SAKARYA UNIVERSITY
INSTITUTE OF NATURAL SCIENCES

DEVELOPMENT OF NANOCOMPOSITE BIODEGRADABLE FILM CONTAINING IRON NANOPARTICLES BIOSYNTHESIZED BY *SACCHAROMYCES CEREVISIAE*

M.Sc. THESIS

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JANUARY 2022

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This thesis has been accepted unanimously / with majority of votes **by the examination committee on**

DECLERATION

I declare that all the data in this thesis was obtained by myself in academic rules, all visual and written information and results were presented in accordance with academic and ethical rules, there is no distortion in the presented data, in case of utilizing other people's works they were refereed properly to scientific norms, the data presented in this thesis has not been used in any other thesis in this university or in any other university.

Jazaer Al-HAYALI

12.11.2021

ACKNOWLEDGEMENT

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LIST OF ABBREVIATIONS

h	:	Hour
min	:	Minute
IONPs	:	Iron oxide nanoparticles
WP	:	Whey protein
WPC	:	Whey protein concentrate
WPB_F	:	Whey protein based film
XRD	:	X-ray diffraction
FESEM	:	Field emission scanning electron microscopy
g	:	Gram
TS	:	Tensile strength
E%	:	Elongation percentage at break
nm	:	Nanometer
FTIR	:	Fourier transform infrared
RH	:	Relative humidity
WVP	:	Water vapor permeability
WS	:	Water solubility
ΔE	:	Total colour change

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SUMMARY

Keywords: Edible films, iron oxide nanoparticles, green synthesis.

The present study has sheds light on the synthesis of iron oxide nanoparticles (IONPs) by *Saccharomyces cerevisiae* yeast as a green method by using the components of yeast cell walls as reducing agents to promote more beneficial effects in life applications. The main objective of the study is to examine the antimicrobial and mechanical properties of whey protein based edible films contained IONPs.

At the present study, IONPs were synthesized by using *S. cerevisiae* from iron chloride ($FeCl_3$) precursor at three different concentrations (0.5, 1.0, and 1.5 mM). These iron oxide nanoparticles and lysed yeast cells residue (YSR) were used with whey protein concentrate and glycerine to prepare nanocomposite edible films contained 0, 0.25, 0.5 and 0.75 mM IONPs. The results have shown successful synthesis of IONPs as hematite (Fe_2O_3) according to the XRD analysis with crystal average size (24-35 nm), while FTIR spectrum indicated the presence of IONPs with the protein matrix of the films. The FESEM images have shown the presence of nanomaterials in range between 43 and 127 nm. The films have shown zero inhibitory effects against the growth of tested bacterial strains. Tensile strength (TS) of the films containing 0.5 mM and 0.75 mM IONPs increased significantly ($P<0.05$) compared to the control films (without IONPs). E% at break and water vapor permeability of the films with IONPs decreased significantly ($P<0.05$) when compared to WPC films with no IONPs. The colour change (Δ) was increased significantly ($P<0.05$) in IONPs contained films as well as the whiting index and yellowing index.

In conclusion, incorporative IONPs into whey protein film showed no influence as antibacterial agent. Yet, IONPs enhanced the mechanical properties of whey protein based edible films.

SACCHAROMYCES CEREVISIAE TARAFINDAN BİYOSENTEZE EDİLEN DEMİR NANOPARTİKLER İÇEREN NANOKOMPOZİT YENİLEBİLİR BİYOÇÖZÜNÜR FİLMİN GELİŞTİRİLMESİ

ÖZET

Bu çalışma, ensüstriyel uygulamalarında faydalı etkileri teşvik etmek için *Saccharomyces cerevisiae* mayasının hücre duvarlarının bileşenlerini indirgeyici ajanlar olarak kullanarak demir oksit nanoparçacıklarının (IONP'ler) sentezine ışık tutmuştur. Çalışmanın temel amacı, IONP'ler içeren peynir altı suyu proteini bazlı yenilebilir filmlerin antimikrobiyal ve mekanik özelliklerini incelemektir.

Bu çalışmada, demir klorürden ($FeCl_3$) *S. cerevisiae* kullanılarak üç farklı konsantrasyonda (0.5, 1.0 ve 1.5 mM) IONP'ler sentezlendi. Demir oksit nanoparçacıkları ve parçalanmış maya hücreleri kalıntısı (YSR), 0, 0,25, 0,5 ve 0,75 mM IONP içeren nanokompozit yenilebilir filmler hazırlamak için peynir altı suyu proteini konsantresi ve gliserin ile birlikte kullanılmıştır. Sonuçlar, XRD analizine göre nanopartiküllerin kristal ortalama boyutu (24-35 nm) ve hematit (Fe_2O_3) olarak IONP'lerin başarılı sentezini gösterirken, FTIR analizlerinden elde edilen spektrum filmlerin protein matrisi içerisinde IONP'lerin varlığını göstermiştir. Aynı zamanda, FESEM görüntüleri, 43 ile 127 nm aralığında nanomalzemelerin varlığını göstermiştir. Filmler, test edilen bakteri suşlarının büyümesine karşı etki göstermemiştir. 0,5 mM ve 0,75 mM IONP içeren filmlerin çekme mukavemeti (TS), kontrol filmlerine (IONP'siz) kıyasla önemli ölçüde artmıştır ($P < 0.05$). Bununla birlikte, IONP'li filmlerin kırılma anında %E ve su buharı geçirgenliği, IONP'siz WPC filmlere kıyasla önemli ölçüde azaldığı görülmüştür($P < 0.05$). Beyazlık indeksi ve sararma indeksinin yanı sıra film içeren IONP'lerde renk değişimi (Δ) önemli ölçüde artmıştır ($P < 0.05$).

Sonuç olarak, peynir altı suyu proteini bazlı filmlerin içerisinde bulunan IONP'ler, antibakteriyel ajan olarak hiçbir etki göstermedi, fakat film içerisine eklenen IONP'ler, peynir altı suyu proteini bazlı yenilebilir filmlerin mekanik özelliklerini geliştirdiği gözlendi.

Anahtar Kelimeler: Yenilebilir filmler, demir oksit nanoparçacıkları, yeşil sentez.

CHAPTER 1. INTRODUCTION

Food is an ultimate essential source of life in humans. It provides all of the body requirements in energy production, structural fabrication and vital processes. On the other hand, foods are rich environment for the growth of microorganisms which can cause pathologies for the consumer [1]. Moreover, environment can cause undesirable changes to the foods, which in some cases can cause serious health problems [2]. Food packaging is quiet solution for food preservation and maintain the desired properties of the food products [3].

Nanotechnology has been introduced to the field of food science, due to the characteristics obtained by nanoparticles (NPs) which can potentiate in various lines of food industry such as processing, flavor or odor enhancement, quality improvement, packaging, etc. [4]. Metal oxide (MO) NPs have shown quite antimicrobial effects and due to this property MONPs have been used in food packaging. Zinc oxide and titanium oxide NPs were among the most extensively used in this field [5]. Iron oxide NPs (IONPs) comprises super-magnetic, non-toxic and biocompatible properties, due to that, IONPs can serve in several potential biochemical applications [6]. Several studies have reported that IONPs have shown to have antimicrobial activity against some strains of pathogenic microbes [7-9].

The green chemistry provides non-toxic approach for synthesizing NPs by using microorganisms [10] and plant extracts [11]. Biosynthesis of metal NPs and MONPs have shown great benefits over the other approaches such as coast-efficiency, simplicity, reproducibility, and production of more sustainable materials [5]. *Saccharomyces cerevisiae* is a non-pathogenic yeast found in many environments of

nature [12]. *S. cerevisiae* has also been used for the synthesis of metal and metal oxide NPs, including IONPs [13].

