BINDURA UNIVERSITY OF SCIENCE EDUCATION

EXTRACTION AND QUANTITATION OF FLAVONOIDS FROM LOCAL FRUITS AND THEIR UTILIZATION IN YOGHURT MAKING

BY

MUGWAZI BEVEN (B1647350)

SUPERVISOR: PROF DZOMBA

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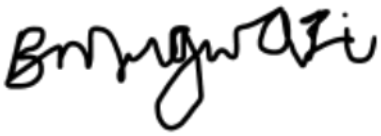
APPROVAL FORM

The undersigned certify that they have supervised, read and recommended for acceptance a research project entitled extraction and quantitation of flavonoids from local fruits and their utilization in yoghurt making.

Submitted by Mugwazi Beven.

In partial fulfillment for Bachelor of Science (Honours) Degree in Chemical Technology.



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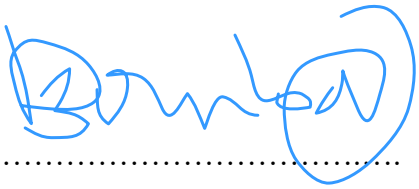

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(Signature of supervisor) (Date)

…. …. 16/10/23

(Signature of chairperson) (Date)

DECLARATION

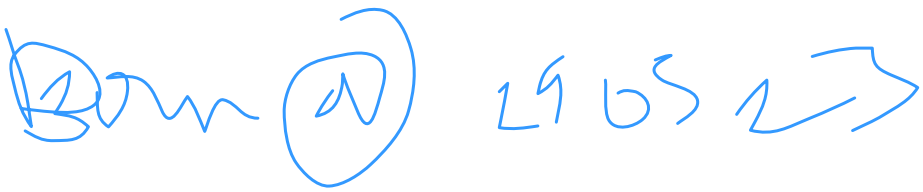
I BEVEN MUGWAZI declare that this project is my own work and the sources that I have used are all acknowledged by the form of references. And I declare that this work has not been submitted before for any examination.

……30/05/23……

(Signature) (Date)

Supervisor

I …Professor P. Dzomba…… declare that I have supervised this project and certify that it can be submitted to BINDURA UNIVERSITY OF SCIENCE EDUCATION.



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DEDICATION

I dedicate this work to my supervisor for his guidance, supervision and academic support. Secondly, many thanks to my parents for their support and encouragement during course of the study.

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Firstly, I would like to give thanks to the Almighty for the spiritual guidance and strength and hopes on trying times. My second gratitude goes to my supervisor Prof Dzomba for his advice, guidance, encouragement and consistent support during the course of the study. My family, I also thank you all for your support especially financial support. My chemistry lecturers at Bindura University of Science Education, I thank you for your academic support. My friends Wilson, Yemurai and Ayatollah they were kept encouraging me during course of the study, I also give thanks to you all. Last but not least I would like to thank our lab technicians for their support, materials and guidance, namely Mr Sada, Mr Dzomba and Mrs Zhou, they offered tremendous support for the success of the project.

Table of Contents

APPROVAL FORM ii

DECLARATION iii

DEDICATION iv

ACKNOWLEGDEMENTS v

ABSTRACT xii

1.2 Statement of the problem 2

1.3 Purpose of the study 2

1.4 Aim 2

1.5 Objectives of the study 2

1.7 Significance of the study 3

CHAPTER 2. LITERATURE REVIEW 4

2.1 Introduction 4

2.2 OXIDATION STRESS AND WAYS TO DELAY THE PROCESS 4

2.3 FLAVONOIDS 5

2.3.1 Flavonoids as antioxidants 5

2.3.2. Chemical structure 5

2.4 *Vangueria infausta* 7

2.5 *Fragaria vesca* 8

2.6 *Strychnos spinosa* 8

2.7 Isolation of flavonoids 9

2.7.1 Chromatography 9

2.7.2 TLC 9

2.8 Yoghurt formulation 11

2.8.1 Adjust Milk Composition & Blend Ingredients 11

2.8.2 Pasteurize Milk 11

2.8.3. Homogenize 11

2.8.4 Cool Milk 12

2.8.5 Inoculate with Starter Cultures 12

2.8.6 Hold/ fermentation 12

2.8.7 Cool 12

2.8.8 Addition of Flavonoids 12

2.8.9 Package 12
2.9 Determination of Antiradical Activity 12
2.9.1 ABTS assay 13
2.9.2 DPPH radical scavenging assay 13
2.9.3 Oxygen Radical Absorbance Capacity (ORAC) assay 14
2.9.4 Ferric reducing antioxidant power 14
2.9.5 Change in pH 15
2.9.6 Viscosity 15
2.9.7 Sensory evaluation 15
CHAPTER 3: METHODOLOGY 16
3.2.1 REAGENTS 16
3.3 Sample collection and preparation 17
3.4 Extraction of flavonoids 17
3.4.1 Liquid solid extraction 17
3.5.0 Analytical TLC 17
3.5.1 Preparative TLC 17
3.6 UV quantification of flavonoids 18
3.7 EVALUATING PARAMETERS OF HERBAL YOGHURT 18
3.7.1 DPPH free radical scavenging activity 18
3.7.2 Stability of a formulation 19
3.7.3 Sensory evaluation 19
CHAPTER 4: RESULTS AND DISCUSSION 20
4.2 YIELD 20
4.3. Analytical TLC 21
4.5 Preparative TLC Results 22
4.6 UV scanning and FT-IR 23
4.6.1 UV Analysis (Quantification) 23
4.6.2 UV Analysis scan 25
4.6.3 FTIR Analysis of *Fragaria vesca* extract 26
4.7 HERBAL YOGHURT 27
4.8 DPPH radical scavenging assay 27
4.8.1 DPPH. Scavenging activity 29

4.9 Sensory properties 29
4.9.1 Panelist's responses 30
4.9.2 Quality properties of herbal yoghurt 30
CHAPTER 5. CONCLUSION AND RECOMMENDATIONS 32
CONCLUSIONS 32
RECOMMENDATIONS 32
REFERENCES 33

List of figures

Figure 2.1General structure of flavonoids. .....**Error! Bookmark not defined.**
Figure 2.2Subclasses of flavonoids..... 6
Figure 2.3 Photographs of the *vanguaria infausta* fruit..... 8
Figure 2.4 Photograph of the *fragaria vesca* fruit 8
Figure 2.5 Photograph of *S. Spinosa*..... 9
Figure 4.1 Analytical TLC chromatograms 21
Figure 4.2 1 Separated flavonoids, 2 Scratched bands, 3 filtrates from the band..... 23
Figure 4.3 Calibration curve for the standard quercetin. 24
Figure 4.4 UV analysis of final extract and quercetin standard..... 26
Figure 4.4 FT-IR analysis of the bands extracts, **A** = quercetin **, B** = sample..... 26
Figure 4.6 Raw yoghurt. (Before addition of flavonoids) 27
Figure 4.7 Herbal yoghurt (After addition of flavonoids) 27
Figure 4.8 DPPH scavenging activity of separated flavonoids..... 28
Figure 4.9 DPPH scavenging activity..... 29

List of tables

Table 3.1 Instruments used and their uses 16
Table 4.1 Sample weight..... 20
Table 4.2 Rf values for detected flavonoids 22
Table 4.3 Panelist's responses 30
Table 4.4 Quality properties of the herbal yoghurt..... 30

Abbreviations

ORAC: Oxygen Radical Absorbance Capacity

DPPH: Diphenylpicrylhydrazyl

SET: Single Electron Transfer

ROS: Reactive Oxygen Species

RNS: Reactive Nitrogen Species

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

PPP: pentose phosphate pathway

DNA: Deoxyribonucleic acid

TLC: Thin layer chromatography

ABTS: 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate)

HAT: Hydrogen Atom Transfer

ORAC: Oxygen Radical Absorbance Capacity

FRAP: Ferric Reducing Antioxidant Power

ABSTRACT

Oxidative stress is generated when oxygen reactive species (ROS) build up in cells and tissues. Multiple diseases can be caused by oxidative stress, which can harm tissues and organs. Oxygen-containing molecules with an unbalanced number of electrons are known as free radicals. Free radicals can interact with other molecules quite easily because of their odd number of electrons. Free radicals can result in lengthy chemical chains in our bodies because of their tendency to interact with other molecules easily. Antioxidant molecules are those that can give an electron to a free radical while maintaining their own stability. The free radical stabilizes and becomes less reactive as a result. Flavonoids due to its capacity to both prevent the formation of free radicals and scavenge existing free radicals without making themselves unstable they act as antioxidants. This study was focusing on formulating an herbal yoghurt rich in flavonoids extracted from local fruits (*strychnos spinosa, Fragaria vesca and Vangueria infausta*) which can help to scavenge free radicals in our body. The fruits used was found from the local forest. From three different fruits chosen the one with better yield was used for the production of herbal yoghurt. Solvent extraction was used to isolate the flavonoids from the fruit pulp. Then separated by TLC, both analytical and preparative TLC. *Fragaria vesca* and *strychnos spinosa* extracts were used in analytical TLC. *Strychnos spinosa* yielded 2 spots with Rf of 0.78 and 0.92 while *fragaria vesca* also yielded 2 spots with Rf with of 0.49 and 0.90. The presenceof flavonoid was further confirmed by UV-Vis spectrophotometer, both the sample and the standard absorb at 385nm. Qualitative analysis was used to identify the presence of flavonoids within in the fruit extract. The results showed that there were flavonoids within the selected fruits. From the three local fruits, *fragaria vesca* was the one with high yield of 41mg/100g and was used for the yoghurt production. *Vangueria infausta* yield was 27mg/100g whilst *strychnos spinosa* had 33mg /100g. Antioxidant activity was investigated by means of DPPH free radical scavenging activity of the herbal yoghurt as a possible therapy for free radical related diseases and oxidative stress. The herbal yoghurt shows better scavenging activity closer to that of the standard. Quercetin was used as a standard and had percentage radical scavenging of 95.4 whilst the beverage had 94.9%. According to the results, herbal yoghurt can be considered a more cost-effective and safe way to control oxidative stress-related disease. The yoghurt's pH, colour, flavour, taste, and stability were evaluated as quality indicators. Fortifying yoghurt using flavonoids from local origin makes it a functional food to fight oxidative stress.

