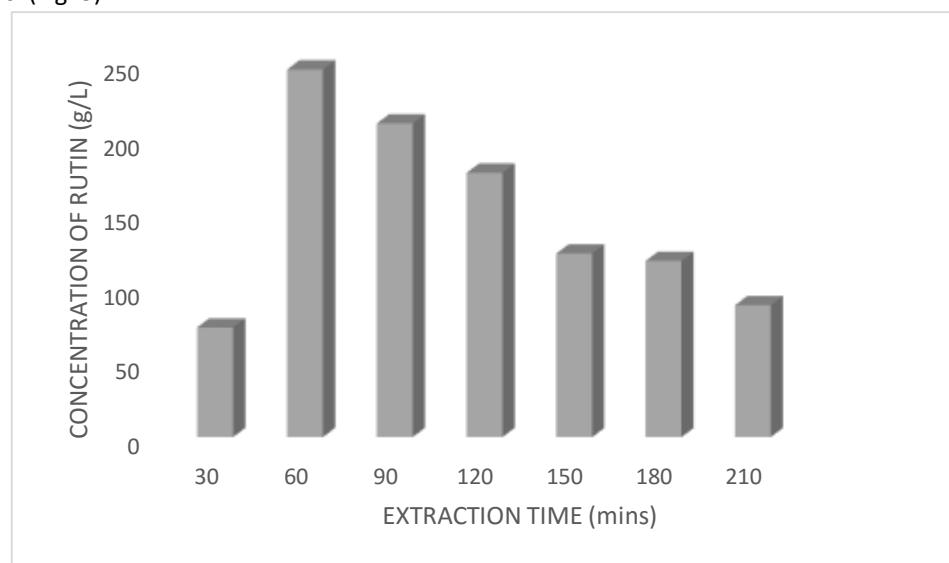


Figure 2. Quantity of rutin extracted at different time using ethanol as solvent

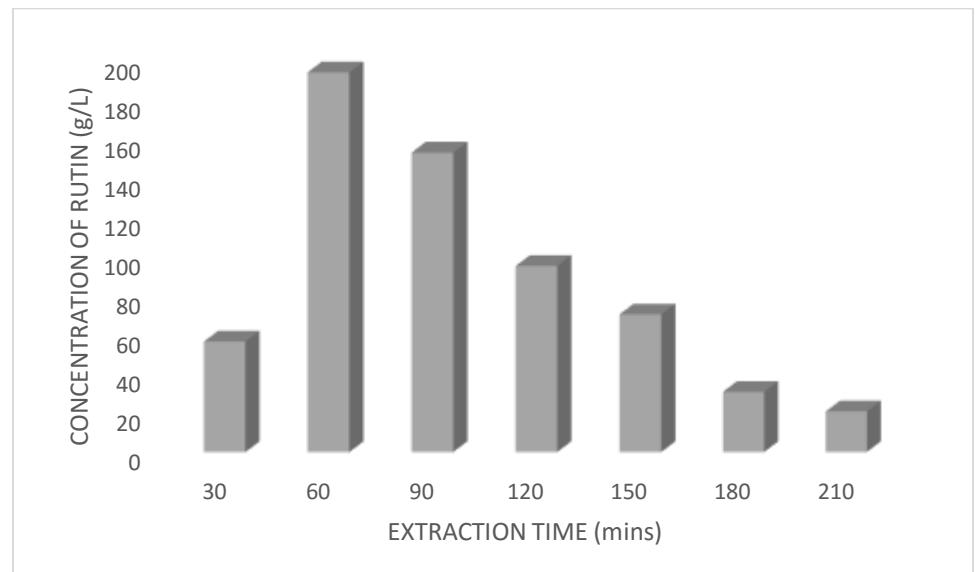


Figure 3. Quantity of rutin extracted at different time using ethanol/water as solvent

Effect of different solvents on extraction of rutin at different time:

For the extraction of rutin from sweet orange mesocarp extract (SOMEX) powder, two organic solvents, ethanol and ethanol/water (60/40 %v/v) were used. Among these ethanol showed the best results and optimum rutin yield of 247 g. while optimum rutin yield for ethanol/water 60/40 %v/v solvent mixture was 195 g (Fig.4)