Whey protein concentrate based films (WPC) have been used extensively in food packaging field as edible films due to the characteristic they offer such as fat, flavor, oxygen and humidity barriers. Furthermore, WPC edible films have shown an enhancement, upon used, in the lifespan of certain foods by delaying the lipid peroxidation that lead to rancidity [14]. WPC films have been studied in combination with other materials to improve their properties [15]. Several studies have shown that WPC films containing MONPs would improve some of the mechanical properties of these films such as in the study of Gohargani et al. which reported an improved of some mechanical properties of the composite samples when TiO_2 NPs were added [16], and in another study the use of silver NPs has shown to enhanced tensile strength and water barrier properties of the films with high percentages [17].

This study was aimed to synthesis IONPs by using *S. cerevisiae* and to investigate the effect of IONPs and lysed yeast cell residue on mechanical, physical and antimicrobial properties of WPC films.

CHAPTER 2. LITRETURE REVIEW

2.1. Food Packaging

The early stage of human's history has recorded that the ancestors were search and collect the food from the environment, disregarding the need of foods protection and storing. However, when people settled in societies the need of food prevention and storing started to appear in these communities. Hence, people have started the search for suitable approaches to prevent the deterioration of foods [3]. Food packing has been found in glass containers dating back to 3000 BCE, as well as clay pots reaching back even further; both have been used for storing food and closed with paraffin, pitch, or cork. In the first century CE, leather bags for alcohol and milk, and also wood jars coated on the interior with pine resin, were in use [18].

The packaging of foods is an important element of process in the food industry because it protects food products from mechanical damage and decelerates biochemical degradation, while also changes produced by microbes. Administering the vapour and gas reciprocity with the outside medium, as well as limiting microbiological and chemical contamination, is the major protective purpose of food packaging, prolonging the quality of foods for long time and dimming the hazards for food safety. To put it another way, packing can prolong the shelf life of fresh items like meats, fruits, and vegetables by preserving the benefits of handling after the progression is completed [19].

The new food packaging concept with antimicrobial properties is designed to minimize quality losses caused by food spoilage [20]. The earliest innovations in the realm of packaging were the technology of electrical packaging, the flexograph print, and the

technology of aseptic and flexible packaging. Furthermore, the use of polypropylene, polyester, and ethylene vinyl alcohol polymers resulted in a considerable shift away from using metals and glass in packaging, in favor of bendable and plastic wrapping. Active packaging and intelligent or smart packaging were introduced in the twentieth century as advancements in packaging [21]. Consumer demands for packaging have resulted in innovative packaging. The packaging industry has developed a variety of niche markets in order to modify market opportunities [22].

Considerable research efforts have been devoted to improve the performance of packaging materials, resulting in a wide selection of materials for any application, capable of responding to the special demands of various food merchandises. Food-packaging systems must be tailored to each packaged foods, considering the following variables: food characteristics (quality performance parameters), quality-loss events (– for example, gas transfer, light permittivity, and so on.), manufacturing specific requirements, and marketing requirements. In addition, food-packaging systems must conform to food-contact component restrictions. Today's packaging materials must also be eco-friendly if it is to be successful. In order to provide maximum ecological effectiveness and decrease the danger of spoiling or damage to the product, packaging plays an important role in its long-term sustainability. A lot of effort is obliged to produce package "sustainable environment" while yet protecting food and delaying decomposition. As a result of the negative public perception of plastic, which is a major source of pollution in the environment, the idea of sustainable packaging has been largely sidelined. In fact, there is a pressing demand for a more appropriate methodology to packaging, one that weighs the undeniable environmental costs of package manufacturing and disposal against the great advantages in expressions of food safety and lowering the risk of waste. Certainly, many customers believe that food packaging is an excessive economic and environmental expense, as well as an unneeded source of solid waste, rather than a helpful waste reduction tool [23].

Over than 25 % of total of food is wasted due to improper packing, according to the World Packaging Organization. As a result, proper packing may help to cut down on

food waste. In addition, the present consumer desire for efficient and high-quality foodstuffs has heightened the influence of packaged foods [24, 25].

2.2. Edible Films

Edible films (EFs) are soluble substances that are placed to the exteriors of foodstuffs in a quiet way that a fine sheet of EF forms straight on the food surface or across separate component layers to inhibit wetness, oxygen, and solute absorption into the foodstuff [26]. Edible coatings were applied on the surface of food products by spraying, dipping, rolling, casting, foaming, and brushing of coating formula. to control mass transfer, , aroma losses, and gas permeability. EF have the potential to increase the freshness and quality of processed foods, and, in addition to preserving the mechanical and rheological properties and colour, [27].

Biodegradability is inherent in EF and coating materials. In fact, coupled with edibility, biodegradability is among the most important advantages of edible covering approach. The film types for agricultural usage (– for example, mulch, tunnels and bales wrapping), groceries packs, sheet coating, or cushion foam are all possible non-food implementations. Many EF functions are comparable to those of artificial packaging films; nevertheless, EF substances must be selected for food packaging purposes based on specific food applications, types of food products, and key quality deterioration mechanisms [28]. The need for films or coatings as principal packaging materials might partly or completely replace traditional packaging materials, reducing the overall consumption of synthetic materials [29]. EFs may improve the overall package structure because of their protective occupations [30].

Natural polymers, including polypeptide, carbohydrates, fats, and resins, are the most common film-forming materials. They could be used separately or in cooperation. The physiochemical properties of biopolymers have a significant impact on the qualities of the films and coatings that result [31]. To ensure edibility, film-constituting substances may be either hydrophobic or hydrophilic, or sometimes both; nevertheless, the

solvents used must be water or ethanol [32]. EFs are mainly composed of polymeric matrix, plasticizers and additives [28].

The most common polymeric matrix materials are hydrocolloids (polysaccharides and proteins) and lipids. Polysaccharides (i.e., alginate, pectin, or carboxymethyl cellulose) are the simplest to obtain and are more suited to forming films or coatings. Film formation is aided by the presence of a significant number of hydroxyl groups and hydrogen bonds.

Plasticizers are essential in EFs, particularly based on sugars and proteins. The latter film forms are frequently fragile and inflexible because of the tight linkages amid polymer molecules. The light-mass molecules, plasticizers, which are added to polymeric substances, constitute films to make them more thermoplastic. To improve flexibility and processability, they can place themselves between polymer molecules and interfere with polymer-polymer interactions [33]. Plasticizers increase the molecular mobility of polymer molecules or the free volume of polymer structures [31].

Additives are used to raise the standard, safety, nutritional value, organoleptic (colour, aroma, and flavor) qualities, efficiency, and economy of foods [34]. EFs can include emulsifiers, antioxidants, antimicrobials, nutraceuticals, tastes, and colorants, and can improve food quality and safety up to the point where the additives interfere with the films' physical and mechanical qualities [35].

2.2.1. Protein-based edible films

Protein EFs are often used to wrap little and individual proportioned meals and are suitable candidates for antibacterial and antioxidant agent carriers. They are effective oxygen barriers, however they are moisture prone [36].

Proteins are made up of unique amino acid sequencing and arrangements. When matched to other film-constituting substances, proteins are distinguished by their

denatured structures, the presence of ionic groups, and amphiphilic behavior. Heat denaturation, chemical interactions, pressure, enzymatic treatments, mechanical treatments, pH alteration, irradiation, salt accumulation or hydrolysis can all be used to change the protein structure. These procedures can be used to modify the physical and functional properties of protein films. In fact, enzymatic, chemical, and physical alterations can be used to modify the characteristics of protein-containing materials to meet the needs of certain applications [37].

Protein films have desirable qualities for packaging foods presentations, for instance these films are transparence and have high membrane characteristics versus carbon dioxide, oxygen, and lipid, despite their hydrophilic nature, which causes water sensitivity and a restricted water vapour membrane. Similarly, protein-based films can be used to incorporate a wide range of substances, including minerals, antioxidants, antimicrobials, antifungals, and flavors [38].

