Microencapsulation of Extracted Functional Components from Industrial by-Product (DDGS)

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by

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I hereby declare that the thesis contain literature survey and original research work by the undersigned candidate, as part of my Master of Technology in Food Technology and Biochemical Engineering studies.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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I am very much pleased to forward this thesis for evaluation.

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Dedicated to my parents, grand-parents and partner in crime

Mr. Samir Ghosh (Baba)

Mrs. Sanghamitra Ghosh (Bu)

Dr. Sukhendu Sekhar Ghosh (Dadu-Baba)

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Abstract

Microencapsulation is a technique that provides effective protection for active agent against chemical reaction, oxidation, or evaporation in food, pharmaceutical, and cosmetics compounds. This technique utilizes polymeric materials to encapsulate solid, liquid, and gaseous components to form microscopic particles with the sealed or semi-permeable capsule. It plays a significant role to produce high-quality functional foods. Microencapsulation is mainly used for protecting the bioactive component from light, heat, oxygen, or other adverse condition and to stabilizing the components. Besides, of this, microencapsulation offers a variety of options such as masking, facilitation of easy handling, and reduced dissolution rate of the active ingredient. The efficiency of encapsulation depends on the choice of shell material and the process of microencapsulation. As the encapsulation technique facilitates the formation of food and pharmaceutical products safer, healthier, and tastier, so the demand for this technique has been growing day by day. The thesis entitled "**Microencapsulation of Extracted Functional Components from Industrial by-Product (DDGS)**" investigates the potential utilization of encapsulated DDGS extract. The motive behind is to investigate functional component of DDGS hence encapsulate it in the form of micro-capsule and used for various beneficial way.

Chapter 1 deals with the literature review on various functional components which are isolated from the different agricultural by-products. Furthermore, it illustrates dried distiller grains with solubles (DDGS). This chapter gives us an idea about the production process, the physical and chemical properties of DDGS. This chapter, described the microencapsulation process, followed by an overview of the microencapsulation process with its importance, techniques, ingredients, and controlled release rate.

Chapter 2 deals with the effects of solvents on solvent extraction of DDGS. This study will help us to understand which solvent gives the highest yield during solvent extraction. This chapter provides us with an idea about three solvents, such as ethanol, methanol, and water, which is mostly used in the solvent extraction method. UV-spectrophotometer was used to analyze the total phenolic content, total flavonoid content, and antioxidant activity of DDGS in three different solvents.

Chapter 3 deals with the effect of different parameters on the extraction of the functional component from DDGS. This chapter illustrates the importance of controlling extraction factors for the batch extraction parameters such as extraction temperature, extraction time, particle sizes, speed of incubator, and the ratio between solid and solvent for extraction of functional compounds from DDGS. This chapter optimizes the most effective parameters for isolating high extraction yield.

Chapter 4 deals with encapsulation and partial characterization of the extracted functional component of DDGS. In this study, two types of coating materials are taken for comparison, which one gives the best recovery yield after encapsulation. This chapter illustrates the gradation of the encapsulated material relatively to the coating material, recovery of the functional components from microcapsule concerning raw DDGS extract, scanning electron microscopy of microcapsules and heat stability of the encapsulated antioxidant.

Chapter 5 deals with the application of encapsulated functional component of DDGS on palm oil. This chapter illustrates the effects of DDGS microcapsule on palm oil by preventing them from rancidity in the presence of air and heat. Acid value and Peroxide value can determine the presence of fatty acid and indicates initial evidence of rancidity of palm oil.

Contents

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List of Abbreviations

AOA	Antioxidant Activity
AOAC	Association of Analytical Communities
AV	Acid Value
CDS	Condensed Soluble
DDGS	Dried Distiller Grains with Solubles
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
G.G	Guar-gum
GAE	Gallic Acid Equivalent
Na-A	Sodium-alginate
PEG	Polyethylene Glycol
POV	Peroxide Value
QE	Quercetin Equivalent
RPM	Revolutions Per Minute
SEM	Scanning Electron Microscope
TFC	Total Phenolic Content
TPC	Total Flavonoid Content
UV-VIS	Ultraviolet–Visible
WDG	Wet Distillers Grains
WDGS	Wet Distillers Grains with Solubles

Chapter 1

Introduction

The term 'by-product' means secondary, reusable wastes. Globally, vast amounts of agricultural wastes were produced from several food processing and agricultural industries. Processing of fruits, oilseeds and vegetables produce high volumes of waste materials. The inedible portion collected from food and agricultural industries ranges from 24% to 30%. The inefficacy to recycle these by-products will significantly subscribe to a prominent loss. The by-products obtained from various agro-industries are the most important and economical source of bioactive and functional compounds especially antioxidants. The functional components are present in foods such as vegetables, fruits, grains, and other food products. The plant by-products are mainly obtain from the non edible part of plants. The non digestible part from plant materials are the rich source of several funtional components such as, Polyphenols, flavonoids, anthocyanins, carotenoids, and vitamins [Selvamuthukumaran and Shi, 2017].

Carotenoids, polyphenol these micronutrients has its health prosperity. Carotenoids are mainly availed as natural color substances; on the other hand, some has Vitamin A benefits. Polyphenols plays a roll as antioxidants; they scavenge free radicals which are responsible for severe diseases and the oxidation of lipids, proteins, etc. Phenolic compounds could be a significant determinant of antioxidant activity(AOA) of food products and works as a natural source of antioxidants [Aguiar et al., 2016].

The antioxidant helps to minimize the oxidative stress of human body considering these compounds can eliminate the chain reaction motivated by free radicals. So, by ab-

sorbing natural antioxidants, the presence of various known diseases may be prevented. The antioxidant fortified functional foods are also gaining more popularity since consumption of natural antioxidant rich foods rare alone not sufficient to reduce oxidative stress [Balasundram et al., 2006].

Extraction of antioxidants can be isolated by using conventional and non-conventional technique. The efficiency of extraction is usually depends on the type of solvents for any conventional extraction methods. There are many drawbacks for convectional extraction such as longer extraction time, solvent cost is higher, evaporation of solvents and the thermal degradation of thermolabile compounds. To overcome these limitations new extraction methods are implemented. These techniques are known as non-conventional extraction techniques. Example of some non-conventional techniques are pulsed electric field (PEF), ultrasound-assisted extraction (UAE), extraction, enzyme-assisted extraction (EAE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) etc. A few of these techniques are considered as 'green techniques' [Aguiar et al., 2016].

The natural antioxidants present in various agricultural by-products are responsive to heat,oxygen, and light which are restricting their application at the food industry. So, to prevent this loss we have to apply a new promising technique, named as microencapsulation. Microencapsulation insulate antioxidants from heat,air,oxygen and light, allowing bioavailability, stability, flavour masking and controlled release. Microencapsulation process is well capable to protect the functional compounds from degradation, and also restrict its release rate, the process of micro particles incorporation affects the original food composition [Jin et al., 2012].

Microencapsulation is a process in which solid, liquid or gaseous materials are grid by a coating material and creating a microparticle. Particles size are classified into three size macro particle (>5000 mm); microparticle (1.0-5000 mm); and nanoparticle (<1.0 mm). Particle size has enormous impudence on food applications sensorial properties. If the particles are more significant than 30 mm, then it may induce a gritty mouthfeel sensation. Evaluation of these particles is generally done under SEM (scanning electron microscopy), TEM (transmitting electron microscopy), etc. Certain key factors should

be in consideration during microencapsulation, like coat material, core material, encapsulation technique, release mechanism, etc. However,control of pH, release of enzyme, the addition of surfactants,photo release ultrasonics,and grinding, may also be release triggers in food products. Although Microencapsulation can reduce the problems associated with degradation of natural antioxidant,bioavailability, and unpleasant flavour [Balasundram et al., 2006].

However, development of microparticles for incorporation into food products is a very complicated and often a trial-error method. Many parameters should be considered when formulating natural antioxidants into food grade microparticles.

1.1 An overview on DDGS

Distillers dried grains with solubles (DDGS) is a main by-product from the ethanol industry. DDGS is a good, economical feed ingredient that produced in large quantities by the dry-grind fuel ethanol industry. DDGS has high nutritional values such as it contain high energy, high protein, and phosphorus. Due to this reason DDGS has more acceptances over more expensive corn, and soybean meal, which are used as animal nutrition. When DDGS is combined to animal feeds it provides excellent animal health, performance, and an excellent food quality. These characteristics have upgrade DDGS to the most popular feed ingredients to use in animal nutrition around the world [Pedersen et al., 2014].

Figure 1.1: Granular corn DDGS (left side) and Powdered corn DDGS (right side).

Globally, bioethanol production increased from about 55.8 million gallons in 2007 to over 235 million gallons in 2016. Grains are the leading substitute for ethanol production

using either a wet milling or dry-grind process. DDGS is produced from cereal grains like whole wheat, corn, soybean, barley, sorghum, and their blends. Traditional ethanol production consists of 6 sequential steps, followed by dry milling, liquefaction, saccharification, fermentation, distillation, and co-product recovery. During the last stage of coproduct recovery, condensed soluble (CDS) are mixed with distillers wet grains to become wet distillers grains with solubles (WDGS) and then dried into DDGS. Distillers Dried Grains with Solubles (DDGS) is produced from the non-fermentable grains which remain after the starch has been converted to ethanol. 100 kg grains produce 32 kg DDGS and CDS, and 32 kg carbon-di-oxide along with 40-liter ethanol. In India, low-quality rice and broken rice is increasingly being used in the ethanol industry. So in the future, a huge amount of rice-based distillery by-products is likely to be available in the country. Distillers Grain with Solubles (DDGS) is a very good origin of protein, fiber, and minerals. But till date, DDGS is considered as waste material and utilized only as animal and poultry feed. DDGS is a good source of nutrients. DDGS contains many organic macromolecules, such as carbohydrates, proteins, vitamins, antioxidant [Zhang, 2013].

1.1.1 Flowchart of DDGS production

The production of ethanol from corn grains has seen a steady increase in the last few years. The dry grind of corn for the ethanol process differs from the wet milling process of corn. Also, it uses little fractionation of the corn kernel components prior to scarification of the starch and fermentation [Bals et al., 2006].

In this process, the whole corn grain is ground by a hammer mill and turns into a coarse powder. The diameter of that particle is about 1mm. In the next step, the mill corn is liquefied in the presence of an enzyme, followed by a saccharification step, where starch is converted into simple sugars. In the next stage, sugars are fermented by yeast. After fermentation, the slurry passes through a stripper where ethanol is recovered. After separating ethanol, the portion which is left over is called the whole stillage. The main components of whole stillage are fiber, oil, protein, other unfermented grains, and yeast cells. Then the whole stillage is centrifuged to produce a liquid fraction called thin stillage

and a solid fraction known as wet distiller's grain (WDG).

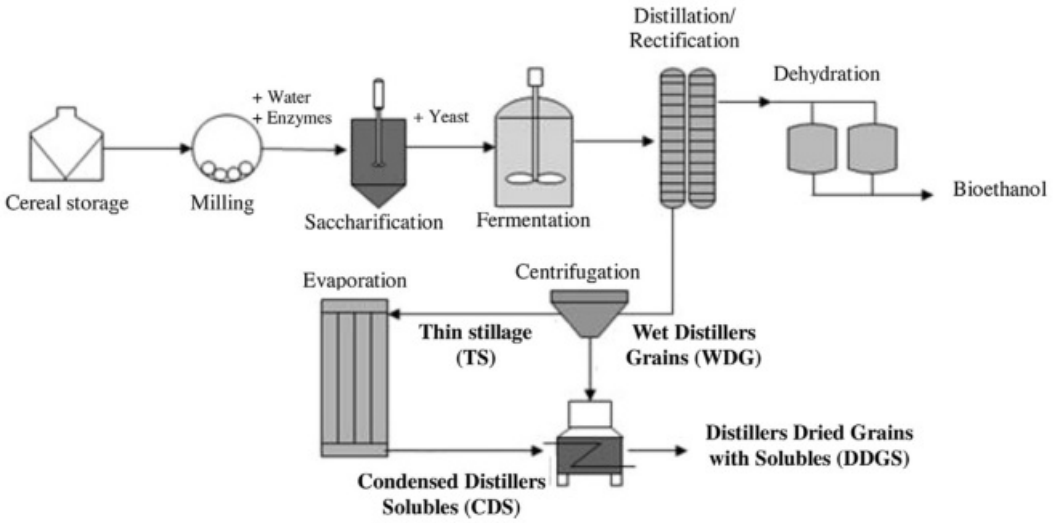

Figure 1.2: Flow diagram of DDGS processing.

A part of thin stillage is recycled to slurry the ground grain. The remaining thin stillage is concentrated through multiple effect evaporators to produce syrup called condensed distiller's solubles (CDS). The WDG is dried to obtain distiller's dried grain (DDG). When CDS is mixed with WDG and dried, it is called distiller's dried grain with solubles (DDGS). The dry solid residue remaining after ethanol production is known as the DDGS.

1.1.2 Basic Properties of DDGS

The main physical properties of DDGS are particle size, shear strength,bulk density, and an angle of repose. Common chemical properties are moisture content, and water activity. The chemical and physical both properties can affect the storage ability and milling properties of DDGS. Moisture content is the main chemical property which can affect it during its storage time. Due to evaluate the amount of "free" water for use by microorganism and chemical agents. Flow ability is measured by an indirect property known as 'shear strength'. The flow ability affects the strength of DDGS and flow problems when DDGS exposed to compressive stress during storage time and milling [Batal and Dale, 2006].

Particle size

Particle size is an essential property for DDGS. Particle size affects other features such as angle of repose and bulk density. The finer particle size directly leads to the greater surface area, and as a result, it can contact more points, which means the air space between two particle is very lesser. Similarly, it can cause greater higher cohesive bulk strength, compressibility, and lower flow-ability [Batal and Dale, 2006].

Bulk Density

Another most important property is bulk density. It can directly affects on quality and on the price of DDGS [Batal and Dale, 2006].

Angle of Repose

An angle that forms between the slope of a pile and a horizontal plane is defined as Angle of repose. It has been formed by dropping the bulk material from some elevation. An angle of repose is a function of physical properties of the particles size, shape, and porosity. The angle of repose varies in between 26.5°- 41.60°[Clementson and Ileleji, 2010].

Shear Strength

The strength of a material against the type of yield or structural failure where the material or component fails in shear is known as shear strength. It is a most important property of DDGS. This property is directly reflected towards flowability of DDGS. The flowability is often restricted by caking and bridging during storage and transportation [Clementson and Ileleji, 2012].

Flow-ability

The relative movement of bulk particles in proximity to neighboring particles or along the wall of the container or storage silo is defined as flow. Flowability of DDGS is important to understand the reliable flow of a particular powder. the difficulties of flowability are mainly related to physical properties of DDGS particle. Flowability is a combination of

the physical properties of materials, environment, and processing techniques which are used for production of that DDGS. Storage condition is also a main factor for flowability of DDGS. The major factors that influence flowability are moisture content, temperature, pressure, humidity, particle size etc [Ganesan et al., 2009].

Color

The color of DDGS has a major quality factor. Color of corn DDGS can vary from very light, golden yellow in color to very dark brown in color. Color of DDGS is measured by Minolta colorimeters that are used for measurement of the human nutrition and animal nutrition industries. Various colorimeters are commonly used to measure color characteristics of DDGS. Lightness or darkness of color is determined by the L$*$ reading. The redness of DDGS is measured by a$*$ reading measures. The yellowness of DDGS is measured by the b$*$ reading measures [Ganesan et al., 2009].

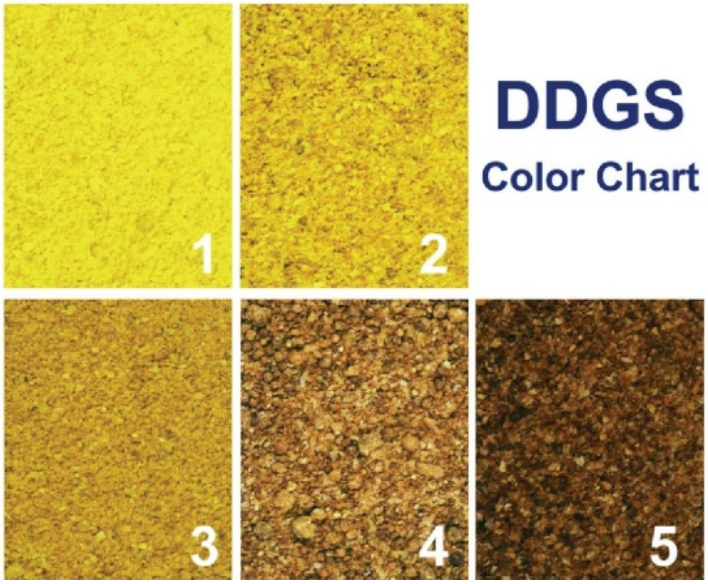

Figure 1.3: Color chart of DDGS.

Smell

The smell of high and golden quality DDGS is sweet and fermented. Dark colored DDGS sources that have been overheated have a burned or smoky smell [Ganesan et al., 2009].

Moisture Content

Moisture content is an important factor that affects flowability, shear strength, cohesive strength and arching of bulk solid. Maximum food products, agricultural products and organic materials tend to lose or to gain water with changing environmental factors. Even small changes in moisture can change the frictional properties of bulk solids very significantly. DDGS is getting from most ethanol plants having their moisture level of approximately 9% to 11%. This moisture content is generally utilized for feed products. Moisture can also be combined with surface properties, and it can change or influence adherence properties between particles and storage silos [Garcia and Rosentrater, 2012].

Water Activity

Water activity is a ratio of the vapor pressure of water in a substance and vapor pressure of pure water at the same temperature. The values Water activity are related to moisture content and it should be restricted to microbe growth. DDGS are stored in bulk amount, in case of potential moisture migration from the environment, especially during the shipping [Ileleji and Rosentrater, 2008].

1.1.3 Antioxidant Capacity of DDGS

DDGS is very much rich in its antioxidant capacity. The five phenolic acids identified in DDGS. They are vanillic, caffeic, p-coumaric, ferulic, and sinapic acids. Among the all quantified phenolic acids, Ferulic and p-coumaric acids accounted for about 85%. The amount of total phenolic acids per gram basis, in DDGS was 4 fold higher and antioxidant capacity was 3 fold higher than that of any other grains. We know that antioxidant is very much heat sensitive, so to protect this property of DDGS we must use a new novel technique that is Microencapsulation process. [Luthria et al., 2012].

