EFFECT OF ULTRASONICATION ON PROPERTIES OF COMPOSITE BIODEGRADABLE CUP FOR FOOD PACKAGING

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Submitted to the Guru Angad Dev Veterinary and Animal Sciences University
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in

DAIRY ENGINEERING

(Minor Subject: Dairy Technology)

By

Ankit Kumar Deshmukh
(L-2019-D-02-M)

Department of Dairy Engineering
College of Dairy Science and Technology
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CERTIFICATE – I

This is to certify that the thesis entitled, **“EFFECT OF ULTRASONICATION ON PROPERTIES OF COMPOSITE BIODEGRADABLE CUP FOR FOOD PACKAGING”** submitted for the degree of **M. Tech.**, in the subject of **Dairy Engineering** (Minor Subject: **Dairy Technology**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Mr. Ankit Kumar Deshmukh** Registration No. **L-2019-D-02-M** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. Gopika Talwar)
Major Advisor
Assistant Professor
Department of Dairy Engineering
Guru Angad Dev Veterinary and Animal
Sciences University
Ludhiana – 141 004 (Punjab)

CERTIFICATE – II

This is to certify that the thesis entitled, **EFFECT OF ULTRASONICATION ON PROPERTIES OF COMPOSITE BIODEGRADABLE CUP FOR FOOD PACKAGING** submitted by **Mr. Ankit Kumar Deshmukh** Registration No. **L-2019-D-02-M**, to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in partial fulfilment of the requirements for the degree of **M. Tech.**, in the subject of **Dairy Engineering** (Minor Subject: **Dairy Technology**) has been approved by the Student’s Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

(Dr. Gopika Talwar)
Major Advisor

(Dr. Preetinder Kaur)
External Examiner
Principal Scientist
Department of Processing &
Food Engineering
PAU, Ludhiana

(Dr. Ramneek)
Head
Department of Dairy Engineering

(Dr. Sanjeev Kumar Uppal)
Dean, Postgraduate studies
Guru Angad Dev Veterinary and
Animal Sciences University
Ludhiana

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Date:-

(Ankit Kumar Deshmukh)

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Name of the Student and Admission No. : Ankit Kumar Deshmukh (L-2019-D-02-M)

Major Subject : Dairy Engineering

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Name and Designation of the Major Advisor : Dr. Gopika Talwar
Assistant Professor

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ABSTRACT

Food packaging is required for the protection of all types of food products. The petroleum plastic packaging material especially the single use cups and serving container, made out of polystyrene is generally utilized in dairy and food industries. It takes huge amount of time to degrade into soil and cause adverse environmental pollution. Many researches have made attempts on making of biodegradable film but very less is done on making stand-able container out of these biodegradable materials. To improve the mechanical barrier and other properties along with applicability of these biodegradable packaging materials in industries, there is need to improve the properties of these materials by new technological interventions. Ultrasonication is one such technology reviewed and reported for successful enhancement of various properties. The present study involves development of biodegradable cup utilizing the ultrasonication pre-treatment. Ultrasonication treatment with varied amplitude (20-60%) and time (1-16 min) was utilized for analysis of various properties of biodegradable cup made out of corn starch, whey protein concentrate, carboxy methyl cellulose and glycerol. Mechanical properties such as tensile strength (3.204 ± 0.287 kgf), puncture strength (1754 ± 150.58 gf) and density (0.1267 ± 0.02 g/mm^3), have increased, whereas other important properties such as water vapour permeability (0.76 ± 0.0031 $g/m^2/h$) and moisture absorption (8.96 ± 0.65 %) have been decreased for ultrasonication pre-treated sample as compared to that of non-ultrasonicated sample while development of biodegradable cup.

Keywords: biodegradability, corn starch, whey protein concentrate, biodegradable-cup, scanning electronic microscopy, tensile strength

Signature of Major Advisor	**Signature of the Student**

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LIST OF ABBREVIATIONS

°C : Degree celcius
AMP : Amplitude
ANNOVA : Analysis of Variance
AOAC : Association of Official Analytical Chemists
ASTM : American Society for Testing of Materials
AU/mm : Absorbance unit per milimeter
CMC : Carboxymethyl cellulose
cm^3 : Gram per cubic centimetre
cp : Centipoise
g : gram
Mbar : Millibar
mg : Milligram
min : Minutes
ml : Milliliter
mm : Millimeter
NaCl : Sodium Chloride
nm : Nanometer
Pas : Pascal second
RH : Relative humidity
sec : Seconds
SEM : Scanning electron microscope
WVTR : Water vapor transmission rate
WPC : Whey protein concentrate
wt% : Percent by weight
µm : Micrometer

CHAPTER I

INTRODUCTION

Food packaging is an essential subject in the field of food technology that concerns the preservation and protection of all sorts of foods and their raw materials, as well as the prevention of oxidation and microbiological decomposition. Petrochemical-based plastics, such as polyolefin, polyesters, and polyamides, have become increasingly popular as packaging materials due to their low cost and availability in large quantities, as well as favorable functionality characteristics such as good tensile and tear strength, good barrier properties to O_2 and aroma compounds, and heat sealability.

Food packaging is required for the protection for all types of food products. India accumulates 5.6 million tonnes of plastic waste every year, out of which 66% account for mixed waste-polybags and pouches used in food packaging (Anon, 2019). On the other hand, biodegradable materials have not only functional requirements but also have important environmental attributes. Biodegradability is not just a functional necessity, but also an essential ecological trait. Thus, the concept of biodegradability benefits from both user-friendly and eco-friendly characteristics, and raw materials are primarily derived from either agricultural feedstock or marine food processing industry wastes, and thus it capitalizes on natural resource conservation with a focus on environmentally friendly and safe environments (Taranathan, 2003). One of the additional advantages is that on biodegradation or disintegration and composting these may act as fertilizers and soil conditioner facilitating the better yield of crops (Kester & Fennema, 1986).

Since 50-60 years ago, scientists have been researching the production of polymers used in food packaging. The growing awareness of the need of environmental conservation and protection prompted the presentation of biodegradable polymers derived from renewable sources as a viable alternative to synthetic polymers for some industrial uses (Munoz et al., 2003). Synthetic polymers are long-lasting and resistant to biodegradation, and their residues contribute significantly to the volume of trash that causes environmental concerns since they are not biodegradable and are difficult to remove and recycle (Carvalho et al., 2007; Mali et al., 2002). Since 1970, it has been found in researches that biopolymer such as polysaccharide and protein are the new alternative utilized instead of synthetic polymers in the manufacture of plastic and plastic film to combat environmental concerns (Garcia et al., 2000). Biopolymers are polymers that occur spontaneously in living organisms. When compared to commodity plastics derived from fossil fuels, the usage of biopolymers will have a less negative impact on the environment (Krochta & De MulderJohnston, 1997). On the opposite, plastics have a very low transfer rate of water vapor and, more significantly, they are completely non-degradable, resulting in environmental contamination and major ecological concerns. As a result, its usage in whatever form or shape must be limited, and may even be progressively phased out, in order to avoid waste disposal issues (Tharanathan and & Saroja, 2001) Currently, there is a paradigm change caused by rising environmental consciousness among everybody to seek out biodegradable and therefore environmentally friendly packaging films and techniques are taken into consideration

Among all of the degradable polymers, corn starch and whey protein concentrate have aroused rising interest for their film-forming properties and prominent biocompatibility (Kuorwel et al., 2011). Various researches have been done for formulation of different biodegradable films using pure starch but the film produced are brittle and hard to handle (Ghanbarzadeh et al., 2011) therefore, corn starch being widely utilized in the production of edible/biodegradable films. Whey protein concentrate has also been utilized in edible films because it is flexible and has high moisture barrier qualities. Carboxy methyl cellulose creates edible films with a clear appearance and strengthens the structure, while glycerol gives the film elasticity. Due to the distinct qualities of each constituent, these were used to design a composite material that could give all of the required attributes to the produced packaging material.

Though, various researches have been made to develop composite biodegrable films and material out of organic materials, but the applicability of these films and biodegrable materials in the food industry as packaging material is very less as they lack in mechanical and barrier properties in comparison to petroleum packaging materials. So, there is necessity of improvement mechanical and barrier properties of these biodegradable and their derived products.

Ultrasonication is one of the innovative strategies which can be used as pretreatment for development of films due to its ability to enhance mechanical properties. Ultrasonication has been reviewed to decrease the water vapour permeability and oxygen permeability. The ultrasonic treatment of starch has been firstly performed in early 1933 (Kardos & Luche, 2001). Since then, many researchers have shown that ultrasonication has a great effect on the properties of gelatinized starch dispersions. After treatment by ultrasound, the starch dispersion showed a decrease in viscosity, and an increase in solubility and clarity due to the increase in numbers of free mobile macromolecules, rather than breakage of starch molecules (Zuo et al., 2009). Studies suggest that ultrasound treatment results in homogeneous solution (Liu et al., 2007). Also, when the whey protein based biodegradable film is prepared with ultrasonication process, reduced water vapor permeability, higher tensile strength has been achieved (Cruz-Diaz et al., 2019). Present study focuses on the behavior of ultrasound treated corn starch dispersion and properties of the cup formed from those dispersions

Though lot of work has been done on ultrasonication of solution for forming biodegradable films but no research review was found related to the effect of ultrasonication on engineering and other properties of a biodegradable cup. To overcome the research gap, the study was planned with following objectives:

1. Process optimization for development of composite biodegradable cup using Ultrasonication
2. To study the properties of developed composite biodegradable cup under objective 1

CHAPTER II

REVIEW OF LITERATURE

This chapter deals with the studies and their conclusion represented by various researcher focusing in topic of our interest that is development of biodegradable films with organic biodegradable material and effect of ultrasonication on the properties of developed composite materials.

2.1 Biodegradable polymers

Naturally produced biodegradable polymers are produced from four major feedstock categories (Tharanathan, 2003). Collagen and gelatin are derived from animal sources, whereas chitin, which is processed into chitosan, is derived from marine sources. However, the other two feedstock categories are garnering the greatest attention from experts and are regarded to be the most promising for future development and expansion. Polylactic acid (PLA) and poly hydroxyl alkenoates can be produced using microbial biopolymer feedstocks (PHA). The last type of agricultural feedstock under investigation at the University of Saskatchewan in Saskatoon, Canada, is biopolymer. This group of polymers is divided into two categories: hydrocolloids and lipids and fats. For many decades, cellulose-based polymers were essential in various of applications, including clothing, food (e.g., sausages), and non-plastics (e.g., varnishes). Meanwhile, these organic polymers have lost substantial market share, mostly to polyolefin. Since the 1980s, a growing number of starch polymers were introduced.

Starch polymers are presently among the most important categories of commercially accessible bio-based products. At first, basic products like pure thermoplastic starch and starch/polyolefin mixes were launched. Because of the inadequate biodegradability of starch/polyolefin mixes, these products had a detrimental influence on the market for biodegradable polymers, which resulted in the creation of copolymers composed of thermoplastic starch and biodegradable petroleum copolymers (Patel et al., 2003).

Significant progress has been achieved in the industrial scale manufacturing of different kinds of biobased polymers based on biotechnology, therefore contributing to the creation of a sustainable bio-based economy. Another technical driver is the advancement of nanotechnology, which opens up new opportunities for bio-based polymers. It is critical to characterize the biodegradability of organic and/or biodegradable polymers.

According to the International Standards Organization (ISO 2005, 2007) and American Society for Testing of Materials (ASTM 1998) the degradable plastics are those that undergo a major change in chemical structure under certain environmental circumstances. These alterations result in a loss of physical and mechanical characteristics as evaluated by conventional techniques. Biobased polymers can be, but are not always, biodegradable polymers. For example, starch polymers are typically biodegradable, but crystalline polylactic acid (PLA) is practically non-biodegradable.

2.1.1 Classification of Biodegradable

I. Biodegradable polymers, according to their source, may be divided into four major groups (Guilbert, 2000; Vilpoux & Avérous, 2004). Agropolymers extracted or separated directly from biomass (i.e., polysaccharides, proteins, polypeptides, polynucleotides). They are renewable and biodegradable polymers that may be treated directly, either plasticized, as fillers, or chemically changed.

II. Polymers derived from traditional chemical synthesis employing sustainable bio - based monomers or combined biomass-petroleum sources, such as bio polyester or PLA.

III. Polymers derived from genetically modified bacteria or microorganisms by fermentation of agricultural products utilised as substrate. curdian gum, Polyhydroxyalkanoates (PHA), pullan, xanthan gum, and bacterial cellulose are a few examples.

IV. Polymers derived traditionally from the petrochemical sector by chemical synthesis, like as polyesteramide (PEA) and polycaprolactone (PCL)

2.2 Composite materials

A materials system comprised of an appropriately organised mixture or combination of two or more elements with an interface separating them that differ in shape and chemical composition and are basically insoluble in each other is known as composite material. The engineering significance of a composite material is that two or more clearly different materials blend to produce a composite material with qualities that are superior, or significant in some other way, to the attributes of the separate components (Smith et al.,2000).

Accordingly, composites are materials created by aligning highly strong and stiff components such as fibers and particles in a binder known as matrix. The mechanical characteristics of the materials in this class are remarkable. One of the components that accommodate stress to integrate a component called reinforcing phase and generate a good binding, called matrix. A matrix material can be made of polymers, ceramics, or metals. The reinforcing phase is another component that is called reinforcement and might be fiber, particle, or laminar.

The qualities of the composite are determined by the attributes of the constituent components as well as their interface compatibility. Many studies have been conducted in order to determine the characteristics that control the mechanical behavior of particle composites. In general, it has been discovered that the reinforcing effect rises with decreasing particle size and increasing matrix adhesion (Marcovich et al., 1998).

2.2.1 Starch

Starch is made up of two primary types of molecules: linear amylose and highly branched amylopectin. Normal starch has around 25% amylose and 75% amylopectin, waxy starches include mostly amylopectin and 0–8% amylose, and high-amylose starches contain 40–70% amylose. A recently produced genetically engineered starch contains more than 90% amylose (Sidebottom et al., 1998). Starch is produced in granules within amyloplasts. Starch granules extracted from various plant sources and organs have varying forms and sizes. Starch granule diameters range from submicron’s to even more than 100 µm. wheat A granules have a diameter of 18 to 33 µm whereas B-granules have a diameter of 2 to 5 µm and Potato starch has a diameter of 15 to 75 µm, maize starch has a diameter of 5 to 20 m, rice starch has a diameter of 3 to 8 m, and amaranth starch has a diameter of 0.5 to 2 m. (Jane, 1994).

Starch is used as a constituent in the production of paper, textiles, adhesives, and meals. The relative quantity of amylose and amylopectin in a starch granule depends on the starch source, like as amyl maize (rich-amylose starch) or waxy maize (rich-amylopectin starch), and film characteristics are affected by the amount of each macromolecule present inside the starch granule (Tharanathan, 2003). There are several sources of starch that may be used to produce bioplastics, and each location can employ the most abundant starch source available locally. Corn (64%) is the most common starch source in worldwide production, followed by sweet potato (13%), and cassava (11%).

Starch, a plentiful and low-cost raw material, has been used in the field of biodegradable plastics, and blend films containing starch are promising materials in the agricultural, medical, and packaging sectors (Lu et al., 2005). Starch is the most significant polysaccharide polymer utilized in the development of biodegradable films because it can create a continuous matrix and is a renewable and plentiful resource (Romero-Bastida et al., 2005; Bertuzzi et al., 2007; Talja et al., 2008). Nonetheless, starch has numerous drawbacks, including a high hydrophilic nature (water sensitivity) and low mechanical characteristics when compared to typical synthetic polymers (Averous & Boquillon, 2004), making it unsuitable for various applications, which including packaging. Demirgoz et al. (2000) developed a film-based material by cross-linking maize starch and cellulose acetate. It was observed that the film had a lower water sorption capacity and a slower breakdown rate in aqueous medium when compared to other starch films.

Bertuzzi et al. (2007) developed the starch-based film and investigated its physicochemical properties. High amylose corn starch (HACS)-based films were produced at low temperatures thanks to a decrease in gelatinization temperature caused by an alkaline pre-treatment of starch. To discover HACS film elaboration conditions to improve film characteristics, the effects of film composition and preparation process on film properties were examined. Films were physically and chemically characterized by their water solubility, water sorption isotherms, opacity, and crystallinity. Film forming suspensions exhibit pseudoplastic and thixotropic behavior. The apparent viscosity of the film-forming gel rose exponentially with HACS content and Arrhenius law sufficiently with temperature change. The alkaline treatment period of HACS to gelatinization has an effect on the film characteristics. It was discovered that when it reached its asymptotic value after 60 minutes of treatment, film solubility and opacity reduced but crystallinity increased. An increase in plasticizer concentration beyond 30% glycerol results in a looser network, increasing film opacity and water sorption.

2.2.2 Whey protein concentrate

Whey protein concentrate has great nutritional and functional qualities and may be produced into edible films and coatings in both denatured and undenatured form, natural state (McHugh et al., 1994; Perez-Gago et al., 1999). Furthermore, these have performed better mechanical and barrier characteristics than competitor's proteins or polysaccharides that are equivalent to the finest synthetic polymer films on the market (Khwaldia et al., 2004). Whey proteins are more unique than other types of proteins. Biopolymers that create films when they pass through conformation denaturation, presence of electrostatic charges, as well as it is amphiphilic in nature

Javanmard et al., (2008) created edible films and coatings from whey protein, a byproduct of cheese production, which served as oxygen, fragrance, oil, and/or moisture barriers. They put the dried peanut kernels into whey protein concentration (WPC-35) – olive oil composite films and kept them at 25 degrees Celsius for four weeks. For 28 days, the moisture absorption, peroxide value (PV) and sensory characteristics of control (unpacked) and packed samples were measured. The greatest moisture absorption (5.40 0.14 percent) was detected in unpacked (control) peanut kernels, while packaging peanuts in WPC-35–olive oil sheets resulted in the lowest moisture absorption. They also discovered that dried peanut packed in WPC-35 films with glycerol: protein ratios (w/w) of 0.7 had superior crunchiness and overall acceptability than control and other treated samples in different film compositions. The PV in treated dry peanut kernels was decreased by WPC-35–oil bags. At intermediate (50%) relative humidity levels, WPC-35–lipid composite films might prevent the development of rancidity in dried peanuts. The addition of oil to WPC films had no influence on the PV of packed peanuts ($P > 0.05$). Weight losses in unpackaged peanuts were substantially larger (10.15 percent w/w) than in peanuts packed at a lesser level (1.05 percent w/w).

Bahram et al., (2014) created an edible whey protein concentration film activated with cinnamon essential oil. Cinnamon fundamental oil (CEO) was mixed into whey protein concentrate (WPC) at 0.8 and 1.5 percent v/v to create an active protein-based film. The effects of CEO on the microstructure, physical, mechanical, and antibacterial characteristics of the films were investigated. The addition of CEO reduced the permeability of water vapor in the films and the solubility in water of the WPC matrix by 38.03 and 29.4 percent, respectively. The films containing CEO also had a reduced affinity for water, with the contact angle increasing to 89.61 percent at 1.5 percent CEO concentration. CEO-containing films exhibited antibacterial efficacy against both gram-positive and gram-negative pathogens, as well as a strong inhibitory impact on fungi. CEO created a heterogeneous fractured structure using films that reduced their tensile strength. It was discovered that the film may be used for food packaging.