CHAPTER 1 INTRODUCTION

1.1 Background of the study

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced as a normal byproduct of biochemical processes in our bodies, as well as a result of increased environmental exposure and higher dietary xenobiotic levels (Nimse and Pal, 2015). In different pathophysiological situations, the ROS and RNS cause oxidative stress. Oxidative stress has more negative effects than positive ones. DNA damage and cell tissue breakdown are both possible effects. Inflammation may also result from this damage. According to Sarma et al. (2021) these factors can cause chronic diseases like diabetes, cancer, obesity, and hypertension. Reactive oxygen species (ROS) influence cancer evolution in apparently contradictory ways through promoting tumorigenesis, supporting cancer cell transformation, or inducing cell death. Tumor cells alter sulfur-based metabolism, NADPH production, and the activity of antioxidant transcription factors to deal with high ROS levels (Hayes et al., 2020). Genetic modifications enable cell survival under high ROS concentrations during initiation by activating antioxidant transcription factors or raising NADPH via the pentose phosphate pathway (PPP) (Hayes et al., 2020).

Tumor cells comply with oxidative stress during progression and metastasis by increasing NADPH in a variety of ways and reductively metabolizing glutamine and folate. Aside from harming DNA, the production of free radicals will also change the structure of important proteins. Increased levels of free radicals, cause oxidative stress, which lowers nitric oxide levels in the body and causes hypertension (Sinha and Kumar, 2015). Obesity-related complications emerge as a result of increased oxidative stress damage to cellular structures and decreased production of antioxidant mechanisms. According to Mana and Jain (2015), oxidative stress can cause obesity by modifying food intake and promoting the accumulation of white adipose tissue (WAT).

By strengthening cellular defenses in the form of antioxidants, oxidative stress can be effectively neutralized (Truong et al., 2018). Because flavonoids are stable molecules, they can donate a free radical an electron and neutralize it. Through their ability to scavenge free radicals, these flavonoids delay or inhibit cellular damage. By boosting the levels of endogenous antioxidant defenses, some substances function as in vivo antioxidants (Halliwell, 2012) In the presence of cofactors like copper, zinc, manganese, and iron, the antioxidant enzymes undergo a multi-step conversion of harmful oxidative products to hydrogen peroxide (H2O2) and then to water. Radical scavenging is the process by which the small-molecule antioxidants neutralize the ROS and eliminate them.The large-molecule antioxidants are enzymes that bind to ROS and stop them from damaging other vital proteins (Nimse et al., 2015). Natural antioxidants from fruits are less volatile and more stable at high temperatures because of their composition and chemical characteristics. This makes them more beneficial to the food production process and more effective in protecting the final product. *Fragaria vesca, Vangueria infausta and Strychnos spinosa* Fruits are good source of flavonoids (Sadau and Eloff, 2014).

1.2 Statement of the problem

Oxidative stress has more harmful effects. It can break down cell tissue and cause DNA damage. This damage can also result in inflammation. These factors can lead to lifelong diseases like diabetes or cancer. The addition of synthetic antioxidants in food successfully solved problems of oxidative stress however they now causes other problems. They have been reported as cancer-causing agents (Danta and Piplani, 2010). This necessitates research to find new substances that can be added to beverages to serve the same purpose without causing problems. Additionally, fewer people can afford the products made from synthetic flavonoids because the majority of them are expensive.

1.3 Purpose of the study

The goal of the study was to discover a means of preventing the oxidation stresses brought on by free radicals. In the current study flavonoids from the fruit of *Fragaria vesca* are utilized as antioxidants that are being added in to yoghurt. The yoghurt serves as remedy for oxidative stresses because it may have ability to scavenge free radicals in our body. Antioxidants are molecules that can lessen the causes or effects of oxidative stress. Although the body makes some endogenous antioxidants, dietary antioxidants may offer an additional line of defense. The most common dietary antioxidants are flavonoids. These flavonoids scavenge/remove ROS before they can harm crucial biomolecules. Support the body's natural defenses, boost catalase, glutathione peroxidase, and superoxide dismutase activity, repair oxidative damage, get rid of damaged molecules, and prevent mutations (Ighodaro and Akinloye, 2018).

1.4 Aim

To assess flavonoid composition in *fragaria vesca, Vangueria infausta and Strychnos spinosa* and utilize them to make herbal yoghurt.

1.5 Objectives of the study

The study addresses the following particular goals:

i. To extract flavonoids from *fragaria vesca, Vangueria infausta and Strychnos spinosa*
ii. To quantify total amounts of flavonoids in the *fragaria vesca, Vangueria infausta and Strychnos spinosa.*
iii. To utilize the extracts rich in flavonoids in the making of yoghurt.

1.6 Research questions

The following questions were addressed in the study:

i. Does *fragaria vesca, Vangueria infausta and Strychnos spinosa* consist of significant amounts of flavonoids?

ii. Can the flavonoids be used to make herbal yoghurt?
iii. What is the anti-oxidant activity of the flavonoids fortified yoghurt?

1.7 Significance of the study

One of the key risk factors for diabetes mellitus, cancers, and cardiovascular diseases is the oxidative damage to cellular components like proteins, lipids, and nucleic acids (Sung et al., 2013). This happens as a result of an imbalance between the generation of oxygen-derived radicals and the organism's antioxidant potential. In place of synthetic antioxidant activity that has negative side effects like liver damage and carcinogenesis, natural antioxidants derived from plant-based ingredients can be used to control the rise in free radical formation and decrease in antioxidant capacity Due to their capacity to scavenge free radicals, the herbal yoghurt produced by addition of flavonoids in beverage is known to have a high oxidative inhibitory capacity (Singh and Hati, 2014). The fruit contains a variety of biologically active phytochemicals, such as isoflavone, saponins, and lecithin, which may have antioxidant properties, lower cholesterol, lower the risk of heart disease, and improve body composition by increasing fat-free mass and reducing abdominal fat mass. Because of this, the current study focuses on using additives like flavonoids to increase the antioxidant and the viability of lactic acid bacteria in cow milk yogurts during refrigeration.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Chronic diseases like cancer, diabetes, and cardiovascular diseases are becoming more common, especially in industrialized nations, which has increased awareness of the importance of diet. (Anderson and Durstine, 2019). According to Sahidhi et al. (2015), plant foods are recognized as an important source of a variety of bioactive compounds, particularly phenolic compounds, which individually or in combination are beneficial to human health. Due to a variety of biological and health-promoting properties, including their ability to exert antioxidant, antimicrobial, anti-inflammatory, antiallergenic, cardio-protective, and vasodilatory effects, flavonoids have attracted a lot of attention. They are also known to help minimize the risk of heart disease, cancer, and diabetes (Da Porto et al., 2013).

2.2 OXIDATION STRESS AND WAYS TO DELAY THE PROCESS

An imbalance between free radicals and antioxidants in our bodies causes oxidative stress. Oxygen-containing molecules with an odd number of electrons are known as free radicals. They can easily interact with other molecules because of their uneven number (Abd, 2012). Because free radicals interact with other molecules so readily, they can trigger long chemical chains in our bodies. These processes are referred to as oxidations. Our bodies undergo the natural and essential process of oxidation. On the other hand, oxidative stress happens when there is an imbalance between the activity of free radicals and antioxidants. When working properly, free radicals can aid in the defence against pathogens. Free radicals can begin harming the fatty tissue, DNA, and proteins in our bodies when there are more of them than can be balanced by antioxidants (Domej and Renner, 2014). Our bodies are largely composed of proteins, lipids, and DNA, so damage to these components over time could result in a wide range of illnesses. These include heart disease, diabetes, inflammatory diseases, high blood pressure, and hypertension. Making sure we are getting enough antioxidants in our diet is one way to prevent oxidative stress. The best way to give our body what it needs to produce antioxidants is to eat five servings of a variety of fruits and vegetables per day.

2.3 FLAVONOIDS

A group of diverse phenolic structures found in natural substances. According to Sangeeth (2016), they can be found in fruits, vegetables, grains, bark, roots, stems, flowers, and tea. There are efforts being made to isolate these natural products because they have beneficial impacts on health that are well known. A class of polyphenolic phytochemicals known as flavonoids consists of flavones, isoflavones, flavonols, catechins, and anthocyanidins (Karak, 2019). Fruits, nuts, vegetables, grains, as well as a variety of herbs and spices, contain them in relatively high concentrations. The polyphenolic plant compounds known as flavonoids are the most prevalent and can be found in almost all plant parts (Shetty, 2013).

Many edible vegetables and fruits contain flavonoids, which are recently reported as being safe and non-toxic compounds. In response to microbial infection, plants are known to produce flavonoids, which are hydroxylated phenolic substances (Karak, 2019). Their actions are influenced by the structure. The structural class, level of hydroxylation, other substitutions and conjugations, and level of polymerization all affect the chemical structure of flavonoids (Kumar and Pandey, 2013). The potential health advantages associated with the antioxidant activities of these polyphenolic compounds have sparked recent interest in these substances. The functional hydroxyl groups in flavonoids scavenge free radicals and chelate metal ions to exert their antioxidant effects. Metal chelation may be essential for preventing the production of radicals that harm target biomolecules (Symonowicz and Kolanek, 2012). Due to their high antioxidant capacity in both in vivo and in vitro systems, flavonoids are believed to have health-promoting properties as a dietary component (Garros et al., 2018). Flavonoids can activate the human body's defence-enhancing enzyme systems. A number of studies have suggested that flavonoids have protective effects against a variety of bacterial and viral infections as well as degenerative illnesses like cancer, cardiovascular disease, and other age-related illnesses. When plant tissues are exposed to various abiotic and biotic stresses, flavonoids also function as a secondary antioxidant defence system.