1.2 An overview on Micro-encapsulation

Encapsulation is a technique of casing liquids, solids, and gaseous substances in microscopic scale sealed capsules. Encapsulation is a well accepted technique in various industrial, pharmaceutical, agricultural areas This rely on the physical and chemical substances of the material to be encapsulated. Food products like cheese,UHT milk, yogurt,muesli bars,mayonnaise,breakfast cereals,health drinks and baked products are fortified with encapsulated components. Encapsulation used to release the conventional ingredients, such as antioxidants,sweetness,vitamins,minerals and flavors etc [Adhikari et al., 2003].

Encapsulation comprise the entrapment of the craving component within a secondary element to reduce the release of active ingredient until a certain factors are achieved.The process encapsulation presents numerous prosperity to the elements being encapsulated. Spray drying, freezing, dehydration, chilling, and organic phase separation. For encapsulation of flavor there are two major methods such as spray drying method and extrusion method. “Extrusion" is defined as “a flavor emulsion forced through a die, with pressures of typically < 100 psi and Temperatures seldom > 115°C" [Reineccius, 1989].

In a review article [JACKSON and Lee, 1991] meet up the properties of microcapsules, contemplated distinct uses by food products, enzymes,lipids, micro-organisms,gases, artificial sweeteners, minerals and vitamins. Microencapsulation methods were summarized under the descriptor of spray cooling , spray chilling and spray drying,extrusion, and multi-orifice centrifugal extrusion.

Aroma release from an encapsulated thing is also done by [Sostmann et al., 2008], showed Proton-Transfer-Reaction(PTR) allows fast appraisal of dynamic aroma component discharge during utilization of variety foods. They practiced this methods for in-vivo assessments of aroma discharge from chewing gum. Mint flavour was incorporated into different chewing gum by applyinh encapsulation method. By using glycerine,maltitol syrup, sorbitol,aspartame and acesulfame K as a material they introduced gum based non-coated chewing gum.

On the other hand [Christelle and Elisabeth, 2013] were found how to encapsulate aroma by using spray drying method Fluid Bed Agglomeration and Coating. They

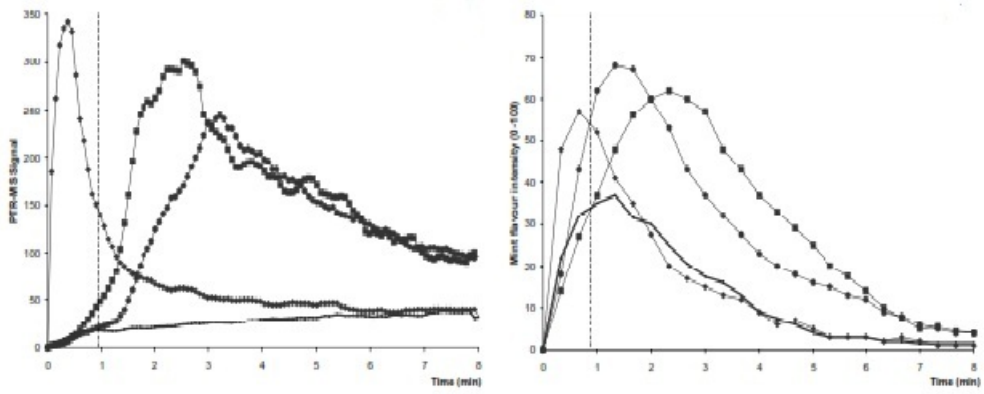

Figure 1.4: Release curves of menthol to the time-intensity curves of mint sensory perception.

produce powdered aroma which are capable to change liquid aroma in the composition of sweet paste. The control release rate of encapsulated flavor were found out by [Madene et al., 2006]. The main aim of their experiment was to describe each method in phrase of the vital chemical and physical principles associate and covers mechanisms of discharge of flavour from food products. Controlled release is defined as a process by which many active ingredients are made available at a desired state and time at a specific rate [Pothakamury and Barbosa-Cánovas, 1995].

Some researchers have desired a better empathetic of the effects the flavour discharge from complicated matrices. An overview of chemistry compatible to flavour discharge has been showed in [Taylor, 1998]. For the matrix systems the release of encapsulated volatile compounds are depends on diffusion of compound through the matrix.The volatile components are transfer to environment from the matrices and tends to degradation of matrix materials [Pothakamury and Barbosa-Cánovas, 1995]. De Roos [de Roos, 2000] evaluated two factors that control the rate of flavour discharge, and volatility of the aroma components in the food system and air phases under equilibrium conditions and the resistance to mass transport from product to air.Generally, release of flavour is decreases with increasing fat level in food system [Guichard, 2002]. The addition of salts in an aroma can increases its volatility; this is in contradiction with the salts effect on small molecules of aroma, where they coax a solubilization effect [Druaux and Voilley, 1997]. Boland et al. [Boland et al., 2004] investigated the discharge of flavour compounds from

starch, gelatine,and pectin gels. They showed that the releasing of flavour was affected by the gel's texture. The gelatin gel assayed large increments in flavour release in presence of animal saliva,but in case of starch and pectin gels it was evaluated that there was a degradation in flavour. Interactions between aromas and protein have been the subject of diverse studies [S Lubbers and Voilley, 1998], showing that covalent binding, hydrogen binding and hydrophobic interactions are all detectable.

1.2.1 Importance of Micro-encapsulation

The micro-capsule has a good capability to hold a matter in the excellent prorated state and to discharge it. The capsules size are varying in many range like micrometer, sub-micrometer,nano-meter millimeters and having different shapes, and these are mainly build upon on the materials and on the methodology used to produce them. The encapsulations of various functioning components are fixed for many reasons:

- Preserving the degradation of core material from the outside environment.
- Diminishing the evaporation of a volatile active ingredient that can be used as the core material.
- Modifying the physical characteristics and enhancing the visual aspect of a material.
- Improved the processing of materials such as flow-ability, texture, solubility, thermal stability etc. [Adhikari et al., 2003]

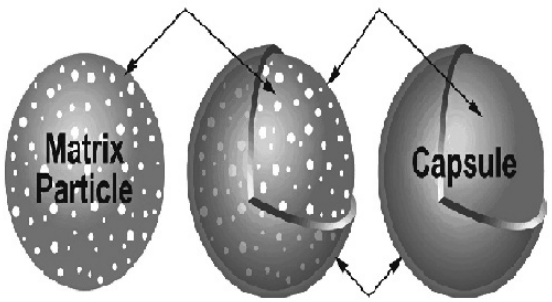

Figure 1.5: Encapsulation morphology.

1.2.2 Classification

Based on size and morphology capsules can be arranged in several way. The size range is from1 µm to scanty millimeters. the capsules whose diameter is measured by nanometer range are called as nano-capsules. Particles whose diameter in between 3 to 8 µm is called micro-particles microcapsules. Particles whose size is larger than 1000 µm are called macro-particles. The formation of microcapsules is basically depended on the distribution of core substance within the system, and the impeachment development of the shell. The microcapsules are assorted into several capricious and the allocation such as:

- Mono-core or single core capsules. In this capsule the shell is around the mono-nuclear shell.
- Polynuclear capsules are circumscribe within a wall with many more core ingredients. This type of capsules are called poly core- or multi-core-type capsule.
- When the core material is dispersed consistently within the shell material then it is known as matrix encapsulation. [Choi et al., 2007]

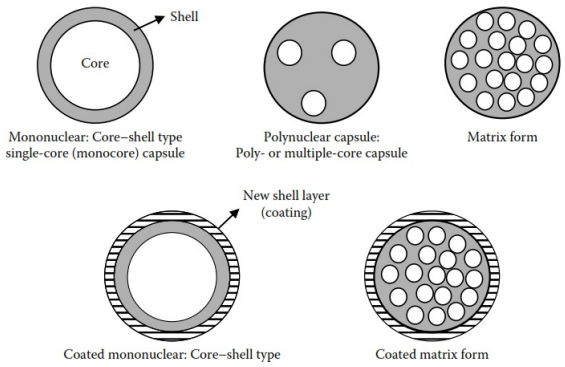

Figure 1.6: Schematic type of Microcapsule.

1.2.3 Techniques of Encapsulation

In a function, the practice of encapsulation deals with three different practices on a time sequence:

- The first practice is constructing a shell barrier over the core ingredients.

- The second practice is retaining the core matters as it is inward the wall matter so that it does not leak out from the shell wall.
- The third practice is deliver the core ingredients at a specific standard of time.

1.2.4 Criteria for Selecting Encapsulation Technology

The choice of microencapsulation method depends on the physical and chemical properties of core and shell materials. For developing the design of micro capsules, there are many technologies and shell materials are available with a wide variety of functionalities. The following things are always kept in mind before designing the encapsulation and encapsulation process:

- The convenient processing situation during the production of final products for the endurance of encapsulated matter.
- The depot situation of encapsulated components before use.
- The physical properties of the encapsulated ingredient.
- The variety of coating material. The shell ingredients elects the physical and chemical properties of capsules. Used polymer must be well proficient of developing a film which is connected with the core ingredients. [Drusch et al., 2007]

1.2.5 Process of Encapsulation

Criteria

The criteria are considered when electing an encapsulation method with its size of capsule, morphology, core and coat materials, equipment, processing techniques, and cost. These precedent are mainly reciprocal and process reliant. There are some exceptions to these general values.

Size

The size of microcapsules from nanometers to millimeters. The particle sizes which are obtained during spray drying process are very tiny and the particles are stands the subordinate end of the sizes for physical machinery. Spray drying of nano-particles is expedient but in case of small ratio only.The capacity to yield low micron droplets for spray drying is also a impediment for coating. In a fluid bed coating the lower limit is 55–72 μm for fluid bed coating.

Materials

Selections of materials are used to eradicate probable methods, or ingredients can be chosen and evaluated based on the pioneer recognition of a process. To further convoluted the inter dependency of methods and ingredients, processing aids are obligatory and considered along with the essential core and coat materials.

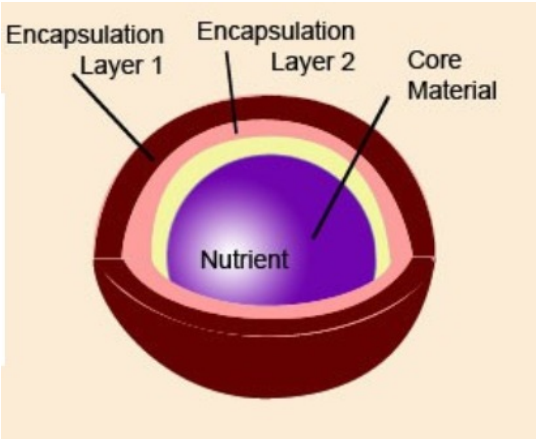

Figure 1.7: Various layers of the Microcapsule.

Shell Materials The shell materials are classified into four parts, such as solvent-based material, water-based materials, molten based materials, and reactive. Spray coating method, atomization methods, and coextrusion methods are the techniques to collect shell materials from the liquid material or as a molten components. Minimal shell material selections are available for the emulsion-based operations. Higher concentrations of wall material within solvent results in higher viscosity and a method that can accommodate

the higher viscosity.

Core Materials Selection of core material is dependent upon thermal stability, viscosity, particle size and shape, density,solubility,and reactivity, etc. By using method of spray coating, we can open core materials to inflated temperatures for elongated periods. In case of spray drying method the concoction remains heated as the mixture stays for pumping to an atomization nozzle. Further processes can be carried out at or below normal room temperature.

Processing Aids

To expedite prosperous operation of microencapsulation some chemical reagents such as solvents, drying aids, surfactants, anticaking agents, plasticizers are needed. For spray drying process water or organic solvents are requisites. At the time of gathering of dried capsules, anticking agents are required to reduce agglomeration,and accelerate the final drying. Surface area is required for liquid suspensions to enhance the suspension stability and to prohibit cluster formation. [Berkland et al., 2004]

Ingredients

For encapsulation the material should be able to entrap, or encapsulate solids, liquids, or gases. There is a myriad of substances along with natural and synthetic root available. But, only a few of them are authorized for food items, pharmaceutical items, and cosmetics utilization. Primitive components used for microencapsulation in the agricultural and food sector are carbohydrate, proteins, lipids, etc.[Oxley, 2012].

Carbohydrate derivatives Starch is tasteless, odorless whitish powder and it is not soluble into organic solvents such as ethanol,methanol and in cold water also. The starch is a polymer of α-d glucose.The general chemical composition of starch is $(C_6H_{10}O)$n. The starch powders swell, lose crystallinity, and release amylose as they dissolved in water. If the amylase content is higher, the swelling power is also lower, as a result the strength of gel will be lower for same amount of starch concentration.

Protein Proteins are formed with linear chains of amino acids called as natural macromolecules and used in many applications including encapsulation method. There are variety of proteins are available. Protein which is extracted from animal as a products (whey proteins, gelatin, and casein) and from vegetables such as pea protein, soy proteins, and cereal proteins are used for encapsulation of active ingredients.

Strategies for the Selection Of Materials

The selection process of coating material is dependent on the selection of the microencapsulation process.

- Performance of the final product .
- The condition of processing during the production of final product.
- The controlled release and mechanisms of the active ingredients.

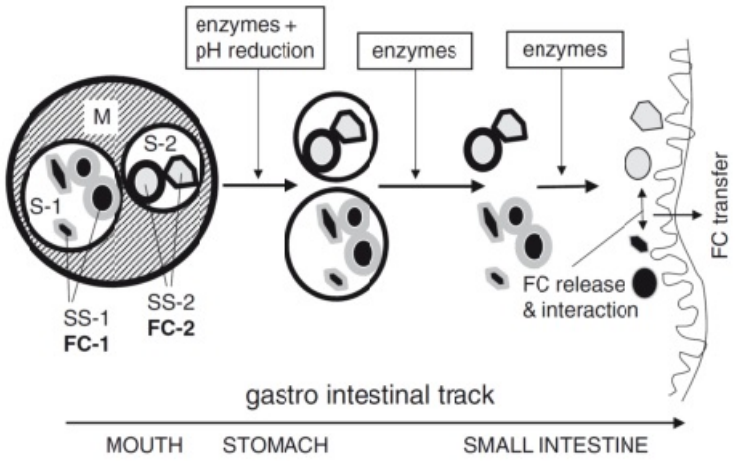

Figure 1.8: Release procedure of core material.

1.2.6 Controlled Release Rate

Controlled release is defined as the demonstration of compounds in acknowledgment to enzyme, pH, temperature, light, osmosis, magnetic fields, ultrasonic, and time. It can be describe as a process by which one or more active ingredients are made accessible

at a yearning site and time at a specific rate. Numerous applications comprise in areas such as food technology,cosmetics, pharmaceuticals, agricultural science, and personal care. This phrasing commonly refers to sustained release, time dependent release, delayed release,pulse release, in oral dose system.

1.3 Conclusion

Nowadays there is an increasing requirement for functional food products and nutraceuticals food products that has collaboration with supply and development of health condition and by regulating of specific immune functions to prevent numerous diseases. DDGS contributes excellent animal health, performance, and food quality forming it one of the most popular feed ingredients worldwide. The amount of antioxidant property is very much high, near about 235mgGAE/100gm. After isolating antioxidant from this, we might not be directly added it to food compounds, to overcome this situation we may adopt the encapsulation technique, for avoiding the destruction of antioxidant due to various environmental factors. We can encapsulate the bioactive compounds by using the freeze-drying method, which is one of the most convenient methods for encapsulation. Several types of processes and factors are there for encapsulation technique. Essential factors are evaluating the size of capsule, morphology of capsules, properties of core material, properties of shell materials, process availability, and material. Numerous types of encapsulating agents are used in food products, such as carbohydrates, proteins, and lipids. Additional researches are required to complete the inspection on encapsulation into the best combination of process, materials and release mechanism.

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Chapter 2

Solvent extraction of Functional Components from DDGS

2.1 Introduction

Finding unique and secure bioactive compounds from natural origins is of vast interest for utilization in natural antioxidants,nutraceuticals and functional foods. Agricultural-industrial by-products arrange a high probable source of functional compounds, such as phenols, flavonoid and antioxidants. It can also be used as utilitrian ingredients in animal nutrition.By-products obtained from Fruit and vegetable are noticeably abundant in antioxidant such as anthocyanin, vitamins, and flavonoids. The practice of agricultural by-products as a origin of antioxidant compounds can be a viable economic alternative [Castrica et al., 2018]. Phenolic compounds are known as secondary metabolites which is evolved from various parts of plants and act as a substitute antioxidant promoter against oxidative depreciation of food products. These components are most generally developing groups of phytochemicals. Phenolic compounds are display an ample range of physiological properties, such as anti-inflammatory, anti-allergenic, anti-microbial, antioxidant, anti-thrombotic, and vasodilator effects. There are countless methods to collect antioxidants from various part of plants [Gabaldón et al., 2010]. The methods are mainly,supercritical fluid extraction(SFE), Soxhlet extraction, subcritical water extraction,maceration, sol-

vent extraction, and ultrasound assisted extraction(UAE). Usually, soluble phenolics are available in higher concentrations in the outer parts of grain's cell and fruits than to the inner muscles. Due to this reason for the isolation of plant antioxidant compounds, solvent extraction method is the most commonly used the technique. The efficiency of extraction based on the solvent which is used for extraction. Polar solvents are mainly used for extracting polyphenols from agricultural by-products [Castrica et al., 2018]. The most suitable solvents for extractions are water, ethyl alcohol, methyl alcohol, acetone, and ethyl acetate etc. Ethyl alcohol is known as a suitable solvent for phenolic compound extraction. For isolating of lower molecular weight polyphenol, methyl alcohol is used because it works very efficiently on that polyphenol. Water has best yield on higher molecular weight flavanols during extraction period. For isolating flavonoids from tea leaves, aqueous solution of ethyl alcohol performed better than aqueous solution of methyl alcohol and aqueous solution of acetone [Wang and Helliwell, 2001]. For solvent extraction of antioxidant, there are some factors such as solvents, temperature, and duration. These parameters are to be developed to generate the highest yield of extract and the maximum extraction quality in terms of the target bioactive compounds and antioxidant power [Bucić-Kojić et al., 2007].

2.2 Objective of the work

The leading by-product from the corn dry-grind process is distillers dried grains with solubles (DDGS). This heightened manufacturing of ethyl alcohol is leaded by a compelling increase in the amount of its by-product. At the dry grind process of corn the fermentation occur and production of DDGS take places. As a nutrition of farm animals DDGS is mainly used.Factors like cultivar,processing condition and growing conditions of plants are responsible for the composition of phenolic compounds presents in plants and plant by-products.During the production of ethyl alcohol the processing situation of corn, has an effects on quality and quantity of phenolic acids which are present in a DDGS.