2.2.3 Carboxy-Methyl Cellulose

Carboxymethyl cellulose (CMC) is the most often used polysaccharide in film mixing as it is non-toxic and has a high viscosity but is also non-allergenic. CMC's many hydroxyls and carboxylic groups contribute to its excellent water-binding and moisture sorption capabilities (Siracusa, 2012). The different biopolymeric materials and cellulose derivatives are widely recognized for their excellent film formation and stability (Minami et al.,2006). Carboxymethylcellulose (CMC) is a cellulose derivative having carboxymethyl substituents (–CH2COOH) attached to a few of the cellulose's hydroxyl groups. CMC can be utilized as an effective addition to enhancing product quality and processing characteristics in a variety of sectors, including food, cosmetics, paper, and textiles [Schmidt et al. (2006)]. CMC addition improves gas barrier property and film strength for biodegradable film production, most likely due to the creation of the film network structure by protein contents and high polysaccharide and firmly connected chemical bonds, respectively (Wang et al., 2011). CMC is also used in edible film formulations owing to its excellent film-forming ability, biodegradability, and non-toxicity (Togrul et al., 2004). Because of its polymeric structure and large molecular weight, it is also utilized as a filler in the manufacture of bio composite films to improve mechanical and barrier characteristics (Almasi et al., 2010).

According to Biswal and Singh (2004), carboxymethyl cellulose (CMC) is mostly utilized in food processing due to its viscosity and water binding characteristics, as well as its soluble clarity. Anionic linear polysaccharides produced from cellulose, such as methylcellulose and carboxymethyl cellulose (CMC), are widely accessible, quickly processed, cheap, and have good film-making characteristics. These polymer-based films usually provide a strong gas barrier as well as moderate to good mechanical characteristics, but they are extremely susceptible to moisture.

2.2.4 Glycerol

Plasticizer agents must be added to increase the film's flexibility. Because of its durability and compatibility with hydrophilic bio-polymeric packing chains, glycerol is one of the most often utilized plasticizers in film-making processes (Cervera et al., 2004). A plasticizer is a substance that, when applied to another material and under particular conditions, changes the material's physical and mechanical characteristics. Plasticizer addition into a film result in a more flexible and stronger film that is less prone to break. The decrease of intermolecular interactions between polymer chains, and therefore overall cohesiveness, allows the film to elongate and lowers its glass transition temperature. This is reflected in a decrease in barrier characteristics to gases, vapors, and film solutes (Banker, 1996). Compatibility of plasticizing agent and the film forming polymer is must and it should have permeability within the solvent-polymer system and at the circumstances employed. To be compatible, it must be miscible with the polymer, which necessitates the employment of molecular processes of a comparable type.

The following plasticizers are often utilized in the field of edible coatings and films:

- monosaccharides, disaccharides, and oligosaccharides (generally glucose syrups or glucose fructose honey)

Polyols are a kind of polymer (principally glycerol and its derivatives, polyethylene glycols, sorbitol)

- lipids and derivatives of lipids (fatty acids, monoglycerides and their esters, acetoglycerides, phospholipids, and other emulsifiers). Plasticizing a hydrophilic polymer-based film is often accomplished by adding a chemical from one of the first two groups, and that of a wax- or fat-based film by adding a molecule from the third group.

Gutiérrez et al. (2015) created Bio-Matrixes for edible films using Corn Starch 80:20 “Waxy”:Regular, “Native,” and Phosphated. Starch films are colorless, tasteless, non-toxic, biodegradable, and cost effective, in addition to having a low oxygen permeability. The cross-linking modification (phosphatation) and strengthening the hydrogen connections inside the granules enhanced the physicochemical characteristics. The objectives of this work were to develop and describe starch corn films 80:20 “waxy”: regular, both native and modified (cross-linked), in order to identify their possible use. It was discovered that films containing modified starch had the highest hydrophilic characteristics, which enhanced their thickness, permeability, and solubility, as well as significant stability in acidic and alkaline media. Finally, the interaction of the starch-plasticizer resulted in the films' physicochemical characteristics and water vapor barrier capabilities.

Ghanbarjadeh et al. (2011) created a corn starch-based film to improve the barrier and mechanical characteristics of the film, as well as investigate the effects of citric acid and carboxymethyl cellulose. It was discovered that films made from pure starch are fragile and difficult to handle. Chemical changes (e.g., cross-linking) and the use of a second biopolymer with in starch-based composite were investigated as techniques for producing low water sensitivity and reasonably high strength starch-based materials. Casting was used to create corn starch films with various concentrations (0–20 percent, W/W) of citric acid (CA) and carboxymethyl cellulose (CMC). CA and CMC were studied for their impact on water vapour permeability (WVP), moisture absorption, solubility, and tensile characteristics. The water vapour barrier performance and ultimate tensile strength (UTS) improved considerably when the CA percentage rose from 0 to 10% (W/W). When CMC was added at a level of 15% (W/W), the starch films had the lowest WVP values (2.34 107 g Pa^{-1} h^{-1} m^{-1}) and UTS raised from 6.57 MPa for the film without CMC to 16.11 MPa for the film with 20% CMC.

Tongdeesoontorn (2011) showed that adding CMC to cassava starch films enhanced tensile strength while decreasing elongation at break. This was attributed to the effective interaction between cassava starch and CMC. Cassava starch-CMC composite films have the potential to replace traditional packaging, and the films created in this study are thought to be ideal for low moisture food and medicinal goods.

Laohakumjit and Noomhorm (2004) investigated the impact of plasticizers such as glycerol, sorbitol, and polyethylene glycerol 400 (PEG400) on the mechanical and barrier properties of rice starch film. According to the author, the film formed from sorbitol and glycerol plasticizer starch is homogeneous, smooth, transparent, and contains less insoluble particles than unplasticized film. PEG 400 did not create a sufficiently plasticized film, and when the concentrations of sorbitol and glycerol were raised, the softness and stickiness of the film increased.

Sukhija et al. (2016) created a biodegradable film of whey protein, psyllium husk, and lotus rhizome starch (3 percent, 4 percent and 5 percent starch concentration). They also tested a new biodegradable film made from a combination of, whey protein concentration, lotus rhizome starch and psyllium husk, which was shown to be superior than lotus rhizome starch alone. When compared to lotus rhizome starch film, biodegradable film had greater tensile strength, reduced water vapor permeability, and higher solubility.

Sonia, (2008) developed biodegradable composite cup with corn starch (5-7 %), whey protein concentrate-35 (1.5-3%), carboxy methyl cellulose (0.5-2%) and glycerol (2-4%) concentration range has been selected. Layering method have been selected for cup manufacturing. 4- 9 numbers of layers of slurry coating on silicon mould have been resulted in cup formation. Properties such as thickness, puncture strength, tensile strength, water vapor permeation rate, biodegradability, moisture and lightness were analyzed. The best properties have been found in cup formed with corn starch (7 %), whey protein concentrate-35 (3%), carboxy methyl cellulose (2%) and glycerol (4%) with 9 layers of coating over silicon mould.

2.3 Ultrasonication

Sonication is the technique of agitating particles in a sample by using sound energy (García-Vaquero et al., 2017) or Ultrasonication is defined as a portion of the sound spectrum between 20 kHz and 10 MHz produced by a transducer that transforms electrical or mechanical energy into high frequency acoustical energy (Wang & Cheng, 2009). Ultrasound generates sound waves with frequencies that beyond the range of human hearing (> 20 kHz). There are two types of ultrasounds: high-intensity, low-frequency ultrasound (20–100 kHz; 10–1000 W/cm^2) and low-intensity, high-frequency ultrasound (100 kHz–1 MHz; 10 W/cm^2) (Welti-

Chanes et al., 2017). During ultrasonication, energy is transmitted to samples by a process known as acoustic cavitation, which refers to the creation, growth, and rapid collapse of gas bubbles. As a result, heat (up to 5000 °C) and pressure (up to 20 MPa) may be created in a very short period of time (a few microseconds), resulting in the rupture of cell membranes, denaturation of enzymes, the creation of micro-channels, and the generation of free radicals (Pingret et al., 2013). Also the components of can be easily modified to new structure and functionality (Sullivan et al., 2018).

2.3.1 Mechanism of ultrasonication

When ultrasound is applied to liquid systems, it generates acoustic cavitation, which is the process of bubble creation, growth, and final collapse. The bubbles fluctuate and collapse as the ultrasound waves propagate, causing the thermal, mechanical, and chemical consequences. Mechanical effects such as collapse pressure, turbulence, and shear stresses are examples of mechanical effects (Yusaf & Al-Juboori, 2014). The cavitation zone's actions produce extraordinarily high temperatures (5,000 K) and pressures (1,000 atm) (Soria & Villamiel, 2010). Depending on the ultrasonic frequency, locally created alternating positive and negative pressures cause material expansion or compression, culminating in cell rupture. Ultrasound causes water hydrolysis inside oscillating bubbles, resulting in the generation of H+ and OH free radicals that can be captured in some chemical reactions, such as free radical scavenging by amino acids of enzymes involved in structure stability, substrate binding, or catalytic functions. This sonication disruption effect is strongly resisted by homogeneous liquids (Ercan & Soysal, 2011) Bubbles generated during sonication therapy are classified into two categories based on their structure:

(1) Stable cavitation bubbles are non-linear, generating enormous bubble clouds with equilibrium size during pressure cycles.

(2) Internal (transient) cavitation bubbles are non-stable bubbles that rapidly collapse and disintegrate into smaller bubbles.

These tiny bubbles disintegrate fast, yet during bubble stretching, the mass-transfer boundary layer is thinner and the interfacial area is larger than during bubble collapse, implying that more air enters the bubble during the stretching phase than seeps out during the collapse phase (Tiwari & Mason, 2012).

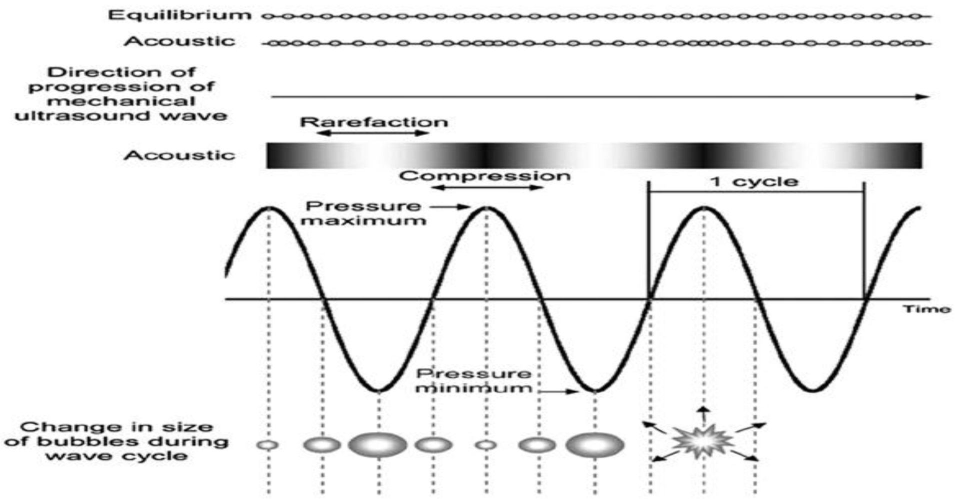


Fig. 2.1 Ultrasonication mechanism

(*Source: Soria & Villamiel, 2010*)

2.3.2 Effect of ultrasonication amplitude and time variation on physico-chemical mechanical and thermal properties of composite biodegradable films

Abral et al. (2019) utilized ultrasonication for enhancing the properties of sago starch-based film they reported that starch granules having amylopectin-rich fractions, such as sago starch, may stay insoluble and unaffected, therefore reducing the film's characteristics. During the gelatinization process, an ultrasonication probe was utilized for 2.5, 5, and 10 minutes. Ultrasonication reduces the amount of incomplete gelatinized granules in the film, resulting in a more compact structure and reduced moisture vapor permeability than the untreated film. The longest time produced the clearest and most thermally resistant film. Starch granules having amylopectin-rich fractions, such as sago starch, may stay insoluble and unaffected, therefore reducing the film's characteristics. During the gelatinization process, an ultrasonication probe was utilized for 2.5, 5, and 10 minutes. Ultrasonication reduces the amount of incomplete gelatinized granules in the film, resulting in a more compact structure and reduced moisture vapour permeability than the untreated film. The longest time produced the clearest and most thermally resistant film. When compared to non-sonicated film, the 5 minute period enhanced tensile strength by 227 percent and decreased moisture absorption by 29.83 percent. After 10 minutes of ultrasonication, the melting temperature rose by 7% compared to the non-sonicated film.

Liu et al. (2018) investigated the effect of ultrasonication on sweet potato starch and sweet potato starch-based film. Ultrasonication treatment of amplitude level 30, 40, 50, 60 and 70% is provided for 5 min to each suspension with 20 kHz ultrasonic processor. The sweet potato starch lauric acid composite films' microstructure, surface morphology, water vapor permeability, and mechanical characteristics were studied. They stated that the inclusion complex might be aided by low power density ultrasound. creation, and the high-power density ultrasound would annihilate amylose-lipid complex structure. Components used to create films after ultrasonic treatment, they became more compatible. Prior to filming, an ultrasonic treatment may improve the light transmission and TS, whereas ultrasonic therapy may decrease the composite films' water vapor permeability and E values. They found that the film with ultrasonic treatment of 40% amplitude level shown greatest tensile value and least water vapor permeability values. Also, they concluded that a low power density ultrasound of 40% was shown to be beneficial in both increasing amylose-lipid complex formation and enhancing the characteristics of the films.

Csiszar et al. (2016) studied the effects of sonication on the characteristics of both the cellulose nanocrystals and their aqueous suspensions on nanocrystalline cellulose (NCC) suspension for 0, 1, 2, 5, and 10 minutes. Furthermore, a variety of nanocellulose films were created and evaluated using sonicated suspensions. Laser diffraction analysis and transmission electron microscopy results demonstrated that sonication not only dissolved the large NCC aggregates (Dv50 14.7 lm) into individual nano whiskers with average length and breadth of 171± 57 and 17 ± 4 nm, respectively, but also deteriorated the nanocrystals and generated shorter and thinner particles (118 ± 45 and 13 ± 3 nm, respectively) after 10-min sonication. The optical haze of suspensions was reduced from 98.4 percent to 52.8 percent with increasing duration from 0 to 10 minutes using ultrasound-assisted disintegration to nano-sized cellulose whiskers. The sonication of the suspensions greatly aided in the production of films with minimal haze (high transparency) and good tensile characteristics. The haze reduced as the time of sonication increased, and the tensile strength steadily increased. Regardless of sonication, all films had an excellent oxygen transfer rate in the range of 5.5–6.9 cm3/m2 day.

Liu et al. (2021) studied ultrasonication effect on developed composite film using maize starch, steric acid and sodium carboxy methyl cellulose. The sample were labelled according to ultrasonic treatment as ST-LPD, ST-HPD, MT-LPD, MT-HPD, LT-LPD, and LT-HPD for samples treated with 5 minutes at 30%, 5 minutes at 70%, 15 minutes at 30%, 15 minutes at 70%, 30 minutes at 30%, and 30 minutes at 70%, respectively. They observed that the hydrophobic and hydrophilic components become more compatible during the ultrasonication process. An ultrasonic duration of 15 minutes at 70% amplitude resulted in improved tensile and moisture barrier characteristics for the polymer sheet. After ultrasonic treatment, the bio- composite film demonstrated outstanding characteristics such as increased light transmittance, a more uniform and smoother surface, acceptable tensile qualities, and moisture resistance. Although an LT-HPD treatment damages the polymer structure to some extent, using ultrasound with the appropriate action duration and amplitude can enhance the characteristics of polymer film.

Kadam et al. (2013) found in the study of preparation and characterization of whey protein isolate film reinforced with TiO2 coated with SiO2 nanoparticles, that the whey protein isolate films maintained their structural integrity when subjected to sonication processing, and the addition of TiO2@@SiO2 nanoparticles improves their mechanical characteristics. The storage modulus (E) rose as the amplitude of the sonication increased, however the inclusion of nanoparticles resulted in a decrease in E value, perhaps due to phase separation. The samples were treated with ultrasonication of amplitude level 0, 10, 50 and 100 %.

Cheng et al. (2010) studied the effect of ultrasonication on properties of maize starch-based film. The film forming dispersion were given 20 kHz ultrasonication treatment for 0, 2.5, 5, 10, 15 and 30 minutes. They reported that ultrasonic treatment could efficiently break swelling starch granules and ghosts in dispersions, increase solubilized amylose quantities, and improve polymer free mobility in starch dispersions, resulting in a significant drop in apparent viscosity and rise in solubility even in 10% starch dispersions. Even at high concentrations, the ultrasound-treated film-forming dispersions' behaviors promote film casting or spraying. Ultrasound-treated gelatinized maize starch dispersions can produce films with high transparency, better moisture resistance, and a stronger structure. The results also reveal that the resultant films with ultrasonic treatment have a lower strain at break, which can be addressed by adding certain plasticizers such as glycerol.

Wu et al. (2021) created cassava starch film by treating it with acetic acid and utilizing ultrasonication. The effects of ultrasonication on the morphology, microstructures, and characteristics of starch–acetic acid films were studied using different ultrasound power levels ranging from 200 W to 750 W. SEM reveals a coherent and compact structure of the ultrasonicated films. The crystalline index was lowered by acid treatment and enhanced by ultrasonication, according to X-ray diffraction research. The tensile strength and elongation at break of the films rose initially and subsequently declined as the ultrasonic power level increased. Ultrasonication also increased the opacity, water barrier effectiveness, and water adsorption of the films. Thus, our findings demonstrate that ultrasonication may be utilized to alter the morphology, microstructure, and performance of starch–acetic acid films to better satisfy application requirements.

Asrofi et al. (2018) studied the properties of tapioca starch-based plastic which is reinforced with 10% by volume fraction of water hyacinth fiber. While gelatinization ultrasonication treatment of 0, 15,30 and 60 min has been given to suspension. The findings of this investigation show that by exposing the gelatinizing mixture to ultrasonic vibration for 30 minutes, a bio composite with optimum characteristics may be created utilizing tapioca starch and WHF. Tensile strength (TS) and tensile modulus (TM) rose by 83 and 108 percent, respectively, after this vibration time. A subsequent 60-minute vibration only increased the TS by 13% and the TM by 23%. Moisture resistance of the bio composite rose by about 25% following vibration, reaching a maximum after 30 minutes. The vibrated bio composites' thermal resistance was also improved.

Kang et al. (2020) employed ultrasonication to enhance the complexation of amylose and fatty acids in various stages. The physicochemical characteristics and film-forming capabilities of amylose-lipid inclusion complexes were studied. They investigated that the capacity of liquid fatty acids to form complexes with amylose molecules was higher than that of solid fatty acids. XRD indicated the development of the V-amylose complex. The melting enthalpies (H) of starch-lipid inclusion complexes treated with ultrasound were greater than those of untreated complexes.

After ultrasonic treatment, the surface texture of the corn starch-fatty acid composite films became smooth. The ultrasonicated sample of films had reduced elongation at break and water vapor permeability, as well as higher tensile strength, than the untreated samples.

Cruz-Diaz et al. (2019) explored high power ultrasound and microbial transglutaminase addition, and their combination, to study protein structure and crosslinking modification in order to modify the characteristics of whey protein concentrate edible films. They were compared against untreated and heat-treated films (from heat-treated solutions). Some film characteristics were altered by ultrasound treatment. The water vapour permeability of ultrasound-treated films was somewhat lower than that of untreated and heat-treated films. In contrast to untreated samples, ultrasound treatment enhanced the tensile and puncture strengths of the films.