2.3.1 Flavonoids as antioxidants

According to Kusumawati and Indrayanto (2013), antioxidants are molecules that can donate an electron to a free radical while maintaining their stability. As a result the free radical is able to stabilize and become less reactive.

2.3.2. Chemical structure

According to Gulcin (2020), flavonoids are a class of complex compounds with a central oxygenated heterocyclic ring and a three-ring structure with two aromatic centers. The classification of flavonoids is based on the substitutions that they undergo. Flavonoids are C15, benzo--pyrone derivatives with phenolic and pyrane rings. In addition to the conjugation between the B- and C- rings, flavonoids differ in how their hydroxyl, methoxy, and glycosidic side groups are arranged. According to Kumar and Pandey (2013), hydroxyl groups are typically formed as 3-O-glycosides in foods as a result of addition, methylation, and glycosylation during metabolism.

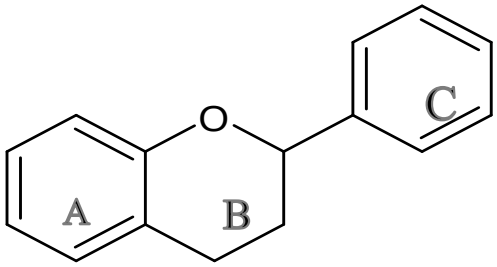


Figure 2.1 General structure of flavonoids (Kumar and Pandey, 2013).

2.2.3 Sub classes of flavonoids

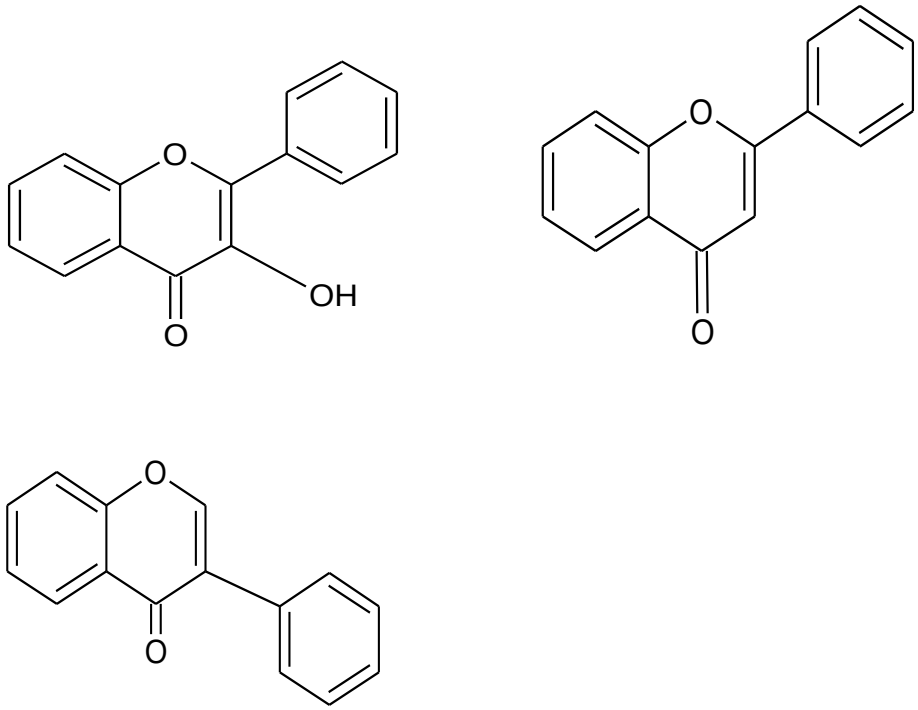


Figure 2.2 Subclasses of flavonoids (Guven and Simsek, 2019)

Flavonoids can be divided into a variety of subgroups based on the carbon in the C ring that the B ring is attached to as well as the degree of unsaturation and oxidation of the C ring (Waghmare et al., 2017). Isoflavones are flavonoids that have a B ring joined to the third position of a C ring. Neoflavonoids are those in which the B ring is linked in position 4. Those in which the B ring is linked in position 2 can be further divided into a number of subgroups based on the structural

properties of the C ring. Anthocyanins, flavones, flavonols, flavanols, and flavanones are examples of these subgroups.

Isoflavones:
They have structural similarities to estrogens though they are not steroid. Because of this, isoflavones bind to estrogen receptors (Kumar and Pandey, 2013). Most of them are present in legumes like soyabeans (Saewan and Jimtaisong, 2013).

Flavonols:
Flavonols are the most prevalent type of flavonoids (Gai et al., 2014). The three most prevalent flavonols are quercetin, kaempferol, and myricetin found in onions and fruits.

Flavones:
Flavones are widely distributed as glucosides in leaves, flowers, and fruits. The polymethoxylated flavones are abundant in citrus fruit peels. They have a ketone at the C ring's position 4 and a double bond between positions 2 and 3. A hydroxyl group is located in position 5 of the A ring in the majority of vegetable and fruit flavones. Flavones are mostly obtained from olives (Kumar and Pandey, 2013).

Anthocyanins:
These are the most prevalent and significant class of water-soluble pigments found in nature. Many fruits and vegetables derive their blue, purple, red, and orange colors from them. Chemically, they are made up of an oxygenated heterocycle with two aromatic rings connected by three carbons (Butnariu and Grozea, 2022).

Flavonones
Are found in all citrus fruits, especially grapes, oranges and lemons. These flavonoids include hesperitin, naringenin, and eriodictyol. Due to their capacity to scavenge free radicals, flavonones have been associated to many health advantages. Citrus flavonoids exhibit significant pharmacological actions as antioxidants, anti-inflammatory, blood lipid-lowering, and cholesterol-lowering agents. Also known as dihydroflavones, flavonones. The main structural difference between the two subgroups of flavonoids is that they have a C ring that is saturated, causing them differ from flavones in that the double bond between positions 2 and 3 is saturated. (Panche et al., 2016).

2.4 *Vangueria infausta*

The medlar, is a species of plant in the family Rubiaceae. The fruits are consumed by humans and have a pleasant apple-like flavor. In Africa, the African medlar is a common food source. This little-known fruit may enhance dietary intake, increase food security, promote rural development, and aid in a sustainable use of land. The fruit pulp can be preserved for later use or it can be

consumed raw. Polyphenolic compounds saponins, flavonoids, and tannins are the phytocompounds found in V. infausta fruit (Gwatidzo et al., 2018).

Figure 2.3 Photographs of the *vanguaria infausta* fruit

2.5 *Fragaria vesca*

Commonly known as alpine strawberries, woodland strawberries or wild strawberries. It is a perennial herbaceous plant that grows in the wild and belongs to the rose family and yields edible food (Vinayak et al., 2021). About 180 mg of flavonoids are present in 100 grams of strawberries. The flavonols, quercetin, and kaempferol are among the flavonoids found in strawberries. These and other polyphenols act as inhibitors of carcinogenesis by lowering the bioavailability of carcinogens.

Figure 2.4 Photograph of the *fragaria vesca* fruit

2.6 *Strychnos spinosa*

The nutritious significance of the African endemic fruit tree known as the spiny monkey orange (Strychnos spinosa) has led to its extensive consumption by humans as well as animals. Various S. spinosa plant parts are commonly utilized in folk medicine to manage human and animal health and wellbeing. Strychnos spinosa has a variety of nutritional and phytochemical components,

particularly flavonoids and alkaloids, which have been shown to have beneficial impacts on health. S. spinosa portions are regularly used by various ethnic groups in Africa as a source of food and energy as well as for various folk medical treatments for people and animals. The fruit Strychnos spinosa is a rich source of nutrients, vitamins, and minerals that support wellbeing and good health (Aremu and Moyo, 2022).

Figure 2.5 Photograph of *S. Spinosa*

2.7 Isolation of flavonoids

2.7.1 Chromatography

Open column chromatography, analytical thin-layer chromatography, preparative thin-layer chromatography, high-speed counter-current chromatography, medium-pressure liquid chromatography, centrifugal preparative thin-layer chromatography and high-pressure preparative liquid chromatography are common chromatography techniques used to isolate flavonoids from plant matter (Santos et al., 2012).

2.7.2 TLC

An affinity-based technique called thin layer chromatography (TLC) is used to separate the components of a mixture. TLC is a separation technique with a wide range of applications, including both qualitative and quantitative sample analysis. TLC can be used to analyze almost any class of substance, including lipids, nucleotides, glycosides, carbohydrates, fatty acids, alkaloids, lipids, steroids, and nucleotides (Stahl, 2013). In TLC, the stationary phase is an inert plate surface, commonly made of glass, plastic or aluminum, coated with a thin coating of an adsorbent substance, most frequently silica gel or aluminum oxide. The sample is placed vertically into a closed chamber with an organic solvent (mobile phase) after being spotted onto one end of

the TLC plate. The sample components migrate over a range of distances depending on their varied affinities for the stationary and mobile phases as the mobile phase is moving up the plate by capillary action. The plate is taken out of the developing chamber when the solvent reaches the top of it and dried. The retention factor (Rf) value of each component is calculated as spots representing the separated components on the plate.

2.7.2.1 TLC PROCESS AND PRINCIPLES

The basic idea behind TLC is the same as that of classical chromatography. Mixture components are separated between a fixed stationary phase and a liquid mobile phase by differences in the affinities between the two phases.