Extraction of these phenolic compounds from DDGS is a good alternative for having natural antioxidant for human consumption. The dissolving power of polyphenolic matters

and their diffusion power to solution, based on their chemical structure that may differ from simple to highly polymerized compounds. So, the preference and selection of solvent is one of the essential factor in the solvent extraction process. The most often used solvents in the isolation of phenolics are methanol, ethanol, and water. Besides, this temperature has its effect on antioxidant activity. This thesis aimed to investigate the efficiency of three extraction solvents such as methanol, ethanol, and water with temperature influence on extraction of TPC, TFC and AOA of DDGS extracts by using UV-spectrophotometer.

2.3 Characteristics of various solvents

The most suitable solvents for solvent extraction are methyl alcohol, ethyl alcohol , and water. The factors should be designed when choosing a solvent are boiling and freezing point, polarity, viscosity, reactivity, stability to heat, cost, and safety. The boiling point should be low to expedite removal to solvent from the products. The solvent are chemically non react able with the extracted matter, and it should be easily decompose [Spigno et al., 2007]. Water, ethanol, and methanol have been largely used to extract compounds like antioxidant from many agricultural material and plant-based foods. Water is capable of dissolving a variety of different substances, so water is called the "universal solvent." The molecule of water has a polar structure with oxygen and hydrogen atoms. One side has a positive electrical charge, and the other hand had a negative charge. Thus these structure of water molecule allow them to become attract to other different types of molecules. The polar molecules and ions interact with the partially positive and negative ends of water molecule, with positive charges attracting negative charges. Hydration shells avow the particles to be dissolve evenly in water [Nawaz et al., 2006]. Ethanol or ethyl alcohol is the second most key solvent after water. Due to presence of hydroxyl group, ethanol is very polar in nature. For this reason, ethanol attracts both polar and ionic molecules. On other hand the ethyl group which is present in ethanol is non-polar. As a result ethanol is able to attract non-polar molecules also. Ethanol is the least toxic of the alcohols. Methanol has been generally found to be more efficient in the extraction of lower molecular weight polyphenols [Pinelo et al., 2005].

2.4 UV-Vis Spectrophotometer

The spectrophotometer is a backbone of any modern laboratory. The ultraviolet spectrophotometry is the technique for identification and measurement of organic and inorganic compounds in a vast range of processes and products.The another name of UV spectroscopy is Electronic spectroscopy, comprise the elevation of the electrons from ground state to the excited state [Hesse et al., 2005]. This instrument studies the changes in electronic energy levels within the molecule arising due to the transfer of electrons from π- or non-bonding orbitals. It commonly provides knowledge about π-electron systems, conjugated unsaturations, aromatic compounds, and conjugated non-bonding electron systems, etc. Every time a molecule has bond, the atoms in a bond have their atomic orbitals merged to form molecular orbitals which can be occupied by electrons of different energy levels. The electrons in a molecule can be of one of three types: σ (single bond), π (multiple-bond), or non-bonding (n- caused by lone pairs). These electrons when imparted with energy in the form of light radiation get excited from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO), and the resulting species is known as the excited state Or antibonding state [Althoff et al., 2017].

2.4.1 Principle of UV-Vis Spectroscopy

UV spectroscopy obeys the Beer-Lambert law, which states that, when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with a thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution. The expression of Beer-Lambert law is A= log(I0/I) = E.C.L [Where, A= absorbance, I0=intensity of light incident upon the sample cell. I= intensity of light leaving sample cell, C= molar concentration of solute, L=length of sample cell in cm, E= molar absorptivity]. From the Beer-Lambert law it is clear that greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption [Metha, 2012].

2.4.2 Working principle of UV-Spectrophotometer

Working principle and instrumentation of UV spectrometers can be evaluated synchronously. The modern UV spectrometer has the following parts

Light Source

As a source of light in spectrometer tungsten filament and hydrogen deuterium lights are used. These light sources are covered whole UV region. The tungsten filament lamp emit the red radiation at the rate of 375nm. But for hydrogen deuterium light it is lower than 375nm.

Monochromator

Monochromators are mainly produced by the composition of slits and prisms. In recent days the utilization of double beam spectro photometers are increasing. The various wavelengths of the light source which are separated by the prism are selected by the slits such rotation of the prism results in a series of continuously increasing wavelength.

Sample and reference cell

Two divided beams is passed through the sample solution and the reference solution respectively. Both sample and reference solution is contained in the cells. These cells are made of either silica or quartz nor Glass, because it absorbs light in UV region.

Detector

Generally, two photocells are presented in a UV spectroscopy.between two photo cell,One is used for receiving the beam from the sample cell and the second one is receive the beam from the reference cell. The intensity of the radiation from sample cell is lower than the beam of the reference cell.

Amplifier

The alternating current generated in the photocells is transferred to the amplifier. The amplifier is connected to a mini servo motor. Generally,the intensity of current which is

generated in the photocells is of very low; the main principle of amplifier is to amplify the signals many times so we can get clear and recordable signals.

Recording devices

Generally, the amplifier is connected to a pen recorder which is coupled to the computer. Computer collects all the value which was generated during the analysis and produces the spectrum of the desired compound [Sooväli et al., 2006].

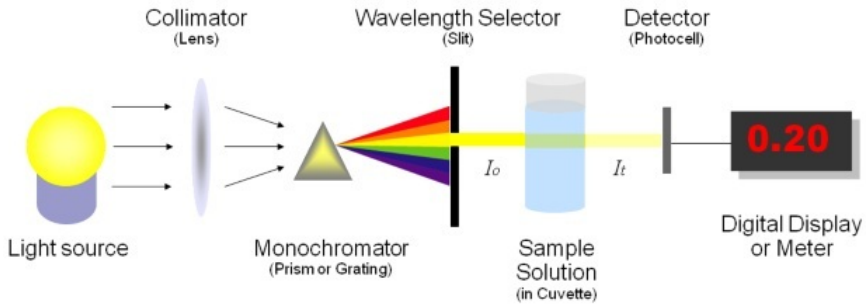


Figure 2.1: Schematic diagram of instrumentation of UV spectrometer.

2.5 Methodology

2.5.1 Sample preparation

10gm of distiller dried grain with soluble has been taken. The particle size of the sample is 150 micron. The sample then poured in 35ml of ethanol, methanol and distilled water respectively in different conical flask. It has been then for 48hours in shaking condition (120rpm) at 30°C. Then the extraction has been centrifuge at 12,000 rpm for 15minutes in a cooling centrifuge. The volumes of extracts were made up to 100ml with ethanol, methanol and distilled water respectively. Then the extraction was heated at 45°C, 55°C, 65°C for the time interval of 0 minutes,15 minute,30 minutes, 45 minutes, and 60 minutes. Then the samples has been kept in a refrigerator.

Chemicals and Reagents

Gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), quercetin, sodium nitrite ($NaNO_3$), aluminum chloride ($AlCl_3$), sodium hydroxide, DPPH(2,2-diphenyl-1-

picrylhydrazyl), methanol ethanol, and distilled water.

2.5.2 Total Phenolic Content(TPC)

Folin and Ciocalteu reagent has been used as to determine The total phenolics of the extracts, with slight modifications. The reading of samples and standards has been measured by using a UV- spectrophotometer at 750 nm against the reagent blank. The 1ml test sample has been mix with 9ml of water and 1ml of Folin-Ciocalteu's phenol reagent. 10 ml of aqueous solution of sodium carbonate (7% w/v in water) was mixed to the solution after 5 min, and the volume was made up to 25ml with distilled water. The mixture was kept in dark environment for 45 minutes, and then the absorbance of blue color was measured at 750 nm from different samples. The phenolic content(TPC) has been determined as mg gallic acid equivalents GAE/100g of DDGS on a standard curve of gallic acid [Bonoli et al., 2004].

2.5.3 Total Flavonoid Content(TFC)

For determining the total flavonoid content(TFC) of the samples, the aluminum chloride colorimetric method was used. For estimation of total flavonoid, quercetin reagent has been used to generate the standard calibration curve. The quercetin stock solution was prepared by dissolving 25 mg quercetin in 25ml ethanol, then the standard solutions has been prepared by serial dilutions using distilled water. An amount of 1ml diluted standard quercetin solutions and extracts of sample was separately mixed 4ml of distilled water followed by 0.5ml of 5% sodium nitrite solution. After 5minutes 0.3ml of 10% aluminum chloride has been added to it, volume was made up to 10ml. After mixing, the solution was kept in room temperature for a few minutes. The absorbance of the reaction mixtures was measured against blank at 510 nm wavelength with a UV-Vis spectrophotometer. The available concentration of total flavonoid content in the samples has been determined from the calibration graph and expressed as mg quercetin equivalent (QE)/100g of DDGS [Stankovic et al., 2011].

2.5.4 Determination of Antioxidant Activity

2, 2-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method is used for evaluating the antioxidant potential of an extract. This is the simplest method, to measure the antioxidant activity of any compound. DPPH is a stable free radical, due to the delocalization of the spare electron on the whole molecule. The delocalization on the DPPH molecule determines the occurrence of a violet color. When DPPH make a reaction with hydrogen donor, the reduced form of DPPH has been generated, accompanied by the disappearance of violet color. The absorbance diminution therefore depends linearly on the concentration of antioxidant. The spectrophotometric method has been used to determine the antioxidant capacity of any compound [Nishizawa et al., 2005].

DPPH Assay

3.94mg DPPH was dissolved in to 1ml of methanol. Then the 150µl solution was taken out from it and mixed in 50ml of methanol. 200µl sample was mixed with 4.8ml of DPPH solution. The mixture has been vigorously shaken and allow to settle for 30minutes in dark condition. Then the absorbance has been measured at 539 nm. For control 200µl ethanol, methanol and distilled water mixed with 4.8ml of DPPH solution. The following equation determined the inhibition of DPPH [Singh and Singh, 2008].

$$\%Inhibition = \frac{Ac - As}{Ac} * 100 \tag{2.1}$$

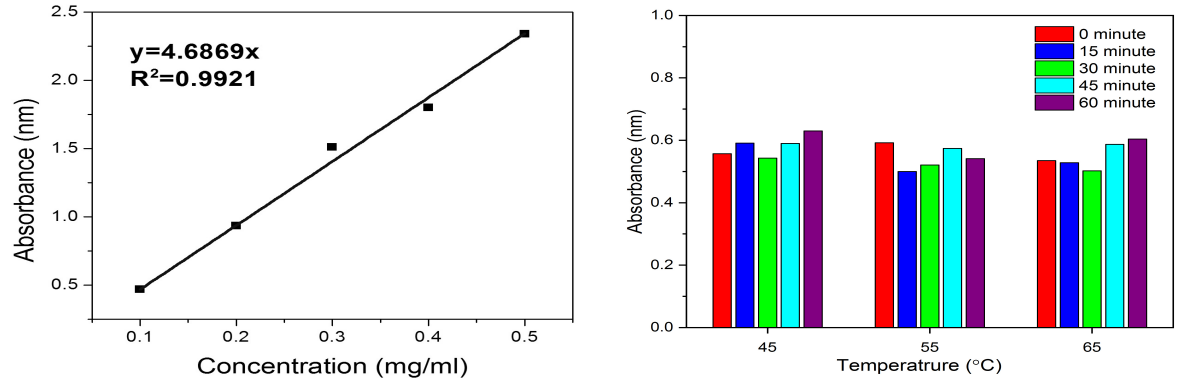
where Ac=absorbance of control at 539nm; As= absorbance of the sample at 539nm.

2.6 Results and Discussion

2.6.1 Total Phenolic Content

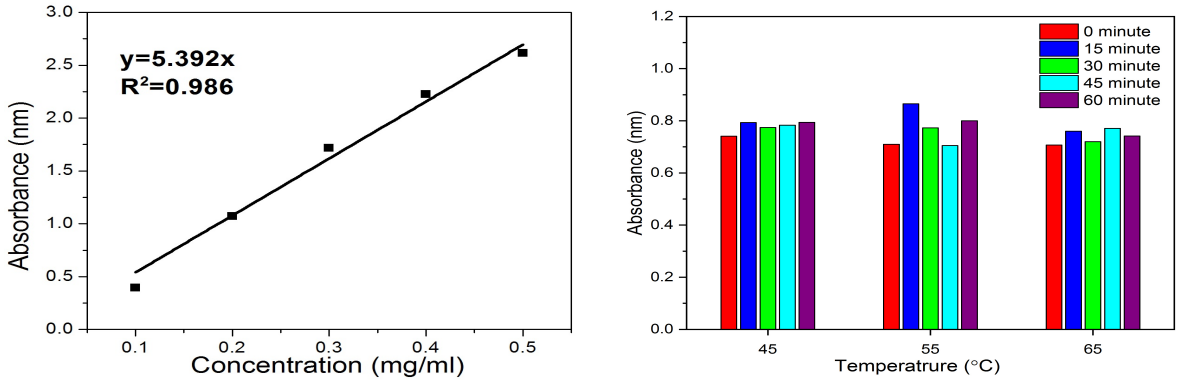
In this experiment, Total phenolic content of the methanol, ethanol and distilled water extract has been determined from a linear gallic acid standard curve (y = 4.6869x, R^2 = 0.9921; y = 5.392x, R^2 = 0.986; y = 3.4971x, R^2 = 0.9833). Fig. 2.2(b) shows the

absorbance values for methanol extract. Fig. 2.3(b) and Fig. 2.4(b) shows the absorbance values for ethanol and distilled water extract of DDGS.



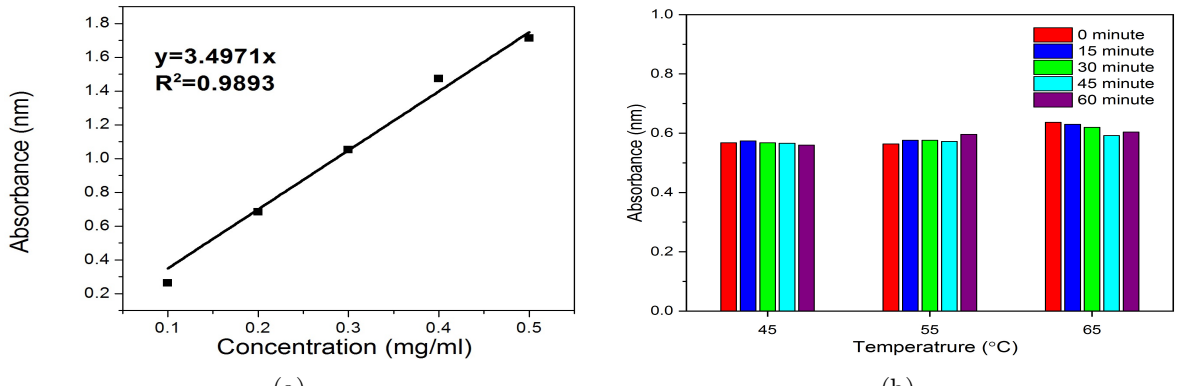
(a) (b)

Figure 2.2: (a) Standard Curve for Gallic Acid, (b) Absorbance of Methanol extract in different temperature.



(a) (b)

Figure 2.3: (a) Standard Curve for Gallic Acid, (b) Absorbance of Ethanol extract in different temperature.



(a) (b)

Figure 2.4: (a) Standard Curve for Gallic Acid, (b) Absorbance of Distilled Water extract in different temperature.

The values from Table 2.1 shows that the amount of TPC is fluctuated due to heat effects. At 55°C, the amount of TPC was degraded for both methanol and ethanol extraction, but for distilled water, the amount of TPC was increased at this temperature. Among the solvents, the Highest phenolic content was found in distilled water extraction at 65°C for 15minutes heat treatment interval (177.2 mgGAE/100g DDGS).

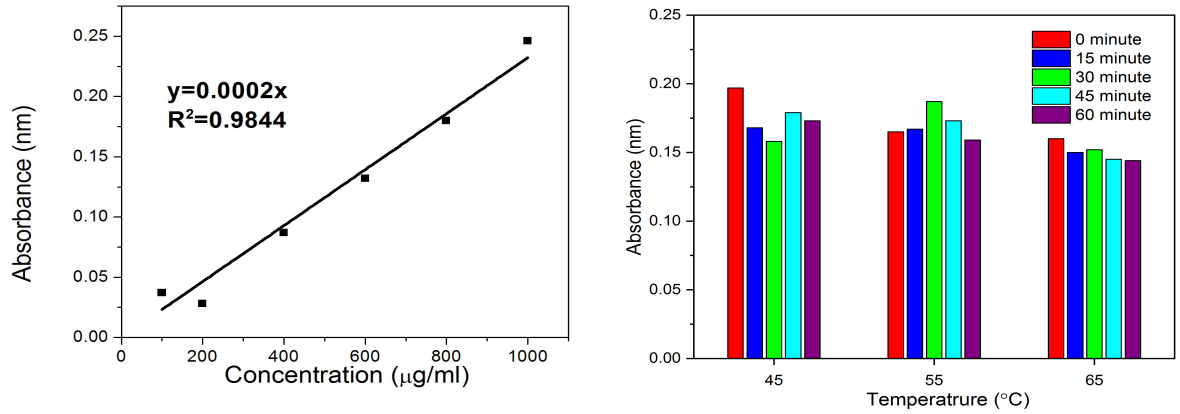
Table 2.1: Comparison of Total Phenolic Content for various solvent

Temperature	**Time(minutes)**	**TPC (mgGAE/100gm)**		
		Methanol	**Ethanol**	**Distilled water**
45°C	0	118.8	137.4	162.4
	15	126	147	164.1
	30	115.8	143.5	162.4
	45	125.8	145.2	161.8
	60	134.4	147.2	160.1
55°C	0	126.3	131.6	161.2
	15	106.6	160.4	164.7
	30	111.1	143.3	164.7
	45	122.4	130.7	163.5
	60	115.4	148.3	170.4
65°C	0	114.1	131.1	182.1
	15	112.6	140	164.7
	30	107.1	133.5	177.2
	45	125.2	142.9	169.2
	60	128.8	137.6	172.7

2.6.2 Total Flavonoid Content

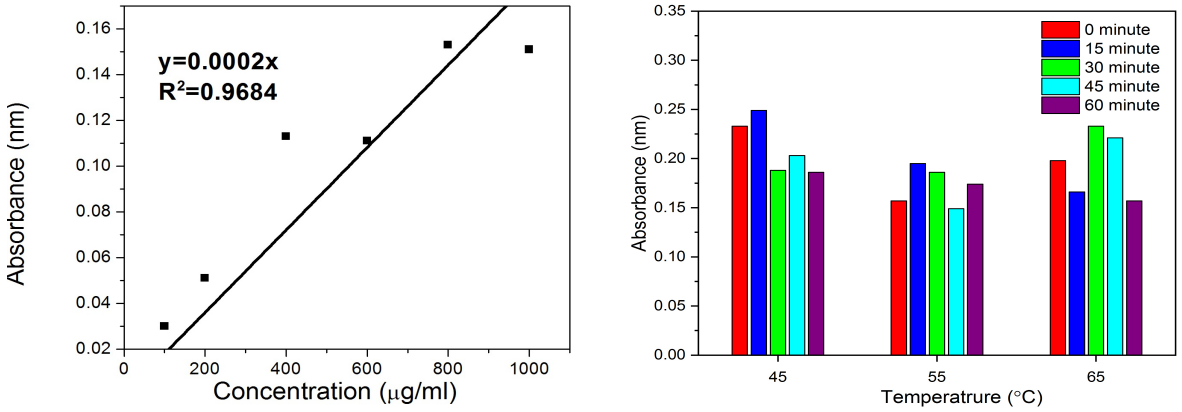
In this experiment, TFC of the methanol, ethanol and distilled water extract was determined from a linear quercetin standard curve (y = 0.0002x, R^2 = 0.9844; y = 0.0002x, R^2 = 0.9684; y = 0.0003x, R^2 = 0.9835). Fig. 2.5(b) shows the absorbance values for methanol extract. Fig. 2.6(b) and Fig. 2.7(b) shows the absorbance values for ethanol and distilled water extract of DDGS.