Gautam & Kumar (2016) developed biodegradable film using whey protein isolate, glycerol and titanium di oxide and studied various mechanical and physicochemical properties as function of ultrasonication amplitude 0,20,40,80 and 100% amplitude. They investigated that when the sonication level increased, the water vapour permeability, moisture content, moisture absorption, and water solubility reduced. Mechanical experiments indicated that nanoparticles had a plasticizing impact on the bio-film. Sonication of whey protein isolate films solution improved tensile strength and elongation at break considerably. It was discovered during the differential scanning calorimetry analysis that raising the sonication levels resulted in a reduction in the melting point of the film.

Ji et al. (2020) prepared composite films with Polyvinyl alcohol (PVA)/sodium carboxymethyl cellulose (CMC)/nano-ZnO/multilayer graphene nanoplatelet (xGnP) using ultrasonication-assisted solution for casting. The capabilities of these films (water vapour permeability (WVP), mechanical characteristics, biodegradability, and antibacterial activity) were studied as a function of the ZnO NPs: Ultrasonication time and xGnP mass ratio. They discovered that ultrasonication improved the mechanical properties of composite films and, in combination with traditional film preparation methods and at an ultrasonication time of 30 minutes, the pure PVA/CMC film exhibited a strain of 165.60 8.88 percent, which was 31.57 percent higher than that observed previously. At the same time, ultrasonic treatment enhances biodegradability considerably. In a nutshell, it was determined that it was well suited for the development of composite films with improved mechanical, antibacterial, biodegradability, and barrier characteristics.

CHAPTER III

MATERIALS AND METHODS

This chapter contains all of the experimental details used in the current study "**EFFECT OF ULTRASONICATION ON COMPOSITE BIODEGRABLE CUP FOR FOOD PACKAGING**," including preliminary trials to choose a range of ultrasonication time and amplitude. The technique for producing a biodegradable cup was followed. It also includes information on the various equipment and devices used throughout the research.

The current study is divided into three sections: selection of ultrasonication amplitude level and time duration for preparation of slurry used for development of biodegradable cup, measurement of various physico-chemical and engineering properties and comparing it with cup formed with non-ultrasonicated casting solution, to study the effect of ultrasonication on the properties of cup developed utilizing ultrasonication treatment.

3.1 Material required

3.1.1 Starch source

The need for natural or organic sources of food and packaging is growing day by day. Starch is well-known for its ability to create films to be used in food and packaging sectors. Starch is a type of polymeric carbohydrate made up of anhydro glucose units. Most starches contain two forms of glucose polymers: amylase, a linear chain molecule, and amylopectin, a branched polymer of glucose. Starch is a polymeric carbohydrate made up of number of anhydro glucose units. Because of its low cost, renewability, and strong mechanical qualities, starch has been widely used in industrial foods as well as in manufacturing biodegradable films to partially or totally replace plastic polymers. High amylose starch, such as corn starch, is a useful source for film formation. Free stand-able films can be made by soaking gelatinized amylose in water and drying it. Ordinary corn starch is composed of about 25% amylose and 75% amylopectin. At relative humidity less than 100 percent, the films formed by high amylase maize starch had essentially low oxygen permeability.

Corn starch: corn starch used in research study was purchased from central drug house (P) Ltd. Product code-030261, batch no.-060616

3.1.2 Whey protein concentrate source

Whey protein is nutritious and has film-forming properties. Whey protein has ability to generate a clear, soft, and elastic water-insoluble film with great aromatic substance barrier function, grease barrier property, and oxygen barrier property under low humidity. WPC-35 (Whey Protein Concentrate 35%) were purchased from Standard sports nutrition, Jaipur, Rajasthan

3.1.3 Plasticizer

The use of plasticizers overcomes the brittleness of starch films and improves their flexibility and extensibility. Due to the excessive interactions between the polymer molecules, the use of only food grade biopolymers in the development of edible/biodegradable films results in brittle and stiff properties. Polyols (glycerol, sorbitol, and poly (ethylene glycol)) are extensively utilized as plasticizers in the creation of hydrophilic films. Plasticizers must be compatible with film-forming polymers, lower intermolecular pressures, and promote polar polymer chain mobility. Glycerol increases film extensibility while decreasing elasticity and water vapor barrier characteristics, which leads to the use of glycerol in cup formation.

Glycerol-AR grade were purchased from Merck Specialties Pvt. Ltd., Mumbai India

3.1.4 Additive

Carboxymethyl cellulose (CMC) is the most commonly used polysaccharide in film mixing as it has a high viscosity and is non-toxic and also non-allergenic. Various hydroxyl and carboxylic groups present in carboxy methyl cellulose contribute to its excellent water binding and moisture sorption characteristics. Carboxymethyl cellulose (CMC) is non-toxic to humans and is utilized as a very effective additive to improve product quality and processing properties in a wide range of applications, including food, cosmetics, and medicines, as well as goods for the paper and textile industries. The use of CMC tends to boost the film strength and gas barrier properties. CMC (Generally recognized as safe material) was chosen as an addition because it forms a transparent solution. Carboxymethyl cellulose (CMC) is a film/cup additive that provides stable thermo-emulsions and is utilized as a reinforcement agent.

For research study to form biodegradable cup the carboxymethyl cellulose (CMC) - were purchased from Merck Specialties Pvt. Ltd., Mumbai India

3.2 Instruments

3.2.1 The instrument utilized for the development of biodegradable cup are as follow:

1. **Ultrasonication-** Ultrasonic vibration was provided with Cole-Parmer ultrasonic processor Model No. CPX750.

Fig. 3.1: Ultrasonicator

Table 3.1 Ultrasonicator specification

Ultrasonicator	
Make	Cole Parmer instruments, U.S.A.
Model	CPX 750
Frequency	20 kHz
Power	230V~AC 50/60Hz 6.3A Max
Probe model	CV 334
Probe serial number	2018100967

2. Hot plate with magnetic stirrer

Casting solution for preparation of biodegradable cup was heated and stirred using hot plate with magnetic stirrer

Fig. 3.2: Hot plate with magnetic stirrer

3. Hot air oven

Developed biodegradable cups was dried using hot air oven

Fig. 3.3: Hot air oven

3.2.2 The instrument utilized for the study of various properties are as follow:

1. **Viscometer**

   Viscosity of casting solution is measured by Brookfield viscometer

Fig. 3.4: Viscometer

Table 3.2 Viscometer specification

Viscometer	
Make	Brookfield Engineering Laboratories, U.S.A.
Item	DV2TLVTJ0
Serial number	8570384
Voltage	90-260 V~
Frequency	50/60 Hz
Power	150VA
Probe serial number	2018100967

2. Digital Micrometer

The Digital Micrometer is used for the measurement of thickness. mechanical properties (tensile and puncture strength).

Fig. 3.5: Mitutoyo Digital micrometer

Table 3.3 Mitutoyo Digital micrometer specifications

Digital micrometer	
Make	Mitutoyo
Model	293-831-30
Measuring range	0 to 25 mm/ 0” to 1”
Resolution	0.0001 mm/ 0.00005”
Instrument error (@ 20° C)	± 2µm (excluding quantizing error)
Accuracy	± 0.0001 in

3. Weighing Balance

Weight was measured using weighing balance for density, solubility, water vapour transmission rate and moisture absorption analysis.

Fig. 3.6: Weighing balance

Table 3.4 Weighing balance specifications

Weighing balance	
Make	Mettler Toledo
Model	JE1002GE/A04
Max. measure	1200 g
Power	12 V~ 0.84 A
Division	0.01g

4. Spectrophotometer

Spectrophotometer is used for opacity analysis

Fig. 3.7: Spectrophotometer

Table 3.5 Spectrophotometer specifications

Spectrophotometer	
Make	Analytikjena
Model	Specord210 plus
Optical design	1200 g
Wavelength reproducibility at 360.9 nm	≤0.02 nm
Wavelength range	185-1200 nm
Scan speed	Up to 12000 nm/min
Wavelength accuracy at 360.9 nm	±0.5 nm

5. Texture analyzer

Using texture analyzer the puncture strength and tensile force of developed biodegradable cups was measured.

Fig. 3.8: Texture Analyzer

Table 3.6 Texture Analyzer specifications

Texture analyzer	
Make	Stable Micro Systems, Surrey, UK
Model	TA-XT plus
Serial number	41068
Voltage	100v-240v
Power	120va

6. Hunter Colormeter

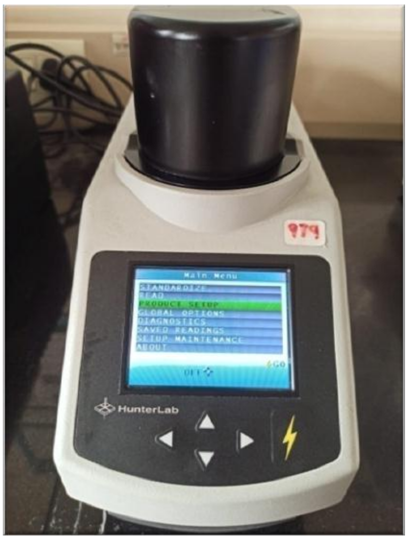


Fig. 3.9: Hunter colormeter

Table 3.7 Hunter colorimeter specifications

Spectrophotometer	
Make	Hunter Associates Laboratory Inc., U.S.A.
Model	ColorFlex EZ
Port Diameter/View Diameter	31.8 mm (1.25 in) illuminated/ 25.4 mm (1 in) measured
Spectral Resolution	< 3 nm
Reporting Interval	10 nm
Photometric Range	0 to 150 %
Light Source	Pulsed Xenon Lamp
Flashes per Measurement	1 flash
Measurement Time	< 1 second from button push to measurement 2 seconds from button push to data display
Minimum Interval Between Measurements	3 seconds

3.2.3 Morphological Analysis

Scanning Electron Microscope (SEM)

The morphological analysis for the developed biodegradable cup was done by Scanning Electron Microscope which was outsourced from Central Instrumentation facility, Lovely Professional University, Phagwara, Punjab.

Fig. 3.10: Scanning electron microscope

Table 3.8 Scanning electron microscope specifications

SEI resolution	1.0nm (15kV), 1.3nm (1kV), During analysis 3.0 nm (15 kV、 probe current 5 nA)
Magnification	25 to 1,000,000
Accelerating voltage	0.1kV to 30kV
Detectors	Secondary Electron, Back Scattered Electron
Specimen stage	Eucentric, 5 axes motor control
X-Y	70mm×50mm
Tilt	-5° to ～＋70°
Rotation	360°endless
WD	1.0mm to 40mm

3.2.4 Thermal property analysis

Thermogravimetric Analyzer (TGA)

The thermal property for the developed biodegradable cup was analysed by thermogravimetric analyzer (TGA) which was outsourced from Central Instrumentation facility, Lovely Professional University, Phagwara, Punjab.

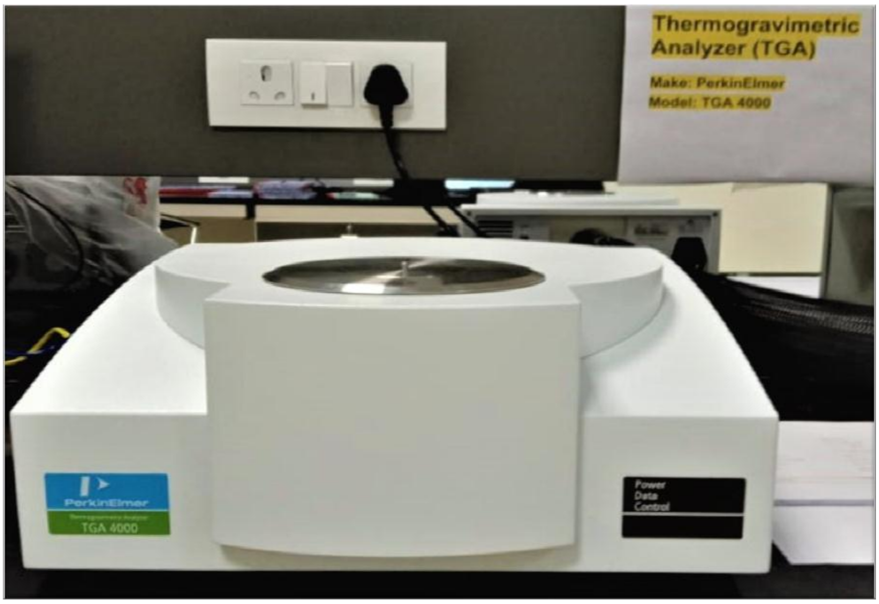


Fig. 3.11: Thermogravimetric Analyzer

Table 3.9: Thermogravimetric Analyzer specifications

Make	Perkin Elmer
Temperature range	15°C to 1000°C
Scanning rates	0.1 to 200°C/min
Temperature precision	±0.8°C
Balance measurement range	1500 mg
Balance Accuracy	Better than 0.2% of total sample and pan
Cooling times	1000 to 100°C in less than 8 minutes and 1000 to 30°C in under15 mines

3.2 Ingredients

For the study of effect of ultrasonication on composite biodegradable cup, the standards of the ingredients were taken from Sonia, 2018. Out of three best combinations of ingredients, the one which have been found best as per reported by Sonia, 2018 were used for the study of "effect of ultrasonication on composite biodegrable cup for food packaging". Standards of base materials which are adapted from research study of Sonia, 2018 are listed in table 3.10.

Table 3.10: Various ingredients and standards

S. No	Ingredient and standards	Level (%)
1	Corn starch	7%
2	Whey protein concentrate	3%
3	Carboxy methyl cellulose	2%
4	Glycerol	4%
5	No. of layers	9

3.3 Formation procedure for biodegradable cup

3.3.1 Slurry formation

Slurry is required for the development of cup.The first step is to weigh the ingredients (Corn starch, Whey Protein Concentrate-35, Carboxymethyl cellulose). The starch powders were first heated in water at 50 °C with constant stirring. Afterwards, whey protein concentrate-35 and carboxy methyl cellulose were added to the solution of starch on hot plate heater with magnetic stirrer until it achieved complete gelatinization (solution A) and then mixing glycerol in water separately (solution B). Thereafter both the solutions were mixed and the resultant dispersions were again heated to 90 °C to induce gelation. The resulting solution was cooled down to room temperature (40 °C) to avoid the generation of air bubbles during the pouring process.

3.3.2 Selection of level of amplitude and time duration of ultrasonication treatment for development of biodegradable cup

Factorial design was used to design the ultrasonication treatment with two variable that is amplitude level and time duration. Three amplitude level and 5-time variations were taken for each a variaable as shown in table 3.2.

Table 3.11: Amplitude and time variations of ultrasonication treatment

S. No.	Amplitude level		Time variations	
Control sample		0%		0 min
1	1	20%	1	1 min
2			2	2 min
3			3	4 min
4			4	8 min
5			5	16 min
6	2	40%	1	1 min
7			2	2 min
8			3	4 min
9			4	8 min
10			5	16 min
11	3	60%	1	1 min
12			2	2 min
13			3	4 min
14			4	8 min
15			5	16 min

3.3.3 Procedure for biodegradable cup formation

Biodegradable cups were produced using the film formation process developed by Sonia (2018) and Chandla (2017) with minor process modifications for incorporation of ultrasonication treatment. Filmogenic casting solution was prepared by dissolving corn starch (7%), carboxymethyl cellulose (2 %), and whey protein concentrate-35 (3%) in distilled water for 10 minutes with constant stirring. The starch powder was first heated in water at 50 °C with constant stirring. The starch solution was then supplemented with carboxymethyl cellulose. Glycerol (4.0 g/100 g starch) was added as plasticizer, and the resultant dispersions were reheated to 90 °C to allow gelation to occur. To avoid the development of air bubbles during the pouring process, the mixture was left to cool at 40 °C. Layering method (layer-by-layer) was used to produce films in a silicon cup obtained from a local store in Ludhiana, Punjab, India. Layered (manually applied) film-forming solution was applied on silicon cup. The mixture solution is layered onto the silicon mould using brush manually. Each layer is dried for 30 minutes at 60°C on forced circulation hot air oven. After the final drying the cup is removed from silicon mould(Fig 3.12). The process is as shown flow diagram (Fig 3.13 & 3.14).

Fig 3.12 Silicon mould used for layering method for formation of biodegradable

Weighing the CS, WPC and CMC

↓

Mixing of weighed ingredients with hot water on magnetic stirrer till complete gelatinization (**Solution A**)

↓

Mixing of Glycerol in water (**Solution B**)

↓

Heating the mixture solution (A& B) to 90^{O} C

↓

Ultrasonication with different variations of amplitude and time

↓

Resting the slurry to cool down 40^{o} C in desiccator

↓

Layering the slurry with brush on silicone mould

Drying at 60 ^{O}C after each layer for 30 minutes and then adding on another layer

↓

Removing the biodegradable cup after final layer and drying

CS* Corn Starch, WPC* Whey Protein Concentrate and CMC* Carboxymethyl cellulose

Fig. 3.13: Procedure of layering method for development of biodegradable cup

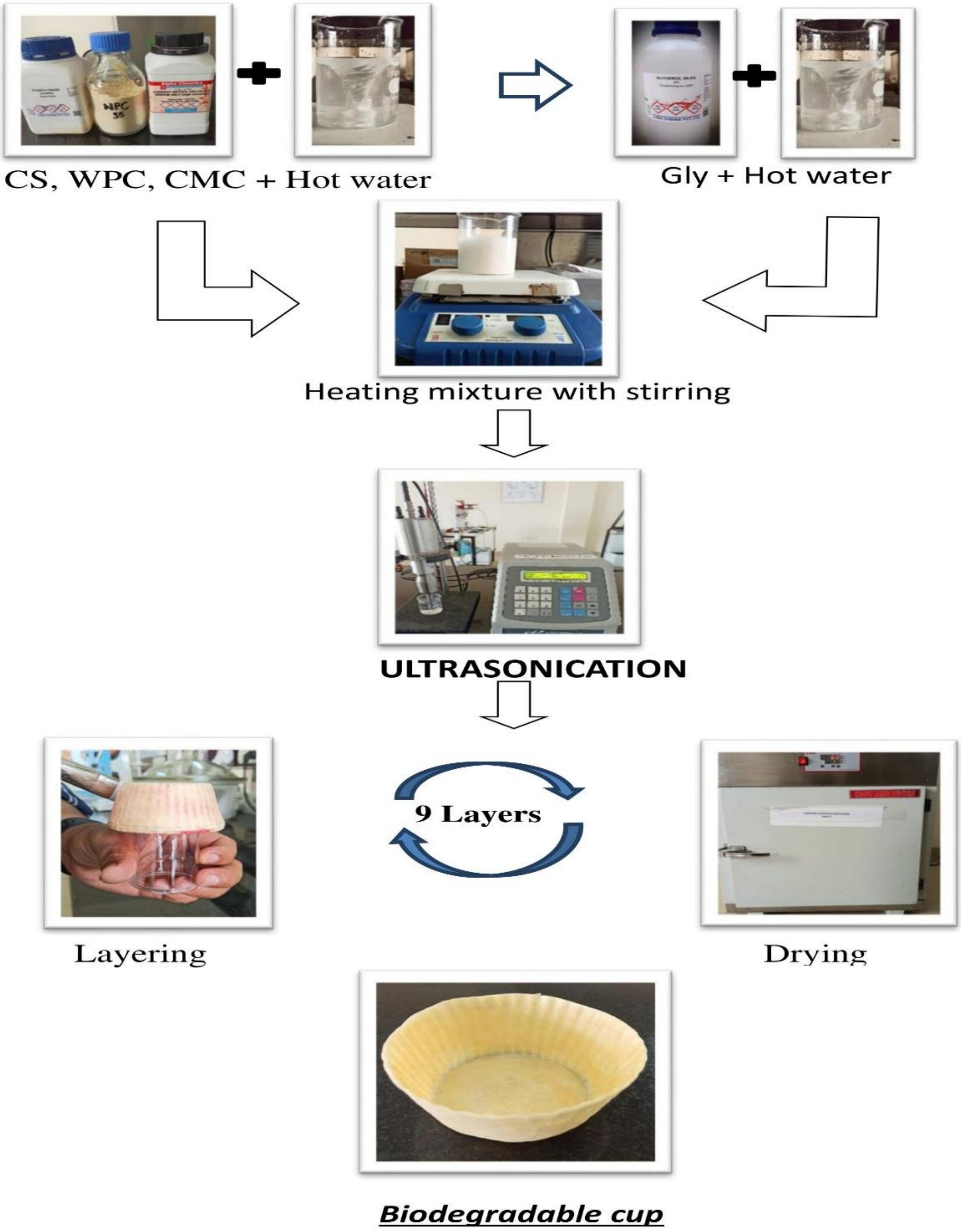


Fig. 3.14: Visual representation of method for development of biodegradable cup

3.3 Viscosity analysis

The viscosity of casting solution measured according to reference method stated by Wang et al., 2007.The viscosity of test solutions was determined at 40 degrees Celsius using a Brookfield Viscometer (DV2TLVTJ0 viscometer; Brookfield Engineering Labs Inc., Stoughton, MA, USA, (Fig 3.4) (Spindle: No. 4; Speed: 100 r.p.m.). The temperature of casting solution is maintained using thermostatic water bath.