2.2.1.1. Films based on milk proteins

Milk proteins, including whey and caseinate proteins, have been intensively researched due to their high nutrient properties and diverse functional qualities that are critical for the creation of EFs. Because of their random coil structure and capacity to create substantial inter-molecular hydrogen, electrostatic, and hydrophobic interactions, caseinates can easily form films from aqueous solutions, increasing interchain cohesion. Furthermore, EFs made from milk proteins were observed to be flavorless, tasteless, and flexible, ranging from transparent to translucent depending on the composition.

2.2.1.1.1. Whey protein-based edible films

As a by-product of the cheese manufacturing process, whey proteins (WP) are those that remain in the milking fluid after the caseins were been congealed at a pH of 4.6 and a temperatures of 20 °C [39]. The WP is made up of a variety of proteins, the most

important of which are alpha-lactalbumin, beta-lactoglobulin, immunoglobins, and bovine serum albumin [40].

At low-to-intermediate relative humidity, the WPs create good oxygen, fragrance, and oil barrier coatings. Furthermore, when employed as layers on foodstuff, film splitting sheets of hetero-geneous (produced from diverse constituents) food, or film molded into bags for food constituents, the mechanical qualities of WP films are acceptable to ensure toughness. The protein concentration of industrially manufactured whey protein concentrates (WPC) ranges from 25 to 80 %. Whey protein isolates (WPI) are made from WPC and have a protein level of roughly 90%. They are made by an ion-exchange process [41].

Whey proteins-based EFs have grown in popularity as people become more conscious of environmental issues. In addition to serving as an edible packaging material, these films can also be used as a carrier for antioxidants, nutraceuticals, or antimicrobials. These films do not jeopardize the desired main membrane and mechanical characteristics of wrapping films, and so offer value for end-use marketable submissions [42].

When heated over 65 °C, the spherical configuration of β-lactoglobulin denatures. This oxidizes free sulph-hydryls, promotes hydrophobic bonding, and facilitates disulphide bond interchange by exposing the hydrophobic and sulph-hydryl groups. As a result, water-insoluble EFs might be made. Heat denaturation, which promotes micellar protein dissociation, aids the interaction between casein and whey proteins. Plasticizers added to the denatured film solution may enhance material flexibility, but they also enhance water vapour permeability (WVP). For effective functionality, the production of EFs based on WPC is compared to that of other protein products [43].

2.2.1.1.2. Casein-based edible films

With approximate concentration of 3 percent in milk, casein accounts for 80 percent of the overall component of milk proteins (Lacroix and Vu, 2014). Caseins are soluble and can form films that resist conformational changes either or both coagulate even in extreme temperatures, allowing the film of protein to stay established throughout an extensive range of pH, temperature, and salt content. Owing to its arbitrarily coiled shape and capacity to create significant intermolecular hydrogen, hydrophobic, and electrostatic interactions, from aqueous caseinate solutions, films may be readily formed, as a consequence, interchain cohesiveness is increased. Those latter coatings are soluble in water, but become insoluble when subjected to a buffer solution with a pH of 4.6 [44].

Casein films for packaged foods have a reduced oxygen permeability and high strength, but they are less flexible and more moisture sensitive than other protein films. By altering the casein-based films according to environmental conditions, they might be employed in a range of commercial applications. Pectin may be incorporated to casein film formulations t, depending on the application and desired film characteristics [45].

2.2.1.2. Films based on egg proteins

After water, eggs albumen is the next most essential component of fluid egg white. It comprises about 10% of the overall mass of fluid egg white. Egg albumen has five major protein fractions: ovomucoid, ovotransferrin, ovalbumin, ovomucin, and lysozymes [40].

Egg white proteins unfold at 60°C, exposing intracellular sulph-hydryl groups that might cause disulphide bond construction and increasing the hydrophobic nature of the surface [46]. pH, salt content, sucrose, and preheat management can all alter the conformational changes of egg white proteins. Ovalbumin develops s-ovalbumin, another stable form of the protein with a forward conformational change, as a result of thiol interactions with temperature and time [47].

The usage of egg whites in coating material is of unique nutritional significance due to egg whites' antioxidant properties. Egg whites are used to make edible packages because they are clear and translucent, and their characteristics are comparable to those of other proteins [27].

2.2.1.3. Other protein based films

Gelatine is a protein with heavy molecular mass generated by hydrolysing collagen at standardized conditions [48]. Gelatine has lately attracted a lot of interest as a starting material for edible coatings due to its availability, biocompatible, component of the economy, and ability to produce composite films. When compared to commercial petroleum-based films, gelatine films were found to have high oxygen barrier qualities but low water and water vapor barrier capabilities [49]. Nevertheless, gelatine films' characteristics can be increased through blending and/or combining with several materials Fan et al. (2018) reported that water resistance of salmon skin gelatine combined with zein protein increased due to the hydrophobicity of zein protein [50]. When gelatine is used to coat foods, it can help minimize the amount of water lost by a variety of items, including bananas, apples, shrimp, and pork [51].

Soy protein is another type of proteins used in the fabrication of EFs. Most soy proteins can be classified as globulin. Soy protein-based films can be prepared at either acidic or alkaline pH; however, soy protein isolate films prepared at alkaline pH have better mechanical properties. Interest in soy protein films and coatings containing natural antimicrobials for food preservation is increasing [27].

Several other proteins involved in manufacturing of EFs such as myosin, actin, keratin, wheat proteins, and zein protein [40].

2.3. Nanotechnology and Nanoparticles

Nanotechnology and nanoscience are modern approaches which gained a great interest due to the widespread applicable activates. It all started in 1959 after the publishing

of Feynman's work entitled "There's Plenty of Room at the Bottom" which followed by a revolutionary advancement in the field of nanomaterials and its applications [52]. The area of nanotechnology is concerned with the development and use of chemical, physical, and biomedical fields with structural dimensions ranging from individual molecules or atoms to submicron, as well as the assimilation of such nanomaterials into complex networks [53].

The term "nanotechnology" refers to systems that is applied on a nanoscale and has practical applications. It is described as the manipulation or reorganization of atomic scale and molecular scales between 1 and 100 nm in size [54]. Metallic nanoparticles (NPs) have different physical and chemical properties from bulk metals [55]. The physicochemical characteristics of nanostructures are often altered due to the quantum entrapment of electrons in tiny nanostructures and a term of improvement of outer edge atoms or ions to those contained inside a specific particle. As a consequence of the increased effective surface area of nanostructures, the number of empty coordination sites, defects, and crystal lattice stresses increases. As a consequence, the surface atoms and ions have a distinct coordination environment, resulting in altered physical and chemical characteristics for a variety of nanostructures. The following are some examples of such changes: alteration of spectrometric characteristics, heat resistance, specific gravity, dissolution rate, material performance (tension, flexural, flexibility), electrical properties, surface tension, altered responding to magnetisation, alteration of crystalline structure (some crystal structures are only stable at the nanoscale), or enhanced catalytic properties of nanostructures [56]. The menial nanoparticles find application in several areas including catalysis, biomedical, biosensor, solar cell, ceramic, textile, electronics, water treatment and polymer nano-composite. The production of nanoparticles occur either top-down (breaking) or bottom-up (building), [57].

2.3.1. Classification of nanoparticles

Nanoparticles are broadly classified in to three classifications [58, 59]:

One dimension nanoparticles: One-dimensional (1D) nanoparticles are those with three dimensions on the nanoscale and additional dimension outside of that range. Carbon nanotubes nanowires, nanofibers of metal oxides, metal nanorods, polymers or metal nitrides are all forms of 1D nanoparticles. Carbon, boron, or transition metal nanotubes are distinguished 1D materials having a high aspect ratio and exceptional electrical characteristics.

Two dimension nanoparticles: 2D nanoparticles have a nanometer-scale dimension and a micrometer-scale dimension. They are the most extensively studied class of NPs and have a wide range of applications due to their extraordinary characteristics. Hexagonal boron nitride, graphene, metal carbides, metal chalcogenides, metal nitrides, metal thin films, graphitic carbon nitride, and nanoplates are the benchmark 2D nanomaterials.