By comparing the overall values from Table 2.2, it can be evaluated that the amount of TFC is degraded due to heat effects. At 45°C, the amount of TFC was high for all solvents. Among the solvents, the Highest flavonoid content was found in ethanol extraction at 45°C for 15minutes heat treatment interval (1245mgQE/100g DDGS).



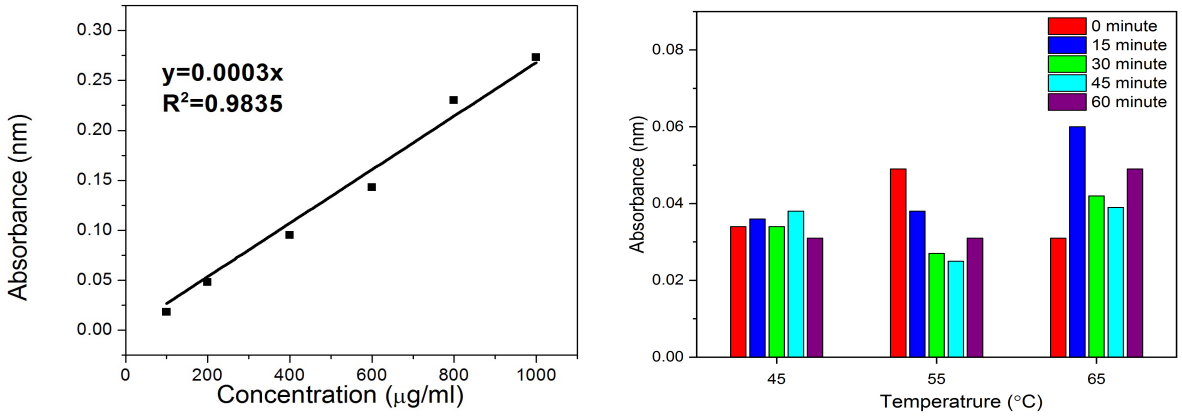
(a) (b)

Figure 2.5: (a) Standard Curve for Quercetin, (b) Absorbance of Methanol extract in different temperature.



(a) (b)

Figure 2.6: (a) Standard Curve for Quercetin, (b) Absorbance of Ethanol extract in different temperature.



(a) (b)

Figure 2.7: (a) Standard Curve for Quercetin, (b) Absorbance of Distilled Water extract in different temperature.

Table 2.2: Comparison of Total Flavonoid Content for various solvent

Temperature	Time(minutes)	TFC (mgQE/100gm)		
		Methanol	Ethanol	Distilled water
45°C	0	985	1165	113.3
	15	840	1245	120
	30	790	940	113.3
	45	895	1015	126.6
	60	865	930	103.3
55°C	0	825	785	163.3
	15	835	975	126.6
	30	935	930	90
	45	865	745	83
	60	795	870	103
65°C	0	800	990	103
	15	750	830	200
	30	760	1165	140
	45	725	1105	130
	60	720	785	163

2.6.3 Antioxidant Activity by DPPH Assay

Antioxidant activity of DDGS is shown in Table 2.6 for different solvent extractions. By evaluating the inhibition percentage, it can be said that antioxidant activity gradually decreases with increasing temperature. At 45°C inhibition percentage is high for all solvent extractions. The highest inhibition percentage 59.32 is shown for ethanol extraction at 55°C for 15minutes heat treatment interval.

Table 2.3: Absorbance of DPPH assay for Methanol Extraction

Temperature	Time (minutes)	Absorbance (nm)
45°C	0	0.11
	15	0.1
	30	0.099
	45	0.105
	60	0.104
55°C	0	0.107
	15	0.102
	30	0.103
	45	0.106
	60	0.103
65°C	0	0.103
	15	0.098
	30	0.104
	45	0.107
	60	0.103
Methanol + DPPH		0.24

Table 2.4: Absorbance of DPPH assay for Ethanol Extraction

Temperature	**Time (minutes)**	**Absorbance (nm)**
45°C	0	0.141
	15	0.140
	30	0.141
	45	0.136
	60	0.135
55°C	0	0.137
	15	0.133
	30	0.153
	45	0.137
	60	0.148
65°C	0	0.146
	15	0.140
	30	0.134
	45	0.137
	60	0.139
Ethanol + DPPH		0.327

Table 2.5: Absorbance of DPPH assay for Distilled Water Extraction

Temperature	**Time (minutes)**	**Absorbance (nm)**
45°C	0	0.101
	15	0.109
	30	0.103
	45	0.103
	60	0.094
55°C	0	0.102
	15	0.095
	30	0.099
	45	0.098
	60	0.097
65°C	0	0.085
	15	0.099
	30	0.094
	45	0.094
	60	0.094
Ethanol + DPPH		0.192

Table 2.6: Comparison of antioxidant activity by DPPH Assay for various solvent

Temperature	**Time(minutes)**	**Antioxidant Activity (%innhibition))**		
		Methanol	**Ethanol**	**Distilled water**
45°C	0	54.16	56.88	47.39
	15	58.33	57.18	43.22
	30	58.75	56.88	46.35
	45	56.25	58.41	46.35
	60	56.6	58.71	51.04
55°C	0	55.41	58.1	46.87
	15	57.5	59.32	50.52
	30	57.08	53.21	48.43
	45	55.83	58.1	48.95
	60	57.08	57.74	49.47
65°C	0	57.08	55.35	55.72
	15	59.16	57.18	48.43
	30	56.66	59.02	51.04
	45	55.41	58.1	51.04
	60	57.08	57.49	51.04

2.7 Conclusion

In this investigation, we assessed total phenols content (TPC), total flavonoids content (TFC) and antioxidant activity (AOA) of DDGS extraction. After investigation, it was observed that final results for every extractions were fluctuated with increasing time and temperature. Sometimes the values were increasing, and sometimes they were decreasing concerning time and temperature. But from the above results, it can be said that the ethanol is a more suitable solvent for extracting polyphenol compounds from DDGS than other two solvents. For ethanol extraction, the amount of flavonoid and antioxidant amounts were higher than methanol and distilled water extraction. So from these results, we can choose ethanol for further DDGS extractions.

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Chapter 3

Effects of different parameters on extraction of Functional Components from DDGS

3.1 Introduction

Corn is the most plenteous sources of bioactive components, including phenolic acid, carotenoids, ferulic acid, and vitamin-E. The presence of these bioactive components in corn is mainly responsible for increasing polyphenol amount in DDGS. Bioactive compounds are contributing antioxidant capacity and potential health benefits to DDGS. The total amount of the bioactive components in DDGS is 2-4 times greater than corn and any other grains. Therefore, DDGS is an excellent source of bioactive phytochemicals. The larger part of polyphenol in DDGS is found in the cell wall matrix as in insoluble condition [Hill et al., 2005].

Polyphenols are the primary type of chemicals which are usually found in plants and its derivatives. The absorption power of polyphenol in gallic acid is very high. Quercetin glucosides are mainly used for flavonoid absorption. Catechins, flavanones, these two components are also used for the absorption of polyphenols. Polyphenols are polyhydroxylated phytochemicals. This polyhydroxylated phytochemical has much variety of compounds,

with similar structures. They can be classified into three classes, phenolic acids, flavonoids, and stilbenoids. These three classes again subclassified into flavonols, isoflavones, flavanones, flavones, flavanols, hydroxybenzoic acids, anthocyanins, lignans, and stilbenes. Polyphenols present in plants are secondary metabolites, which are produced by primary synthesis. Polyphenols have various structures, with a basic structure having a benzene ring which is linked to one or more hydroxyl ions [Acosta-Montoya et al., 2010].

Phenolic extracts of agricultural by-products are a mixture of different phenols. These phenols are selectively soluble in various solvents. The extraction of phenolic compounds from agricultural, industrial by-products is influenced by their physical characteristics, sample size, chemical nature, extraction time, extraction temperature, shaker speed, and storage conditions. The solvent extraction method is ofently used for extracting phenolic compounds from agricultural by-products. Solvent extraction defined as a method of separation by applying a solvent to extract the targeted solute from the solid component. The solubility is determined through the intermolecular interaction between the solute molecules and the solvent. The total efficiency of extraction can be optimized by adding a certain amount of aqueous solution of different organic solvents which is depending on the matrixes. The solvent extraction method is very easy to handle. Solvent extraction depends on the type of solvent, polarity of solvents, extraction time and temperature, sample to solvent ratio, speed of shaker, etc [Barros et al., 2007]

3.2 Objective of the work

The main aim of this work is to get the maximum yield of phenolic content, flavonoid content and antioxidant power in the highest quality and concentration from DDGS extraction. The extraction efficiency is influenced by various factors such as solvent type, extraction temperature, particle size, extraction time, speed of shaker, and solid to solvent ratio. Nevertheless, the solvent type has a major importance in extraction efficiency. Solvent extraction is often used for isolation of antioxidant and extraction yield is dependent on the solvent, due to the different antioxidant potentials of compounds with its different polarity. The yield is increased if the polarity of the solvent is increased during the time

of extraction [Ribeiro et al., 2006]

From our previous work, we are already choosing ethanol as a solvent for DDGS extraction process. In this work, we have to optimize the other factors such as temperature, time, particle size, etc for high yield from DDGS. During solvent extraction increasing the temperature of the solvent may increase the yield of antioxidants extraction. Extraction efficiency may be increased in favor of high temperature by making the diffusion coefficient higher. Extraction time is also a major factor; after a certain period, the extraction may be losing its highest yield [Durling et al., 2007]

The speed of shaker is key factor for polyphenol extraction. Increasing speed of shaker may de degrade the quality of antioxidants. Selection of particle size is also mandatory for high yield of extraction. Because, if the particle size of the solid is not small, there may be substantial diffusional resistance to transport of the solute within the solid. So, break down the solid into particles is mandatory to enhance the extraction rate [Astill et al., 2001]

3.3 Effect of various factors during Solvent Extraction of DDGS

3.3.1 Methodology

Sample preparation

The DDGS were collected from IFB Agro Pvt Ltd. Then the sample was ground to the small particles by using a domestic grinder. Then the DDGS powder was separated into various particle sizes such as 850 μms, 500 μms,250 μms, 150 μms, and 75 μms by using the sieving method in a sieving machine.

Chemicals and Reagents

Gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), quercetin, sodium nitrite ($NaNO_3$), aluminum chloride ($AlCl_3$), sodium hydroxide, DPPH(2,2-diphenyl-1-

picrylhydrazyl), methanol ethanol, and distilled water.

Extraction of polyphenols from DDGS

Extraction of polyphenol from DDGS was done at five different factors, to get the best yield from it.

3.3.2 Effect of Temperature and Particle size

10gm of distiller dried grain with soluble was taken in a different conical flask with different particle size. The particle sizes of the samples were 850 μms, 500 μms, 250 μms, 150 μms, and 75 μms. Then in each conical flask 35ml of ethanol was poured. Keep it for 48hours in shaking condition (80 rpm) at 20°C, 30°C and 40°C respectively. Then collect the extract by using Whatman filter paper. Then centrifuge the extract at 12,000 rpm for 15minutes in a cooling centrifuge. The volume of extracts for each particle was made up to 100ml with ethanol.

3.3.3 Effect of Time and Particle Size

10gm of distiller dried grain with soluble was taken in a different conical flask with different particle size. The size of the particles was 850 μm, 250 μms, and 150 μms. Then 35ml of ethanol was poured in each conical flask. Keep it at 30°C in shaking condition (80 rpm) for 5hours, 24hours and 48 hours respectively. Then collect the extract by using Whatman filter paper. Then centrifuge the extract at 12,000 rpm for 15minutes in a cooling centrifuge. The volume of extracts solution for each particle was made up to 100ml with ethanol.

3.3.4 Effect of Speed, Time and Particle Size

10gm of distiller dried grain with soluble was taken in a different conical flask with different particle size. The size of particles was 150 μm, and a mixture of 150 and 250 μms (5gm each). Then 35ml of ethanol was poured in each conical flask. Keep it at 30°C for 24 hours and 48 hours (150-μm particle only) respectively in different speed (100 rpm,120

rpm, and 200 rpm). Then collect the extract by using Whatman filter paper. Then centrifuge the extract at 12,000 rpm for 15minutes in a cooling centrifuge. The volume of extracts solution for each particle was made up to 100ml with ethanol.

3.3.5 Effect of Solute and Solvent Ratio

A mixture of 150 and 250 μms (5gm each) size DDGS was taken in a three different conical flask. Then 35ml, 45ml, and 55ml ethanol were poured in three conical flasks for extraction of DDGS. Keep it at 30°C for 24hours at 120 rpm speed. Then collect the extract solution by using Whatman filter paper. Then centrifuge the extract at 12,000 rpm for 15minutes in a cooling centrifuge. The volume of extracts solution for each particle was made up to 100ml with ethanol.

3.3.6 Effect of Final Temperature, Time, Speed, Particle Size and Solute-Solvent Ratio

A mixture of 150 and 250 μms (5gm each) size DDGS was taken in a three different conical flask. Then 35ml, 45ml, and 55ml ethanol were poured in three conical flasks for extraction of DDGS. Keep it at 30°C for 24hours at 120 rpm speed. Then collect the extract solution by using Whatman filter paper. Then centrifuge the extract at 12,000 rpm for 15minutes in a cooling centrifuge. The volume of extracts solution for each particle was made up to 100ml with ethanol.

3.4 Determination of TPC, TFC and AOA of DDGS extraction

3.4.1 Total Phenolic Content (TPC)

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, with slight modifications [Bonoli et al., 2004]. Sample and standard readings were made using a UV- spectrophotometer at 750 nm against the reagent blank. The 1ml test sample

was mix with 9ml of water and 1mlof Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of sodium carbonate solution (7% w/v in water) was added to the mixture, and the volume was made up to 25ml with distilled water. The reaction was kept in the dark for 45 min, and the absorbance of blue color from different samples was measured at 750 nm. The phenolic content was calculated as mg gallic acid equivalents/100gm DDGS on a standard curve of gallic acid.

3.4.2 Total Flavonoid Content (TFC)

For determining the total flavonoid content of the samples, the aluminum chloride colorimetric method was used [Stankovic et al., 2011]. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 25 mg quercetin in 25ml ethanol, then the standard solutions of quercetin were prepared by serial dilutions using distilled water. An amount of 1ml diluted standard quercetin solutions or extracts was separately mixed 4ml of distilled water followed by 0.5ml of 5% sodium nitrite solution. After 5minutes 0.3ml of 10% aluminum chloride was added to it, volume was made up to 10ml. After mixing, the solution was kept in room temperature for a few minutes. The absorbance of the reaction mixtures was measured against blank at 510 nm wavelength with a UV-Vis spectrophotometer. The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalents/100gm DDGS.

3.4.3 Determination of Antioxidant Activity by DPPH Assay

3.94mg DPPH was dissolved in 1ml of methanol. Then the 150µl solution was taken out from it and mixed in 50ml of methanol. 200µl sample was mixed with 4.8ml of DPPH solution. The mixture was shaken vigorously and allow to stand for 30minutes in dark condition. Then the absorbance was measured at 539 nm. For control 200µl ethanol, methanol and distilled water mixed with 4.8ml of DPPH solution. The following equation determined the inhibition of DPPH [Singh and Singh, 2008].

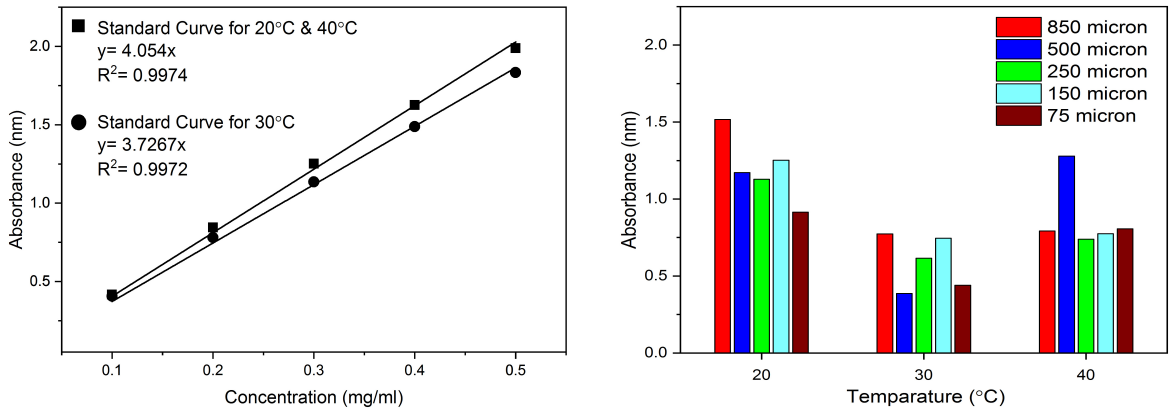
$$\%Inhibition = \frac{Ac - As}{Ac} * 100 \tag{3.1}$$

where Ac=absorbance of control at 539nm; As= absorbance of the sample at 539nm.