3.4 Standability and capacity of cup

To test the cup's standability, it was placed on a horizontal slab at 25°C for 5 days and checked to see whether it collapsed. The cup's capacity was measured in grams as well as the volume it could hold. The cup was filled to the full with water and then poured into a graduated cylinder to determine the volume. To determine the gramme weight, an empty cup was placed on the weighing scale, followed by a cup filled with water till the brim and weighed in weighing scale. The difference between w_2 and w_1 represents the possible weight.

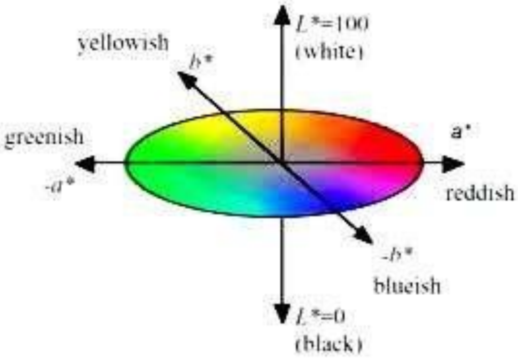
3.5 Thickness of biodegradable cup

The thickness of the cups was measured using digial micrometer (Fig. 3.5) (Mitutoyo 2046F) with a 1m resolution. Calculations were based on the average of three thickness measurements taken at various places on each film sample.

Before testing, all cup specimens were conditioned for 48 hours in a humidity test room at 50% relative humidity (RH) and 25°C.

3.6 Color of biodegradable cup

The Hunter colorimeter (Fig. 3.9) was used to measure the color of cup samples in terms of "L" values "a" value and "b" value (Hunter Associates Laboratory Inc., Reston, VA., USA). Before taking sample measurements, the equipment was standardized using white and black tiles. Following value depicts L* (lightness, 0 = black, 100 = white), a* (negative values indicate the amount of green color, positive values indicate the amount of red color), and b* (negative values indicate the amount of blue colour, positive values indicate the amount of yellow color.



(Source: Bora, 2017)

Fig. 3.15: "L", "a" and "b" values graphical representation

3.7 Opacity

Opacity of cup samples are calculated according to standard method of Ren et al. (2017), opacity is measured using spectrophotometer (Fig. 3.7), cup sample were cut down into rectangular pieces, put in the sample into cuvette, such that it covers the width and length of cuvette, blank cuvette was taken as reference. The opacity was calculated as

$$\text{Opacity} = \text{Abs}_{600} / X$$

Where Abs_{600} is absorbance at 600 nm and X is thickness of respective sample.

3.8 Density

The composite biodegradable cup sample was divided into specimens of varying sizes. A thickness gauge was used to measure the thickness, which was computed as the average of one point in the center of the specimen and four more spaced around it with an accuracy of 1 mm. The mass was determined using an electronic scale with a 0.1 mg resolution; s is the specimen area; and Equation mentioned below was used to get the film density. Before testing, the samples were kept at a constant temperature (23 °C) and humidity (50 %). We performed the aforementioned processes three times for each cup specimen to acquire the mean values (Li et al., 2019).

$$\rho = \frac{m}{s \times d}$$

Where,

ρ = density

m = the mass of specimen,

s = the surface area

d = the thickness of the specimen

3.9 Solubility

The solubility of cup sample in water was determined using the standard method given by Romero-Bastida et al. (2005). Biodegradable cup stripes of 20 mm by 20 mm rectangular samples (n = 3) were cut from the films. The materials' original dry weight was determined by drying them in an oven at 105 °C for 24 hours (MAC India). Following the first weighing, the samples were submerged in a flask containing 80 ml of distilled water at 25°C and agitated slowly for 1 hour. The enlarged samples were then removed and dried at 60 °C until they reached a consistent weight. Weight loss is calculated as a percentage loss of weight in one hour. The solubility was determined using the equation below;

$$\text{Solubilty (\%)} = \frac{\mathrm{W_i} - W_f}{\mathrm{Wi}} \times 100$$

Where,

W_i = Initial weight of dried sample

W_f= Final weight

(a) (b)

Fig. 3.16(a) Biodegradable cup samples for soloubility (b) Setup for solubility analysis

3.10 Water vapor transmission rate

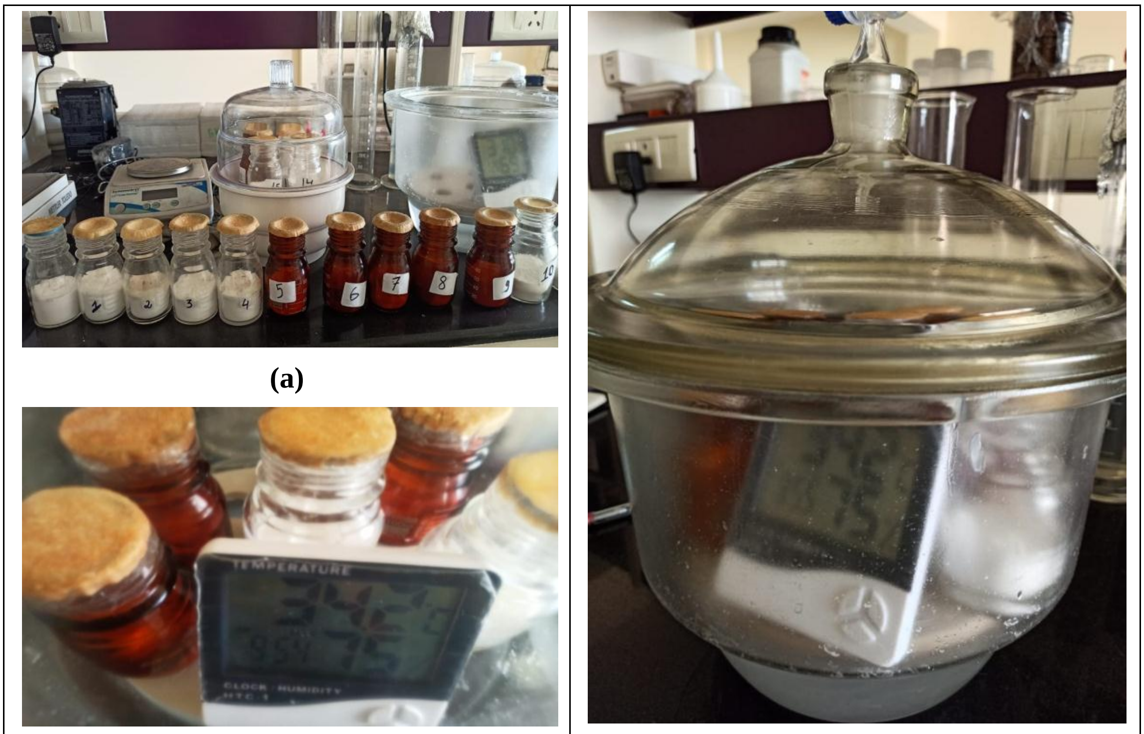
Water vapor transmission rate was measured according to the standard method proposed by Othman et al., (2017). The water vapor transmission of the biodegradable cup was measured using circular mouth reagent bottle. The biodegradable cup samples are cut into circular pieces having diameter greater than the mouth of jar. Cup is filled with anhydrous calcium chloride as a desiccant with 0% relative humidity. Following that the Circular cup sample was fixed on the mouth of jar with liquid paraffin sealing. After that the jars were placed into the desiccator contain Sodium Chloride saturated solution which maintained 75% relative humidity inside the desiccator. Thermometers were installed within the apparatus to validate the temperature (equipment environment). Every 24 hours, the weight of the cup was measured until the variation in mass was less than 10%. Water vapor transmission rate (WVTR) were calculated using the formulae below.

$$WVTR = \frac{\Delta m}{\Delta t \times A}$$

Where,

$\Delta m / \Delta t$ = moisture gain per unit time

A = surface area



(a)

(b) **(c)**

Fig. 3.17: (a) Biodegradable cup sample, fixed on mouth of jar, (b) & (c) Top and side view of desiccator in which humidity is maintained

3.11 Moisture content

The AOAC standard of analysis were used to determine the moisture content (MC) of the biodegradable cups AOAC (1990). Pieces of each (1.0 g) were dried in an oven at 120°C for about 6 hours, or until the weight became consistent. The provided findings are the average of three samples in each case. The percentage of moisture content was determined as follows.

$$\text{Moisture content (\%)} = \frac{(M_i - M_f)}{M_i} \times 100$$

Where; M_i is the initial mass of the wet sample and M_f is the final mass of the dried sample

3.11 Moisture absorption

Moisture absorption is expressed as percentage weight gain in the sample. Moisture absorption is calculated as per the standard method suggested by Abral et al. (2018). Non-treated and ultrasonicated cup samples of rectangular size, 1.5 cm x 1 cm were dried in a drying oven until constant weight, then kept in a closed room with 75% RH for 5 hours. After 300 minutes, the samples were removed and weighed on a precision balance to the closest 0.1 mg. The moisture absorption (percentage) in the sample was estimated using the following equation (Abral et al., 2018):

$$Moisture\ absorption\ (\%) = \frac{W_h - W_o}{W_o} \times 100$$

Where,

w_h = weight after absorption of moisture

w_0 = weight of samples after drying

3.12 Mechanical Properties

3.12.1 Tensile strength

Tensile strength of a biodegradable cup was evaluated using a texture analyzer TA.XT2i according to the reference method of ASTM (2001), D882-12, 2012. (SMS, Surrey, England). Cup samples were cut into 2 mm wide by 8 mm long strips and placed between the texture analyzer grips. The starting grip separation was set at 50 mm, and the speed was set at 5.0 mm/s. Tensile strength was calculated directly from the stress strain curves using the Texture Expert programme.

Fig. 3.18: Biodegradable cup sample placed in grip separator of texture analyzer for tensile force analysis

3.12.2 Puncture strength

Using the same equipment texture analyzer TA. XT2i, puncture tests were performed to measure puncture strength (N) and deformation (mm). With the assistance of a tape, samples with diameters of 40 mm were mounted to the plate of the equipment with a hole of 20 mm diameter (3M Scotch, Brazil). A 5 mm diameter cylindrical probe was pushed perpendicular to the Cup samples surface at a constant speed of 1 mm/s until it went through the film. Force–deformation curves were captured. Force and deformation were measured at the rupture site. Three samples were examined for each test.

3.13 Biodegradability

Biodegradability of the composite biodegradable sample was measured according to the standard method of Shafik et al., (2004) with slight modification. Cup samples of dimension of 2 X 2 cm^2 were buried at the depth of 8 cm in a container containing soil. The pot was positioned in the laboratory, and the soil's wetness was maintained by spraying water at predetermined intervals. The degradation of the cup samples measured in terms of weight loss percentage. Weight of the cup samples was checked at regular intervals by carefully extracting the sample from the soil. Soil from the surface of the sample was removed with the help of the brush. The weight loss percentage of the cup samples over time was utilized to determine the biodegradation rate.

3.14 Thermal properties

3.14.1 Thermo gravimetric analysis (TGA)

Thermogravimetric analyzer (Perkin Elmer TGA 4000) was used to perform thermo gravimetric testing. A 4 mg to 6.2 mg of the biodegradable cup samples were heated from room temperature to 120^{o}C at a rate of 10^{o}C/min with a nitrogen flow of 30ml/min. The weight loss of the materials was computed on a dry basis, and the various deterioration phases were identified. To guarantee reproducibility, analyses were carried out in triplicate.

3.15 Morphological characteristics

Scanning electron microscopy was used to investigate the exterior surface of a biodegradable cup. Before observation, cups were broken and suspended in solvent before being mounted on an aluminum stub with double backed cellophane tape and coated with gold palladium (60:40, w/w) in an auto fine coater, JEOL Smart Coater. FE-SEM: JEOL JSM-7610F Plus, was used to analyze all of the biodegradable cup samples. At a moisture level of 5-6 percent, the cups' surface morphological features were absorbed. At various magnifications, the starch samples were analyzed. Prior to analysis, all cup samples were kept in a desiccator with silica gel (0% RH).

3.16 Statistical analysis

The data are represented as average of at least three replicates. The data was subjected to statistical analysis using SPSS 17.0 software. The experimental data was analyzed using two-way analysis of variance (ANOVA) and values in various tables are expressed as mean value ± standard deviation. Differences were considered at a significance level of 90 % ($p<0.1$).

CHAPTER IV

RESULTS AND DISCUSSION

The results of present study were to investigate the effect of ultrasonication treatment on the properties of developed biodegradable cups. The study was framed in two phases. The first phase includes the development of a biodegradable cup utilizing ultrasonication treatment as a source for property augmentation. The next phase involves comparative study of properties between ultrasonicated and non-ultrasonicated cup samples. The research work encompasses study of physical and mechanical properties of cups as well as morphological and thermogravimetric analysis. The results collected during these investigations are presented and discussed, in this chapter.

4.1 Selection of level of ultrasonication provided to casting solution for biodegradable cup formation

Ultrasonication treatment involve two parameter that is amplitude level and the duration of ultrasonication. Level of amplitude and time duration is selected on the basis of research work done earlier and preliminary trials undertaken. Amplitude level of 20%, 40% and 60% for time duration of 1, 2 ,4 ,8 and 16 min has been selected for the study of effect of ultrasonication on properties of biodegradable cup. The research involved 15 variations combination of varying amplitude and time, one control sample in which no ultrasonication was done. Table 4.1 depicts all the variation taken for the research work.

Table 4.1 Amplitude and time variation of ultrasonication for casting sample

Sample	Amplitude	Time (min)
Control sample	0	0
1	20%	1
2	20%	2
3	20%	4
4	20%	8
5	20%	16

Sample	Amplitude	Time (min)
6	40%	1
7	40%	2
8	40%	4
9	40%	8
10	40%	16
11	60%	1
12	60%	2
13	60%	4
14	60%	8
15	60%	16

4.2 Viscosity

Table 4.2 presented viscosity values for ultrasonicated and non-treated casting solution samples. The non-treated or control sample found to be highest in viscosity value i.e. 2326 ± 14.8 cp. Viscosity of the casting solution decreased with rise in ultrasonication treatment. The viscosity of control sample was 2326 ± 14.8 cp. Viscosity was significantly decreased up to 686 ± 28.70, 592 ± 26.98 cp for 20 and 40 % for 16 min. The least viscosity was observed with 60% and 16 min which was 536 ± 12.32 cp. The collapse of cavitation bubbles, causes severe heating in the remaining bubbles and significant mechanical consequences. This effect should cause fewer mobile polymers to break into tiny fragments ((Mason & Lorimer, 1988; Kardos & Luche, 2001). It is common knowledge that when casting or spraying a starch-based film, the starch dispersion should be heated first. When starch granules are heated in the presence of abundant water, they undergo a transition from order to disorder known as gelatinization (Lionetto et al., 2006). Gelatinization leads to elevate the viscosity significantly. Also, the non-solubilized part of gelatinized starch suspension, commonly known as “ghost” appear which make it difficult to form good film with such suspension. Viscosity of casting solution is important in determining the further characteristic in developing cup. Viscosity assessment of all the sample including control sample has been done. The observations for viscosity of casting solution shows that there is gradual decrease in the values of viscosity with increase in ultrasonication treatment. Fig. 4.1 shows the decline curve for viscosity value for different amplitude levels and time. The value of viscosity for all the amplitude level i.e., 20, 40 and 60% gradually decrease with time. The same behaviour has been observed by cheng et al., 2010, who reported decrease in the viscosity of maize starch suspension by ultrasound. There have been some similar outcomes revealed that non solubilized part diminished on gelatinized waxy maize starch (Iida et al., 2008) and wheat starch (Seguchi et al., 1994) by ultrasonication. This phenomenon might be explained by the fact that power ultrasound's high mechanical impact and heat disrupt the cross-link between polymers (mostly amylose/amylopectin), which if not treated resulting in the formation of ghost element (Debet & Gidley, 2007). In addition, similar results have been reported for starch protein complex, it was observed there is decrease in viscosity value for whey protein concentrate-pectin complex suspension through ultrasonication treatment. Also, in another experiment Zisu et al. (2010) reported protein suspension viscosity is reduced by particle size reduction through ultrasonication. Ultrasonication cause the reduction in size of complex by the virtue of cavitation, turbulence and shear force which cause decreased viscosity value (Le Henaff, 1997). All the sample examination has been performed in triplicate and table shows the average value.