Three dimension nanoparticles: Although 3D nanomaterials lack nanometer-scale dimensions, their internal morphology and characteristics are nanometer-scale. They are primarily self-contained, template-free or substrate-free materials having a large surface area, high electrical conductivity, and high catalytic properties. Graphene aerogel, graphene foam, graphene sponges, metal foams, and metal nano-flowers are all examples of common three-dimensional nanostructures.

The NPs has other classifications as following [60]:

According to origin:

- − Natural
- − Anthropogenic

According to chemical composition:

- − Inorganic substances
- − Organic substances

– Elements of the living kingdom

2.3.2. Metal oxide nanoparticles

Because of the quantum confinement and greater obtainability of surface atoms than inside atoms for participation in any reaction, metal oxide nanoparticles (MONPs) display distinctive chemical, physical, electronic, and optical characteristics in comparison to their bulk equivalents. Metal oxide nanoparticles are used in a variety of ways depending on their surface area, photocatalytic activity, size, crystallinity, anti-corrosiveness, shape, conductivity, and stability [61]. Metal oxide NPs with strong controlled properties during synthesis are particularly wanted for commercialization. MONP synthesis methods can be split into two classes in general: Ball milling, sputtering, laser ablation, electrospraying, and electron beam evaporation are examples of physical procedures, whereas sol–gel, polyol, hydrothermal, co-precipitation, microemulsion technique, and chemical vapor deposition are examples of chemical methods [62].

2.3.2.1. Iron oxide nanoparticles

Iron oxide nanoparticles (IONPs) are involved in the biological system and serve a vital function in meeting daily needs. Numerous nanoparticles support advancements in the area of biological application. Iron oxide nanoparticles are used extensively in a variety of applications, including gas sensor, electrochemical, magnetic, energy storage, magnetic storage and cancer therapy, as well as biological treatments. One of the several varieties of NPs, hematite (iron oxide) is a common, natural, and ecologically benign material [63].

There are dissimilar phases of IO crystallites demonstrated as hematite (α-Fe$_2$O$_3$), maghemite (γ -Fe$_2$O$_3$), goethite FeOH (OH) and magnetite (Fe$_3$O$_4$) [64]. Hematite is anticipated to exhibit the most stable n-type semiconductor characteristics. Currently, researchers are focusing their attention on the biological application of stable hematite iron oxide. The reason for selecting this specific α-Fe$_2$O$_3$ (hematite) IONPs is solely

owing to its inexpensive cost and non-toxicity. The band gap of this hematite IONPs is 2.1–2.2 eV [65].

The effect of eco sustainable IONPs on biological systems can be investigated. Numerous researchers have made various attempts in biomedical applications in this regard [66]. The application of magnetic and anti-ferromagnetic IONPs in biological applications dates back more than four decades. Using nanoparticles as antibacterial agents, it is possible to destroy certain bacterial species while inflicting no harm to the host body cells. IONPs have lately been employed as sensors for metabolites, hyperthermia, and biomolecules, as well as for toxicity and magnetic nano-toxicology research and development [63].
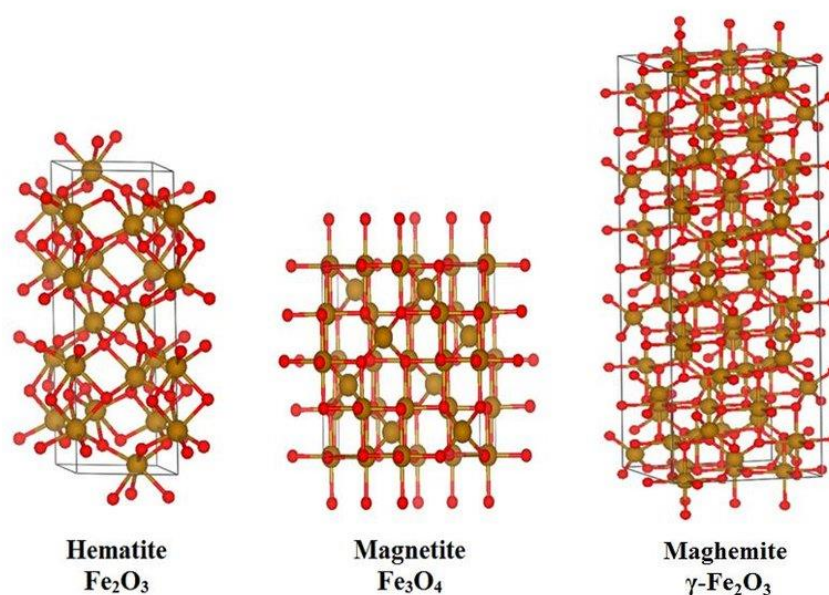

Figure 2.1. Crystal structures of IONPs [67].

2.3.3. Green synthesis

The term "green nanostructured manufacturing" describes the process of synthesizing various nanostructure employing biomolecules including plant sources, microbes, and a variety of biomass resources such as agricultural wastes, fruit peel trash, egg end up wasting, and others [68].

It has been determined that nanoparticles manufactured by biological processes have distinct features that distinguish them from nanoparticles made by physical and chemical methods. NPs can be manufactured using a variety of physical and chemical techniques [69]. Nevertheless, these procedures are capital intensive and have numerous drawbacks, such as the consumption of poisonous solvents, the formation of dangerous side products, and the inaccuracy of the exterior construction, among others [70]. The component ambiguity and lack of predictability of chemical methods, which are generally composed of several chemical substances or molecules, have the potential to increase particle reactivity and toxicity, as well as to harm human health and the environment, as a result of their composition [71].

Green synthesis produces particles that are unique from those generated by conventional physical and chemical processes. When it comes to the synthesis of metallic or oxide NPs, green biosynthesis, which would be a bottom-up technique, is comparable to chemical reduction in that an extraction of a natural substance, such as leaf of vegetation or flowers, is used in place of a chemical reductant. There is enormous potential for the generation of NPs in biological organisms. Through biogenic reduction, metal precursors are converted to their corresponding NPs in an environmentally benign manner [72], sustainable [73], free of chemical contamination [74], inexpensive [75] and this is suitable for large manufacture [76]. Additionally, the biologic generation of NPs enables the recycling of precious metal compounds found in waste streams, including gold and silver. Due to their rarity, these metals have varying values [77].

The biological molecules that stabilize NPs, which are primarily proteins, enzymes, sugars, and even whole cells, make it easy for NPs to interact with other biological molecules and, As a consequence, as previously indicated, boost the antibacterial efficacy of NPs by optimizing their interaction with microbes [78]. Due to the biological growth of NPs, separating them from the reaction medium or increasing their concentrations by centrifuge is straightforward [79]. When contrasted to chemically manufactured silver NPs, biogenic silver NPs had 20 times greater

antibacterial activity [80]. For the purpose of producing NPs, plant extracts are chosen for their added value as compared to other bioactive materials. In addition to exhibiting reducing agent capabilities, the algal cells of *Spirulina platensis* also exhibit pharmacological and nutraceutical properties, which made them an excellent choice for this study [81].

Single-celled microorganisms and extract of eukaryotic organisms transform metal precursors into NPs of specific shapes throughout the reaction phases [82]. Additionally, biological entities possess encapsulating and stabilising molecules that function as growth regulators and hinder the process of accumulation [83]. The size and structure of NPs are influenced by the nature of biotic factors and their concentrations in addition of organic reducing agents [84]. Furthermore, the size and shape of NPs are greatly influenced by factors such as pH, temperature, salt concentration, and exposure duration in the development medium [85]. For the creation of nanomaterials, bio-reduction of metal precursors occurs in vitro or in vivo. Enzymes, proteins, carbohydrates, and phytochemicals such as flavonoids, phenolic, terpenoids, cofactors, and others, on the other hand, mainly act as reducers and stabilizers [82, 83].

Bacteria, yeast, fungi, algae, and plants have all been used to produce NPs *in vivo* [71, 83]. *In vitro* synthesis, which entails purifying bioreducing mediators and combining them into an aqueous-solution of the necessary metal originator in a regulated manner, is generally done with biological extracts. At room temperature, the reaction occurs spontaneously [86] however, further heating and stirring may be required on occasion [87].