3.5 Results and Discussion

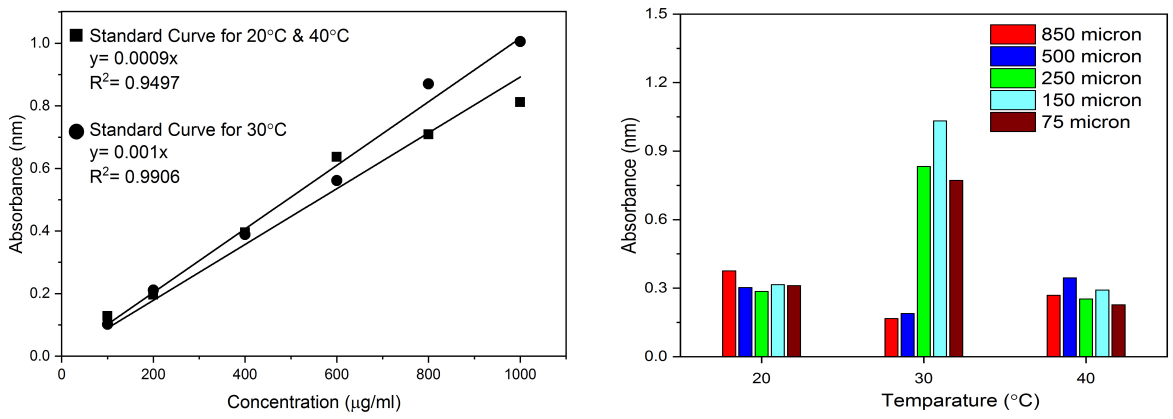
3.5.1 Effect of extraction Temperature and particle size on DDGS extraction

From Table 3.1, it has been evaluated that the extraction gives best yield at 30°C. Total Phenolic Content and Total flavonoid content is high at 30°C for all particle size except 500μm. The values of TPC and TFC are 200mgGAE/100gm DDGS and 1032mgQE/100gm DDGS respectively for 150 μm particle size of DDGS. Increasing temperature from 30°C to 40°C degrades the extraction yield, due to the decrease of the phenolic compound because of hydrolysis and polymerization. For DPPH assay it shows that at 20°C it gives the best antioxidant activity. But as 30°C gives good yield for both TPC and TFC, So we choose this temperature for further extraction and for optimizing other parameters. After this experiment, we eliminate 500μm and 75μm particle size, due to the low yield of TPC and TFC of 500μm particle and lack of availability of 75μm particle size of DDGS.



(a) (b)

Figure 3.1: (a) Standard Curves for Gallic Acid, (b) Absorbance values for calculate TPC of DDGS extract with variation of particle size in different temperature.



(a) (b)

Figure 3.2: (a) Standard Curves for Quercetin, (b)Absorbance values for calculate TFC of DDGS extract with variation of particle size in different temperature.

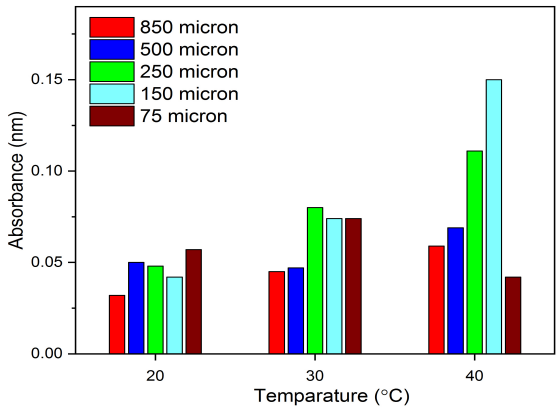


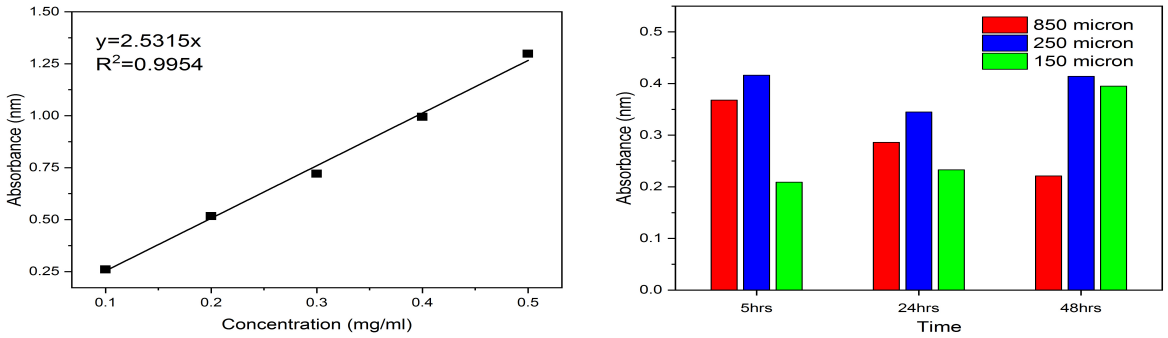
Figure 3.3: Absorbance values for calculate %inhibition of DDGS extract with variation of particle size in different temperature.

Table 3.1: Percentage of Antioxidants for various particle size with different Temperature

Temperature (°C)	**Particle Size (micron)**	**TPC (mgGAE/100gm DDGS)**	**TFC (mgQE/100gm DDGS)**	**DPPH assay (%inhibition)**
20	850	187.05	208.5	89.15
	500	144.5	168	83.05
	250	139	158.5	83.72
	150	154	175	85.76
	75	225.7	345	80.67
30	850	207.4	167	68.3
	500	103.5	189	66.9
	250	165	833	43.66
	150	200	1032	47.88
	75	118	772	47.88
40	850	195.3	298	76.61
	500	157.5	191.5	62.37
	250	182.2	281	49.15
	150	191.1	324	85.76
	75	198.8	252	89.15

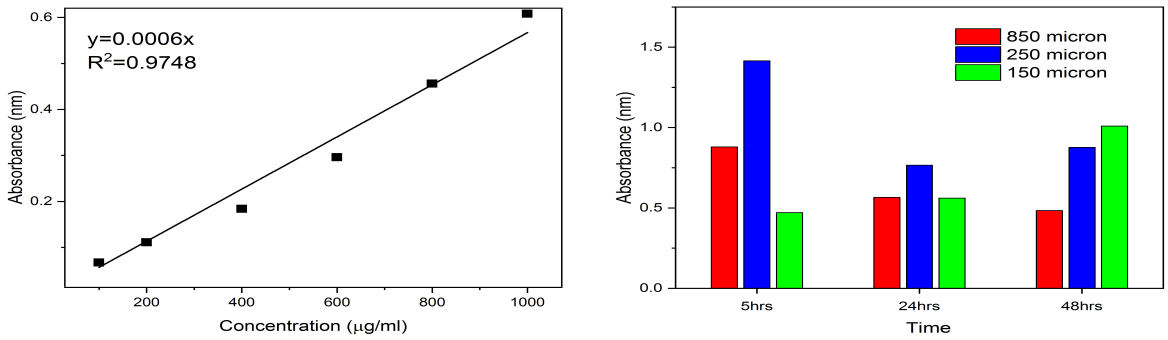
3.5.2 Effect of extraction Time and particle size on DDGS extraction

By evaluating overall values of TPC, TFC and antioxidant activity from Table 3.2 of DDGS we can eliminate 5 hours as extraction time.



(a) (b)

Figure 3.4: (a) Standard Curve for Gallic Acid, (b) Absorbance values for calculate TPC of DDGS extract with variation of particle size in different time.



(a) (b)

Figure 3.5: (a) Standard Curves for Quercetin, (b) Absorbance values for calculate TFC of DDGS extract with variation of particle size in different time.

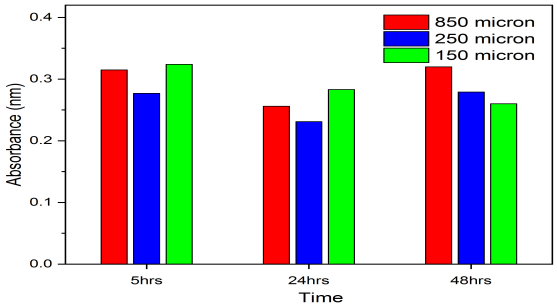


Figure 3.6: Absorbance values for calculate %inhibition of DDGS extract with variation of particle size in different time.

Table 3.2: Percentage of Antioxidants for various particle size with different Time

Temperature (°C)	**Time**	**Particle Size (micron)**	**TPC (mgGAE/100gm DDGS)**	**TFC (mgQE/100gm DDGS)**	**DPPH assay (%inhibition)**
30°c	5hrs	850	72.65	733	21.64
		250	82.15	1179	31.09
		150	164	1570	19.42
	24hrs	850	112.9	94.3	36.31
		250	136.2	1276	42.53
		150	184	1870	29.6
	48hrs	850	43.65	403	20.39
		250	82.6	730	30.59
		150	156	3362	35.32

Along with that, we can also eliminate 850-μm particle size for all temperature, as it does not give the best yield during any period of extraction time. The highest value was given by 150μm particle size for all extraction period. The amount TPC, TFC both are high for 150μm particle DDGS along with the high antioxidant activity. But by evaluating all the values of the experiment, it is not cleared that which temperature is best between 24 hours, and 48 hours for the DDGS extraction. So, further experiment is needed for time optimization.

3.5.3 Effect of extraction Speed, Time and particle size on DDGS extraction

Extraction speed is a major factor for polyphenol extraction. From the values of Table 3.6, it is cleared that at the speed of 120 rpm, for 24 hours the mixture of two particles (150+250 μm) gives the best yield for DDGS extraction. Highest TPC and TFC values were 269.9mgGAE/100gm DDGS and 1187mgQE/100gmDDGS respectively. From the values of Table 3.6 it can be concluded that, when the speed was increased from 100 rpm to 120 rpm, TPC and TFC both were increased. This thing may denote that proper mixing and close contact between DDGS particle and ethanol. But at 200 rpm both TPC and TFC were decreased very rapidly. Increasing rpm from 120 to 200 may attribute that mixing was vigorous and so fast for contacting between particles and ethanol. So from this result, we fixed 120 rpm as a speed, 24 hours as extraction time and a mixture of 150 and 250-μm particle as particle-size for DDGS extraction.

Table 3.3: Absorbance values for calculate TPC of DDGS extract with variation of particle size and speed of incubator (rpm) in different time

rpm	**Time**	**Particle size (micron)**	**Absorbance (nm)**
100	24hrs	150	0.272
		150+250	0.466
	48hrs	150	0.085
120	24hrs	150	0.67
		150+250	1.056
	48hrs	150	0.624
200	24hrs	150	0.41
		150+250	0.422
	48hrs	150	0.364

Table 3.4: Absorbance values for calculate TFC of DDGS extract with variation of particle size and speed of incubator (rpm) in different time

rpm	**Time**	**Particle size (micron)**	**Absorbance (nm)**
100	24hrs	150	0.357
		150+250	0.42
	48hrs	150	0.348
120	24hrs	150	0.5
		150+250	1.069
	48hrs	150	0.55
200	24hrs	150	1.005
		150+250	0.913
	48hrs	150	0.76

Table 3.5: Absorbance values for calculate %inhibition of DPPH assay of DDGS extract with variation of particle size and speed of incubator(rpm) in different time

rpm	**Time**	**Particle size (micron)**	**Absorbance (nm)**
100	24hrs	150	0.077
		150+250	0.055
	48hrs	150	0.47
120	24hrs	150	0.08
		150+250	0.067
	48hrs	150	0.078
200	24hrs	150	0.121
		150+250	0.111
	48hrs	150	0.132

Table 3.6: Percentage of Antioxidants for various particle size and speed of incubator(rpm) in different time

Temperature (°C)	rpm	Time	Particle Size (micron)	TPC (mgGAE/100gm DDGS)	TFC (mgQE/100gm DDGS)	DPPH assay (% inhibition)
30	100	24hrs	150	111.7	595	36.36
			150+250	191.4	700	54.54
		48hrs	150	34.9	580	61.15
	120	24hrs	150	171.2	555	57.44
			150+250	269.9	1187	64.36
		48hrs	150	79.7	611.1	58.51
	200	24hrs	150	104.7	1116	35.63
			150+250	107.8	1014.4	40.95
		48hrs	150	46.5	422	29.78

3.5.4 Effect of Solute solvent ratio on DDGS extraction

From Table 3.8, it is proven that the extraction yield will be higher with increasing the amount of solvent concerning the solute. Table 3.7 shows the absorbance values for calculate TPC, TFC and % inhibition of DDGS extract in presence of different concentration of ethanol. TPC, TFC, and antioxidant activity all were in higher amount when the amount of solvents was increasing. These things indicate that if we increase the amount of solvent concerning the solute, the solute will be more dissolved in it and gives the best yield during extraction time. Highest TPC and TFC values were 213mgGAE/100gm DDGS and 1139mgQE/100gmDDGS respectively The highest values were obtained at the ratio of 10:55 (solute : solvent) from Table 3.8.

Table 3.7: Absorbance values for calculateTPC TFC & % inhibition of DDGS extract with different concentration of solvent

rpm	Time	Particle Size (micron)	Particle Weight (gm)	Ethanol amount (ml)	Absorbance (nm) for TPC	Absorbance (nm) for TFC	Absorbance (nm) for DPPH assay
120	24	150+250	10	35	0.41	0.911	0.145
				45	0.475	1.028	0.141
				55	0.557	1.139	0.140

Table 3.8: Percentage of Antioxidants for different concentration of solvent

rpm	Time	Particle Size (micron)	Particle Weight (gm)	Ethanol amount (ml)	TPC (mgGAE/100gm DDGS)	TFC (mgQE/100gm DDGS)	DPPH assay (% inhibition)
120	24	150+250	10	35	157	911	43.35
				45	181	1028	44.92
				55	213	1139	45.31

3.5.5 Effect of Final Factors on DDGS extraction

A mixture of 150 and 250μm particle gives the highest yield of extraction at 30°C, for 24hours in the presence of 55ml ethanol. During this extraction speed of incubator was 120 rpm. The values for TPC and TFC were 230.4mgGAE/100gm DDGS and 1037mgQE/100gm DDGS and the %inhibition rate was 56.59.

Table 3.9: Absorbance values for calculte TPC, TFC & %inhibition of DDGS extract with optimized extraction factors

rpm	**Time**	**Particle Size (micron)**	**Particle Weight (gm)**	**Ethanol amount (ml)**	**Absorbance (nm) for TPC**	**Absorbance (nm) for TFC**	**Absorbance (nm) for DPPH assay**
120	24	150+250	10	55	0.651	0.934	0.102

Table 3.10: Percentage of Antioxidants for DDGS extraction with optimized extraction factors

rpm	**Time**	**Particle Size (micron)**	**Particle Weight (gm)**	**Ethanol amount (ml)**	**TPC (mgGAE/100gm DDGS)**	**TFC (mgQE/100gm DDGS)**	**DPPH assay (% inhibition)**
120	24	150 +250	10	55	230.4	1037	56.59

3.6 Conclusion

This study shows the importance of various extraction condition such as temperature, time, particle size, shaker speed, and solute-solvent ratio to obtain an extract with the highest value of TPC and TFC along with the highest antioxidant activity. It can be concluded that the optimum factors for maximum polyphenols extraction from DDGS were as follows: temperature 30°C, extraction time 24hours, particle size mixture of 150 and 250 μms, shaker speed 120 rpm and solute-solvent ratio 10:55. By fixing all these factors we can get high extraction yield as TPC and TFC values were in high amount and antioxidant activity also gives a satisfactory appraisal. So, for future work, we can use this extract as a source of natural antioxidant which is derived from industrial by-product DDGS. By applying new methods, we can entrap this antioxidant and may add it with various products to reduce oxidation. For approaching these methods, further studies are needed.

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Chapter 4

Encapsulation and Partial Characterization of Extracted Functional Components of DDGS

4.1 Introduction

The investigation of bioactive compounds from plant sources or agricultural waste has increased day by day. Isolation of these bioactive compounds from these materials has its beneficial effects. It can lower the risk of a specific disease, which may occur due to oxidative damage. An antioxidant is one of the essential compounds which can be isolated from a plant source and fortified in functional foods to improve its quality. Antioxidant has some properties such as antiradical, anti-inflammatory, anti-carcinogenic, anti-atherosclerotic properties by which it can be effective to reduce oxidative damage on the human body. It can also inhibit several types of human disease like cancer, cardiovascular disease, etc. Consumption of plant-based foods may not be enough for having natural antioxidants. But functional foods which are fortified with antioxidants can provide a higher amount of antioxidants. But these natural antioxidants are highly sensible; it can easily be degraded by the effect of natural factors like heat, and light. So preventing them from these factors are very challengeable [Manousaki et al., 2016].

Microencapsulation is one of the most acceptable techniques to overcome the stability problems of antioxidants. This technique has been applied by the food industries to protect natural antioxidants. Microencapsulation is defined as compacting solids, liquids or gaseous matter in small, sealed capsules. These capsules can release their contents at controlled speed under some specific conditions. These capsules can protect antioxidant from the light and oxygen and prevent its degradation [Saénz et al., 2009]. Microencapsulation is mainly depended on the type of core material, shell material, and size of capsules. The choice of shell material is important because it influences the stability and efficiency of microencapsulation. There are many materials which can be used as shell material like, starch, guar gum, gum Arabic, gelatin, sodium alginate, etc. Chemical structure of alginate is as similar to polysaccharide components of the extracellular matrix. Alginates have been applied for microencapsulation. Alginate structure is stable at acidic condition, but it is easily degraded under mild alkali conditions. Guar gum is also used for coating material in the microencapsulation process, due to its easy availability, low cost and for excellent thickening property. Among various microencapsulation techniques, spray and freeze-drying are the most commonly used methods. The freeze drying method depends on the dehydration of frozen products by a sublimation process. During this process, products are not in contact with high temperatures so the freeze-dried products can protect their nutritional quality [Ahmed et al., 2010].

The efficiency of the microencapsulation process depended on different techniques like protecting the encapsulated antioxidant from various environmental factors; stability of the encapsulated antioxidant for a long duration; release of the encapsulated antioxidant from the shell under specific conditions. This process is suitable for encapsulation of natural antioxidants that will be used in food, oil, and pharmaceutical industries [Venkatesan et al., 2009].

4.2 Objective of the work

Developments of antioxidants now gradually changed. Preferred of antioxidant is also changed from synthetic into a natural antioxidant. Among all natural antioxidants, plant

antioxidants are the non-toxic, healthy, beneficiary, with a broad application prospect in food and other fields. These natural antioxidants are easily degradable as they are sensitive towards light and heat. Besides this, their bitter taste is also responsible for limiting uses of them in food products. Microencapsulation process can be implemented to prevent this bitter taste of antioxidant. For this microcapsule, the antioxidant is used as core material. The primary aim of this study is to encapsulate the extracted antioxidant from DDGS and also stabilized this antioxidant inside the coating material of microcapsule. The coating material should be nonreactive with the core material. Generally, both hydrophilic and hydrophilic polymers are used for encapsulation [Jun-xia et al., 2011]. Sodium alginate and guar-gum used as a coating material because both are hydrophilic. Heat treatment of microcapsules is also performed to determine the degradation rate of antioxidant as well as the recovery rate of antioxidants after encapsulation. This study evaluated the total phenolic content, total flavonoid content, and antioxidant activity of DDGS microcapsules obtained by different coating material using freeze drying. This study also analyzed the scanning electron microscopy of microcapsule to evaluate its structure.