2.7.2.2 RETENTION FACTOR (Rf)

The movement of compounds along the TLC plate is measured using the retention factor. Rf is calculated as the distance travelled by the component divided by the distance travelled by the solvent. Its value is always in the range of 0 and 1. Generally speaking, the slower a chemical migrates up the TLC plate, the more strongly it binds to the stationary phase adsorbent. Non-polar substances have a tendency to migrate along the plate more quickly than polar substances due to the fact that TLC adsorbents are normally polar, whereas polar substances move more slowly and have lower Rf values. The temperature, adsorbent, solvent system, and amount of material detected are the variables that impact the retardation factor. One of the quickest, cheapest, and simplest chromatographic techniques is TLC. TLC is the one of the quickest, most straightforward, most affordable chromatographic methods.

2.7.2.3 Visualization:

After separation, the TLC plates are taken out of the developing chamber, dried, and seen using an ultraviolet transilluminator at 365 nm (Gwatidzo et al., 2018). The plate is kept in a jar with iodine crystals if the organic compounds are colorless so that they can absorb the iodine vapour for about two minutes and the spots become visible (Cai & Carolina, 2014). After the run, several distinct colors were noticed on a plate, each one represented a different chemical. Visualization would be fairly simple if the extract contains dye or inks. After the run, several distinct colors were noticed on a plate, each one represented a different component. Visualization would be fairly simple if the extract contains dye or inks.

2.7.2.4 Analysis:

Under a UV transilluminator, the distance of a distinct band is determined. Retardation factors (Rf) are measured against the solvent front and compared to the typical retardation factor values provided in literature (Patil, 2020). Alternately, a reference chemical with a known Rf value might be used in conjunction with the samples to be separated.

2.7.2.4 Analytical TLC

Analytical TLC is utilized to do a qualitative study when different chemicals are tested on a TLC plate and viewed under UV light. Due to the preliminary nature of the identification of secondary

metabolites and other compounds contained in the sample, only a small amount of material is used.

2.7.2.5 Preparative TLC:

According to Rabel and Sherma (2017), preparative thin layer chromatography (PTLC) is used to separate and isolate quantities of material that are larger than those typical for analytical TLC. The sizes of the processed amounts range from 10 mg to more than 1 gram. On the TLC silica plate (coated 1-3 mm silica) for scraping out the band of interest, materials to be separated are frequently applied as long streaks during preparative TLC as instead of spots. After development, certain components may be recovered by scraping the sorbent layer off the plate in the region of interest and eluting the separated material from the sorbent using a powerful solvent. The substance that is extracted from the layer might need to be further purified using TLC or other chromatographic techniques, or its purity might be sufficient for elemental analysis on spectrometry-based identification and structure determination, for use in biological activity or chemical synthesis studies, or as a standard reference material for comparison with unidentified samples. UV spectrophotometer, FTIR, and mass spectrometer can be used for further process of secondary metabolites isolated, filtered and concentrated from the bands.

2.8 Yoghurt formulation

2.8.1 Adjust Milk Composition & Blend Ingredients

To get the required amount of fat and solids, milk composition can be adjusted. Often dry milk is added to increase the amount of whey protein to provide a desirable texture. At this time, additives are introduced such as stabilizers.

2.8.2 Pasteurize Milk

Pasteurization of the milk mixture takes place at 85°C for 30 minutes or 95°C for 10 minutes. The whey proteins are denatured using a high heat treatment. As a result, the proteins can create a gel that is more stable and prevents the separation of the water during storage. Due to high heat treatment, the quantity of spoilage organisms in the milk is further diminished, improving the growth conditions for the starter cultures. To ensure that the starter cultures (Lactobacillus bulgaricus and Streptococcus thermophiles) remain active in the yogurt after fermentation and serve as probiotics, milk is pasteurized before they are introduced. If the yogurt is pasteurized after fermentation, the cultures will be in activated form.

2.8.3. Homogenize

The mixture is homogenized to properly mix all of the components and enhance yogurt consistency.

2.8.4 Cool Milk

The milk is cooled to 42°C in order to bring the yogurt to the ideal growth temperature for the starter culture.

2.8.5 Inoculate with Starter Cultures

The starter cultures are added into the cooled milk.

2.8.6 Hold/ fermentation

The milk is held at 42°C until a pH of 4.5 is reached. This helps the fermentation to progress to form a soft gel and the characteristic flavor of yogurt. This procedure could require several hours.

2.8.7 Cool

To prevent further fermentation, the yogurt is cooled to 7°C.

2.8.8 Addition of Flavonoids

The addition of flavonoids varies based on the type of yogurt. For set style yogurt, the fruit is placed in the bottom of the cup, the yogurt is then filled with the inoculated yogurt, and the yogurt is allowed to ferment inside the cup. For swiss style yogurt, the fruit extract is blended with the fermented, cooled yogurt prior to packaging. This is where fruit extract (flavonoids) is being added. The addition of fruit extract might also add flavor to the product.

2.8.9 Package

The yogurt is pumped from the fermentation vat and packaged as desired

2.9 Determination of Antiradical Activity

Based on their chemical reactions and reaction mechanisms, antiradical activity assays are separated into two groups. According to Zeghad et al. (2019), the two categories are Single Electron Transfer and Hydrogen Atom Transfer Based Assays. The two reactions have the same final outcome, but they differ in their kinetics and the potential of side reaction. While SET-based methods determine a potential antioxidant's capacity to transfer one electron to reduce any compound and radicals, HAT-based methods assess an antioxidant's capacity to quench free radicals by hydrogen donation to form a stable compound.

(HAT) $X\blacksquare + AH \longrightarrow XH + A\blacksquare$

AND

(SET) $X\blacksquare + AH \rightarrow H^- + AH\blacksquare^+$

$AH\blacksquare \rightarrow A\blacksquare + H_3O^+$

$X^- + H_2O^+ \rightarrow XH + H_2O$

$M(III) + AH \rightarrow AH^+ + M(II)$

2.9.1 ABTS assay

In the ABTS assay, the radical cation 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS•+) is generated, and its maximum absorbance is at 414, 645, 734, and 815 nm (Aadil et al., 2014). The test sample is introduced to quench the ABTS+ radicals. The absorbance, which is proportional to unquenched ABTS+ is then measured after a predetermined reaction time in order to ascertain the test sample's antiradical activity. Results are given as a percentage of antiradical activity (Aadil et al., 2014).

Advantages:

- It is a simple process that proceeds quickly and produces reliable results.
- ABTS+ can dissolve in both aqueous and organic solutions, regardless of the ionic strength (Shahidi and Zhong, 2015).

Disadvantages:

- Requires a special preparation where the production of ABTS•+ by enzymes or a chemical reaction is necessary (Aadil et al., 2014).

2.9.2 DPPH radical scavenging assay

Free radical DPPH is strongly absorb at 517 nm. The DPPH radical is quenched in the presence of antiradical compounds, which reduces the intensity of the absorption at 517 nm (Aadil et al., 2014). The DPPH radicals' purple colour is changed to a light yellow hydrazine. The ability to determine a substance that donates either hydrogen atoms or electrons in a homogeneous system is provided by DPPH, which operates in both electron transfer (SET) and hydrogen transfer (HAT) systems.

Advantages:

- It produces stable organic nitrogen radicals, which have a deep purple color in the region between 515 and 520 nm.
- It is a fast and easy process
- Affordable approach for showing out how effective foods are in fighting free radicals.

Disadvantages:

- Some antioxidants interfere with the results because their spectra overlap with DPPH's at 515 nm (Moo-Huchin et al., 2014).
- When oxygen or light is present, it decreases the DPPH radical's absorbance at 515–520 nm after the reaction with an antioxidant

2.9.3 Oxygen Radical Absorbance Capacity (ORAC) assay

According to Santos-Sánchez et al. (2019), the method is based on the inhibition of peroxyl-radical-induced oxidation that is initiated by the thermal decomposition of azo-compounds like 2,2-azobis(2-amidino-propane) dihydrochloride (AAPH). ORAC represents typical radical chain-breaking activity by hydrogen-atom transfer and measures the inhibition of peroxyl radical-induced oxidation (Apak et al., 2016).

Advantages:

- Makes use of a biologically significant source of radicals and is the only technique to calculate the length of inhibition time.
- This method can be used to assess the antioxidant capacity of both lipophilic and hydrophilic components independently because it can use a variety of free radical oxidants and measure a variety of substances, such as antioxidants against peroxyl and hydroxyl radicals.

Disadvantages:

- Does not evaluate the capacity to prevent all reactive oxygen species, such as singlet oxygen and superoxides but only the ability to inhibit peroxyl and hydroxyl radicals.

2.9.4 Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay measures the ability of antioxidants in an acidic medium to convert ferric ion (Fe^{3+})-ligand complex to the quite blue ferrous (Fe^{2+}) complex. According to Azad et al. (2018), the percentage of absorbance at 593 nm that increases indicates the presence of antioxidant activity. Results are expressed as micromolar Fe^{2+} equivalents or in relation to an antioxidant standard. To maintain iron solubility and promote electron transfer, the FRAP assay is conducted under lower pH conditions than other ET-based methods. A change in the dominant reaction mechanism will result from this raising the redox potential. Although tripyridyltriazine (TPTZ) serves as the iron-binding ligand in the original FRAP assay, other ligands, such as ferrozine, have also been used to assess the ascorbic acid-reducing power of various compounds. The most widely used ferric reagent in FRAP assays in recent years has been potassium ferricyanide. In the latter case, Prussian blue is generated as the

final byproduct, which is measured spectrophotometrically and reveals the reducing power of the antioxidants tested (Munteanu and Apetrei, 2021). Prussian blue can be produced in two different ways with the same results. Antioxidants can either reduce the ferricyanide to ferrocyanide, which binds the free Fe3+ in the solution and produces Prussian blue, or reduce the ferricyanide to ferrocyanide.

Advantages

- The FRAP assay does not need specialized equipment and is easy, quick, and economical.
- The FRAP assay provides a quick, low-cost, and adaptable method for calculating the total antioxidant activity.
- It was originally used to measure the reducing power in plasma but its use has expanded to include determining the antioxidant activity of other biological fluids, foods, and plant extracts.