2.3.3.1. *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is a species of yeast that can be abundantly found in ecological systems, although it is primarily farmed for use in the wine and food industries. When administered in sufficient numbers, live *S. cerevisiae* colonies have been shown to provide health benefits to the subject. *S. cerevisiae* has been used as a

functional food and nutritional supplement for a long time. The physiological activities and health impacts of bioactive secondary metabolites of *S. cerevisiae*, including as naringenin, reticuline, artemisinin, and other pigments, have been demonstrated in humans. Typically, *S. cerevisiae* has been utilized as an immunological booster, neuroprotective, antidiabetic, antioxidant, anti-inflammatory, antitumoral agent, and antimalarial [88].

S. cerevisiae is one of the most extensively studied single celled model organisms in molecular and cell biology, since its cell structure and functional organization bear a high degree of resemblance to those of higher-level species. Additionally, yeast has a rapid growth rate and is readily grown [89].

It has been reported that *S. cerevisiae* has the ability to produce metal NPs. For example, gold ions have been shown to be attached to the cell walls of dead yeast S. cerevisiae cells and then decreased *in situ* [90]. In different experiment, [91] revealed that *S. cerevisiae* yeast was able to produce spherical antimony trioxide (Sb_2O_3) nanoparticles. Spherical amorphous iron phosphate NPs with broad size distribution of 50 – 200 nm were formed within *S. cerevisiae* yeast cells exposed to $FeCl_3$ solution [92]. Other investigations have demonstrated the biosynthesis of nearly spherical extracellular TiO_2 nanoparticles by *S. cerevisiae* [93].

2.4. Nanotechnology and Edible Films

Acquiring hybrid composites from the combination of distinct biopolymers, biopolymers and lipids, or natural polymers and solid materials has been one of the greatest commonly used approaches for tailoring film properties and thus developing EFs that meet the desired functional features and requirements for use as food packaging (non-soluble material includes hydrophobic proteins, fibers, organic, and/or inorganic nanoparticles) [94].

The primary objective is to maximize the unique properties of each component while also using their collaboration when feasible [95]. Composite films have a

heterogeneous structure, which means they are composed of several sheets or a continous matrix with specific additional phases [95, 96]. Multi-layered films and coatings often have superior mechanical and barrier efficiency than emulsion-based films and coatings, but they need further process of distributing or lamination and drying for each layer, which can result in layer delamination [97].

A study has synthesized bionanocomposite films from fish gelatin (FG) with sphere nanofiber (CSNPs) ranging in size between 40 to 80 nanometers. The addition of CSNPs to FG film enhanced the water vapor membrane, tensile strength (TS), and deformability, which was attributed to the particles' uniform distribution within the biocomposite matrix at low volume fraction (below 8%, w/w), which enhanced the hydrogen - bonded engagement between CSNPs and FG. Additionally, the inclusion of CSNPs caused a significant reduction of WVP, culminating in a 50percentage reduction with 6percent (w/w) fill. When compared to a control film containing 0% CSNPs, composite films exhibit decreased transparent at 600 nm but exhibit higher UV radiation barrier properties. The results of this research indicate that bionanocomposite technology may be utilized to improve the characteristics of biocomposite films composed of fish gelatin [98].

CHAPTER 3. MATERIALS AND METHODS

3.1. Materials

The chemical materials and the microbial strains that have been used in the study are listed in Table 3.1. along with their provider.

Table 3.1. Materials used in the study.

No.	Material	Supplier
1	*Aspergillus niger*	Department of Food Engineering / Sakarya University
2	*A. sydowii*	
3	Calcium sulphate ($CaSO_4$)	Merck, Darmstadt, Germany
4	*Cronobacter sakazakii*	Department of Food Engineering / Sakarya University
5	*Escherichia coli*	
6	Glycerol	Merck, Darmstadt, Germany
7	Iron chloride ($FeCl_3$)	
8	*Listeria monocytogenes*	Department of Food Engineering / Sakarya University
9	*Pencilium expansum*	
10	*Salmonella* Enteritidis	
11	*Staphylococcus aureus*	
12	Tryptic soy agar (TSA)	Merck, Darmstadt, Germany
13	Tryptic soy broth (TSB)	
14	Whey protein concentrate 80%	Milkas, İstanbul
15	*Williopsis saturnus var*. saturnus	Department of Food Engineering / Sakarya University

3.2. Equipments

The tools and instruments that have been used in the study are listed in Table 3.2. along with their models.

Table 3.2. Tools and instruments used in the study.

No.	Instrument	Model
1	Colour testing device	PCE-CSM 7
2	Field emission scanning electron microscope	Jeol JSM-6060-LV
3	Fourier transform infrared spectrometer	Shimadzu IR Prestige-21 spectrometer, Japan.
4	Mechanical testing machine	Instron Universal Testing 3367, USA.
5	Micrometer	Pisttsburg, USA.
6	UV-Vis Spectrophotometer	Shimadzu UV-Visible 160A, Japan.
7	Water bath	JSR, JSSB-30T, Korea.
8	X-ray diffraction	PANalytical X'pert Pro MRD Diffractometer

3.3. Biosynthesis of IONPs from *S. cerevisiae*

The biosynthesis of IONPs was carried out in a modified method of the original method of Zhu et al. (2017). *S. cerevisiae* was grown in TSB with yeast extract (0.6%) at 30°C for 48 h. After incubation, from this culture 100 µL were transferred to 40 ml TSB in centrifuge tubes and left for incubation at 30°C for 48 h. Following incubation, the tubes were centrifuged at 9000 rpm at 4°C for 10 min and washed three times with 30 mL of sterilized distilled water (DW). The pellet containing pure yeast cells was dispersed by 40 mL of three concentrations (0, 0.5, 1.0, or 1.5 mM) of iron chloride ($FeCl_3$). After the tubes were incubated in a shaking incubator (120 RPM) at 27 °C for 72 h the tubes were centrifuged at 9000 rpm at 4°C for 10 min and the cells were washed three times with sterilized DW (30mL).The yeast cells contain IONPs in 40 mL sterile DW was ultrasonicated (19920 Hz) for 2 min to extract IONPs from yeast cells. Homogenized suspension was stored in a freezer at -18°C.

Figure 3.1. The prepared IONPs at 0, 0.5, 1.0 and 1.5 mM from *S. cerviseai.*

3.4. Characterization of IONPs

3.4.1. UV-Vis spectrum of IONPs

The absorbance of the solutions containing IONPs was measured by using Shimadzu UV-Visible 160A spectrophotometer equipment in range of 200-760 at 1 nm measuring interval. The solutions of IONPs were prepared by dissolving IONPs powder in DW.

3.5. Preparation of whey protein concentrate-based edible film

The edible films were prepared by a modified method from the method of [99]. Seven grams of whey protein concentrate were added to 60 mL of DW. After addition of 40 mL of the prepared suspensions (0, 0.5, 1.0, or 1.5 mM IONPs and yeast cell wall components) to the film solution, pH of the film solution was adjusted to 8.0 with 0.5 N NaOH. The solution was heated in a shaking water bath (JSR, JSSB-30T, Korea) at 90 °C for 30 minutes. During the last 5 min of heating, 2.0% (w/v) glycerol was added at the final concentration as a plasticizer. The final IONPs concentrations in the film solutions were 0, 0.25, 0.5 and 0.75 mM. DW (without the presence of IONPs and yeast cell wall) was used to prepare the WPC-0mM film. Air bubbles within the film solutions were eliminated by using vacuum pump for 20 min. The prepared film solutions were casted in pre-sterilized Teflon dishes (63.59 cm^2 in size) and kept to dry

for 48 h at 24 °C, and 50% RH. After drying, the films were peeled and stored for 72 h under the same conditions.