4.3 Methodology for Microenapsulation of DDGS

The DDGS were collected from IFB Agro Pvt Ltd. Then the sample was ground to the small particles by using a domestic grinder. Then the DDGS powder was separated into 250 microns, and 150 microns by using the sieving method in a sieving machine.

4.3.1 Chemicals and Reagents

Gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), quercetin, sodium nitrite ($NaNO_3$), aluminum chloride ($AlCl_3$), Sodium Hydroxide, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, ethanol, polyethylene glycol (PEG), sodium alginate, guar gum, calcium chloride, glacial acetic acid, chloroform, saturated potassium iodide solution, 0.1N sodium thiosulphate solution, neutral alcohol, 0.5% phenolphthalein, N/10 potassium hydroxide solution (KOH), N/10 oxalic acid solution and distilled water .

4.3.2 Extraction Procedure of DDGS

A mixture of 150 and 250 microns size DDGS particles was taken in a conical flask. Then ethanol was poured in a conical flask for extraction of DDGS. Sample/solid ratio was 10:55(gm/ml). Keep it at 30°C for 24 hours at 120 rpm speed. Then collect the extract solution by using Whatman filter paper. Then centrifuge the extract at 12,000 rpm for 15minutes in a cooling centrifuge.

4.4 Microcapsule Preparation with DDGS Extraction

4.4.1 Sodium-alginate Coated Microcapsule

Extracted antioxidant from DDGS was mixed with polyethylene glycol at a ratio of 1:2(v/v). This mixture was mixed with coating material Sodium alginate(Na-A) containing 1%, 2% and 3% solid respectively, at a ratio of 1:2(v/v). Then the mixture was spread on a calcium chloride solution. Then the solution was filtered after 2 hours. After that freeze-drying process was performed to dry up the solid for 12 hours. Then they were frozen at -18°C.

4.4.2 Guar-gum and Sodium-alginate Coated Microcapsule

0.5% guar-gum (G.G) was added with 1%,2% and 3% sodium alginate(Na-A) at a ratio of 1:1(v/v) to produced three different coating material. Extracted antioxidant from normal DDGS was mixed with polyethylene glycol (PEG) at a ratio of 1:2(v/v). Then the mixture of antioxidant and PEG was mixed with coating material at a ratio of 1:2(v/v). Then the mixture was spread on a calcium chloride solution. Then the solution was filtered after 2 hours. After that freeze-drying process was performed to dry up the solid for 12 hours. Then they were frozen at -18°C.

4.4.3 Effects of Heat on Microencapsulated Antioxidant

Guar-gum(0.5%) and sodium alginate(1%,2% & 3%) coated samples were kept in hot air oven at 80°C for 30 minute, 60 miutes, and 90 minutes. After that, the samples were kept in desiccators for 2 hours. Then they were frozen at -18°C.

4.5 Sample Preparation

4.5.1 Sample Preparation by Vortex Method

Approximately 0.5gm encapsulated sample was weighed and mixed with 10ml methanol stirred for 5 minutes in a regular vortex mixer. They were then frozen at -18°C for 90 minutes and thawed at room temperature. The sample was stirred in a vortex mixer for 2 minutes and centrifuged at 6000 rpm for 15 minutes [Vidal et al., 2013].

4.5.2 Sample Preparation by Sonication Method

0.5 gm of encapsulated powder was dissolved in 10ml of Methanol, mixed for 1 minute and sonicated twice for 20 minutes. The sample was then centrifuged at 6000 rpm for 15 minutes [Aliakbarian et al., 2015].

4.6 Characterization of Microcapsule

4.6.1 Total Phenolic Content (TPC)

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, with slight modifications. Sample and standard readings were made using a UV- spectrophotometer at 750 nm against the reagent blank. The 1ml test sample was mix with 9ml of water and 1mlof Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of sodium carbonate solution (7% w/v in water) was added to the mixture, and the volume was made up to 25ml with distilled water. The reaction was kept in the dark for 45 min, and the absorbance of blue color from different samples was measured at 750 nm. The

phenolic content was calculated as mg GAE/5ml of DDGS based on a standard curve of gallic acid [Bonoli et al., 2004].

4.6.2 Total Flavonoid Content (TFC)

For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving quercetin in ethanol, then the standard solutions of quercetin were prepared by serial dilutions using distilled water. An amount of diluted standard quercetin solutions and samples were separately mixed with distilled water followed by mixed with 5% sodium nitrite solution. After 5minutes 10% aluminum chloride was added to it, volume was made up. Then the solution was kept in room temperature for a few minutes. The absorbance of the reaction mixtures was measured against blank at 510 nm wavelength with a UV-Vis spectrophotometer. The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/5ml of DDGS [Stankovic et al., 2011].

4.6.3 Antioxidant Activity by DPPH Assay

3.94mg DPPH was dissolved in 1ml of methanol. Then the 150µl solution was taken out from it and mixed in 50ml of methanol. 200μl sample was mixed with 4.8ml of DPPH solution. The mixture was shaken vigorously and allowed standing for 30minutes in dark condition. Then the absorbance was measured at 539 nm. For control 200μl ethanol mixed with 4.8ml of DPPH solution [Singh and Singh, 2008].

4.6.4 Scanning Electron Microscopy (SEM) of Microcapsules

Internal morphological properties of DDGS microcapsules were observed by scanning electron microscopy. The sample was fixed on a stub. Then the sample was coated with the gold layer in a vacuum evaporator. Digital images were stored at an accelerating voltage of 15.0 KV [Arshad et al., 2018].

4.7 Results and Discussion

4.7.1 Sodium-alginate (Na-A) Coated Microcapsule

Total phenolic content of the sodium-alginate coated(1%,2% and 3%) microcapsule was determined from a linear gallic acid standard curve (y = 3.6307x, R^2 = 0.9938). Fig 4.1(a). Shows the absorbance amount for total phenolic content. The absorbance of DDGS extracts for TPC was 1.737 nm. In this study, the total flavonoid content of the sodium-alginate coated microcapsule was evaluated by using the aluminum colorimetric assay. The total flavonoid content was determined from the quercetin standard curve (y =0.0009x, R^2 = 0.9548). Fig 4.1(b) Shows the absorbance amount for total flavonoid content. The absorbance of DDGS extracts for TFC was 2.584 nm. Antioxidant activity was determined by DPPH assay, and for this analysis absorbance of the control and DDGS extracts were 0.067 nm and 0.019 nm respectively. Fig 4.1(c) Shows the absorbance amount for DPPH assay.

Table 4.1 Shows that total phenolic content, total flavonoid content and antioxidant activity of encapsulated samples. The heights TPC value obtained for 3%sodium alginate coated powder (sonication method) and the value was 4.40mgGAE/5ml DDGS extraction. Percentage of inhibition is also highest for 3%sodium alginate coated powder(sonication method). The value was 62.68%. The heights TFC value obtained for 2%sodium alginate coated powder (sonication method), and the value was 42.25mgQE/5ml of DDGS extract.

From Fig 4.2, we can say that the sonication method gives a good yield over the vortex method. For every sample, the sonication method gives good yield on total phenolic content, total flavonoid content and as well as on percentage inhibition. During the sonication method, uniform agitation was performed by a sound wave, due to this reason the coating material was broken down properly and recovery of antioxidant from microcapsule was made correctly. But again it shows that recovery percentage was increased for the sonication method with an increasing percentage of the coating material.

The recovery percentage was calculated by comparing TPC and TFC value of DDGS extracts. So from this, we can say that the antioxidant leaches out quickly as the lower

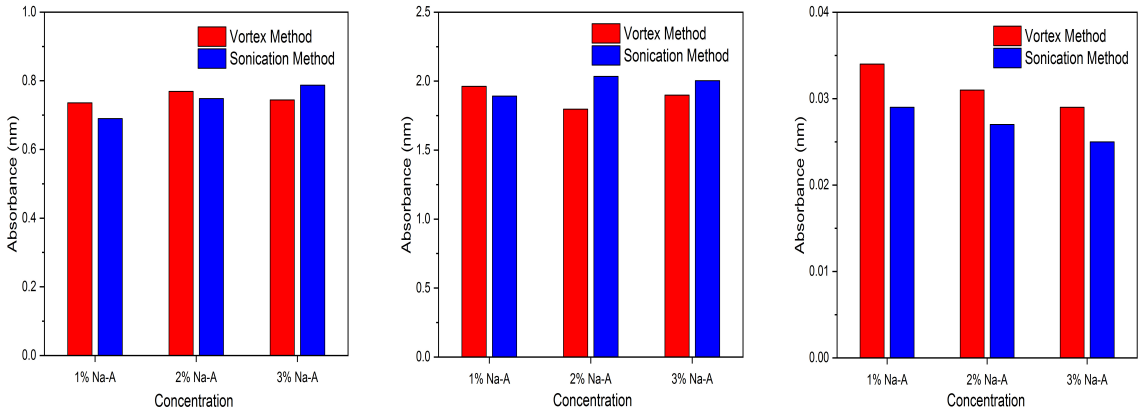


Figure 4.1: (a) Absorbance of TPC, (b) Absorbance of TFC (c) Absorbance of DPPH for different sample preparation method from sodium alginate coated microcapsule.

Table 4.1: Percentage of Antioxidant in Sodium-alginate coated DDGS microcapsule and in DDGS extract

Sample Name	**Methods of sample preparation**	**Total Phenolic Content mgGAE/5ml Extract**	**Total Flavonoid Content mgQE/5ml Extract**	**DPPH % inhibition**
1% Na-A	Vortex	2.8085	26.511	49.25
1% Na-A	Sonication	3.5565	39.35	56.71
2% Na-A	Vortex	2.5615	24.14	53.73
2% Na-A	Sonication	3.9289	43.122	59.7
3% Na-A	Vortex	2.4309	25.02	56.71
3% Na-A	Sonication	4.4075	42.25	62.68
Extract of DDGS	-	9.7725	58.65	71.64

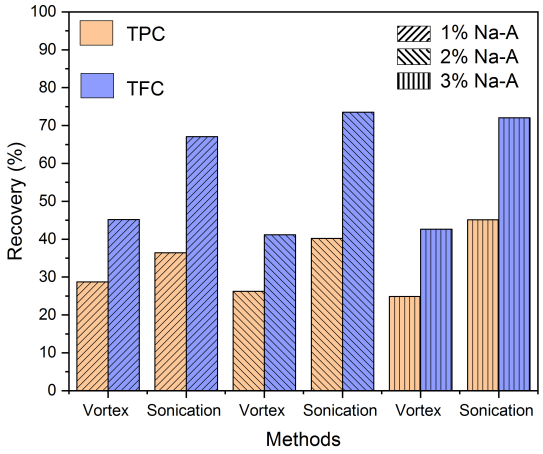
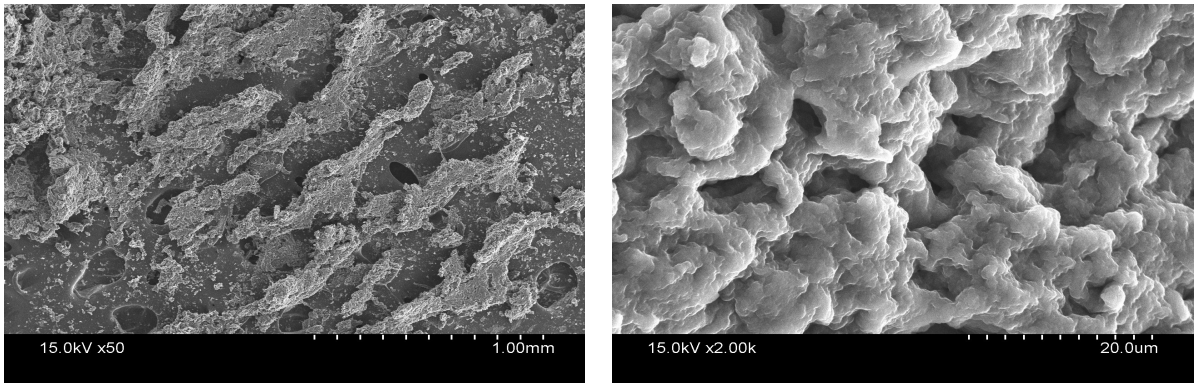


Figure 4.2: Recovery percentage of TPC and TFC in Na-A coated microcapsules

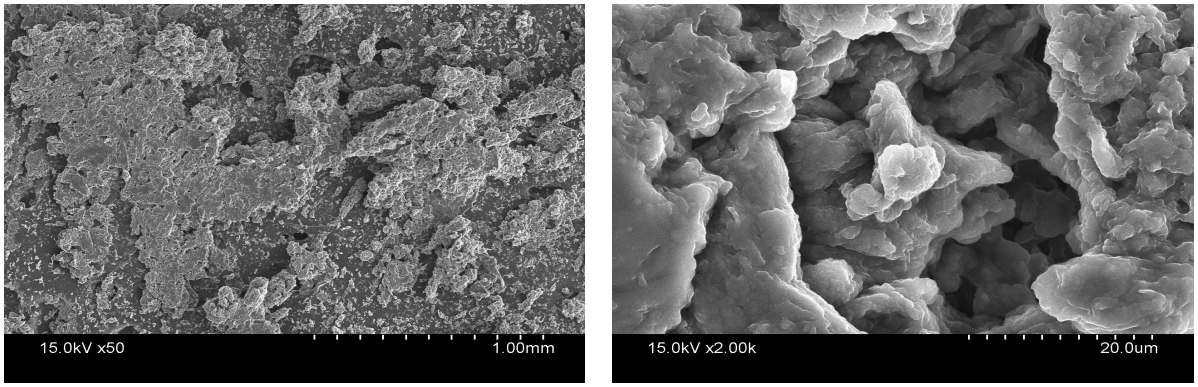
percentage coating material was unable to hold the encapsulated antioxidant inside it. As was made by lower concentration so the coating material broken down quickly, because it

was unable to resist the vibration of sounds during sonication method. As a results there was degradation took place in antioxidant so it felt effects on values of TPC, TFC, and percentage inhibition.



(a) (b)

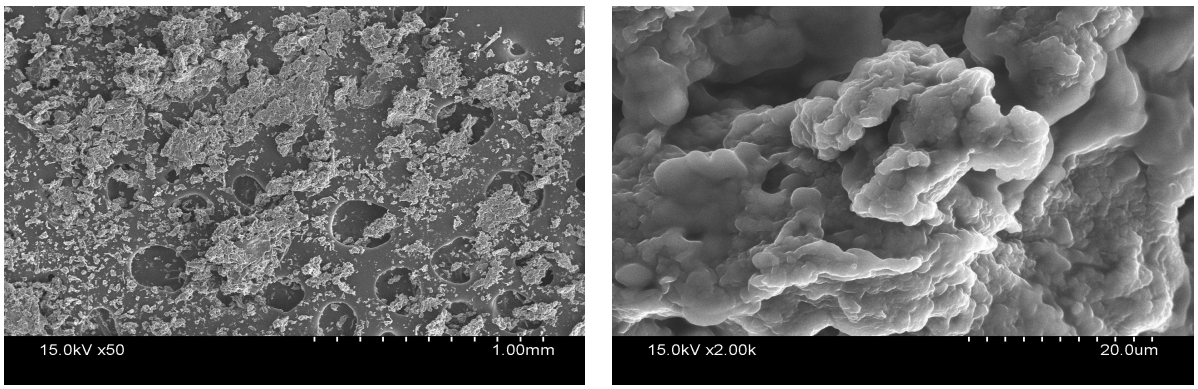
Figure 4.3: SEM image of (a) DDGS microcapsule with 1% sodium-alginate coating, (b) Internal structure of DDGS microcapsule with 1% sodium-alginate wall material



(a) (b)

Figure 4.4: SEM image of(a) DDGS microcapsule with 2% sodium-alginate coating, (b) Internal structure of DDGS microcapsule with 2% sodium-alginate wall material

Fig. 4.3(a) and 4.3(b) represent the internal SEM structures of DDGS microcapsule composed of 1%sodium alginate as coat material. Fig. 4.4(a) and 4.4(b) represent the internal SEM structures of DDGS microcapsule composed of 2%sodium alginate as coat material. Fig. 4.5(a) and 4.5(b) represent the internal SEM structures of DDGS microcapsule composed of 3%sodium alginate as coat material. The SEM images showed that most of the microcapsules were cloudy and congested with each other. The structures were mostly ropy in shapes. There is no void space inside the capsule; this characteristic of microcapsules formed due to the freeze-drying method.



(a) (b)

Figure 4.5: SEM image of(a) DDGS microcapsule with 3% sodium-alginate coating, (b) Internal structure of DDGS microcapsule with 3% sodium-alginate wall material

4.7.2 Guar-gum and Sodium-alginate Coated Microcapsule

Total phenolic content of the 0.5%guar-gum and sodium-alginate(1%,2% and 3%) coated microcapsules were determined from a linear gallic acid standard curve (y = 3.666x, R^2 =0.983). Fig. 4.6(a) Shows the absorbance amount for total phenolic content. The absorbance of DDGS extracts for TPC was 1.835 nm. In this study, the total flavonoid content of the sodium-alginate and guar-gum coated microcapsule was evaluated by using the aluminum colorimetric assay. The total flavonoid content was determined from the quercetin standard curve (y =0.0009x, R^2 =0.9696). Fig. 4.6(b) shows the absorbance amount for total flavonoid content. The absorbance of DDGS extracts for TFC was 2.575 nm. Antioxidant activity was determined by DPPH assay, and for this analysis absorbance of the control and DDGS extracts were 0.135 nm and 0.044 nm respectively. Fig. 4.6(c) shows the absorbance amount for DPPH assay.

Table 4.2 shows that total phenolic content, total flavonoid content and antioxidant activity of encapsulated samples. The heights TPC value obtained for 0.5% guar-gum and 3%sodium alginate coated powder (sonication method), and the value was 8.24mg-GAE/5ml DDGS extraction. Percentage of inhibition is also highest for 3%sodium alginate coated powder(sonication method). The heights TFC value obtained for 0.5% guar-gum and 3%sodium alginate coated powder (sonication method), and the value was 55.39mgQE/5ml of DDGS extract.