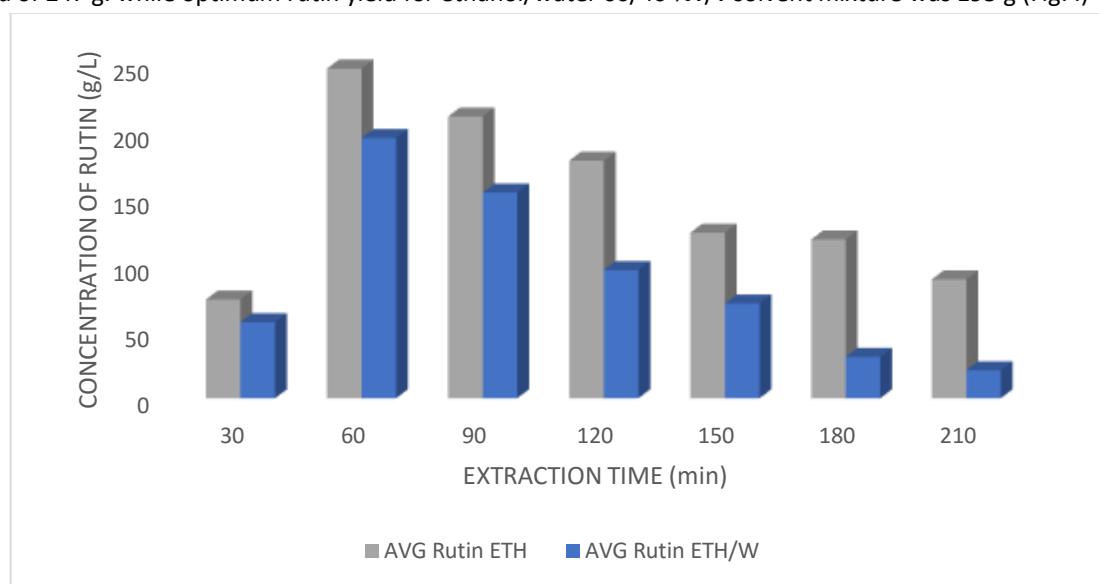


Figure 4. Comparison of effect of different solvents on extraction of rutin at different time