3.6. Characterization of Nanocomposite Films

3.6.1. X-Ray diffraction spectra of the nanocomposite WPC films

The x-ray diffraction (XRD) technology was used to determine the crystal configuration and size of IONPs in the film structure using PANalytical-X'pert-Pro-MRD Diffracto-meter at Sakarya University, Turkey. The measuring was done at 2θ range from 30 to 80 degree. The speed of scanning was 0.4 degrees in minute at room temperature. The Debye-Scherrer equation was used to calculate the average size of crystals of IONPs [100].

3.6.2. Morphology of the nanocomposite WPC films

The morphological characteristics were observed by Jeol-JSM-6060-LV FESEM device at 20 kV voltages accelerating in high vacuum. Horizontal and vertical visualization of the film powders were taking at different magnification. Fourier transfer infrared (FTIR) spectrum of each film powder was obtained by using Shimadzu-IR-Prestige-21 spectrometer.

3.6.3. Antimicrobial activity of the nanocomposite WPC films

The disc diffusion assay was used for the examination of antimicrobial properties of the prepared edible films. The discs with a diameter of 10 mm were used for the growth of bacteria on TSA, containing TSA plates inoculated with 10^7 CFU/g of *E. coli*, *C. sakazakii*, *S. aureus*, *L. monocytogenes*, *S.* Enteritidis, *W. saturnus* var. saturnus, *A. niger*, and *A. sydowii*, and *P. expansum*. Tested bacteria, yeast and mold cultures were incubated at 35, 25, and 25 °C for 2, 3 and 5 days, respectively.
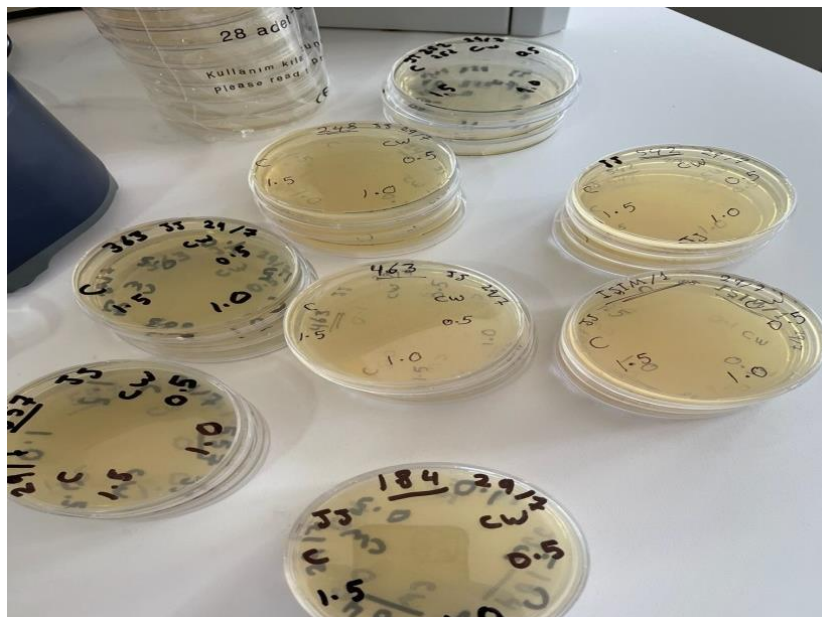
Figure 3.2. The plates of bacterial growth that used in the study.

3.6.4. Mechanical properties of the nanocomposite WPC films

The thickness of films was measured at eight different locations by using a micrometer (Pisttsburg, USA). Tensile strength (TS) and percent elongation at the break (E%) of the films were determined using Instron Universal Testing 3367 (USA). Each film was cut to strip with dimension of 15×100 mm and kept under 25 °C and 50 % RH until analysis. The measuring was carried out by placement of the strip between the device's jaws with pulling speed of 200 mm/s [99]. The maximum force applied to the specimen at the time of fracture and the strain at fracture were determined using the Bluehill 2 (Norwood, MA, USA) program.

3.6.5. Water solubility of the nanocomposite WPC films

The solubility of the films in water (WS%) was measured according to the method described by [99]. The film samples were cut into fragments (area= 2×2 cm), and the fragments were dried at 105 °C for 24 h. After drying, the fragments were flooded in 50mL DW at 23 °C for 24 h. When the 24 h passed, the fragments were filtered and dried at 105 °C for another 24 h. The WS% was calculated by using the mathematical equation (3.1) described next page:

$$\%WS = \left[(m_1 - m_2) \times (m_1)^{-1} \right] \times 100 \tag{3.1}$$

where m_1 is the dry weight of the fragment before flooding in water and m_2 is the dry weight of the fragment after flooding in water.

Figure 3.3. Fragments of films during water solubility experiment.
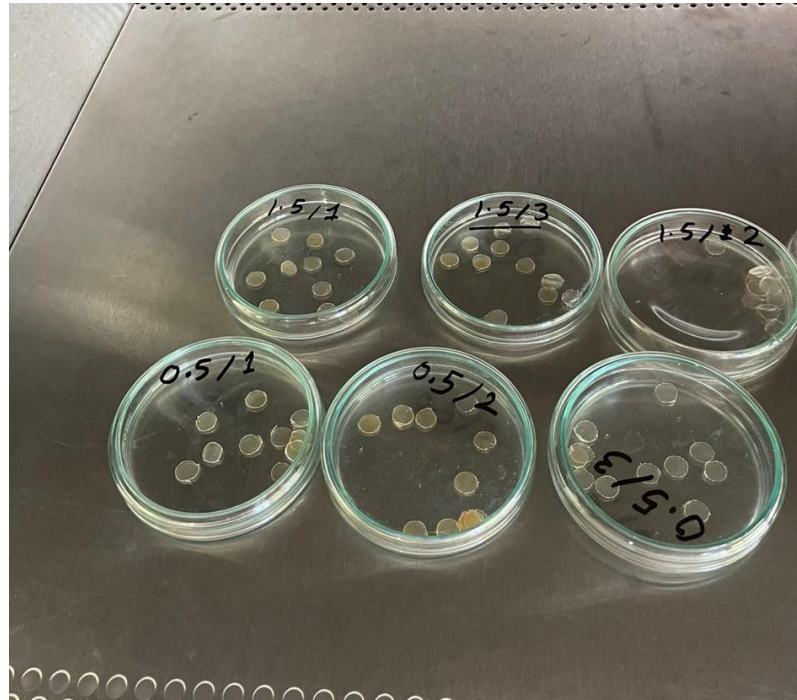
3.6.6. Water vapour permeability of the nanocomposite WPC films

The water vapor permeability (WVP) of the prepared edible films was measured according to the standard ASTM 96-80 assay [100]. Ten grams of $CaSO_4$ were added to glass beaker and sealed by the prepared films (Adhesive tape used on the edges to insure the sealing). Then the beakers were placed in a chamber at 37 °C, and 85% RH. After that the weight of the beakers was monitored each hour until 10 hours. The water vapour transmission rate (C) was calculated by drawing line in the points between the time per day on the X axis and weight of beaker on the Y axis, the slope of the line was equal to C (g/day). WVP was calculated by using the mathematical equation (3.2) described below:

$$WVP = (C \times T) \times (A \times \Delta P)^{-1} \, g.mm \, / \, m^2.day.kPa \tag{3.2}$$

where T is the thickness of the film (mm), A is the film's area (63.59 cm^2), ΔP is the partial vapor pressure variance across the two sides (5.94 kPa, a constant number calculated based on temperature and humidty in the chamber).

3.6.7. Colour of the nanocomposite WPC films

The determination of the colour properties was achieved by using PCE-CSM 7 devise at six different locations within the film on the bases of Hunter colorimetric system. The total colour change (ΔE), whiteness index (WI) and yellowing index (YI) were calculated according to the mathematical equations (3.3), (3.4) and (3.5) respectively:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \tag{3.3}$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \tag{3.4}$$

$$YI = \frac{142.86b}{L} \tag{3.5}$$

where L*, a* and b* are the lightness, redness, and yellowing parameters respectively (the star represents the values of standard white plate and the letters alone represents the values of measured films). Values of standard plate were; L* = 94.60, a* = 0.40, and b* = 0.30.