Disadvantages

- According to Benzie and Devaki (2018), FRAP results could differ depending on the analysis time observed in the reaction between antioxidants and Fe3+, which varied from a few minutes to a few hours. Because different antioxidants require different detection times, a single-point absorption end point might not represent an entire reaction**.**

2.9.5 Change in pH

A pH meter with calibrated glass electrodes is used to measure the pH levels of yogurt during the fermentation process. Yogurt is titrated against a sodium hydroxide solution to ascertain its titratable acidity. The titratable acidity is expressed as grams of lactic acid per 100 grams of yogurt.

2.9.6 Viscosity

Using the Rheometer plus viscometer, the viscosity of suspensions containing various suspending agents is measured in order to assess the effects of various shear rates on the rheology of the suspensions.

2.9.7 Sensory evaluation

The sensory qualities of the prepared yogurt are examined using the hedonic scale in order to assess the panelists' reactions. The appearance, texture, flavor, stability, and general acceptability of samples of the yogurt, each sample with different amount of flavonoids as well as control yogurt were evaluated.

CHAPTER 3: METHODOLOGY

3.1 INTRODUCTION

This chapter outlines the steps involved in preparing samples for data collection, as well as the instruments and their models, which will be discussed in chapter four.

3.2 MATERIALS

3.2.1 REAGENTS

Iodine, quercetin standard, aluminium chloride, ethanol, aluminium chloride, acetone, petroleum ether, ferric chloride, fruit samples (*Fragaria vesca*, *Vangueria infausta* and *Strychnos spinosa*).

Table 3.1Instruments used and their uses

Instrument	Use
Blade	For cutting TLC plates
TLC Developing chamber	For preparative TLC
Buncher funnel	For filtering sample
UV	Detecting of flavonoids in samples
Thermoscientific Scientific Nicolet FT-IR	For detecting flavonoids
Thermo Scientific Nicolet 6700 FT-IR spectrometer	For absorbance determination
Laboratory shaker	Shaking the samples during extraction procedure
Blender	For crushing
TLC plates	For separation of fruits extract
Analytical balance	for measuring mass of reagents
UV- Visualization detector set at 365nm	For visualizing spots

Blades	To cut the TLC plates

3.3 Sample collection and preparation

Fresh fruits of *Fragaria vesca*, *Vangueria infausta* and *Strychnos spinosa* were collected from a nearby forest in Marondera west district, Mashonaland East province. Three types of them of about 1kg each were collected and washed thoroughly. A blender was used to ground the fruits so that solvent can penetrate for better extraction. The suspension was then taken into reagent containers, labelled and refrigerated for further use.

3.4 Extraction of flavonoids

3.4.1 Liquid solid extraction

For an extraction process at room temperature, 20 g of crushed fruit pulp without seeds were mixed with 200 ml of analytical grade, absolute ethanol in a 250 ml volumetric flask. A laboratory shaker was used to shake the samples for four hours. After that, the mixture was filtered to separate the extract and solutes from the crude material using Whatman's number 1 filter paper and a Buchner funnel. The residue were collected and an additional 100 ml and 50 ml of absolute ethanol were used in the extraction procedure again. The ethanol extract was produced by combining the filtrates that were collected throughout the entire process. In preparation for future investigation, the obtained samples were then kept in a refrigerator.

3.5.0 Analytical TLC

The chromatographic analysis was conducted using TLC plates, 10 x 5 cm aluminum silica gel. The method of (Gwatidzo et al., 2018) was used with a few minor modifications. The plates were heated up first to activate them and then they were allowed to cool down to about room temperature. From one plate edge, a pencil-drawn baseline was created. On two different TLC plates with pencil-drawn baselines, the two extracts were spotted using thin capillary pipettes. The plates were put in a chamber for development along with some test solvents. Numerous trial runs were conducted to find the mobile phase that displayed the best separation. A 500 ml beaker with a tapped plastic sheet on the top served as the airtight chamber. Water: ethanol (2:7v/v) was used as a solvent which produces the best separation results. Up to 1.5 cm from the TLC plate's top end, the solvent front was permitted to move. The plate was taken off, and a pencil was used to mark the position of solvent front. The plates were dried by air, sprayed with a solution of 1% ethanolic aluminum chloride to identify flavonoid-related spots, and then dried again with air. The dried TLC plates were observed using a 365 nm UV/Vis lamp. Yellow or green spots with flavonoids were marked in their precise positions, and retention factors were calculated (Gwatidzo et al., 2018).

3.5.1 Preparative TLC

A technique reported by Gwatidzo et al., (2018) was applied with only minor modifications. 20 x 20 cm glass-coated silica gel preparative TLC plates were used in this process. On a pencil drawn baseline that was 1.6 cm from the edge of the relevant TLC plates, a concentrated band of ethanol

extracts was applied and it was then allowed to dry. The dried plates were placed in a development chamber (5L) with ethanol acting as the mobile phase. Rf values were calculated after the plates allowed to dry at room temperature. The bands that tested positive from analytical TLC for the flavonoids were scratched and redissolved in 2 ml of ethanol. The solvent was then evaporated and the dried samples were then stored in a refrigerator (0–5°C) for further analysis. The bands which tested positive as from analytical TLC for the flavonoids were scratched and re-dissolved in 2 ml of ethanol, then solvent evaporated and stored in a fridge (0-5^{o}C) waiting for further analysis. The presence of flavonoids was further confirmed by scanning the dried powdered sample on a UV spectrophotometer and using FT-IR. Quercetin spectrums, a standard flavonoid, were compared to the results.

3.6 UV quantification of flavonoids

Exactly, 0.1 mL of aluminum chloride and 0.1 mL of ethanol were mixed with 2 mL of the extract and incubated for 40 minutes at room temperature. The intensity of the extracts that formed after incubation was measured between 300 and 500 nm. The flavonoids content of the extracts was determined as the quercetin equivalent, depending on spectra match (5-200 mg/L) from a calibration curve for ether quercetin. The stock quercetin solution was made by dissolving 200 mg of quercetin in 50 mL of ethanol. The standard quercetin solutions were made by serial dilutions of the stock solution using ethanol (2.5 mg-100 mg/mL). Standard quercetin solutions or extracts in a volume of 1 mL were separately mixed with 1 mL of 2% aluminum chloride. The mixture was then incubated at room temperature for 60 minutes. The absorbance of the reaction mixtures was measured against a blank at 420 nm using a UV-Vis spectrophotometer. The calibration plot was used to determine the concentration of total flavonoid content in the test samples, which was then expressed as mg of fresh fruit material equivalent to quercetin standard.

3.7 EVALUATING PARAMETERS OF HERBAL YOGHURT

Yoghurt used for the analysis was yummy banana flavoured yoghurt Dairibord product.

3.7.1 DPPH free radical scavenging activity

Using the modified method (Shori and Baba, 2014), 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was used to measure the antioxidant activity of yogurt samples. In order to perform the test, the samples had to be extracted in ethanol, and then 0.5 mL of this extract was added to a 4.0 mL ethanol solution containing 50 mg of DPPH. The volume was made to 10 mL by the addition of water and samples were incubated in the dark at room temperature for 30 min. A spectrophotometer was used to measure the absorbance at 517 nm. The percent inhibition of the DPPH free radical is used to express the DPPH scavenging activity. The following equation was used to determine the percentage of yogurt samples that inhibited the DPPH free radical:

Free radical scavenging ability%= (A1-A2)/A1X100

A1= Absorbance of individual DPPH solution. A2= is the absorbance in the presence of tested samples at 30 min.

3.7.2 Stability of a formulation

Stability of formulation was evaluated by storing the formulation for several days at 4 °C

3.7.3 Sensory evaluation

In order to assess the panelists' response, the prepared yogurt undergo sensory analysis using the hedonic scale. Yogurt samples with varying amounts of flavonoids and control yogurt were examined for appearance, texture, flavor, and overall acceptability within 14 days of storage at 4 °C. Eight panelists were chosen to rate the samples using a 5-point hedonic scale, four of whom were men and four of whom were women. The panelists received approximately 10 mL of yogurt per cup. Excellent (5), Very Good (4), Good (3), Average (2) and Poor (1) were the sensory scores used. The panelists were also asked to offer some suggestions for the sensory parameter in terms of appearance, texture, and flavor and overall acceptability of all yogurt samples.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

The results are displayed as tables, diagrams, and graphs. Extractable flavonoids have proven to be an excellent source of medication for many illnesses (Panchal and Parvez, 2019). Extracts for human consumption must predominantly use ethanol or water as solvents due to their safety for human consumption. Some solvents may need specialist equipment to be removed from the extracts because they are poisonous and some of them are carcinogenic. The substances that need to be extracted also influence the chosen solvent. In this work, ethanol was used for extraction due to its wide range of solubility in extracting polar to non-polar groups. Due to the increased demand for efficient and safe extraction methods that do not endanger human health, ethanol is desirable (Hyun et al., 2013). The yield obtained from these 3 different fruits, *Fragaria vesca* produces higher yield followed by *strychnos spinosa* and finally *Vangueria infausta.* This might have been due to the difference on how rich each fruit is or solubility of compounds in the extracting solvent. The extract rich in flavonoids from the bands were examined by scanning within the range of 300 nm–500nm using UV spectrophotometer and FT-IR to further confirm the presence of the flavonoids.

4.2 YIELD

Percentage yield of *fragaria vesca* is more than of the *strychnos spinosa* and *Vangueria infausta*. Due to different in yield, the researcher decided to use the *fragaria vesca* extract for the yoghurt making. *Vangueria infausta* is the one with least yield.