From the Fig. 4.7, we can again say that the sonication method gives a good yield

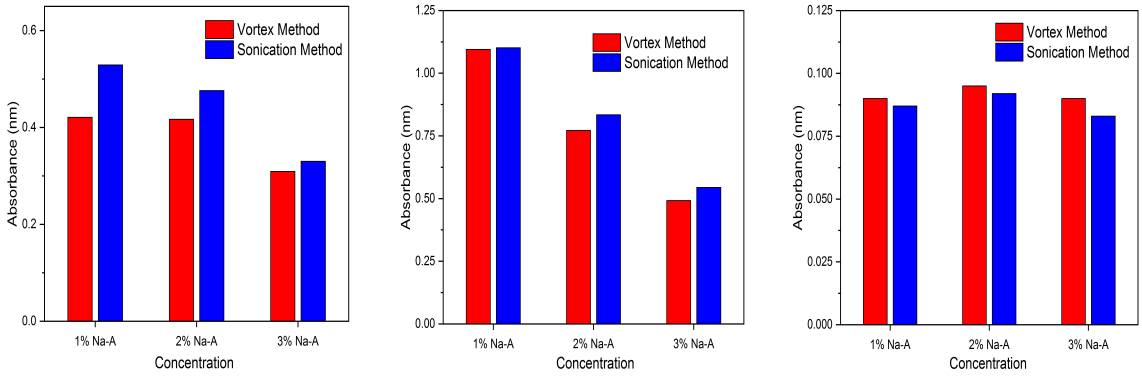


Figure 4.6: (a) Absorbance of TPC, (b) Absorbance of TFC (c) Absorbance of DPPH for different sample preparation method from sodium alginate and Guar gum coated microcapsule.

Table 4.2: Percentage of Antioxidant in guar-gum & Sodium-alginate coated DDGS microcapsule and in DDGS extract

Sample Name	**Methods of sample preparation**	**Total Phenolic Content mgGAE/5ml Extract**	**Total Flavonoid Content mgQE/5ml Extract**	**DPPH % inhibition**
1% Na-A + 0.5% G.G	Vortex	4.32	45.81	33.33
1% Na-A + 0.5% G.G	Sonication	5.43	46.07	35.55
2% Na-A + 0.5% G.G	Vortex	6.66	49.75	29.62
2% Na-A + 0.5% G.G	Sonication	6.61	53.74	31.85
3% Na-A + 0.5% G.G	Vortex	7.69	50.107	33.33
3% Na-A + 0.5% G.G	Sonication	8.24	55.399	38.51
Extract of DDGS	-	10.54	60.28	67.4

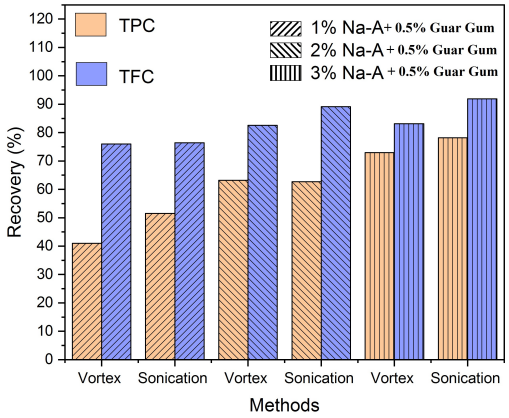
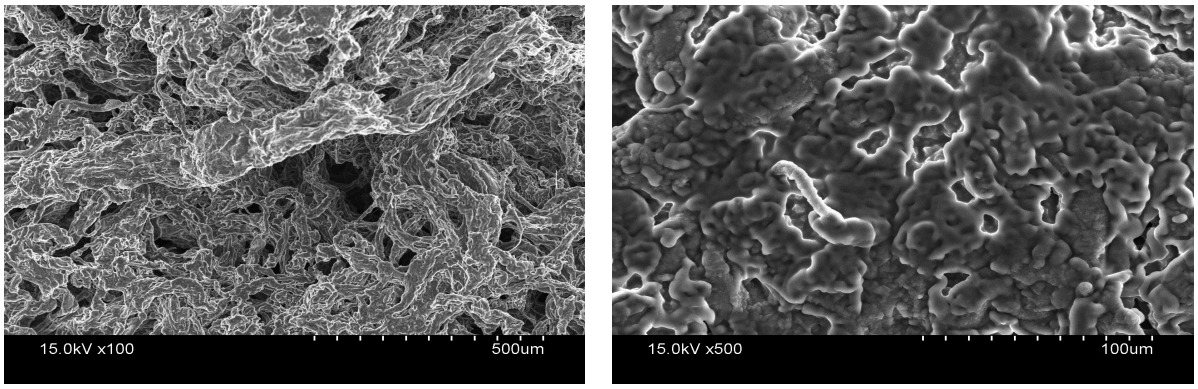


Figure 4.7: Recovery percentage of TPC and TFC in Guar-gum & sodium-alginate coated DDGS microcapsules

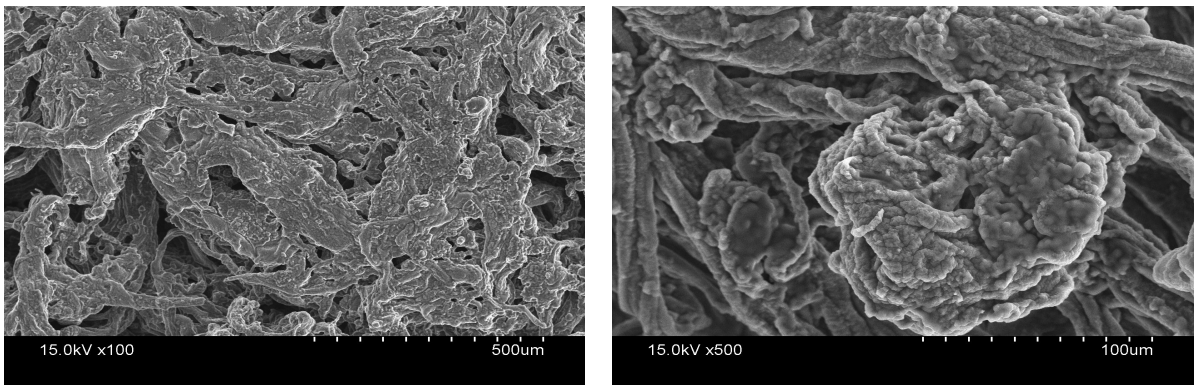
over the vortex method. For every sample, the sonication method gives good yield on total phenolic content, total flavonoid content and as well as on percentage inhibition.

During the sonication method, uniform agitation was performed by a sound wave, due to this reason the coating material was broken down properly and recovery of antioxidant from microcapsule was done perfectly. From this recovery percentage, we can say that by adding guar-gum with sodium alginate gives better coating yield over sodium alginate as a coating material. But again it shows that recovery percentage was increased for the sonication method with an increasing percentage of the coating material. The antioxidant leaches out quickly from lower percentage coated microcapsule. But by observing previous values, it's proven that still, the mixture of two component as coating material took more time for broken up, as a result, degradation of antioxidant is lower than previous.



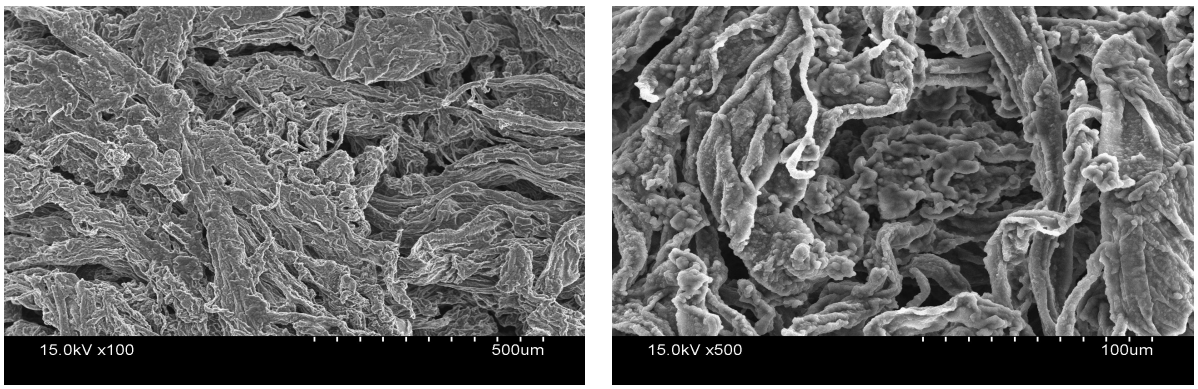
(a) (b)

Figure 4.8: SEM image of(a) DDGS microcapsule with 0.5% G.G and 1% sodium-alginate coating, (b) Internal structure of DDGS microcapsule with 0.5% G.G and 1% sodium-alginate wall material.



(a) (b)

Figure 4.9: SEM image of(a) DDGS microcapsule with 0.5% G.G and 2% sodium-alginate coating, (b) Internal structure of DDGS microcapsule with 0.5% G.G and 2% sodium-alginate wall material.



(a) (b)

Figure 4.10: SEM image of(a) DDGS microcapsule with 0.5% G.G and 3% sodium-alginate coating, (b) Internal structure of DDGS microcapsule with 0.5% G.G and 3% sodium-alginate wall material.

Fig. 4.8(a) and 4.8(b) represent the internal SEM structures of DDGS microcapsule composed of 0.5% guar-gum and 1%sodium alginate as coat material. Figures 4.9(a) and 4.9(b) represent the internal SEM structures of DDGS microcapsule composed of 0.5% guar-gum and 2%sodium alginate as coat material. Figures 4.10(a) and 4.10(b) represent the internal SEM structures of DDGS microcapsule composed of 0.5% guar-gum and 3%sodium alginate as coat material. The SEM images showed that most of the microcapsules were in cluster form. There is no void space inside the capsule; this characteristic of microcapsules formed due to freezing drying method.

4.8 Effects of Heat on DDGS Microcapsules

4.8.1 Effects of heat treatment for 30 minutes

Total phenolic content of the 0.5%guar-gum and sodium-alginate(1%,2% and 3%) coated microcapsules after heat treatment at 80°C for 30 minutes was determined from a linear gallic acid standard curve (y = 3.744x, R^2 =0.976). Fig. 4.11(a) shows the absorbance amount for total phenolic content. The absorbance of DDGS extracts for TPC was 1.913 nm. The total flavonoid content of the sodium-alginate and 0.5% guar-gum coated microcapsule was evaluated by using the aluminum colorimetric assay. The total flavonoid content was determined from the quercetin standard curve (y =0.0006x, R^2 =0.9834).

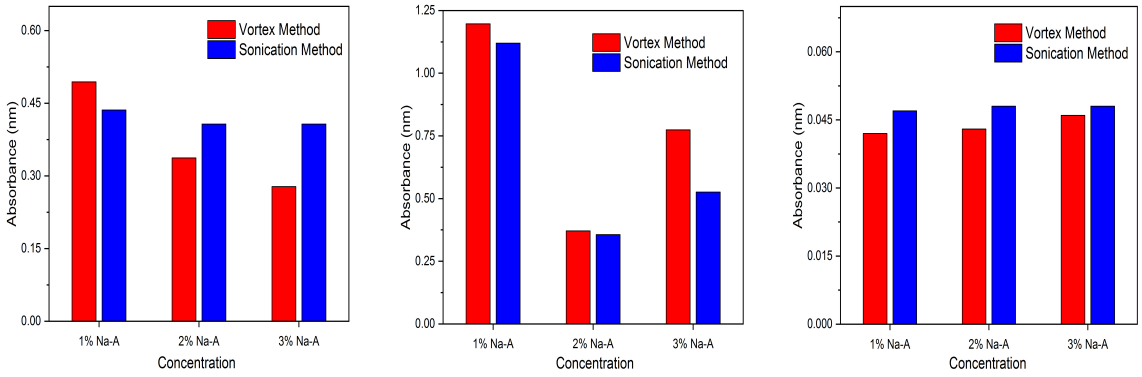


Figure 4.11: (a) Absorbance of TPC, (b) Absorbance of TFC (c) Absorbance of DPPH for different sample preparation method from sodium alginate and Guar gum coated microcapsule for 30 minutes heat treatment.

Table 4.3: Percentage of Antioxidant after 30 minutes of heat treatment in guar-gum & Sodium-alginate coated DDGS microcapsule and in DDGS extract (without heat treatment)

Sample Name	**Methods of sample preparation**	**Total Phenolic Content mgGAE/5ml Extract**	**Total Flavonoid Content mgQE/5ml Extract**	**DPPH % inhibition**
1% Na-A + 0.5% G.G	Vortex	2.916	22.48	40.84
1% Na-A + 0.5% G.G	Sonication	2.573	21.02	33.8
2% Na-A + 0.5% G.G	Vortex	2.42	14.26	39.43
2% Na-A + 0.5% G.G	Sonication	2.036	13.52	32.39
3% Na-A + 0.5% G.G	Vortex	2.35	28.53	35.21
3% Na-A + 0.5% G.G	Sonication	1.62	19.57	32.39
Extract of DDGS	-	10.56	63.811	67.6

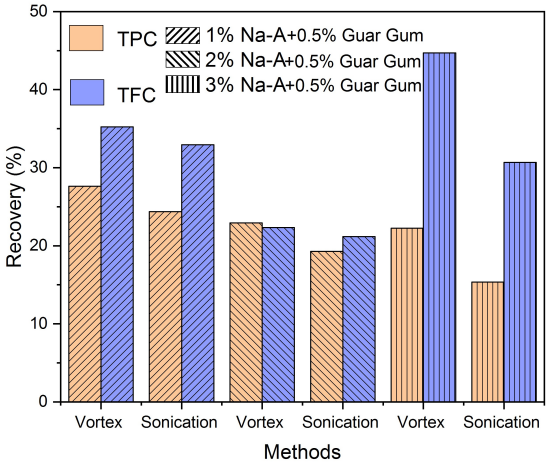


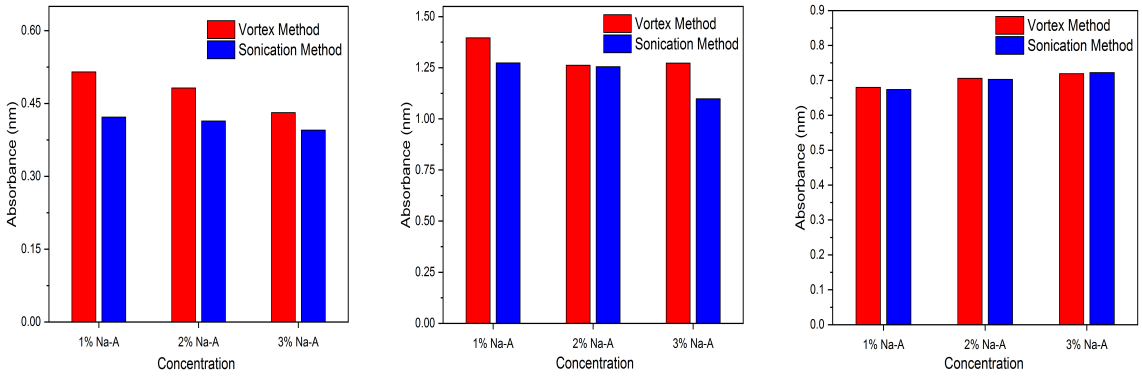
Figure 4.12: Recovery percentage of TPC and TFC in Guar-gum & sodium-alginate coated DDGS microcapsules with 30 minutes of heat treatment.

Fig. 4.11(b) shows the absorbance amount for total flavonoid content. The absorbance of DDGS extracts for TFC was 1.817 nm. Antioxidant activity was determined by DPPH assay, and for this analysis absorbance of the control and DDGS extracts were 0.071 nm and 0.023 nm respectively. Fig. 4.11(c) shows the absorbance amount for DPPH assay. From Table 4.4, we can say that the vortex method gives a good yield over the sonication method. For every sample vortex method gives good yield on total phenolic content, total flavonoid content and as well as on percentage inhibition. As we know antioxidant is heat sensitive and its start degradation in the presence of heat. Here, after heat treatment already some antioxidant part is already degraded, again when we try to break the coating material of microcapsule we used vortex and sonication method. During the sonication method due to sound vibration again some heat was produced, and due to this reason again some degradation of antioxidant happened during the extraction of antioxidant from microcapsule. So TPC, TFC both values were in lower for sonication method than the value obtained by the vortex method. Percentage inhibition was also low for the sonication method.

4.8.2 Effects of heat treatment for 60 minutes

Total phenolic content of the 0.5%guar-gum and sodium-alginate(1%,2% and 3%) coated microcapsules after heat treatment at 80°C for 60 minutes was determined from a linear gallic acid standard curve (y = 3.730x, R^2 =0.991). Fig. 4.13(a) shows the absorbance amount for total phenolic content. The absorbance of DDGS extracts for TPC was 1.321 nm. The total flavonoid content was determined from the quercetin standard curve (y =0.0007x, R^2 =0.9752). Fig. 4.13(b) shows the absorbance amount for total flavonoid content. The absorbance of DDGS extracts for TFC was 1.679 nm. Antioxidant activity was determined by DPPH assay, and for this analysis absorbance of the control and DDGS extracts were 0.822 nm and 0.297 nm respectively. Fig. 4.13(c) shows the absorbance amount for DPPH assay.

From Table 4.4, we can say that after heat treatment (at 80°C for 60 minutes) TPC and TFC values were increased than previous heat treatment. This result shows the typical



(a) (b) (c)

Figure 4.13: (a) Absorbance of TPC, (b) Absorbance of TFC (c) Absorbance of DPPH for different sample preparation method from sodium alginate and Guar gum coated microcapsule for 60 minutes heat treatment.

Table 4.4: Percentage of Antioxidant after 60 minutes of heat treatment in guar-gum & Sodium-alginate coated DDGS microcapsule and in DDGS extract (without heat treatment)

Sample Name	**Methods of sample preparation**	**Total Phenolic Content mgGAE/5ml Extract**	**Total Flavonoid Content mgQE/5ml Extract**	**DPPH % inhibition**
1% Na-A + 0.5% G.G	Vortex	3.241	45.9	17.27
1% Na-A + 0.5% G.G	Sonication	2.603	42.39	18
2% Na-A + 0.5% G.G	Vortex	3.886	55.38	14.11
2% Na-A + 0.5% G.G	Sonication	3.408	55.08	14.47
3% Na-A + 0.5% G.G	Vortex	4.255	49.9	12.53
3% Na-A + 0.5% G.G	Sonication	3.066	45.44	12.16
Extract of DDGS	-	8.601	58.25	63.86

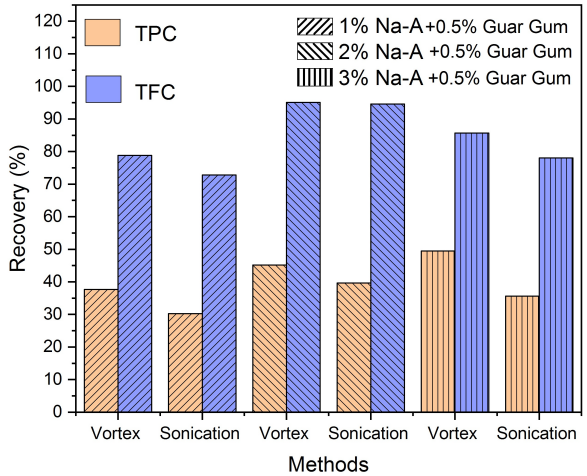


Figure 4.14: Recovery percentage of TPC and TFC in Guar-gum & sodium-alginate coated DDGS microcapsules with 60 minutes of heat treatment.

nature of antioxidant, which was extracted from DDGS. It proves that after certain time heat treatment can increase the TPC and TFC values of the microcapsule. But the recovery percentage of those values was not at a satisfactory level. Therefore further analysis neede. In this study again vortex method gives a good yield over the sonication method. The same phenomenon happens due to the production of heat in this method.