3.7. Statistical analysis

The results were repeated three times for each test (N=3) and statistically processed by using the SPSS program version 26.0 of IBM company. The values are expressed in the form of mean±standard deviation (SD) and the means comparisons were analysed by using analysis of variance (ANOVA) and the post-Tukey's test to determine the differences between the groups. The study groups classified as WPC (zero amount of

IONPs and yeast cell residue), WPC-No IONPs (zero amount of IONPs), WPC-0.25 mM IONPs, WPC-0.5 mM IONPs and 0.75 mM IONPs.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Characterization of IONPs

4.1. UV-Vis spectrum

The clear solution of FeCl3 has become reddish-brown color at the end of 72 h of incubation with biosynthesis of IONPs by *S. cerevisiae*.The solutions were prepared by dissolving films in DW, Figure 4.1. shows the absorbance of IONPs solutions corresponding to wavelength. Positive association has found between IONPs concentration and absorbance of the solutions. The absorption curves of all IONPs has shown an intense peaks in the range 450-600 nm which is close to hematite data in other works [101-103]. The peaks around 446 nm corresponds to the surface plasmon resonance (SPR) band of hematite [104].

Figure 4.1. UV-visible spectrum of IONPs biosynthesized by *S. cerevisiae*.
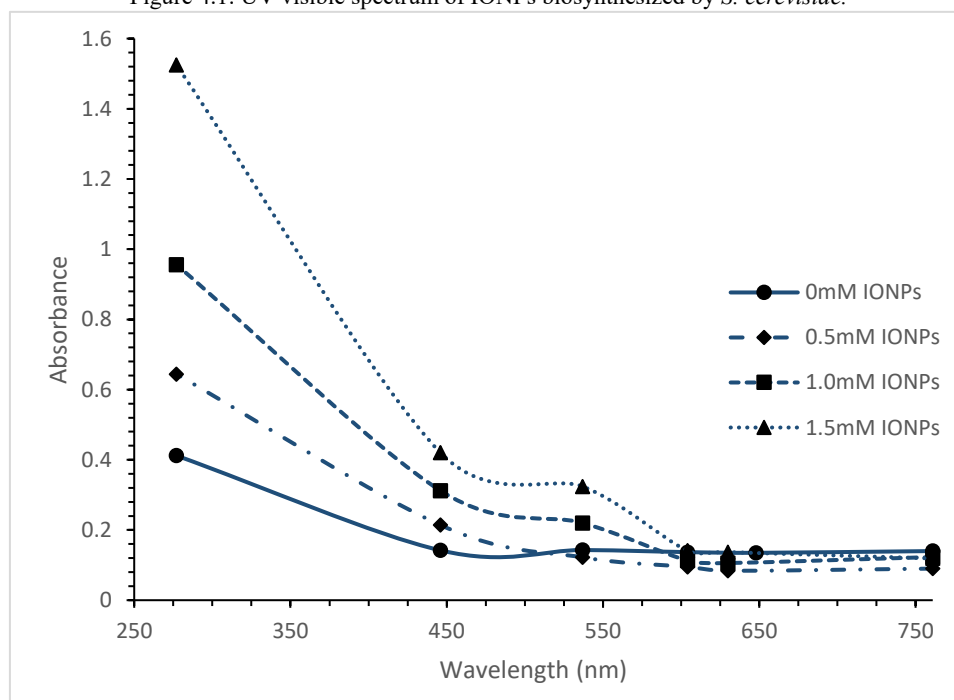
4.2. Characterization of the nanocomposite WPC films

4.2.1. XRD analysis of the nanocomposite WPC films

X-ray diffraction analysis was applied to determine the crystalline nature of synthesized IONPs. All the patterns exhibit the characteristic XRD pattern of hematite (Fe_2O_3) nanoparticles (ICDD card no. 33-0664). X-ray diffractograms of synthesized NPs showed characteristic peaks at a diffraction angle of 2h at 24.125, 33.115 and 35.612 (Figure 4.2.). The crystal sizes appeared 65.5, 52.44 and 84.35 nm, In addition, miller indices appeared as (012), (104) and (110), respectively which matched with the structure of rhombohedral α-Fe_2O_3 [101].
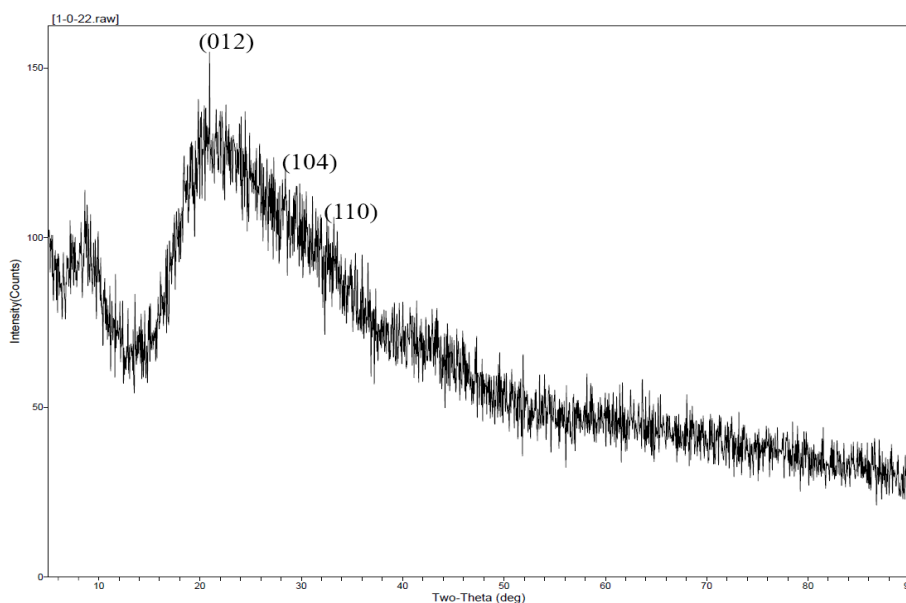

Figure 4.2. XRD diffractogram of IONPs.

4.2.2. FTIR analysis of the nanocomposite WPC films

Figure 4.3. shows FTIR spectrum band of WPC (no IONPs and yeast cell residue), WPC-0 mM (no IONPs) and nanocomposite films of WPC with IONPs at 0.25 mM, 0.5 mM and 0.75 mM. The FTIR spectrum peaks at 541, 890, 922, 1039, 1400, and

1637 cm^{-1} demonstrated the formation of IONPs [105]. The region at 3200-3300 cm^{-1} belongs to the bonds stretch of hydroxyl and amine groups of the protein. Region at 2900-3000 cm^{-1} represent the bond stretch of C-H. The broad peak at 3278 cm$_{-1}$ belongs to the –OH joined to amine group of the protein and the peak at 1637 cm-1 represents the primary amide group while the peak at 1541 cm^{-1} represents the secondary amide within the protein molecule [106, 107]. The peak at 541 cm^{-1} belongs to the Fe-O bond which indicates the existence of iron oxide [108]. There are small variations in the wave number of certain peaks among samples, which result from the interactions between IONPs and the WP in the films. Bandwidth in the spectrum is closely related to the degree of crosslinking in the protein film. Because the more frequent the cross-linking between the chains, the less free -OH groups are present in the structure. The bandwidth of 3231 cm^{-1} representing the -OH groups in the film structure containing IONPs was observed to be lower than that of control film (3235 cm^{-1}). This can be shown as another proof that iron nanoparticles increase cross-linking in the film [100].