Table 4.1Sample weight

Sample	Mass of sample and container/g	Mass of a container/g	Mass of sample/g
strychnos spinosa	22	2	20
Vangueria infausta	23	3	20
Fragaria vesca	22.5	2.5	20

The weighed samples were then each sequentially extracted with ethanol. Air drying was used to concentrate the filtered solutions. Below are the calculations of %yield of extracted material from a 20g sample of each fruit

%Yield = mass of extract obtained (g) / initial sample mass (g) ×100

Vangueria infausta (0.5523g/20g) ×100

2.7%

*Strychnos spinosa (*0.6631g/20g) ×100

3.3%

Fragaria vesca, (0.8253g/20g) ×100

4.1%

4.3. Analytical TLC

Analytical TLC was carried out using the following solvent systems

1. Ethanol: water (2:6v/v);
2. Hexane: petroleum ether (3:7 v/v)

The chromatograms shown in Fig 4.1 obtained when ethanol:water (2:6v/v) were used as a solvent and is the one gave the best separation. Extract of *strychnos spinosa* and *fragaria vesca* fruits with better concentration of flavonoids was used in this process. In this case *strychnos spinosa* (monkey orange), left spot and *Fragaria vesca* (Wild strawberry) extract, right spot was used. The flavonoids spots were encircled in white.

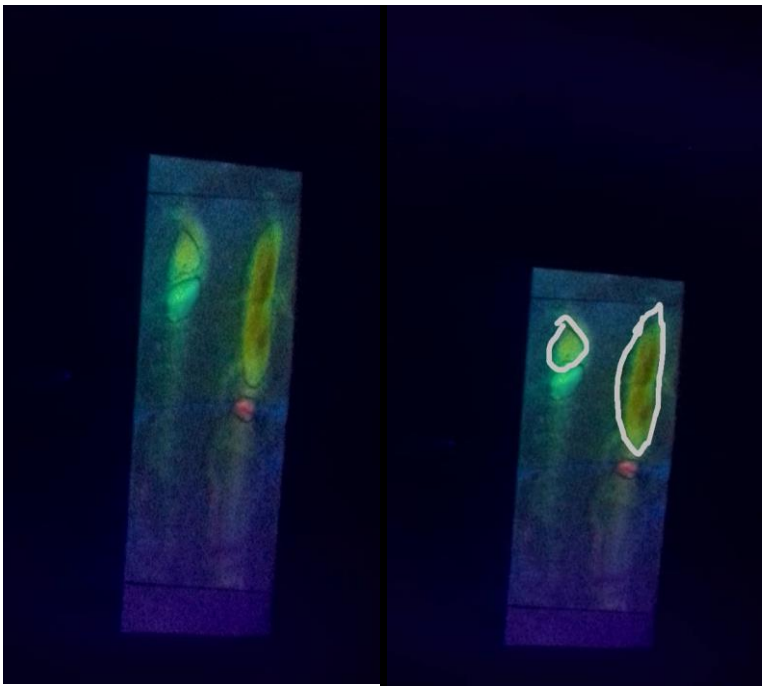


Figure 4.1 Analytical TLC chromatograms

Analytical TLC chromatograms for *strychnos spinosa* and *fragaria vesca* fruit extraxts, sprayed with 1% ethanolic aluminium chloride and visualized under UV Vis at 365nm is shown on Fig 4.1. Ethanol: water (2:6) v/v) was used as a solvent in this process.

4.4 Rf values for detected flavonoids

The Rf values presented are those from ethanol: water (2:6v/v), solvent system because the results from other solvent were not clearly reviewed.

Table 4.2Rf values for detected flavonoids

	Fragaria vesca	***strychnos spinosa***
First spot	**0.49**	**0.78**
Second spot	**0.90**	**0.92**

4.5 Preparative TLC Results

In this process only *Fragaria vesca* extract was used because it produces the best separation results and its yield is better than that of *strychnos spinosa.* The ethanol:water (2:6v/v) solvent system was used for preparative TLC. Surgical blades were used to scrap off flavonoid-rich bands, which were then dissolved in 5 ml of ethanol and filtered using Whatman filter paper for purification.

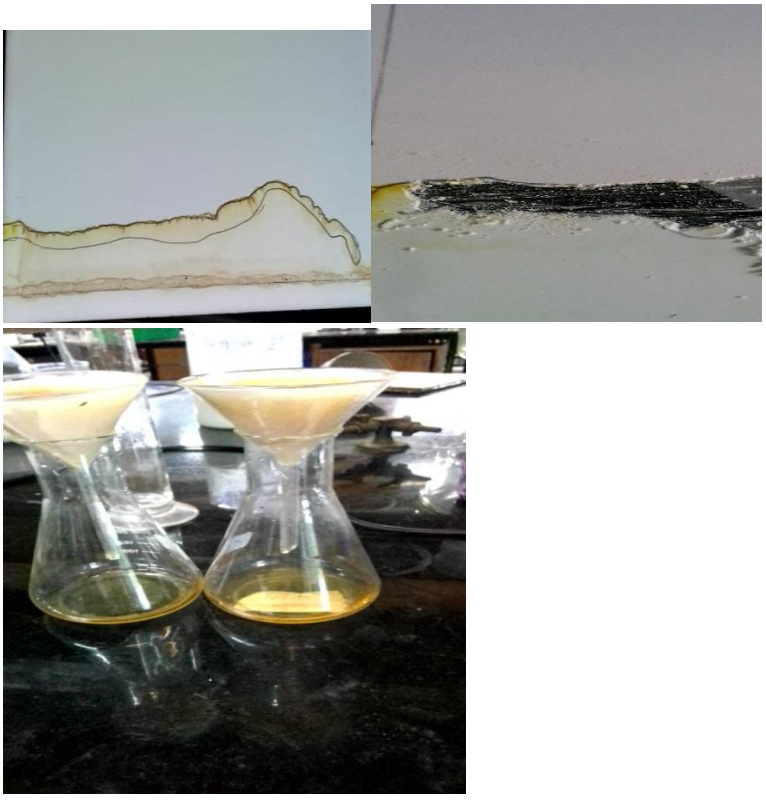


Figure 4.2 1 Separated flavonoids, 2 Scratched bands, 3 filtrates from the band.

4.6 UV scanning and FT-IR

The extracts from the scrapped bands are then dissolved in ethanol for purification. The extract was then concentrated and taken for UV and FT-IR analysis.

4.6.1 UV Analysis (Quantification)

A UV spectrophotometer was used to measure the amounts of flavonoids in each extract utilizing the calibration curve. The estimated flavonoid concentrations per quercetin equivalent are shown below

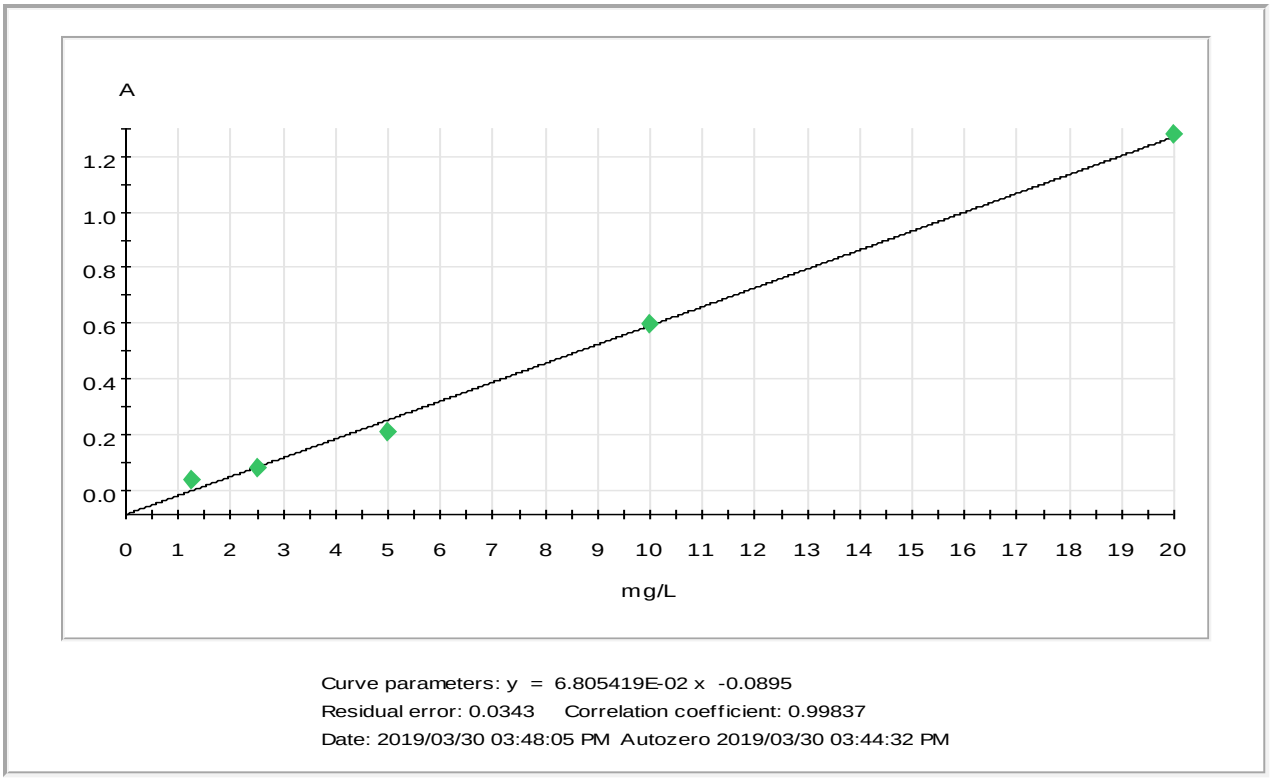
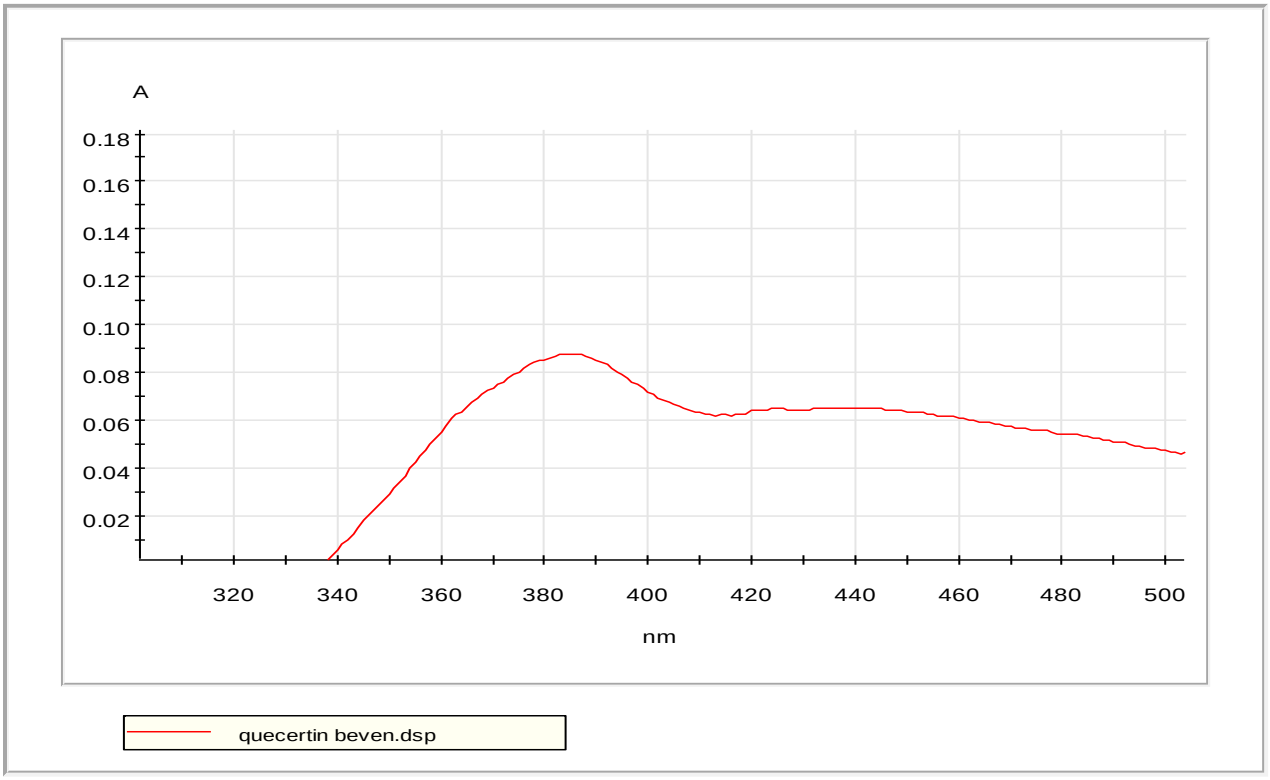