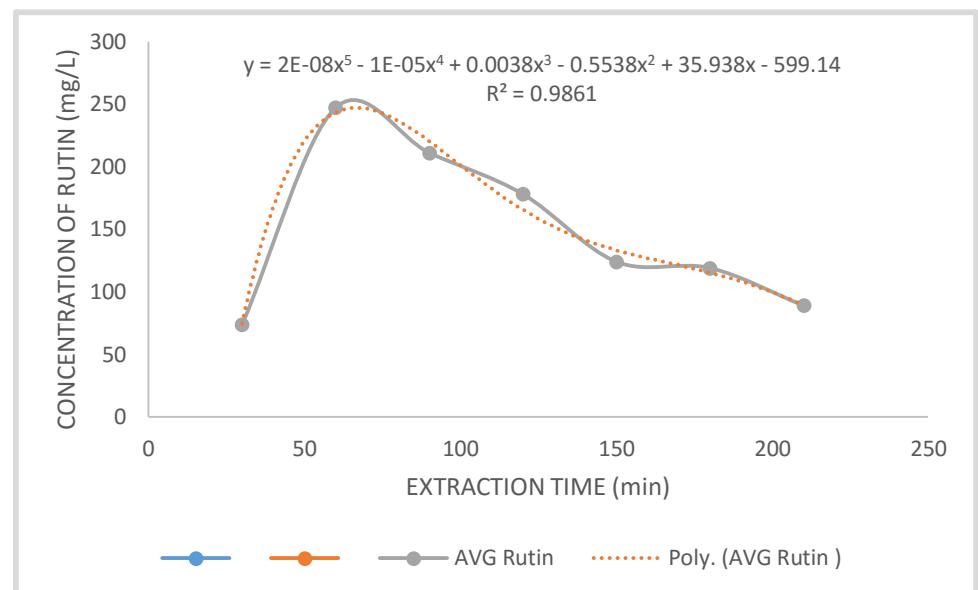


Figure 5. Effect of yield extract using ethanol with extraction time on rutin

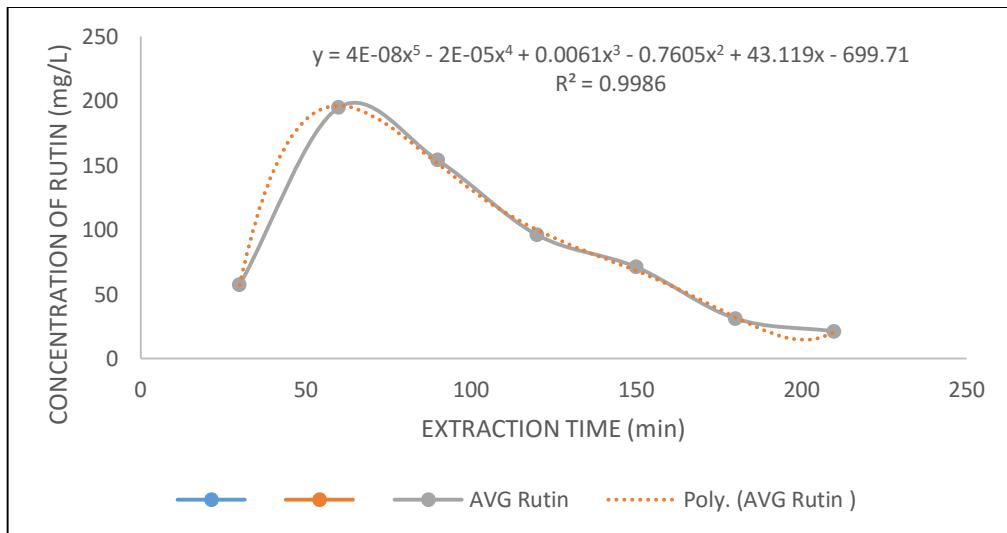


Figure 6. Effect of yield extract using ethanol/water with extraction time on rutin

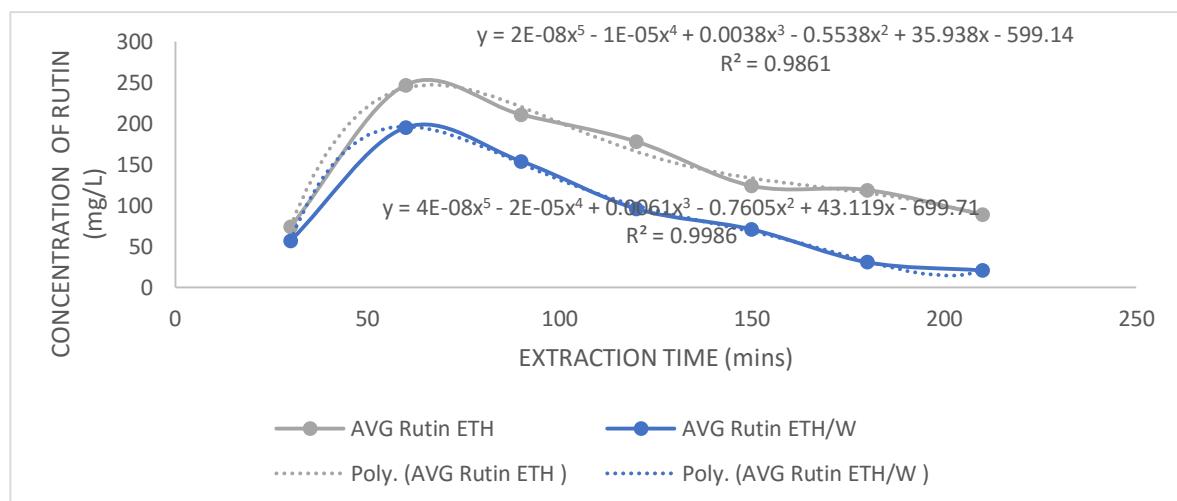


Figure 7. Comparison of effect of yield extract with different solvents on extraction of rutin at different time