Table 4.2: Viscosity values of for ultrasonicated and non-treated casting solution sample

Time (min)	**Viscosity (cp)**		
	Amplitude (%)		
	20%	**40%**	**60%**
1	2214 ± 30.59^{pc}	2118.67 ± 65 ^{pb}	1854 ± 27.27^{pa}
2	1841.67 ± 25^{oc}	1667 ± 28.80^{ob}	1514.67 ± 12.5^{oa}
4	1259 ± 32.89^{nc}	1116.67 ± 23.32^{nb}	946.67 ± 135na
8	868 ± 36.76^{mc}	739.67 ± 95^{mb}	668 ± 10.19^{ma}
16	686 ± 28.70^{lc}	592 ± 26.98^{lb}	536 ± 12.32^{la}
Control sample 2326 ± 14.8^{qd}			

N=3, Values are the average of ±standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

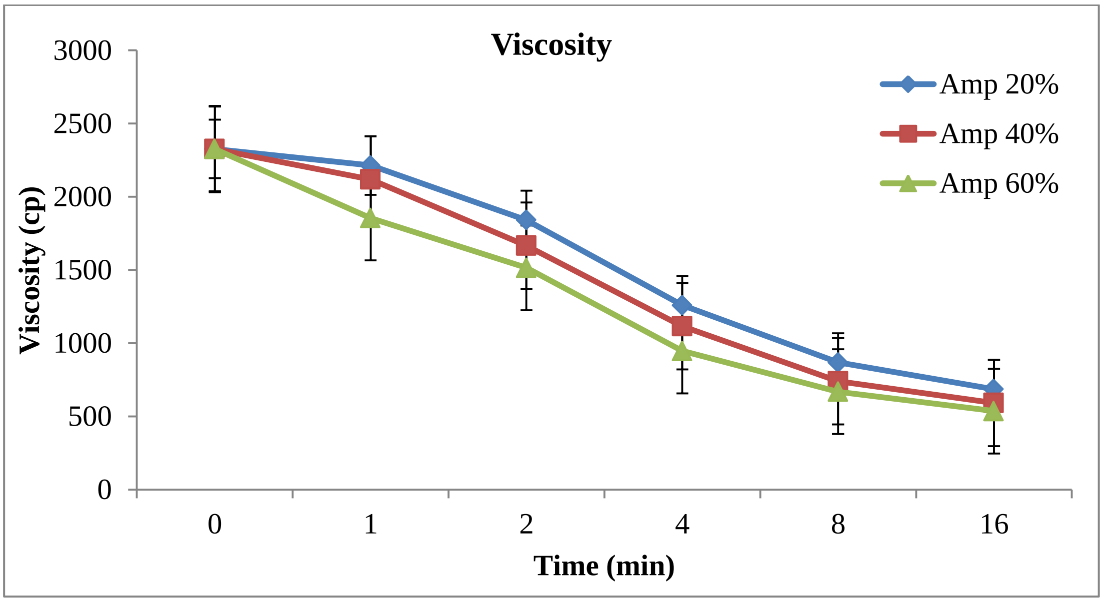


Fig. 4.1: Viscosity values of ultrasonicated and non ultrasonicated samples

4.3 Standability of formed cup

Standability of cup defines the ability of the formed cup to stand without any external support. Which shows the capability of polymer matrix that it is enough strong to remain erected in as such form without any support, all the cup formed sonicated and non-sonicated all are found stand able

4.4 Thickness

The thickness of the film measured with the digital Mitutoyo micrometer for all the sample. Control sample found thickest among all the sample. Thickness of the control sample was 0.813 ± 0.023 mm. The thickness of the Ultrasonicated sample decreased significantly as the ultrasonication intensity is increased. The thickness decreased up to 0.608 ± 0.016 mm, 0.56 ± 0.081 mm and 0.572 ± 0.016 mm for 20, 40 and 60% amplitude levels for the time duration of 16 minutes. Table 4.3 depicts the thickness values of the all the sample

Thickness of film influence or determine different characteristics of film, like opacity, water vapour permeability. The decrease in thickness can be understood with the fact the ultrasonicated sample formed more denser and compact structure. Also, the viscosity of casting suspension decreases as amplitude and duration of treatment increased. Due to reduced viscosity of casting suspension, thickness of every single layer reduces which led to overall decrease in thickness of cup formed. The thickness of control or non-ultrasonicated sample was 0.813 mm, which is less than all the ultrasonicated sample. Fig. 4.2 shows the plot for thickness value against the time for different amplitude level. The thickness of ultrasonicated sample ranges in between 0.56 to 0.781 mm. the similar finding has been observed on rice starch and chitosan-based film, film thickness of ultrasonication treated film was lesser than non-sonicated sample (Brodnjak & Todorova, 2018). Also, in whey protein-based film similar behaviour was found on ultrasonication thickness of film get reduced (Cruz diaz et al., 2019).

Table 4.3: Thickness values of for ultrasonicated and non-treated biodegrdable cup samples

Time (min)	**Thickness (mm)**		
	Amplitude (%)		
	20%	**40%**	**60%**
1	0.776 ± 0.035^{pc}	0.76 ± 0.021^{pb}	0.718 ± 0.028^{pa}
2	0.719 ± 0.021^{oc}	0.697± 0.029^{ob}	0.671 ± 0.014^{oa}
4	0.652 ± 0.014^{nc}	0.667 ±0.037^{nb}	0.613 ± 0.024^{na}
8	0.626 ± 0.049^{cm}	0.617 ± 0.024^{mb}	0.609 ± 0.035^{ma}
16	0.608 ± 0.016^{lc}	0.56 ± 0.081^{lb}	0.572 ± 0.016^{la}
Control sample 0.813 ± 0.023^{qd}			

N=6,Values are the average ±standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

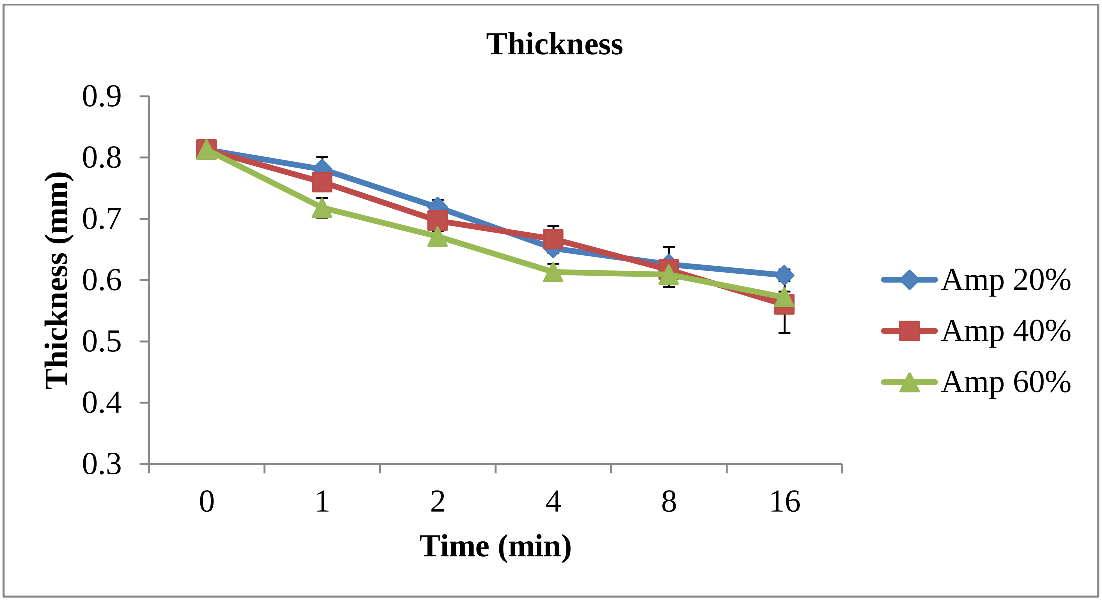


Fig. 4.2: Thickness values of ultrasonicated and non-ultrasonicated samples

4.4 Tensile strength

Table 4.4 shows the value for tensile strength for sample sonicated at different amplitude level and time duration. The non-treated control sample has been observed for least tensile strength 0.932 ± 0.086 kgf. This implied that due to the poor compatibility of the components, a less compact polymer structure of CS/WPC/CMC/GLY composite film developed from the film-forming suspension without ultrasonication. At 20, 40 and 60% amplitude level the maximum tensile strength achieved was 2.39 ± 0.302, 2.615 ± 0.055 and 3.204 ± 0.287 kgf, which was attained by treatment duration of 8 min, 4 min and 2 min respectively. Clearly, the ultrasonic treatment increased the tensile strength value, suggesting that the changes in the polymer matrix were caused by ultrasonication, resulting in a more compact and constricted polymer matrix between ingredients of film forming suspension during drying of cups. In other words, the mechanical force and shock waves used in ultrasonic therapy may allow for more molecular contact between the film-forming components, resulting in a polymer film with a higher tensile strength. The elimination of ghosts in the film-forming suspension, resulted in a more homogenous matrix of the ultrasonically treated samples, resulting in more efficient reinforcement of the film strength (Abral et al., 2018). Also, given to their chemical closeness, this was most likely produced by an interfacial contact between the CS and the CMC

(Ghanbarzadeh et al., 2010). Other researchers discovered a similar outcome in a sago starch-based polymer film after ultrasonic treatment, with an enhancement in tensile characteristics (Abral et al., 2019). The destruction of starch ghosts with ultrasonic vibration cause release of entrapped amylose, increase in amylose content increases the tensile strength (Tharanathan, 2003). The tensile strength value for sample at 20 % amplitude increase till the time duration of 8 min. the value has been decreased for 20% amplitude 16 min sample, similarly for 40% and 60% sample the decrease in value is exhibited at time duration of 4 min and 2 min, respectively. Fig. 4.3 depicts the pattern for tensile behaviour of samples of different amplitude level and time duration. The maximum value for tensile strength has been observed at 60% amplitude level for 2 min of time duration.

Table 4.4: Tensile force values for ultrasonicated and non-treated biodegradable cup samples

Time (min)	**Tensile force (kgf)**		
	Amplitude (%)		
	20%	**40%**	**60%**
1	1.06 ± 0.045^{mb}	2.218 ± 0.102^{mc}	2.602 ± 0.061^{md}
2	1.362 ± 0.041^{nb}	2.284 ± 0.240^{nc}	3.204 ± 0.287nd
4	2.12 ± 0.167^{nb}	2.615 ± 0.055^{nc}	2.819 ± 0.061nd
8	2.39 ± 0.302^{nb}	1.974 ± 0.092^{nc}	2.681 ± 0.086nd
16	1.55 ± 0.089^{mb}	1.454 ± 0.104^{mc}	2.182 ± 0.078^{md}
Control sample 0.932 ± 0.086^{la}			

N=3, Values are average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

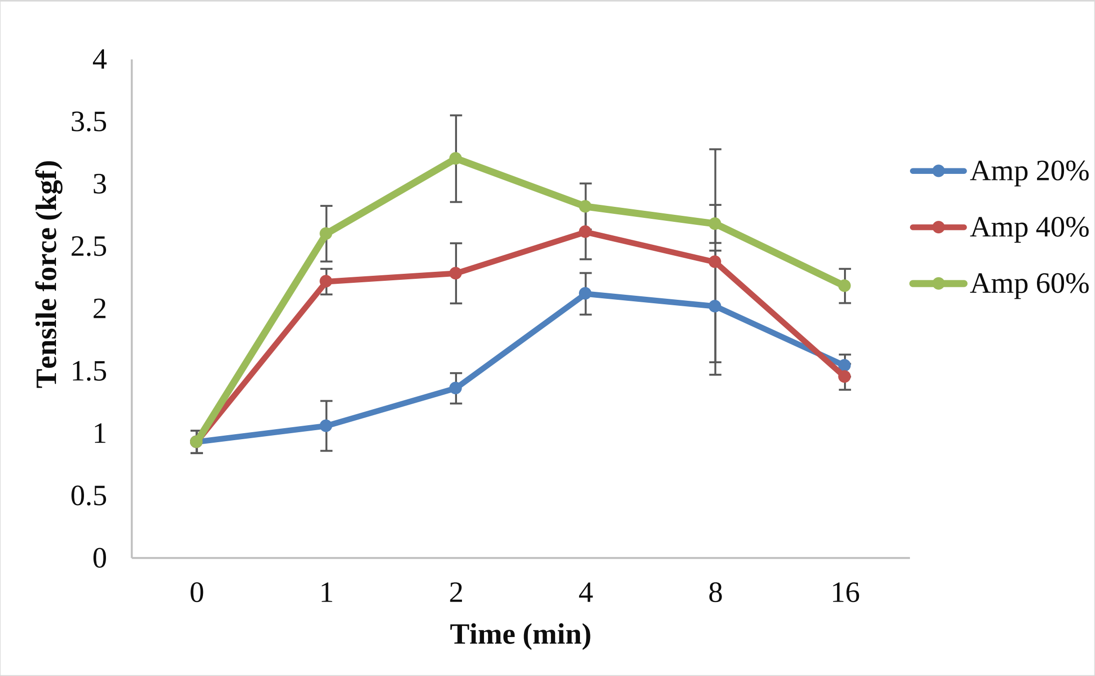


Fig. 4.3: **Tensile force values of ultrasonicated and non-ultrasonicated biodegradable cup samples**

4.5 Puncture Strength

The puncture strength for all the sample has been analyzed by texture profile analyzer. Increase in the ultrasonication cause increase in puncture force. Fig.4.4 shows graph of puncture force against the time for different amplitude samples The control sample require the puncture force of 645 ± 70.2 gf. The sample treated with 20 % amplitude with duration of 1 min had puncture force value of 670.64 ± 50.22 gf, puncture force required for 20% and 16 min treated sample is significantly increased 1339 ± 176 gf. Similarly for 40 and 60% amplitude treated cup samples highest puncture force is achieved for 16 min duration that is 1679 ± 301 and 1754 ± 150.58 gf respectively. Table 4.5 depicts all the values for puncture force for different sample. The aforementioned result is supported by the fact that the denser matrix cause increase in puncture force. Puncture force is related to distribution of amylose and amylopectin content into the matrix, however, amylose is chiefly responsible for denser network as amylose films are stronger than amylopectin films because their network structure is very stable and has strong molecular bond alignment (Stading et al., 1998). However, the glycerol as plasticizer added into a starch network, it diminishes the direct connections and closeness between starch chains and make it flexible, which also decreases the puncture force. The puncture force for non-ultrasonicated sample was found least among the all the Sample.

Table 4.5: Puncture force values (gf) of for ultrasonicated and non-treated biodegradable cup samples

Time (min)	Puncture force values (gf)		
	Amplitude (%)		
	20%	40%	60%
1	670.64 ± 50.22^{mb}	863 ±101.16^{mc}	910 ± 136.92^{mc}
2	847.22 ± 98.12^{nb}	1332 ± 52.14^{nc}	1360.92 ± 165.88^{nc}
4	866 ± 33.65^{nob}	1240 ± 69.17^{noc}	1595 ± 102.8^{noc}
8	936 ± 83.24^{ob}	1494 ± 48.35^{oc}	1634 ± 161.78^{oc}
16	1339 ± 176^{pb}	1679 ± 301^{pc}	1754 ± 150.58^{pc}
Control sample 645 ± 70.2^{la}			

N=3, Values are the average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

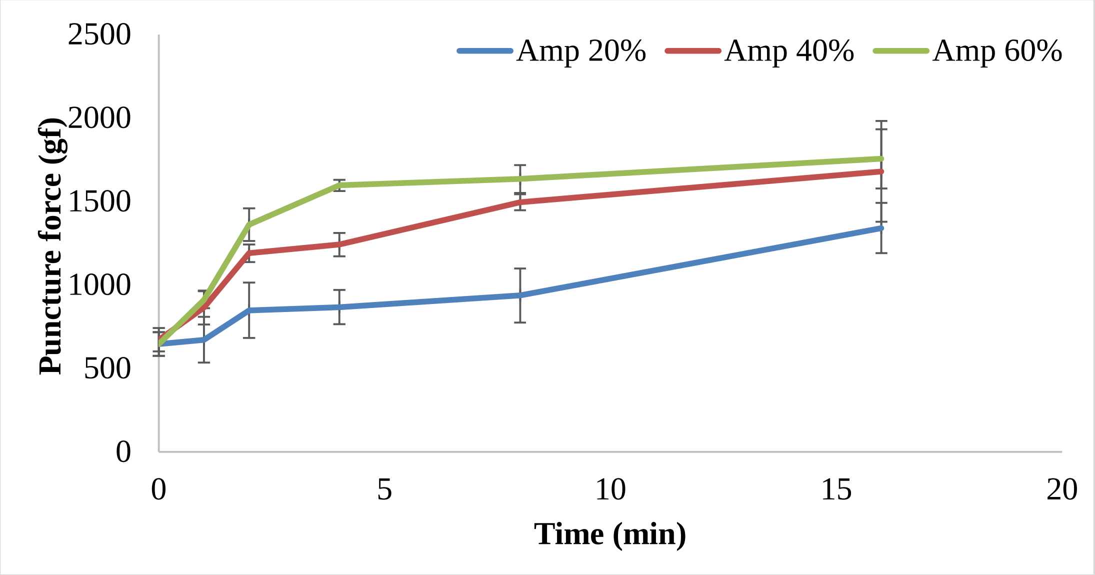


Fig. 4.4: Puncture force values of ultrasonicated and non-ultrasonicated biodegradable cup samples

4.6 Water vapour transmission rate

The water vapour transmission rate for the ultrasonicated samples was less with respect to non-ultrasonicated sample. Water vapor permeability value ranges between 0.76 -1.19 g/m^2 / h for ultrasonicated samples. The control sample had WVTR value of 1.33 ± 0.044 g/m^2 / h, which was significantly decreased up to 0.87 ± 0.00241, 0.79 ± 0.0037 &0.76 ± 0.0031 g/m^2 / h for samples with 20, 40 and 60 % amplitude for 16 min respectively. Table 4.6 shows WVTR values for all the samples. The result implied that the water vapour permeability of the ultrasonicated CS/WPC/CMC/GLY composite material of cup was lower than that of the control sample, suggesting that ultrasonication enhanced the material's moisture resistance. Fig. 4.5 depicts the graph for WVTR values of all the samples. The aforementioned finding may be supported by the fact that higher tightness and compactness in the polymer matrix of ultrasonicated films results in better resistance to moisture penetration, such result has also been reported in earlier studies (Abral et al., 2019). The speed of diffusion, adsorption and desorption, in which water molecules dissolve towards one side of the film, move within the void space between polymeric chains, and then desorb from the polymer surface on the other side of the film, make up the rate of water vapour transporting through a starch film. Chemical structure, amylose-to-amylopectin ratio, crystallinity, ambient humidity, polymer packing and plasticizer are all variables that influence starch film permeability (Chung & Lai, 2007).

Table 4.6: Water vapour transmission rate for ultrasonicated and non-treated biodegradable cup samples

Time (min)	**Water Vapour transmission rate (g/mm^2 /hr)**		
	Amplitude (%)		
	20%	**40%**	**60%**
1	1.1864 ± 0.0045^{pc}	1.09 ±0.035^{pb}	1.01 ± 0.050^{pa}
2	0.9825 ± 0.0121^{oc}	0.97 ± 0.0037^{ob}	0.92 ± 0.0024^{oa}
4	0.96 ± 0.0029^{nc}	0.94 ± 0.0014^{nb}	0.90 ± 0.0028^{na}
8	0.89 ± 0.0030^{mc}	0.83 ± 0.0078^{mb}	0.84 ± 0.0029^{ma}
16	0.87 ± 0.00241^{lc}	0.79 ± 0.0037^{lb}	0.76 ± 0.0031^{la}
Control sample 1.33 ± 0.0442^{qd}			

N=3, Values are the average of triplicates ±standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different (p<0.1)

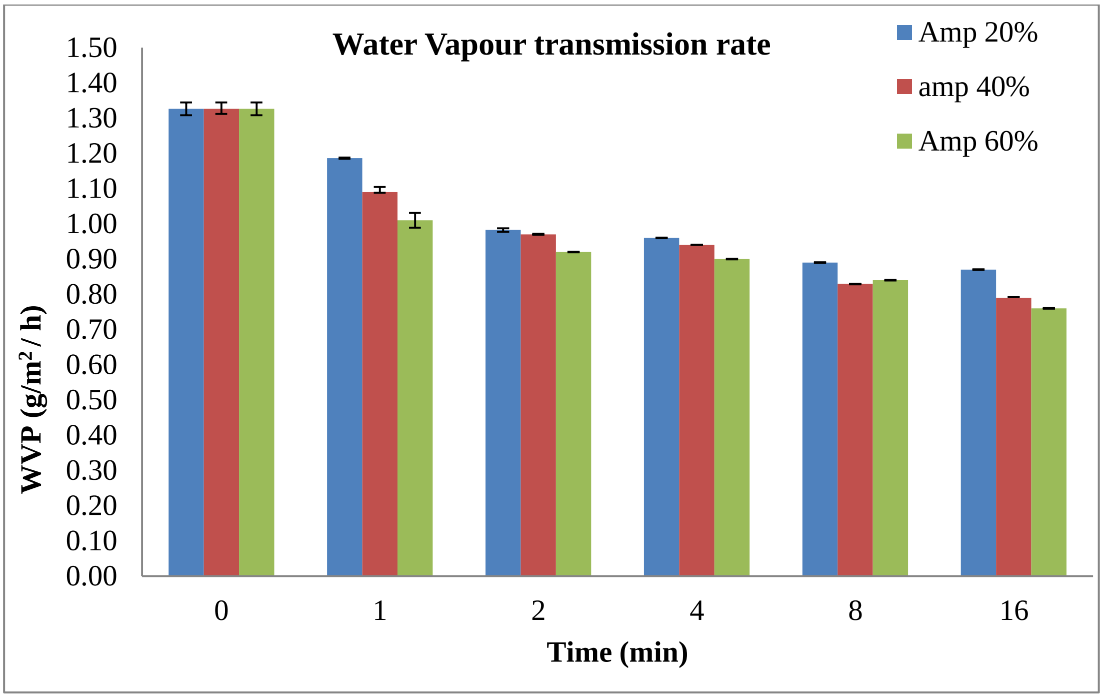


Fig. 4.5: Water vapour transmission rate of ultrasonicated and non-ultrasonicated biodegradable cup samples

However, the presence of CMC may cause water molecules to travel via a convoluted route, limiting moisture penetration (Kristo et al., 2007). Another possibility might be that when the cup casting suspension was ultrasonicated, CMC distributed effectively in the polymer matrix, restricting water molecule transfer (Ghanbarzadeh et al., 2010). Cheng et al. (2010) observed a similar finding, claiming that the film produced from gelatinised maize starch suspensions ultrasonic treatment had reduced water vapour permeability values. Meanwhile, except for sample 14 (ultrasonication amplitude level 60%, time 8 min) sample, the effects of ultrasonic amplitude and treatment duration reveal that higher/longer amplitude/time results in lower water vapour permeability values (Cheng et al., 2010). The increase in water permeation value for the sample no 14 can be elucidated by non-homogeneous structure caused due to manual casting of cup.