Figure 4.3Calibration curve for the standard quercetin.

Sample	Dilution Factor	Ordinate [A]	Concentration [mg/L]
Fragaria vesca	1	0.452	7.952
Strychnos spinosa	1	0.089	2.619
Vangueria infausta	1	0.068	2.308

This was chosen to estimate the concentration of flavonoids in the extracts because the R^2 of 0.9984 indicates good linearity in the range chosen.

4.6.2 UV Analysis scan



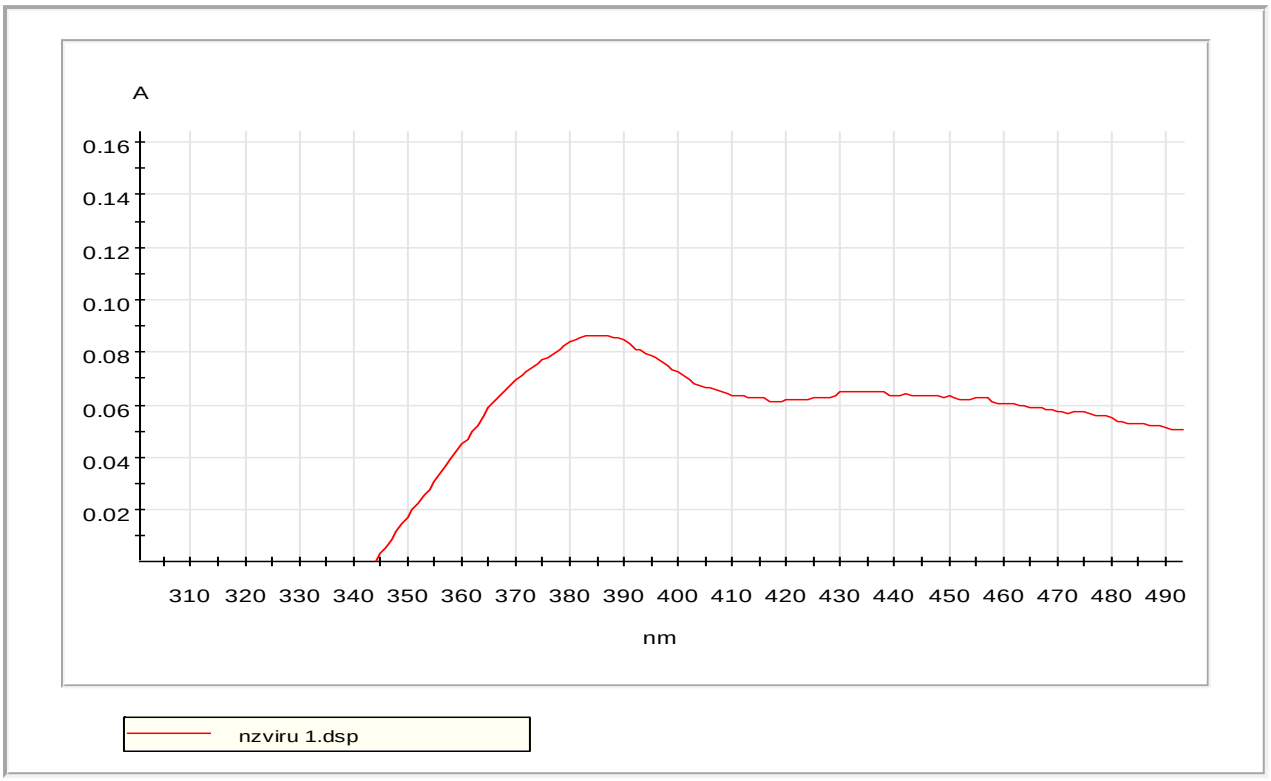
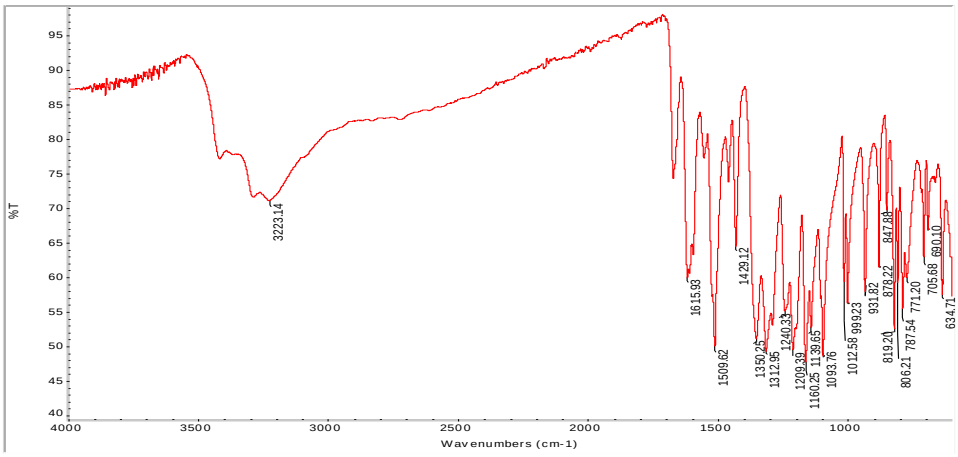


Figure 4.4UV analysis of final extract and quercetin standard (nearly pure flavonoid)

4.6.3 FTIR Analysis of *Fragaria vesca* extract

A



B

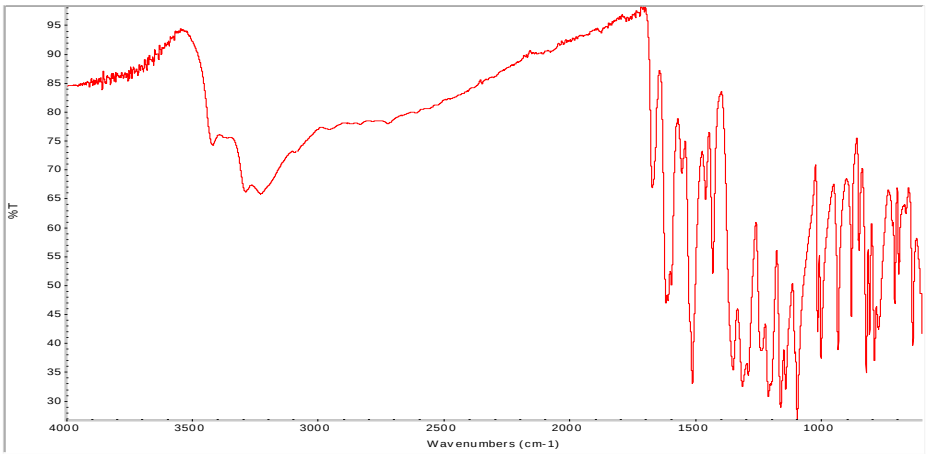


Figure 4.4FT-IR analysis of the bands extracts, **A** = quercetin**, B** = sample *(Fragaria vesca)*

FT-IR and UV Vis analysis further confirm the presence of the flavonoids and quantify the flavonoids in selected local fruit. According to UV and FT IR analysis, flavonoids absorb between 370 and 400 nm, which is in the same range as quercetin. Flavonoid samples were mixed with 0.1 mL of aluminum chloride and 0.1 mL of sodium acetate for the UV-Vis spectrophotometric quantitation method and then incubated at room temperature for 40 minutes. As the hydroxyl groups of flavonoids form complexes with aluminum cations, their chances of interfering with

other phytochemicals is reduced (Dzomba and Mhini, 2021). Since quercetin was used as the reference in this investigation, the data were reported as total flavonoids per quercetin equivalent.