Solvent extraction (soxhlet methods) was employed for this work. The modes of extraction used and the choice of solvent were targeted at extracting more of the natural rutin component of sweet orange mesocarp (SOM). The report shows that rutin is a major flavonoid contained in the mesocarp of a sweet orange consisting of 40.67% of the total flavonoids extracted. Both ethanol solvent and ethanol/water solvent extracted a higher yield of rutin. However, ethanol solvent produced a higher yield of rutin than ethanol/water (60/40, % v/v) mixture solvent at each of the same experimental conditions as shown in Figure 4. Overall yield of rutin obtained from the sweet orange mesocarp extract was 1,667 g. Sheriff et al., (2023) in their work "Comparative flavonoid profile of orange (*Citrus sinensis*) flavedo and albedo extracted by conventional and emerging techniques using UPLC-IMS-MS, chemometrics and antioxidant effects", used four different extraction approaches- (Solvent extraction, Ultrasound-assisted extraction, Pulsed electric fields assisted extraction and High hydrostatic pressure assisted extraction) and ultra-pure water, methanol, ethanol and ethyl acetate as solvents as different solvents for their extraction. However they reported no rutin in the albedo (mesocarp). But a good yield of rutin from orange mesocarp peel has been reported by some previous researchers (Ogali et al., 2008, Millicent et al., 2012, Sok et al., 2018). It was also observed that the optimum yield of Rutin, 247 g using ethanol and 195 g, using ethanol/water, occurred at 60 mins extraction time and at a process temperature of 75°C (Figures 5 and 6). After 60 min the yield start, and continue to decrease. At 210 mins extraction time, Rutin yield for ethanol solvent reduced to 89 g while that of ethanol/water reduced to 21g as can be seen in Figures 5 and 6. The decrease in yield can be attributed to thermal degradation of the compounds. Generally, prolonged heating of flavonoids may result to their degradation or transformation of the compound to other products. Hind et al., (2017) carried out a study to determine the effect of heat processing on the stability and antioxidant activity of six flavonoids and to establish the relation structure–activity–stability of these compounds. Their process conditions are temperature range from 30°C to 130°C and a process time of 2 h. They reported 50% loss of flavonoid content of rutin at a temperature of 90 °C after 2 h process duration. Our result shows that for optimum yield of rutin and for energy economy, extraction of rutin from sweet orange mesocarp using ethanol or ethanol/water mixture, should be performed within 60 min at a temperature of 75°C.

Modeling of extraction of rutin using soxhlet extractor:

Ethanol was found to be the best solvent for the extraction of rutin from sweet orange mesocarp by soxhlet extractor. It was carried out for 210 min at 75°C and an optimum concentration obtained at 60 min with a concentration of 247 g/L. Ethanol/water (60/40 % v/v) solvent mixture extracted optimum rutin of 195 g/L at 60 min.

Modeling of extraction of rutin using soxhlet extractor apparatus was studied in order to describe the rutin from the mesocarp of sweet orange to the bulk of the solvent. The mass transfer coefficient is constant. The solvent in the extractor is perfectly mixed, while the transfer resistance in the liquid phase is negligible and the rutin concentration in the solvent depends only on time. The transfer of the rutin was a diffusion phenomenon and independent of time. By this assumptions, an equation was developed (Meena et al., 2014).

$$E(t) = At^2 + Bt + C$$

$$Y(x) = a_n x^n + a_{n-1} x^{n-1} + a_{n-2} x^{n-2} + \dots + a_1 x^1 + a_0$$

Where a_n , a_{n-1} , a_{n-2} , ..., a_1 are constants, $Y(x)$ = yield extract (g/L of rutin) and x = extraction parameter being studied, ($a_0 \neq 0$).

The final form of proposed equation are: $Y(x) = 2E-08x^5 - 1E-05x^4 + 0.0038x^3 - 0.5538x^2 + 35.938x - 599.14$ with $R^2 = 0.9861$ for ethanol solvent extraction (Fig.5) and $Y(x) = 4E-08x^5 - 2E-05x^4 + 0.0061x^3 - 0.7605x^2 + 43.119x - 699.71$ with $R^2 = 0.9986$ for ethanol/water solvent extraction (Fig.6). The effect of yield extract with different ethanol and ethanol/water solvents on extraction of rutin at different time were compared in Figure 7.

IV. Conclusion

The present study was intended for the optimization of extraction of rutin from sweet orange mesocarp. By the use of different process conditions such as extraction time, different solvents, and temperature the yield of Rutin was investigated. Both ethanol solvent and ethanol/water solvent extracted a higher yield of rutin, however, ethanol solvent produced a higher yield of rutin (1,042 g), than ethanol/water (60/40, % v/v) mixture

solvent (625 g) at the same experimental conditions. Overall yield of rutin obtained from SOMEX is 1,667 g. The yield indicates that sweet orange mesocarp (SOM) is a rich source for a large scale production of rutin. The process can be optimized. The optimum yield of rutin, 247 g using ethanol and 195 g, using ethanol/water, was observed at 60 mins extraction time and at a process temperature of 75°C. After 60 min the yield start, and continue to decrease. At 210 mins extraction time, rutin yield for ethanol solvent reduced to 89 g while that of ethanol/water reduced to 21 g. The decrease in yield can be attributed to thermal degradation of the compounds. Generally, prolonged heating of flavonoids may result to their degradation or transformation of the compound to other products. Empirical models developed using the experimental data followed polynomial functions. Rutin extraction with ethanol and ethanol/water combination for a total of 210 mins and a temperature range of 70°C to 80°C followed fifth (5th) order polynomial.

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