The fact that ultrasonication treatment did not cause a very drastic change in water vapour permeability of composite polymer cups can be attributed to the reasons that, the starch exhibits hydrophilic characteristics therefore resulting in films with low moisture resistance (Petersson & Stading, 2005). Also, the number of polar groups present in the polymer film determines the water vapour permeability. The hydrophilic whey protein films are made up of extremely polar amino acids. Whey powder also includes lactose sugar, a very hydrophilic substance. The hydrophobic or hydrophilic nature of the polymer, the presence of voids or cracks, the type, amount, and compatibility of plasticizer, and the steric hindrance in the film structure all impact the barrier characteristics of films (Santosa & Padua,1999; Lawton, 2004). Plasticizer molecules also enhance the intermolecular gap between polymer array of protein, enabling water particles to flow through the film (McHugh et al., 1994a, b).

4.7 Density

The density of the control sample was least for the 0.0738 ± 0.002 g/mm^3. The density significantly increased with increased amplitude level and time duration. The maximum density observed for 20 % and 40% at 16 min of time duration which was 0.1049 ± 0.0018 and 0.1183 ± 0.0019 g/mm^3. The peak density achieved was with amplitude level of 60 % and 16 min which is 0.1267 ± 0.0002 g/mm^3. Table 4.7 depicts the values for density of all the sample.

The density of composite material is an indicator of compactness in the film structure as it characterizes the intermolecular connections generated within the components. Since the cross-sectional area of the samples was same, the weight and thickness of biodegradable film are proportional to its density, which ultimately refers to the thickness of the cup material. The aforementioned growth pattern in density value could have occurred as a result of ultrasonic vibrations causing a denser network among the composite material's ingredients. The elimination of void air vacuoles may also result in an increase in density as has been confirmed with sem images (Fig 4.14) The increase in numerical value of density for composite cup material is aided by the lowered thickness of the cup material. Another possible explanation is that after ultrasonication, the increased interaction of WPC with glycerol causes a rise in the density of the resultant material, as described on (Abhishaben et al., 2019). Fig. 4.6 shows plot for density values against time values for different amplitude level. All samples’ triplicates were used to reach the result.

Table 4.7: Density values for ultrasonicated and non-treated biodegradable cup samples

Time (min)	Density (g/mm3)		
	Amplitude (%)		
	20%	40%	60%
1	0.0752 ± 0.0015^{mb}	0.0806 ± 0.0006^{mc}	0.0975 ± 0.0007^{md}
2	0.0817 ± 0.0006^{nb}	0.0915 ± 0.0015^{nc}	0.1006 ± 0.0020^{nd}
4	0.0901 ± 0.0003^{ob}	0.0993 ± 0.0011^{oc}	0.1081 ± 0.0013^{od}
8	0.0978 ± 0.0001^{pb}	0.1114 ± 0.0009^{pc}	0.1129 ± 0.0007^{pd}
16	0.1049 ± 0.0018^{qb}	0.1183 ± 0.0019^{qc}	0.1267 ± 0.0002^{qd}
Control sample 0.0738± 0.002^{la}			

N=3,Values are the average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticlly different ($p < 0.1$)

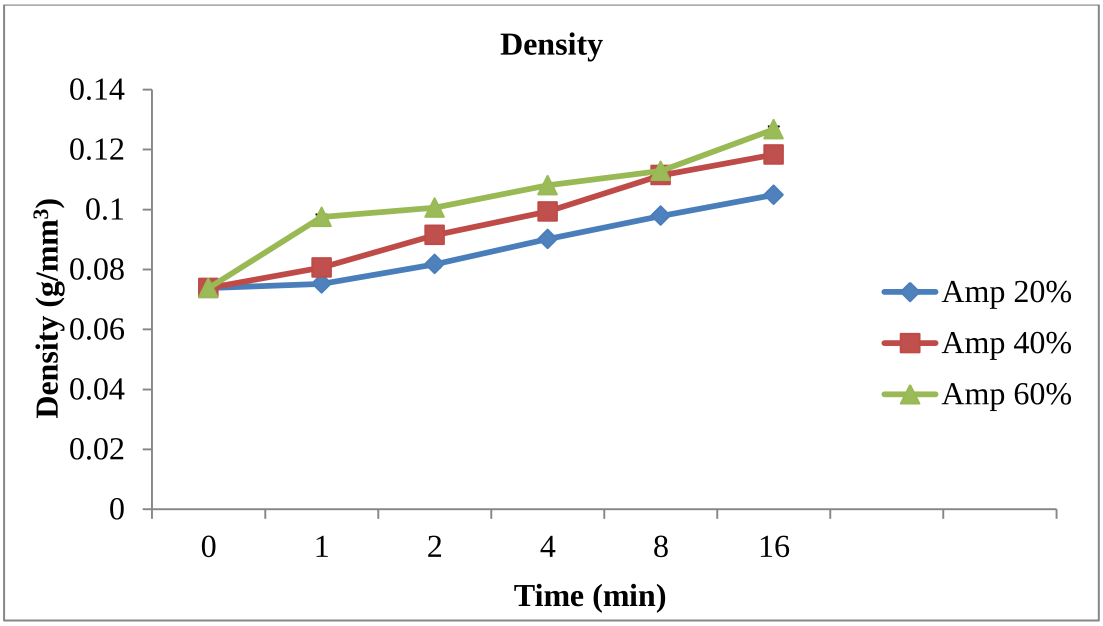


Fig. 4.6: Density of ultrasonicated and non-ultrasonicated biodegradable cup samples

4.8 Solubility

The solubility of control sample was 24.5 % and the maximum value for solubility 42.89% which is attained by sample 10 (Amplitude level 40% and time duration 16 min). Solubility % of biodegradable cup sample increases for 20, 40 an 60

% amplitude level, The solubility % for 1 min samples 31.083 ± 1.516, 31.869 ± 1.632 and 33.612 ± 1.121 % respectively which is increased up to 42.333 ± 0.99, 42.889 ± 0.59 and 42.075 ± 0.691 % for respective amplitude level. Table 4.8 depicts the values for the solubility % of all the sample.

The ability of starch-based films to dissolve in water is an essential characteristic. Water insolubility may be required in some applications to improve product reliability, moisture barrier characteristics, and shelf life. In some situations, though, prior product use, the water solubility of films may be beneficial for food encapsulation, for example. addition of any additive or ingredients (Bertuzzi et al., 2007). The high pressure associated with the collapse of cavitation bubbles causes depolymerization of macromolecules, which causes severe heating in remnant bubbles and causes dramatic mechanical consequences. This process should result in more fragmentation of less mobile polymers (Mason & Lorimer, 1988; Kardos & Luche, 2001). The solubility of all the sample has been examined to check the effect of ultrasonication on solubility of composite cup material. The result obtained shows that with the increase of ultrasonication intensity that is amplitude level and time the solubility of composite cup material increases. The solubility of non-ultrasonicated was found to be least among all the ultrasonicated sample. The forementioned increase in solubility percentage of sample with increase in ultrasonic amplitude and time duration can be attributed with the fact that, the depolymerization and structural weakening of starch granules caused by the oxidation process (Adebowale et al., 2002). Oxidation of starch granule increases the carbonyl and carboxyl group which weaken the structure of starch granule by degrading the amylose molecule into short chains which is soluble in water (Uthumporn et al., 2010).

After the gelatinization of starch, the non-solubilized, which contain inner amylopectin and amylose (Han & Hamaker, 2002). The rise in solubility can be further explained by the fact stated that the non solubilized material is destroyed after ultrasonication which led to increased number free mobile polymer which are ready for hydration reaction. The polymer responsible for ghost (non solubilized fraction) formation might be fully hydrated and cause solubility in water (cheng et al., 2010).

Table 4.8: Solubility of ultrasonicated and non-treated biodegradable cup samples

Time (min)	Solubility (%)		
	Amplitude (%)		
	20%	40%	60%
1	31.083 ± 1.516^{mb}	31.869 ± 1.632^{mc}	33.612 ± 1.121^{mc}
2	33.667 ± 1.09^{nb}	35.012 ± 0.792^{nc}	36.209 ± 1.152^{nc}
4	34.002 ± 0.507^{ob}	37.727 ± 0.92^{oc}	37.913 ± 1.015^{oc}
8	39.778 ± 1.612^{pb}	42.653 ± 1.135^{pc}	42.907 ± 1.01^{pc}
16	42.333 ± 0.99^{pb}	42.889 ± 0.59^{pc}	42.075 ± 0.691^{pc}
Control sample 24.561 ± 1.166^{la}			

N=3, Values are the average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticlly different (p<0.1)

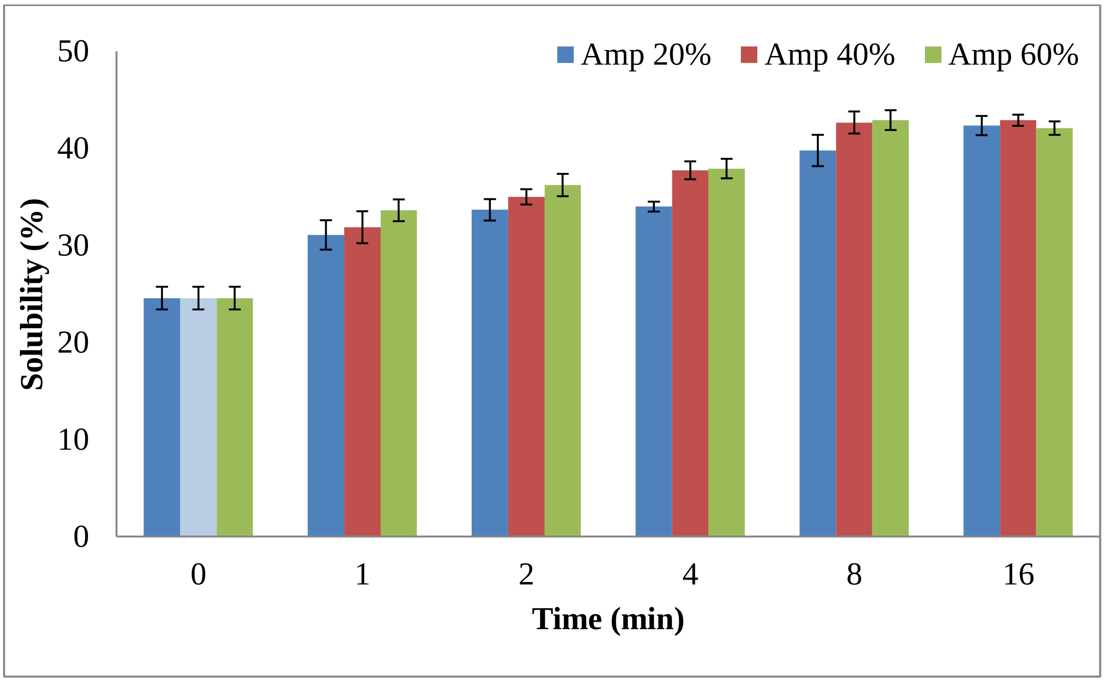


Fig. 4.7: Solubility of ultrasonicated and non-ultrasonicated biodegradable cup samples

The solubility of solely maize starch-based film can reach up to 100% after ultrasonication of 15 min at amplitude level of 20% (cheng et al., 2010). The added CMC in composite material form matrix with starch and resulting in relatively less solubility in water (Ghanbarzadeh et al., 2010). Furthermore, in a study of whey protein suspension, due to the protective effect of lactose present during pressure treatment by ultrasonication solubility of wpc suspension not change significantly (Jambrak et al., 2008). Fig. 4.7 shows the plot for solubility % of all the sample of different amplitude and time duration.

4.9 Moisture content

Moisture content of the biodegradable cup samples decrease with increase in ultrasonication. The moisture content of the control sample was 24.1 ± 0.140 % which was decreased up to 15.51 ± 0.510 % for 20% amplitude and 16 min treatment time, for 40 % amplitude it is decreased up to, 17.34 ± 0.355 % which is attained by 16 min treated sample. The least moisture content percent is observed in sample treated with 60 % amplitude and 16 min duration which is 14.9 ± 0.276 %. Table 4.9 depicts values of moisture content for all the sample. The decreased moisture content percentage of biodegradable cup sample is supported by the fact that ultrasonic vibration might avail the water for drying by destructing bonds the similar has been observed with whey protein isolate, glycerol-based film when amplitude of the ultrasonication treatment is increased (Abhishaben et al., 2019). Fig. 4.8 Shows the plot between moisture content and time for different amplitude treated samples.

Table 4.9: Moisture content % of ultrasonicated and non-treated biodegradable cup samples

Time (min)	Moisture content (%)		
	Amplitude (%)		
	20%	40%	60%
1	23.214 ± 0.152^{oc}	22.727 ± 0.097^{ob}	20.21 ± 0.243^{oa}
2	21.69 ± 0.585^{nc}	20.90 ± 0.610^{nb}	18.96 ± 0.290^{na}
4	20.87 ± 0.336^{mc}	17.34 ± 0.364^{mb}	17.35 ± 0.372^{ma}
8	19.65 ± 0.533^{mc}	19.09 ± 0.409^{mb}	15.52 ± 0.303^{ma}
16	15.51 ± 0.510^{lc}	17.34 ± 0.355^{lb}	14.9 ± 0.276^{la}
Control sample 24.1 ± 0.140^{pd}			

N=3, Values are the average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

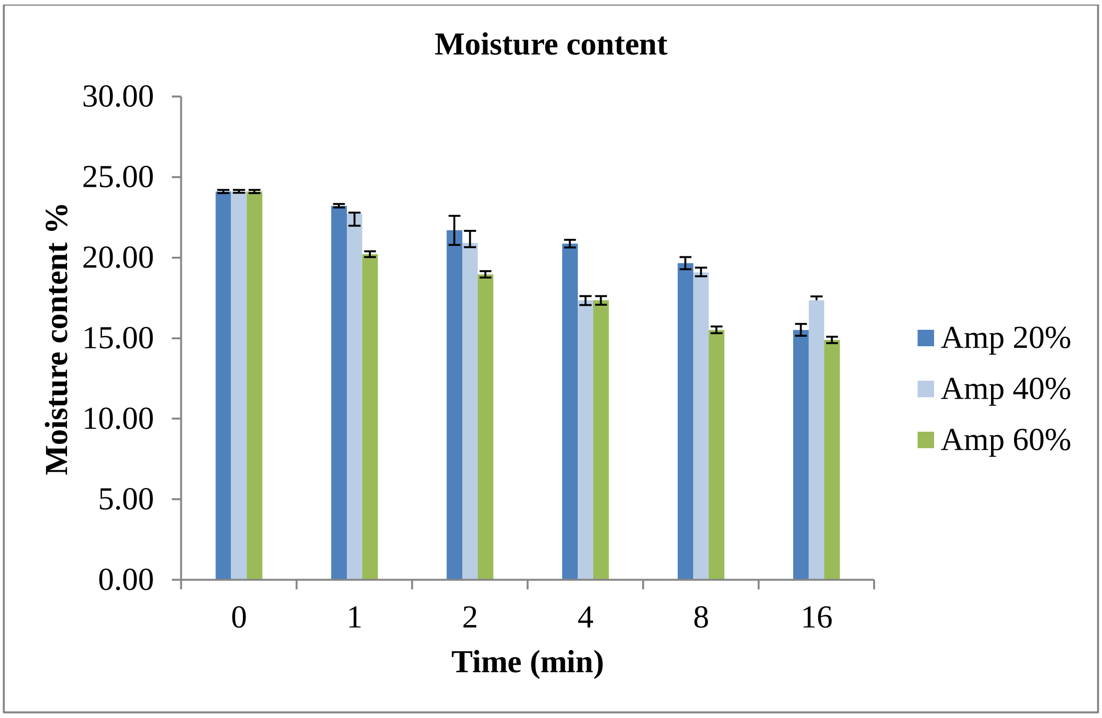


Fig. 4.8: **Moisture content (%) of ultrasonicated and non-ultrasonicated biodegradable cup samples**

4.10 Moisture absorption

Moisture absorption percentage decreases with the increased ultrasonication. The moisture absorption percentage for the control sample was 18.37 ± 0.522 %. The absorption percentage decreases from 17.41 ± 0.42, 16.26 ± 0.346 & 15.94 ± 0.37 % to 11.05 ± 0.17, 10.96 ± 0.197 & 8.97 ± 0.122 % for 20, 40 and 60% from 1 min to 16 min respectively. Fig. 4.9 show the plot for moisture absorption percentage values of ultrasonicated and ultrasonicated sample.

Absorption of the moisture is always among the one of the biggest problems in starch-based films, hence the analysis of moisture absorption and moisture content is considered important, in case of starch based composite packaging material. Moisture absorption test for all sample has been done. Table 4.10 depicts the values for moisture absorption percentage for control and ultrasonicated bio composite cup samples. The result suggests that on ultrasonication help in reducing the moisture absorption of the composite material, as the ultrasonication level increases the moisture absorption decreases. The above-mentioned outcome can be explained by the fact that ultrasonication of the casing composite suspension causes decrease in viscosity, which cause more active depolymerization resulting in filling of cavities, pores. As the number of pores decreases, the diffusion of water molecule in pores also reduces which led to decreased moisture absorption percentage of ultrasonicated sample (Fu et al., 2011). Previous investigations (Garcia-Hernandez et al., 2017; Cheng et al., 2010) had found similar results giving reason, that moisture absorption was reduced due to a reduction in insoluble starch granules and microfractures in the microstructure of the sonicated film. Ultrasonication created a more compact structure, making it more difficult for water molecules to permeate the film and therefore boosting moisture resistance. The similar result has been found with tapioca starch film where ultrasonication effect cause the transformation of natural sample behaviour into a hydrophobic substance improved the resistance of vibrated bio composite to high humidity. Improvements in intermolecular interface bonding, compatibility, and uniformity of samples structure, according to them, could help in resisting water molecules from diffusing into the film matrix (Abral et al., 2018).