4.7 HERBAL YOGHURT

Formulation. (Appearance of the formulated herbal yoghurt)

Figure 4.6Raw yoghurt. (Before addition of flavonoids)

Figure 4.7Herbal yoghurt (After addition of flavonoids)

4.8 DPPH radical scavenging assay

Utilizing the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a frequent and quick method to assess flavonoid's capacity to scavenge free radicals. The antiradical activity of the

separated flavonoids was evaluated by spraying DPPH onto the TLC plates spotted with flavonoids and by UV analysis. The present of flavonoids is shown by decolourization of purple DPPH by antioxidant (flavonoids). The yellow and white colour shown in Fig 4.8 represents the antiradical activity of flavonoids on sprayed DPPH.

DPPH antiradical activity.

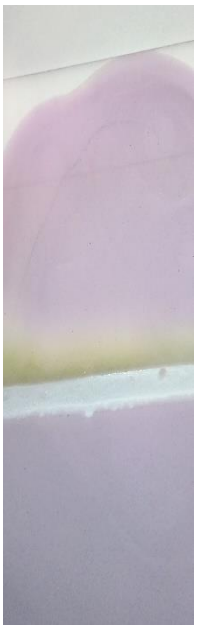


Figure 4.8 DPPH scavenging activity of separated flavonoids

The yellow and white colour show a positive test.

4.8.1 DPPH. Scavenging activity

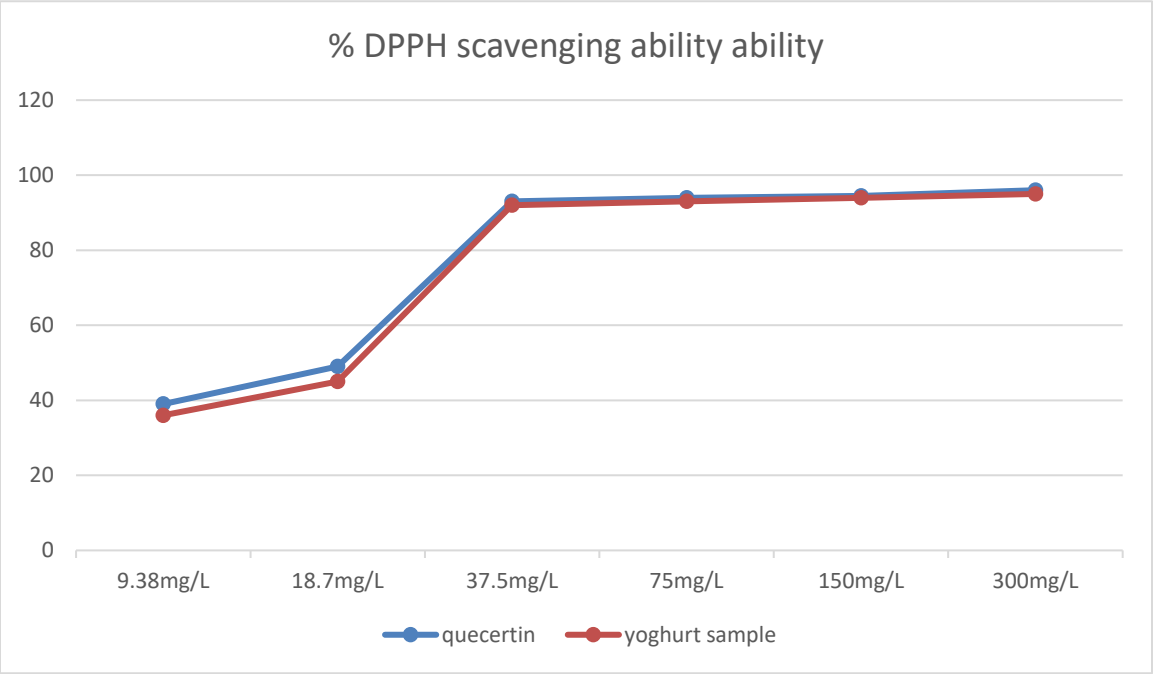


Figure 4.9 DPPH scavenging activity

Antioxidant property of the flavonoids has a stronger relationship with their molecular structure. Flavonoid's ability to exert their anti-oxidative effects through electron donation and hydrogen atom transfer are the two main methods. A hydrogen-donating antioxidant's presence causes DPPH to be reduced in the DPPH assay, forming DPPHH. A DPPH free radical scavenging assay was used to determine the antiradical activity of the herbal yoghurt. A purple free radical solution DPPH absorbs at 517 nm. According to Vijayalakshmi et al. (2013), the odd electron of DPPH is paired up in the presence of an antioxidant compound, reducing the intensity of the absorption at 517 nm. The DPPH radical scavenging activities in fig 4.9 above, of yoghurt sample and quecertin standard were found to be 94.5 and 95.6% respectively at 300mg/L. Quercetin standard showed better antioxidant activity in comparison with herbal yoghurt. From the observed results we can safely say that the herbal yoghurt has scavenging properties and can be a remedy to human health as an antioxidant as it has a percentage radical scavenging ability of 94.5 which is slightly less than that of quercetin at 300mg/L.

4.9 Sensory properties

Product shelf-life was monitored for 16days. Sample stored between 1℃-5℃. Parameters monitored were color, pH, texture, molds presence, viscosity and taste of the beverage. Color is considered as the most important sensory parameter, which can influence consumer's expectations(Spence, 2015)

4.9.1 Panelist's responses

Three-digit random numbers of cups that had been coded were used for the sensory profiles. Excellent (5), Very Good (4), Good (3), Average (2) and Poor (1) were the sensory scores. The panelists were also asked for their recommendations on the sensory parameters of all the yogurt samples, which includes the appearance, texture, flavor, taste, and overall acceptability.

Table 4.3Panelist's responses

Panelist	Appearance	Texture	Test	Flavor
1	5	5	5	5
2	5	5	5	4
3	5	5	5	5
4	4	5	5	5
5	5	5	5	5
6	5	4	4	5
7	5	5	5	5
8	5	5	5	5

4.9.2 Quality properties of herbal yoghurt

Table 4.4Quality properties of the herbal yoghurt

DAY	4	8	12	16
Colour	Cream	cream	cream	cream
Viscosity	Good	good	good	good
Texture	smooth	smooth	smooth	smooth
Taste	Good	good	good	good

pH	Acidic	acidic	acidic	acidic
Molds	None	none	none	none

From table above, there was no detectable colour change up to day 16 at temperatures below 7℃. Colour is considered as the most important sensory parameter, which can influence consumer's expectations (Spence, 2015). For taste, it was good at temperatures below 7℃, whilst at temperature above 20℃, the beverage started to produce sour taste. This sour taste may be caused by continuous fermentation due to high temperatures. Also the pH value decreased at a greater rate as the beverage become more acidic. Since the beverage at 5℃ tasted good and tasted even better when chilled we can safely conclude that the herbal yoghurt needs refrigeration and is best served when chilled. From day 1 up to day 16 no signs of separation were observed so we can conclude that the yoghurt was stable. Since no separation encountered, there is no need for shaking or mixing before use. The viscosity of the yoghurt remains the same when it was refrigerated but it became thicker when it was exposed to room temperatures. So the herbal yoghurt needs to be kept at temperatures below 7℃. From the panelist's recommendation we can label the beverage "Best served when chilled".

CHAPTER 5. CONCLUSION AND RECOMMENDATIONS

CONCLUSION

Production of herbal yoghurt using flavonoids from local fruits can help to improve people's health since the yoghurt has got the free radical scavenging ability. *Fragaria vesca* fruit has proved to be a suitable source of flavonoids in formulating an herbal yoghurt as it has better yield as compared to *Vangueria infausta and Strychnos spinosa.* The flavonoids from the *Fragaria vesca* fruit were added into a yoghurt to make it herbal. Due to addition of flavonoids, the herbal yoghurt has the capacity to scavenge free radicals from the body. Thereby protecting our body's cells from oxidative stress. The yield of the flavonoids from *fragaria vesca* fruit was less than expected. From the previous studies the yield of *fragaria vesca* fruit was 180mg/100g. From the present study the yield was 41mg/100g. This might be caused by extraction methods and the extraction solvent. The scavenging ability of the herbal yoghurt is slightly less than of the quercetin. The herbal yoghurt exhibited the % free radical scavenging activity of 94.6 as compared with 95.6 of the quercetin standard. From the findings we can safely say the beverage can be consumed for its nutritional value also as a remedy for oxidative stress. The beverage produced meet standard characteristics of the raw yoghurt in terms of colour, flavor and taste. The herbal yoghurt produces best properties if stored at temperatures below 7 degrees Celsius. The inclusion of the beverage in our daily diet food may help to reduce the of oxidative stress related diseases.

RECOMMENDATIONS

The fruits used in this study were reported to have better content of phytochemicals such as allegic acids, vitamins and flavonoids. These phytochemicals may add nutritional value of the beverage. This study only focuses on flavonoids quantitation. Leaves of *fragaria vesca* are also reported to have a better content of flavonoids. In future studies it is recommended to focus more on other types of locally found fruits, *fragaria vesca* leaves and even to take a look in other phytochemicals in *fragaria vesca* fruits. Also to identify the specific flavonoid structures which produces highest anti- radical activities since this study only focusing on the extraction and quantification of flavonoids from local fruits.

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