4.8.3 Effects of heat treatment for 90 minutes

Total phenolic content of the 0.5%guar-gum and sodium-alginate(1%,2% and 3%) coated microcapsules after heat treatment at 80°C for 90 minutes was determined from a linear gallic acid standard curve (y = 3.015x, R^2 =0.976). Fig. 4.15(a) shows the absorbance amount for total phenolic content. The absorbance of DDGS extracts for TPC was 1.119 nm. The total flavonoid content of was determined from the quercetin standard curve (y =0.0007x, R^2 =0.9887). Fig. 4.15(b) shows the absorbance amount for total flavonoid content. The absorbance of DDGS extracts for TFC was 1.592 nm. Antioxidant activity was determined by DPPH assay, and for this analysis absorbance of the control and DDGS extracts were 0.293 nm and 0.107 nm respectively. Fig. 4.15(c) shows the absorbance amount for DPPH assay.

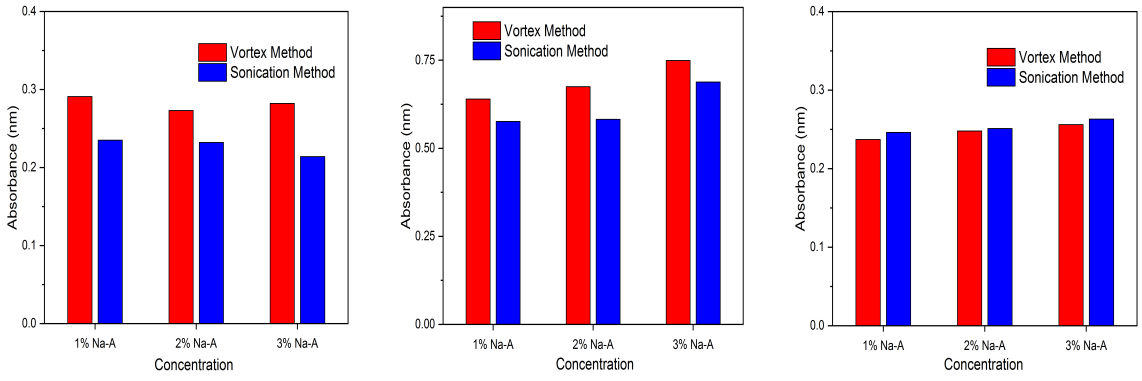


Figure 4.15: (a) Absorbance of TPC, (b) Absorbance of TFC (c) Absorbance of DPPH for different sample preparation method from sodium alginate and Guar gum coated microcapsule for 90 minutes heat treatment.

Table 4.5: Percentage of Antioxidant after 90 minutes of heat treatment in guar-gum & Sodium-alginate coated DDGS microcapsule and in DDGS extract (without heat treatment)

Sample Name	**Methods of sample preparation**	**Total Phenolic Content mgGAE/5ml Extract**	**Total Flavonoid Content mgQE/5ml Extract**	**DPPH % inhibition**
1% Na-A + 0.5% G.G	Vortex	2.258	21.411	19.11
1% Na-A + 0.5% G.G	Sonication	1.803	19.25	16.04
2% Na-A + 0.5% G.G	Vortex	2.152	22.93	15.35
2% Na-A + 0.5% G.G	Sonication	1.74	19.03	14.33
3% Na-A + 0.5% G.G	Vortex	2.33	26.7	12.62
3% Na-A + 0.5% G.G	Sonication	1.6	22.28	10.23
Extract of DDGS	-	9.011	55.23	63.48

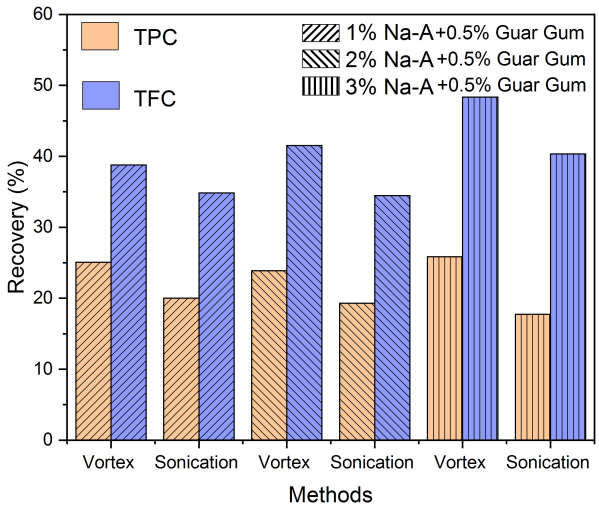


Figure 4.16: Recovery percentage of TPC and TFC in Guar-gum & sodium-alginate coated DDGS microcapsules with 90 minutes of heat treatment.

4.9 Conclusion

The study reviled the ability of DDGS microcapsules comprised of different percentage of sodium alginate and a mixture of 0.5% guar-gum and different percentage of sodium alginate to carry antioxidant after freeze drying. The distinctive of the microcapsules were affected by various type of coating materials. The functional extracts of the sample comprised of 0.5% guar-gum with 3% sodium alginate resulted in higher recovery of Totale phenolic content, flavonoid content, and excellent antioxidant activity. During heat treatment initially TPC, TFC and percentage inhibition of microcapsule was decreased but after that it the amount of all was increased as the time was increased. After 90minutes of

heat treatment, TPC, TFC, and antioxidant activity were low. This result indicates that in the presence of heat after a certain period, the activity of antioxidant starts decaying. So temperature control during encapsulation is a very crucial factor. During the SEM investigation, it is shown that all emulsions were homogenous and cloudy. So, based on all results, it can be concluded that combination of guar-gum with sodium alginate are suitable coat material for the production of DDGS microcapsules with excellent antioxidant abilities that could thereupon lead to the development of food products with increased and improved functional properties.

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Chapter 5

Application of Encapsulated Functional Components of DDGS

5.1 Introduction

Production of oils that meet the world commonplace of consumption of the cosmetics and specialty food industries may be a multi-step method that involves the acquisition of raw materials, extraction, refining, and packaging. Among the various oils, palm oil is one of the most consumed oil worldwide. It is important for both human and animal nutrition. Palm oil plays a major role in most of the food industries [Shahidi and Wanasundara, 2008]. The major component of palm oil is triacylglycerols. Among these triacylglycerols, 40.4 to 56.9% were saturated fatty acid and 43.0 to 62.5% unsaturated fatty acids. Carotenoids were a most important constituent found in palm oil in vast quantity. Palm oil is unable to remain stable as triacylglycerols are present in which can quickly oxidize in the presence of air, oxygen, and in high temperature to form lipid oxidation products [Baharin et al., 2001]. The lipid oxidation is responsible for rancid flavor and odor within the oils which minimize the nutritional quality of oils. Oxidation can be classified into auto-oxidation, enzymatic oxidation, photo-oxidation, and ketonic oxidation [Rossell, 2001]. Auto-oxidation is natural decomposition during storage of edible oils. The first component formed during the oxidation process is peroxides, so

they are called primary oxidation products. Several factors affect the auto-oxidation reaction like unsaturation, presence of elements, temperature, light, moisture, heavy metals, and antioxidant [Young and Woodside, 2001]. Antioxidants are a substance that once introduced into a substrate at low concentration considerably inhibits oxidation of the substrate. Antioxidants are two types such as primary or chain breaking type and secondary or preventive type. The primary type is further classified into two parts natural antioxidants (tocopherol, rosemary, carotene, etc) and synthetic antioxidants (BHA, BNB, TBH, TBHQ, etc). But nowadays natural antioxidants are widely used because of its harmless nature towards human bodies [Singh et al., 2007]. Many studies have established the effectiveness of natural antioxidants in the protection of the oils from lipid oxidation [Taghvaei and Jafari, 2015]. Extract of various essential oils from plants and the functional components which are derived from plants sources are commonly used as natural antioxidants for stabilizing the edible oils. Many studies have been proved that some functional component which is derived from the plant could be considered as proper alternatives to synthetic antioxidants [Taghvaei et al., 2014]. But antioxidants are also heated sensitive; they can easily degrader in the presence of heat. Microencapsulation is the process by which we can overcome the degradation problem and entrap the antioxidant in micro capsule by stabilizing it. After encapsulation, we should apply it on oils and food products to minimize the lipid oxidation, and improve the quality of oils and foods [Kanakdande et al., 2007].

5.2 Objective of the work

Antioxidants are those components which prevent auto-oxidation of oils by donating their hydrogen to free radicals. These free radicals are formed in the propagation stage of auto-oxidation. To prevent this unwanted chemical reaction in oils we have to add antioxidant in it. As antioxidant has a bitter taste, so it can fell effects on oil's taste. To prevent this drawback, we encapsulate the DDGS extract. 0.5% guar-gum with 3% sodium alginate was used as coating material of microcapsule. The study aimed to evaluate the effect of DDGS (extracted functional components) encapsulated microcapsule on palm oil. Evalu-

ation of storage stability was also checked by assessing the acid value and peroxide value of palm oil. Acid value and peroxide value helps to find out if there was any rancidity that occurs in oil during storage time. The application of DDGS microcapsule may be lead to minimize the use of synthetic antioxidants in edible oils, because of their potential adverse health effects on human bodies.

5.3 Methodology

5.3.1 Chemicals and Reagents

Glacial acetic acid, chloroform, saturated potassium iodide solution,0.1N sodium thiosulphate solution, neutral alcohol, 0.5% phenolphthalein, N/10 potassium hydroxide solution(KOH), N/10 oxalic acid solution, 1% starch, palm oil, and distilled water.

5.3.2 Microcapsule Preparation

0.5% guar-gum (G.G) was added with 1%,2% and 3% sodium alginate(Na-A) at a ratio of 1:1(v/v) to produced three different coating material. Extracted antioxidant from normal DDGS was mixed with polyethylene glycol (PEG) at a ratio of 1:2(v/v). Then the mixture of antioxidant and PEG was mixed with coating material at a ratio of 1:2(v/v). Then the mixture was spread on a calcium chloride solution. Then the solution was filtered after 2 hours. After that freeze-drying process was performed to dry up the solid for 12hours. Then they were frozen at -18°C.

5.3.3 Sample Preparation

5mg 0.5% guar-gum with 3% sodium alginate coated DDGS microcapsule was mixed with 50ml of palm oil. In another container 50ml normal palm oil was kept without mixing any microcapsule. Both samples were kept in an incubator at 50°C for 15days in open condition. Remaining palm oil was kept in normal room temperature.

5.3.4 Determination of Acid Value

The acid value of the oils was determined using a titration method according to AOAC Official Method with slight modification. 5gm of the sample was taken in a conical flask. Then 50ml of neutral alcohol was added to it. The mixture of oil and neutral alcohol were heated up at 65°C in a hot water bath for 15minutes. Then 0.5% phenolphthalein was added to the hot solution, and the solution was titrated against 0.1N KOH until pink color observed. The acid value expressed as milligrams of potassium hydroxide, which is required to neutralize the acidic constituent in 1gm of oil [Bai et al., 2017]. The test was again repeated after 15days. The following equation determined the acid value.

$$AcidValue(mgKOH/g) = \frac{(TitreValue \times NormalityofKOH \times 56.1)}{WeightofSample} \tag{5.1}$$

5.3.5 Determination of Peroxide number

The peroxide number of the oils was determined using a titration method according to AOAC Official Method 965.33 with slight modification. 5gm of the sample was taken in a conical flask. Then a mixture of glacial acetic acid and chloroform (3:2;v/v) was added to it and shacked it very gently. Then saturated potassium iodide solution (1 mL) was added, followed by 1 min shaking and adding 100mL distilled water to the mixture. The mixture was then slowly titrated using 0.1 N sodium thiosulphate solutions untill the supernatant aqueous phase shows a slight yellow color.Then 1% starch solution(1ml) was added to it, and very gradually continue the titration, until the blue iodine starch color has been completely removed [Bai et al., 2017]. The test was repeated after 15days. The PV expressed as mill equivalents of oxygen kg-1 oil were calculated according to the following equation

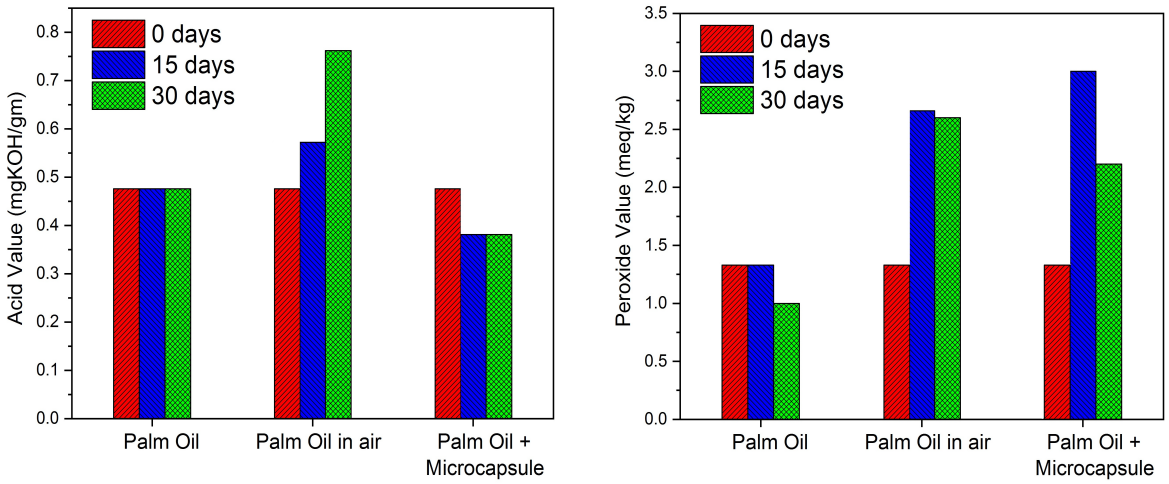
$$POV[meq/kg] = \frac{(V1 - V0) \times C \times 1000 \times T}{m} \tag{5.2}$$

[Where, POV= peroxide value, V1= consumption of 0.1mol/l sodium thiosulphate in

the main test, V0= consumption of 0.1mol/l sodium thiosulphate in the blank test, the c= molar concentration of the sodium thiosulphate solution, T= titer of the thiosulphate solution,m= weighed portion of substance in grams.]

5.4 Results and Discussion

From Fig. 5.1(a), we can understand that DDGS encapsulated microcapsule can control the acid value of palm oil. The initial acid value for normal palm oil was 0.476 mg KOH/gm for Zero-day. This value was remaining unchanged for next 15 and 30days. That indicates there were no traces of hydrolytic splitting of triglycerides during normal storage condition. But the acid values were increased for palm oil which was kept in presence of air. The acid values were 0.572 mg KOH/gm and 0.762 mg KOH/gm for 15 and 30 days respectively. These values were indicate that amount of free fatty acids were increased as hydrolytic splitting of triglycerides occur. But in presence of microcapsule, the acid value of was decreased for palm oil. The acid value was 0.381 mg KOH/gm for both time intervals. This value was lower than the initial acid value of palm oil.



(a) (b)

Figure 5.1: Determination of (a) Acid Value, (b) Peroxide Value.

From Fig. 5.1(b) we can evaluated that the microcapsule has no effects to control the peroxide value of palm oil. The initial peroxide value of normal palm oil was 1.33meq/kg. in presence of microcapsule which was increased upto 3meq/kg for 15 days and 2.2meq/kg

for 30 days. These values were does not met upto satisfactory level and cannot summarize the effect of microcapsule on palm oil.

5.5 Conclusion

In this chapter, the DDGS extracts were encapsulated using combination of 0.5%G.G and 3% Na-A using freeze drying method. The microcapsule are slightly capable to control the hydrolytic splitting of triglyceride in palm oil, which means there was less chance to find free fatty acids into the palm oil in presence of DDGS encapsulated microcapsule. But in case of maintain peroxide value, the microcapsules were not capable. More research work will be needed for evaluating the effects of microcapsules on stability of palm oil. In this experiment may be oil reacts with other component of micro capsule, and as a result value of POV was increased. To understand the actual phenomena more study will be required on this experiment.

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Summary

In this work, it has been summarized that DDGS is a good source of polyphenolic components. The amount of antioxidant present in it is very high. By isolating this component from DDGS, we can use it as a good source of natural antioxidant to minimize the oxidative damage in various food products. For isolating antioxidant from DDGS ethanol shows good potential on it. The extraction efficiency was higher in the presence of ethanol during extraction. Besides, of solvent selection, other parameters were also optimized for getting the best extraction yield from DDGS. As the antioxidant has a bitter taste, and it is also heat sensitive, we cannot use it directly into the food products. To overcome this drawbacks new technique should be adopted to stabilize the antioxidant. Microencapsulation process was used to stabilize the antioxidant in it. Freeze drying method was introduced to produce the microcapsules. Guar-gum and sodium alginate were used for the coating material, and a combination of 0.5% guar-gum and 3%sodium alginate coating provide best recovery yield over other coating material. This coating material also protects the antioxidant up to a certain period and temperature. After reaching that level, it was unable to preserve the antioxidant. As a result, degradation of antioxidant takes places. A combination of 0.5% guar-gum and 3%sodium alginate coating microcapsule provide good recovery yield, so we incorporate it in palm oil, to evaluate its effect on it. After assessing the acid value and peroxide value of palm oil in the presence of microcapsule, we can see that it can control the formation of free fatty acid, but there is no effect on peroxide value of palm oil.

By evaluating all the results, it sounds that further research works are needed to improve the microencapsulation technique for application in food product formulations. Selection of coating material should be improved for stabilizing the antioxidant in it. Spray drying method can be used in the future for formulating hollow and spherical microcapsule particle. Hence, the application of these microparticles on different food products is also examined by using different parameters.