Table 4.10: Moisture absorption % of ultrasonicated and non-treated biodegradable cup samples

Time (min)	Moisture absorption %		
	Amplitude (%)		
	20%	40%	60%
1	17.41 ± 0.42^{pc}	16.26 ± 0.346^{pb}	15.94 ± 0.37^{pa}
2	15.96 ± 0.069^{oc}	14.65 ± 0.651^{ob}	13.95 ± 0.581^{oa}
4	13.24 ± 0.421^{nc}	13.01 ± 0.157^{nb}	11.91 ± 0.592^{na}
8	11.02 ± 0.279^{mc}	11.13 ± 0.61^{mb}	10.69 ± 0.409^{ma}
16	11.05 ± 0.17^{lc}	10.96 ± 0.197^{lb}	8.97 ± 0.122^{la}
Control sample 18.37 ± 0.522^{qd}			

N=3, Values are the average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

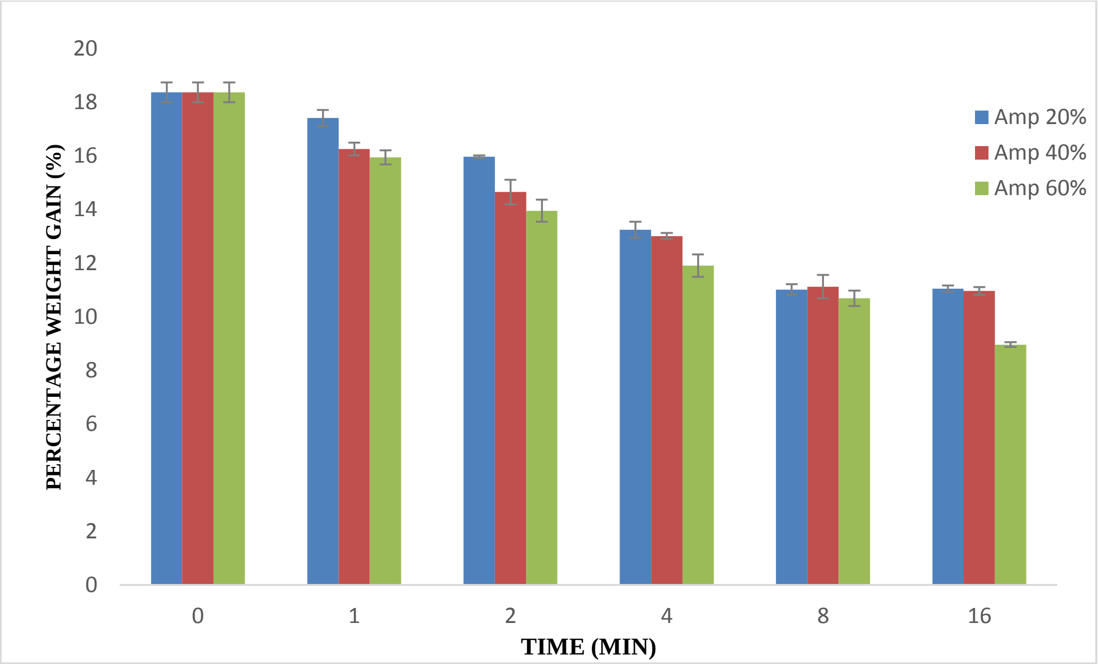


Fig. 4.9: Moisture absorption (%) of ultrasonicated and non-ultrasonicated biodegradable cup samples

4.11 Opacity

Opacity of the packaging material is of prime importance as it determine the product visibility in the package. The opacity of control sample was 3.0171 ± 0.02 which was decreased to the minimum level of 2.56 ± 0.38 due to ultrasonication. The least value was attained by ultrasonication treatment of amplitude 60% for the duration of 8 min. However, the opacity values for cup sample with 40 and 60% at 16 min were slightly increased with time i.e., 2.75 ± 0.316 & 2.70 ± 0.316 respectively. These might be because the dense structure obstructs the light transmission path. The table 4.11 displays the opacity values for all the cup sample.

The outcome from the test suggests that the opacity of cup sample decreases as the ultrasonication amplitude and duration increases. The ultrasonication cause the maximum decrease in opacity value by 15%. the decreasing trend in opacity values was explained by (Cheng et al.,2010) as the high amplitude causes the thorough destruction of the starch ghost and also removes the air bubbles in the film which increase the transparency of the film which ultimately decreases the opacity of film (Cheng et al., 2010). This suggests that the light transmission through the cup sample made from ultrasonic treated CS/WPC/CMC/GLY composite suspension is significantly better than via native corn starch film.

Table 4.11: Opacity of ultrasonicated and non-treated biodegradable cup samples

Time (min)	Opacity (AU/mm)		
	Amplitude (%)		
	20%	40%	60%
1	2.893 ± 0.023^{pc}	2.84 ± 0.31^{pb}	2.88 ± 0.31^{pa}
2	2.85 ± 0.04^{oc}	2.81 ± 0.32^{ob}	2.77 ± 0.32^{oa}
4	2.784 ± 0.22^{nc}	2.79 ± 0.34^{nb}	2.68 ± 0.34^{na}
8	2.67 ± 0.26^{lc}	2.59 ± 0.38^{lb}	2.56 ± 0.38^{la}
16	2.267 ± 0.13^{mc}	2.75 ± 0.316^{mb}	2.70 ± 0.316^{ma}
Control sample 3.017 ± 0.02^{qd}			

N=3, Values are the average of triplicates ±standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p<0.1$)

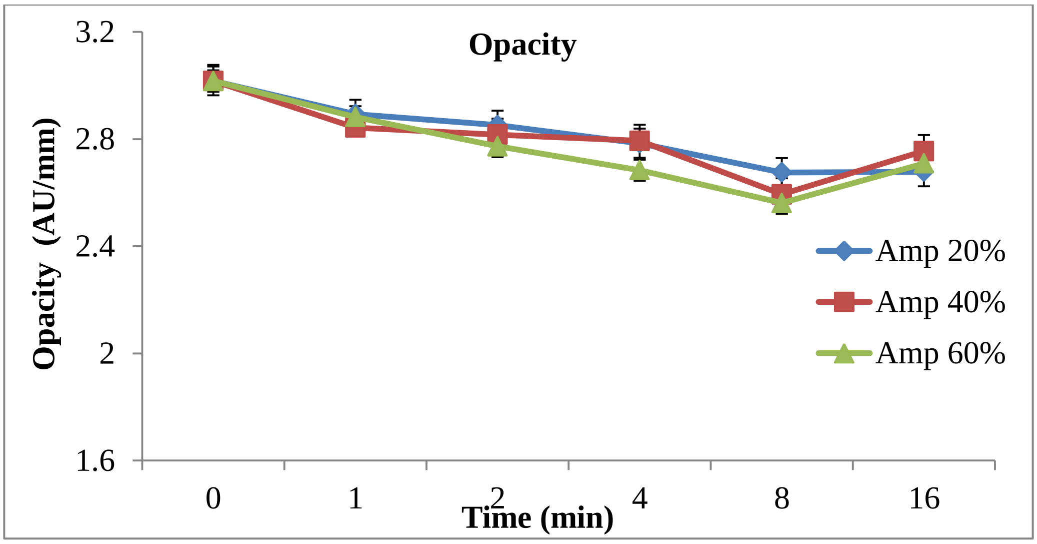


Fig. 4.10: Opacity values of ultrasonicated and non-ultrasonicated biodegradable cup samples

The light transmission of control sample was less. The probable reason for so is the transmission of light might be hampered by imperfections such as agglomerations of incompletely gelatinized starch (Li et al., 2015) and that is removed by ultrasonication cause in reduction of opacity value. Furthermore, the presence of CMC in the starch-based composite enhances the transmission of light there by reduces the opacity (Abdorreza, et al., 2011, Battegazzore et al., 2015).

4.12 Color analysis

The analysis of color is important for any packaging material, it governs the consumer acceptance. Color analysis of all the sample done using hunter colorimeter. Table depicts the values of “L”, “a”, ”b” for all the composite cup material. There is no significant effect found on the color of film by ultrasonication. L value for the sample remain between 52- 57, It might be because of browning of starch due to ultrasonication as reported in previous studies (Pingret et al., 2013). This is also attributed with the “a” value ranging between 5-8. The “b” value indicates the yellowness of the composite cup material which was least for which control sample 20.12 and for ultrasonicated samples, it was ranging between 22-28. Overall, the cup appears bright creamish yellow, Shinny inner surface as the outer surface exposed to hot air.

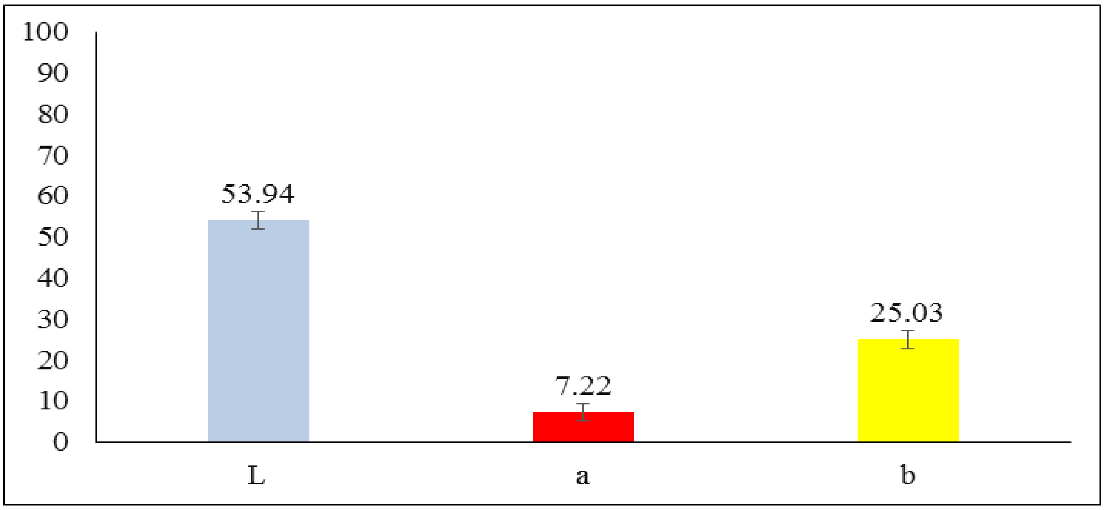


Fig. 4.11: Average “L”, “ a” and “b” values of ultrasonicated cup samples

4.13 Biodegradability

Examining the behavior of soil biodegradation is critical for utilizing bio composites in the environment. Soil biodegradation is the decomposition of materials caused by the action of soil organisms such as microorganisms, fungus, bacteria, and another organism. Table 4.12 shows the percentage degradation in weight of the sample. It was found that the non-ultrasonicated sample took less time to degrade, than the ultrasonicated treated sample. The non-ultrasonicated / control sample vanished within 47 days. Table 4.13 shows the days required for complete degradation of biodegradable cup samples. Maximum time is taken by the sample with time 16 min and 60% amplitude as it took 64 days to degrade up to 93.236%. Whereas samples with 1 to 4 min within 62 days.

The ultrasonication retarded the rate of biodegradation this behavior could be understood and with the fact that the ultrasonicated samples are more compact and less porous. The control or non-ultrasonicated sample as they have pores and less dense structure. The ultrasonication time also slowed the biodegradation rate. The gain in weight of samples in initial days shows the absorption of moisture. These occurrences may be related to the water absorption properties of the bio composite film, in which untreated film absorbs more water than treated film, rendering the former more vulnerable to microorganism attacks. Also previous studies state that the good fiber distribution in the matrix affected moisture, bacteria, and other elements, making their migration into the matrix problematic (Ilyas et al., 2019; Bootklad and Kaewtatip,2013). Another possible explanation for the retardation in biodegradation rate is the kinetic energy from the ultrasonic bath was responsible for this occurrence, which distributed the fibers uniformly within the matrix (Asrofi et al., 2018).

The ultrasonicated sample of cups took almost 70 days to degrade completely. Fig. 4.12 depicts the percentage degradation of weight of ultrasonicated and non-ultrasonicated cup samples in number of days. Sample with higher amplitude and long-time duration takes more time in comparison with lower amplitude percent and short time duration. This conclusion was corroborated by the results of scanning electron microscopy images, where the morphological structure portrays pores with an uneven arrangement. This occurrence may be due to clumping in the matrix (Abral et al., 2018).

Table 4.13: Days required for complete biodegradation of ultrasonicated and non-treated biodegradable cup samples

Time (min)	Days		
	Amplitude (%)		
	20%	40%	60%
1	48.67 ± 0.58^{mb}	52.67 ± 0.58^{mc}	56.67 ± 0.57^{md}
2	49.34 ± 0.58^{nb}	53.67 ± 0.58^{nc}	58.34 ± 0.58^{nd}
4	51.34 ± 1.53^{ob}	57 ± 1^{oc}	63.34 ± 1.52^{od}
8	54.34 ± 1.52^{pb}	61.67 ± 0.58^{pc}	67.67 ± 1.53^{pd}
16	63.68 ± 0.58^{qb}	63.67 ± 1.15^{qc}	68.34 ± 1.53^{qd}
Control sample 42 ± 1^{la}			

N=3,Values are the average ± standard deviation. Average followed by different superscripts (a, b, c, d, in row for amplitude variation and l,m,n,o,p,q in column for time variations) are statisticly different ($p< 0.1$)

Table 4.12: **Day wise percentage weight loss of ultrasonicated and non-treated biodegradable cup samples**

Percent weight loss day wise												
Sample	**Day 0**	**Day 2**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**	**Day 47**	**Day 55**	**Day 62**	**Day 64**
0	0	-6.246	17.20541	23.52941	42.651	68.7841	81.7438	96.3741	*			
1	0	-5.645	13.038	19.362	38.9632	62.5465	76.3641	92.5421	95.2314	*		
2	0	-4.019	10.577	16.901	37.6545	62.0132	72.4554	91.2356	95.03214	*		
3	0	-4.0012	7.699	14.023	35.9741	59.6542	72.6961	89.6345	93.4523	*		
4	0	-4.0642	7.354	13.678	34.2561	58.9814	70.6512	89.6156	93.6621	*		
5	0	-3.961	6.64	12.964	31.6541	57.6325	69.9896	87.321	90.0054	91.4523	95.2314	*
6	0	-5.562	12.638	18.962	41.0345	68.0213	79.632	94.9614	95.3541	*		
7	0	-3.936	8.997	15.321	37.3565	58.6312	68.4618	85.6641	93.2136	*		
8	0	-3.892	8.699	15.023	36.0743	55.1472	67.7945	76.9645	89.1456	93.5541	*	
9	0	-3.9812	7.508	13.832	35.0632	53.8441	65.6685	75.6142	89.0694	92.5694	*	
10	0	-3.878	5.023	11.347	36.5644	52.7745	56.3714	66.9346	79.3641	87.3614	94.3215	*
11	0	-4.581	9.643	15.967	39.9641	65.5354	73.1963	90.6321	92.4344	94.651	*	
12	0	-3.924	7.7121	14.0361	35.4741	61.7687	70.6933	88.1146	91.2841	96.3458	*	
13	0	-3.796	6.3	12.624	33.5821	53.8814	68.7391	73.1777	86.6367	92.0121	93.2111	*
14	0	-3.6428	4.654	10.978	33.0651	51.3361	62.1341	66.1611	79.9364	86.1454	91.6931	94.6142
15	0	-3.596	3.328	9.652	31.3611	49.1782	57.2154	65.7841	75.3614	84.4312	90.7415	93.2364

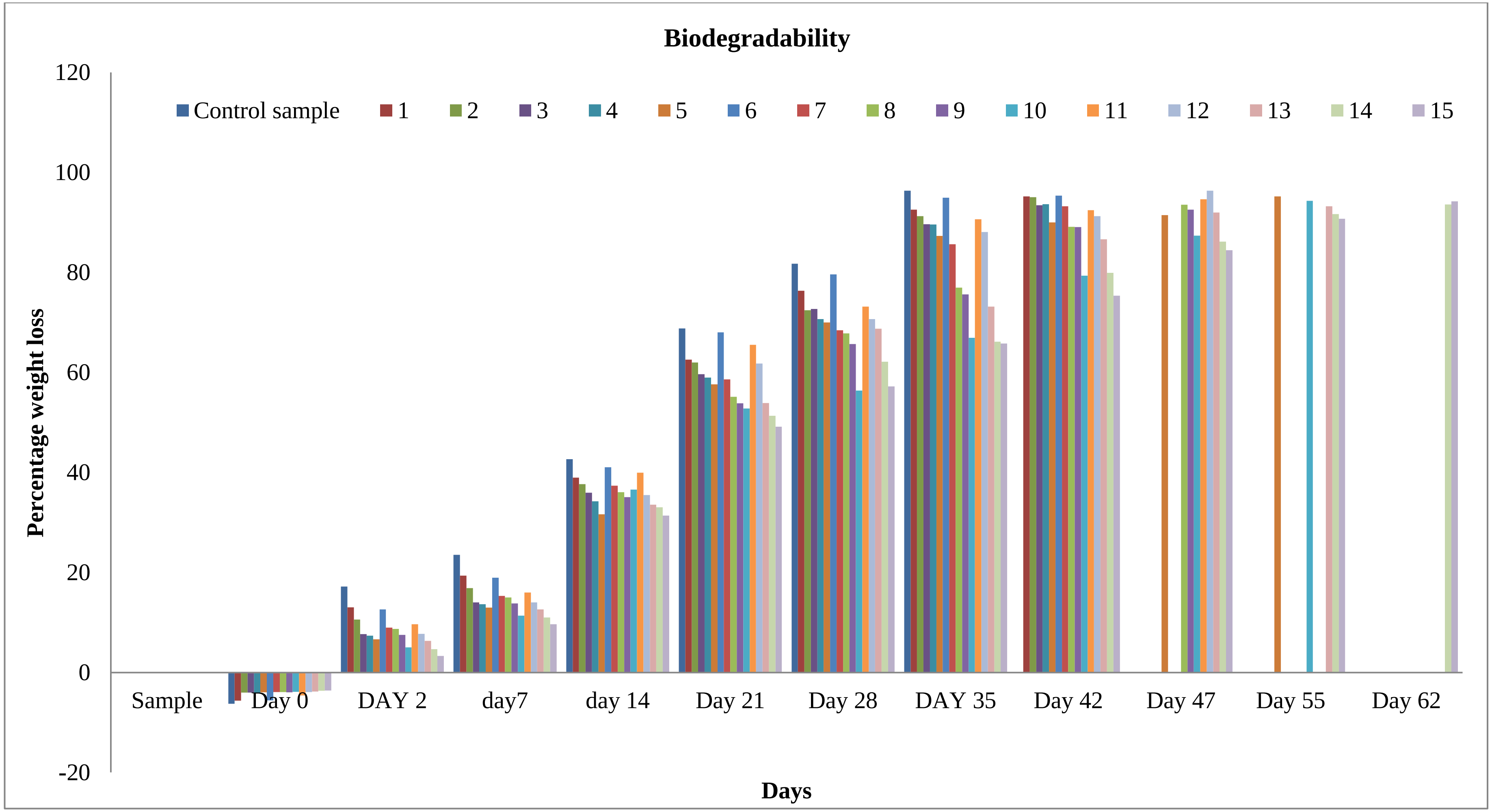


Fig. 4.12: Percentage weight loss vs days plot of ultrasonicated and non-ultrasonicated biodegradable cup samples

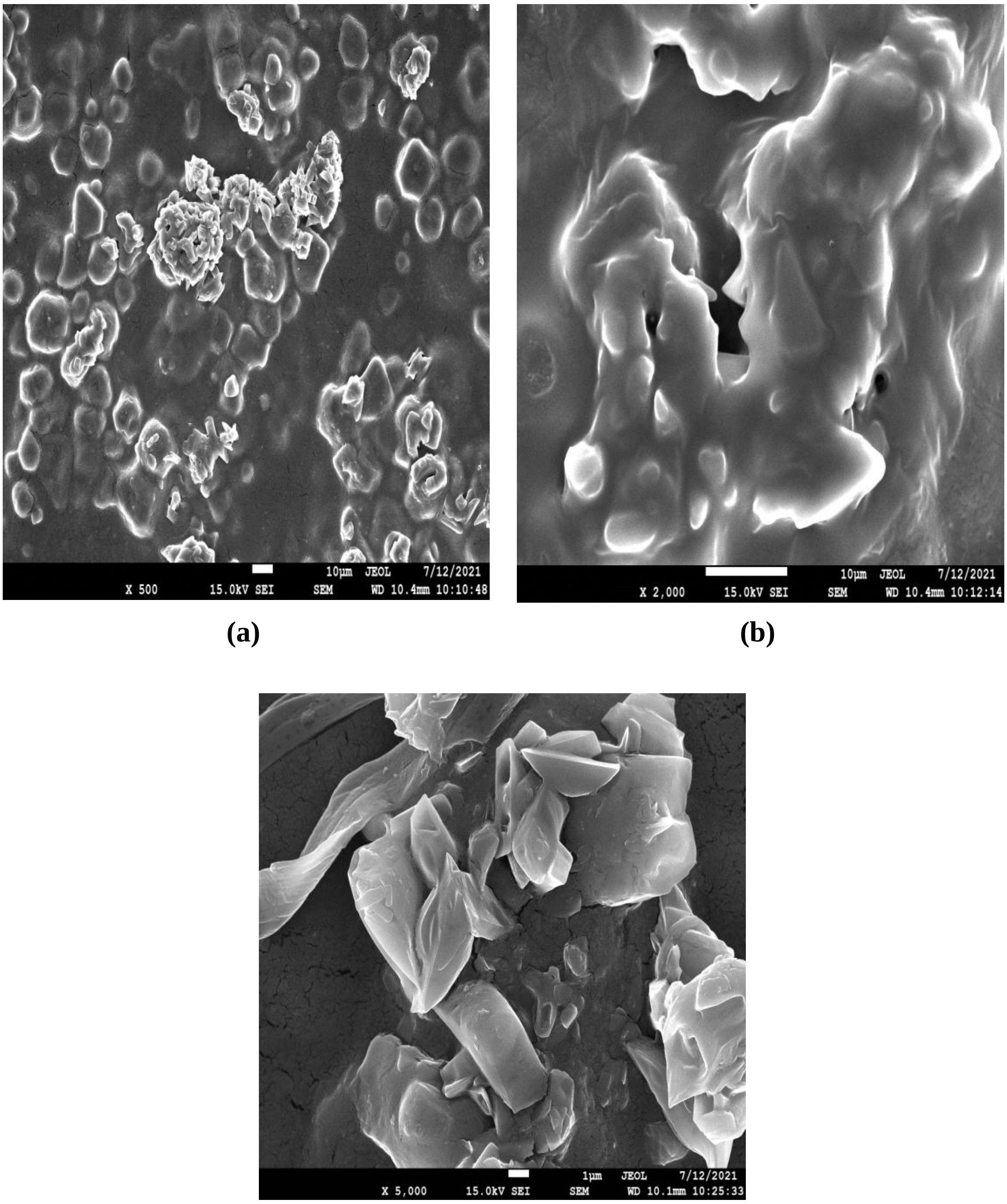
4.14 Morphological characteristics

Morphological analysis of ultrasonicated and non-ultrasonicated sample has been done with scanning electron microscopy imaging technique. Figs. 4.13 and 4.14 depicts scanning electron microscope images for control and ultrasonicated sample. The samples were not treated while development of biodegradable cup has bigger granule size in the matrix and forms rougher surface which is visible in Fig. 5.1. The rough surface is caused by partly gelatinized starch, which prevents fracture growth. Longer ultrasonic duration results in a smoother and more homogenous fracture surface. Also, the void and gap can be seen in images at 2000X. The starch ghost or non- solubilized part of starch are very big in size as can be seen in image 5000X. Whereas the cup samples formed utilizing ultrasonication has more homogeneous, compact and constricted structure, at 2000X it can be seen that voids and gaps present in the matrix are filled by the virtue of ultrasonication. Previously similar results have been reported by Abral et al., (2019), on sago starch-based film prepared utilizing ultrasonication. The similar outcomes were also described by Cheng et al., (2010), with film prepared using ultrasonication while gelatinization of maize starch. They stated that microscopic examination reveals significant rapture of ghost pieces in the dispersion as treatment duration is increased.

4.15 Thermal stability

Thermal stability of biodegradable cups is measured using thermogravimetric analyzer. Figs. 4.15 and 4.16 depicts the thermal degradation curves for control and ultrasonicated samples. When compared to initial weight of sample before testing, this result showed an increase in weight reduction. The weight loss percentage figures of the control sample when compared to that of ultrasonicated biodegradable sample, it is observed that the control sample loosed 23.76% weight in 9.9833 min whereas the ultrasonicated sample lost only 13.72% weight in similar duration of time against the thermal treatment. The degradation temperatures of untreated bio composite samples min were low as it includes fiber agglomeration and porosity formation in the matrix, resulting in decreased thermal stability (Asrofi et al., 2018).

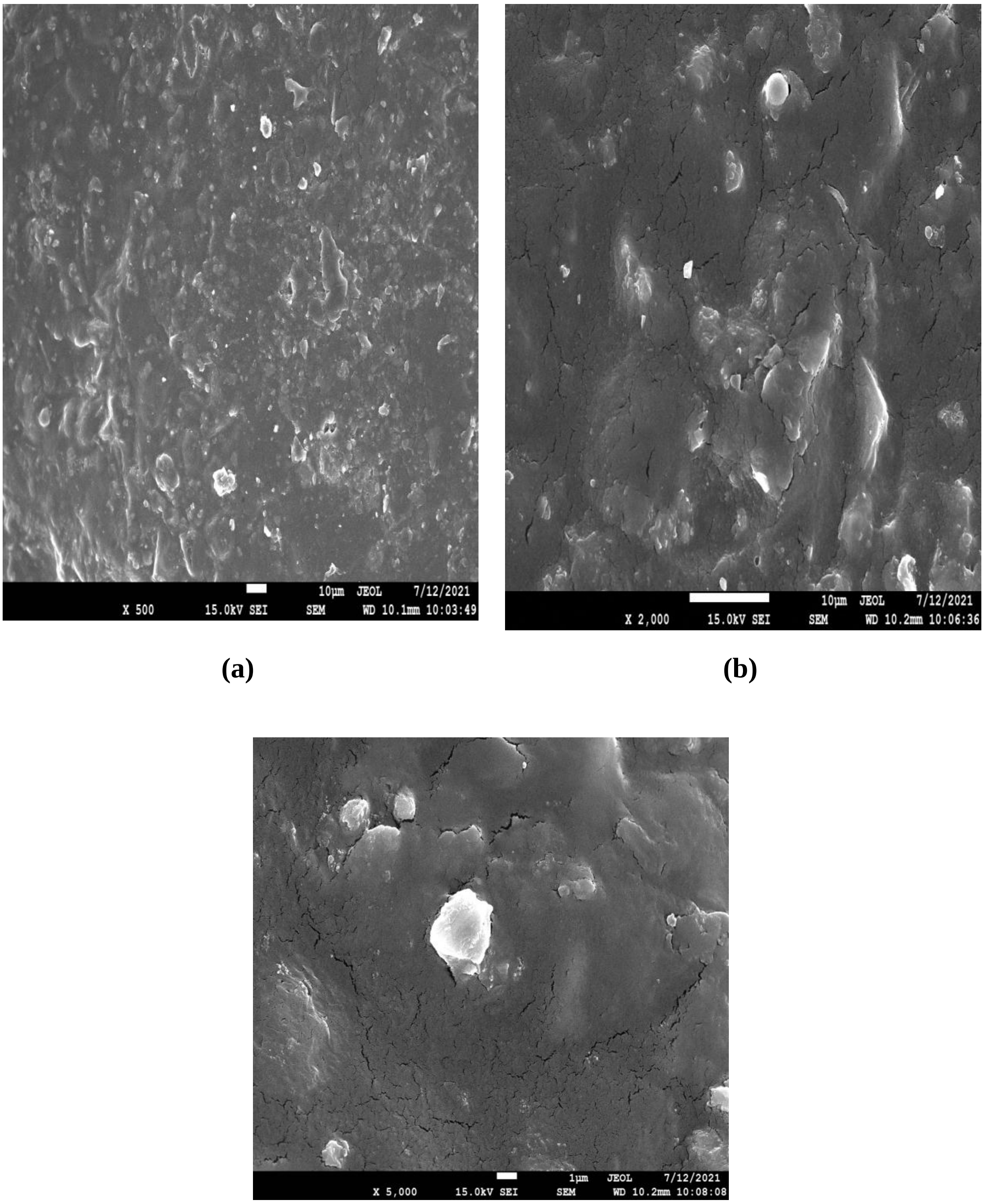
Enhanced vibration duration, on the other hand, increased the thermal stability of the biodegradable cup samples. This finding was supported by a rise in the degradation temperature of the ultrasonicated biodegradable cup samples compared to the non-treated cup samples, which was attributed to enhanced interfacial adhesion between the fibers and matrix as a result of the kinetic energy provided by ultrasonication (Asrofi et al., 2018; Mali et al., 2005).



(a) **(b)**

(c)

Fig. 4.13: Morphological analysis of non-treated /control biodegradable cup a)500X b) 2000X c) 5000X



(a) **(b)**

(c)

Fig. 4.14: Morphological analysis of ultrasonicated biodegradable cup sample

a)500X b) 2000X c) 5000X

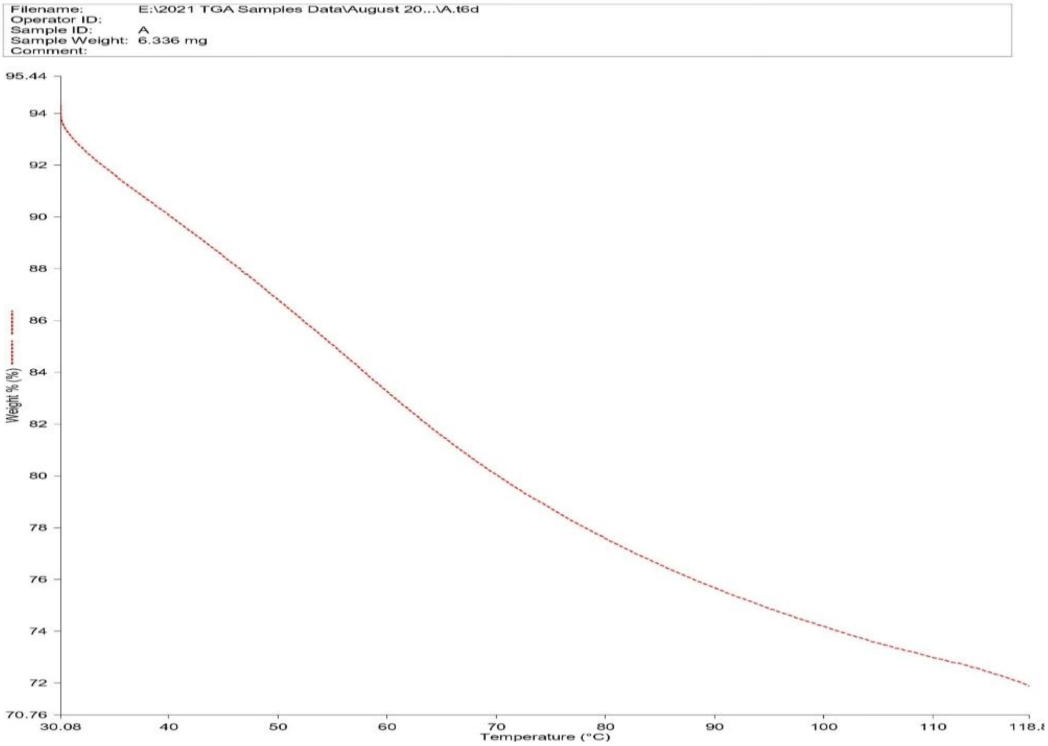


Fig. 4.15: Thermogravimetrical analysis of non-ultrasonicated biodegradable cup sample

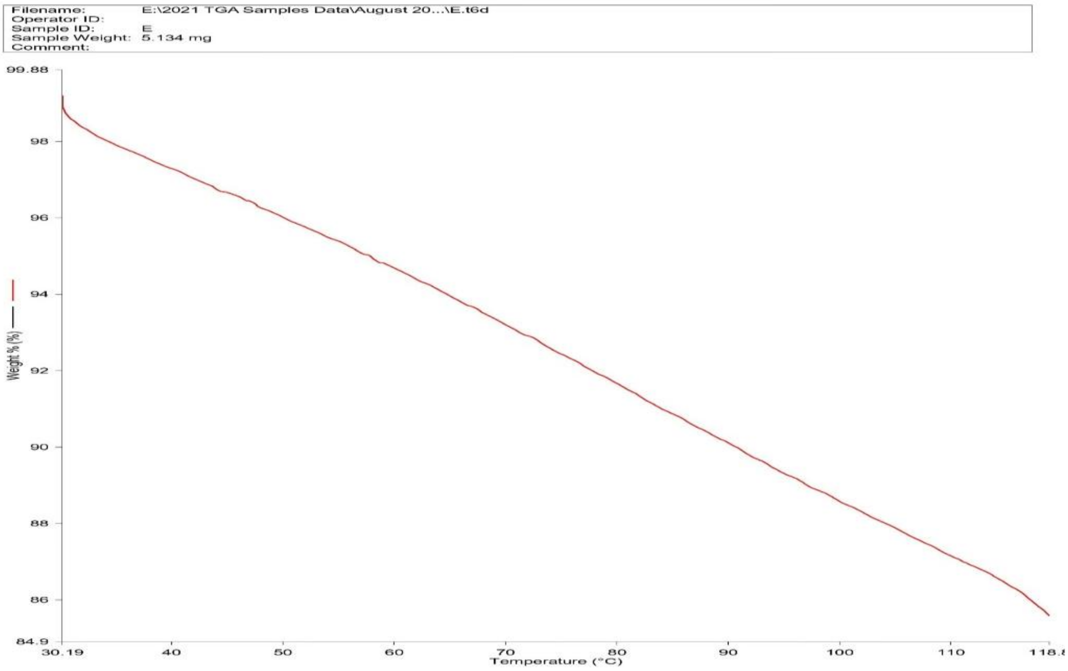


Fig. 4.16: Thermogravimetrical analysis of ultrasonicated biodegradable cup sample

CHAPTER V

SUMMARY AND CONCLUSIONS

The petroleum plastic packaging material which is generally utilized in dairy and food industries takes huge amount of time to degrade into soil and cause adverse environmental pollution. Many researches have made attempts on making of biodegradable film but very less is done on making stand able container out of this out of these biodegradable materials. Also, the applicability of these biodegradable packaging materials are very less in the industries as they lack in mechanical barrier and other properties against plastic packaging material. The current study was carried out to study the effect of ultrasonication on the properties of composite biodegradable cup. For the study different amplitude and time variations were taken. Selection of amplitude level and treatment time duration were done on the basis of reviews of literatures and preliminary trials. Amplitude level of 20%, 40 % and 60% whereas time duration of 1 min, 2 min, 4min, 8 min and 16 min is selected for the study. Various properties such as thickness, density, moisture content, opacity, tensile strength, puncture force, water vapour transmission rate, moisture absorption, solubility and biodegradability in soil were analyzed and compared with non-ultrasonicated biodegradable cup samples.

Following conclusions were drawn at the end of the research study:-

1. For the development of cup the viscosity of the casting solution was of prime importance. Viscosity of the casting solution were analysed using Brookfield viscometer. The viscosity of the ultrasonicated samples control sample was 2326± 14.8 cp which was reduced up to 536 ± 12.32 cp after ultrasonication
2. Thickness of Non ultrasonicated biodegradable cup sample was 0.813 ± 0.023 mm, which was reduced up to minimum level of 0.572 ± 0.016 (sample 15) by ultrasonication of amplitude 60% and 16 min.
3. Density of the biodegradable cup sample was increased up to 0.1267 ± 0.0002 g/mm^3 which was initially 0.0752 ± 0.0015 g/mm^3 for control sample
4. Mechanical properties of biodegradable cup is successfully enhanced by ultrasonication. The tensile and puncture force of the control sample was 0.932 ± 0.086 kgf and 645 ± 70.2 gf. The peak tensile and puncture force observed for

ultrasonicated samples are 3.204 ± 0.287 kgf and 1754 ± 150.58 gf for sample 12 and 15 respectively.

5. Water vapor transmission rate of biodegradable cup sample is reduced to 0.76 ± 0.0031 g/m^{2}h (sample 15), which was 1.33 ± 0.0442 g/m^{2}h for non-ultrasonicated sample.
6. Opacity of the biodegradable cup reduced with ultrasonication the the control sample had the opacity of 3.017 ± 0.02 AU/mm. Minimum value of opacity achieved was 2.56 ± 0.38 AU/mm with sample 14
7. The moisture content and moisture absorption also decreased with increased ultrasonication intensity. Least value of moisture content achieved was 14.9 ± 0.276 %, whereas the least moisture absorption (expressed as percentage weight gain) 8.97 ± 0.12 %, which was 18.37 ± 0.522% for the control sample.
8. Solubility of the biodegradable cup increased with the increasing ultrasonication intensity. The non-ultrasonicated sample had the solubility of 24.561 ± 1.166 % which was increased up to 42.075 ± 0.69 % (sample 15).
9. The rate of biodegradation decreases with increasing ultrasonication intensity. The control sample disappear in soil within 47 days. Maximum time is taken by sample with 60 % amplitude and 16 min time duration which was degraded only 93.2 % in 64 days.
10. Thermogravimetric analysis results shows that the ultrasonicated sample degraded less in same time as compared to control sample and resulted in more thermo stable structure.

Therefore, from the above results it can be concluded that ultrasonication while preparation of casting solution can improve the mechanical barrier as well as several other properties such as density, moisture absorption and resulted in more thermostable structure.

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VITA

Name of student : Ankit Kumar Deshmukh

Father's name : Mr. Vishnu Shankar Deshmukh

Mother's name : Mrs. Ishwari Deshmukh

Nationality : Indian

Date of birth : 10/09/1996

Permanent home address : Nutan Chowk, Shikshak nagar, Bhilai-3,
Distt.- Durg, Chhattisgarh, 490021

EDUCATIONAL QUALIFICATION

Bachelor's Degree : B.Tech (Dairy Technology)

University : Chhattisgarh Kamdhenu Vishwavidyalay,
Anjora, Durg, Chhattisgarh

Year of Award : 2019

OGPA : 7.57/10

Master's Degree : M.Tech (Dairy Engineering)

OCPA : 7.973/10

Scholarship/Awards : National Talent Scholarship (ICAR)