Vibrations observed at 1634 and 1537 cm-1 represent the amide I (C=O, C-N) and amide II (N-H) in the control films, respectively. These peaks were shifted to 1625 and 1539 cm^{-1} for the film with IONPs. In addition, group III (N-H, C-N) of the films containing AgNP stretched at 1235 and 1241 cm^{-1}; however, at the same bandwidth, no peak was observed for the control film. This shift in the peaks may be evidence of the change in the secondary structure of proteins with the increase of intermolecular bonds [109].
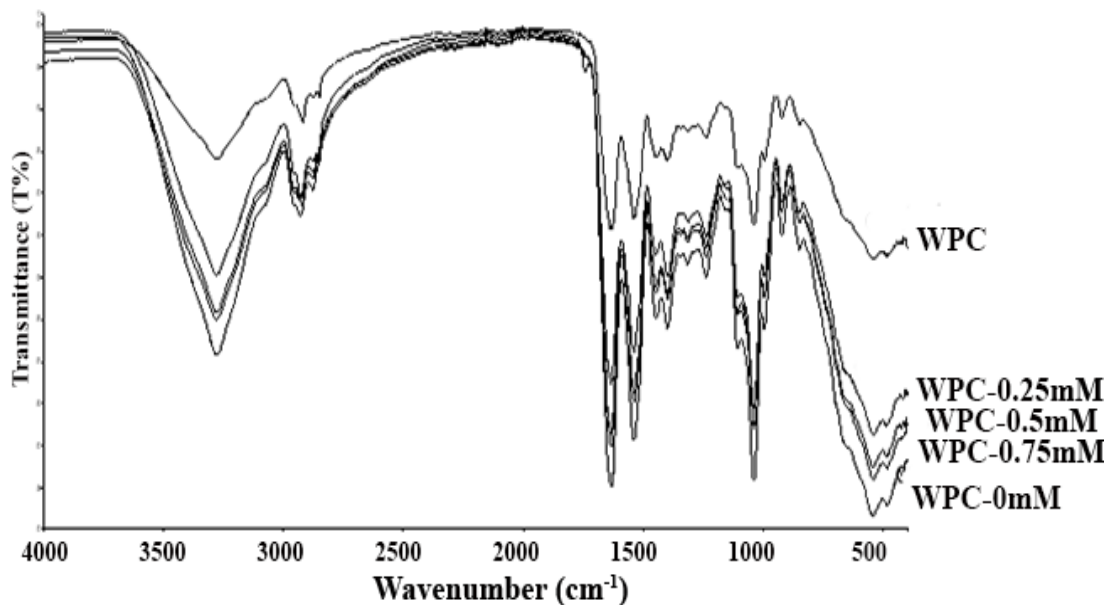
Figure 4.3. FTIR spectrum of whey protein nanocomposite films.

4.2.3. Particle size and morphology of the nanocomposite WPC films

The FESEM micrographs (Figure 4.4.) demonstrate the size of objects within each film. WPC (a) and WPC-0mM (b) images have shown no presence of nano-objects. The SEM images of films contained 0.25mM, 0.5mM, and 0.75mM of IONPs (c, d, and e respectively) have shown the presence of nanometric objects, which give a primer indication that IONPs were successfully combined to WPs in the prepared films. The SEM also shows spherical morphology of IONPs. Mostly the NPs are in sphere form, except for few cubic in shape [110]. The size of nanomaterials at all concentrations of IONPs has shown to be above 50 nm, with wide range of variation, and no agglomeration has detected.
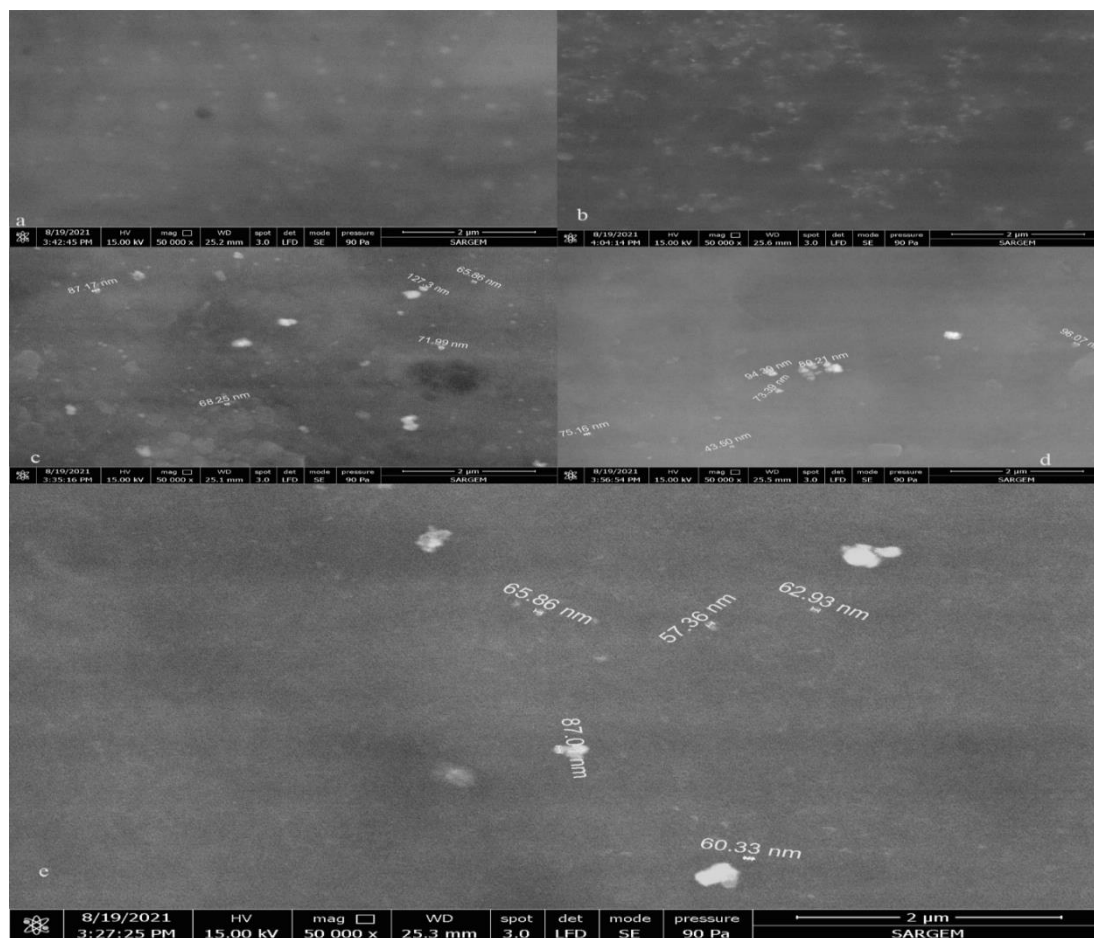
Figure 4.4. FESEM images of WPC film (no IONPs and yeast cell residue) (a), WPC-0mM (No IONPs) (b), WPC-0.25mM IONPs (c), and WPC-0.5 mM IONPs.

4.3.3. Antimicrobial activity of the nanocomposite WPC films

The activity determined by measuring the zones of inhibition in mm. Neither WPC and WPC-0mM films, nor IONPs contained films have exhibited antimicrobial activity. All of the prepared films have shown zero inhibition zones against the growth of all types of cultured strains. These results contradicts the findings of other workers, who attributed the activity of iron oxide nanocomposite against the growth of bacteria to the ability of iron oxide for inducing oxidative stress and destroying the cell membrane of unicellular organisms [8, 111]. This property of iron oxide is vanished in our nanocomposite films, this could happen as result of the interactions between the IONPs and protein molecules in the nanocomposite film. This interaction might change the charge on the surface of nanomaterial which plays significant role in its property as antimicrobial agent [112].

4.3.4. Mechanical properties of the nanocomposite WPC films

The mechanical properties of edible films are expressed by tensile strength (TS), elongation percentage at break (E%) and the thickness of the film. Thicknesses of all films were around 0.12 mm. The TS values of WPC films contained IONPs were significantly higher (P<0.05) compared to the nanocomposite films (Table 4.1). The incorporation of IONPs with WP has increased its TS significantly at 0.5 mM (4.40±0.19 MPa) and 0.75 mM (5.82±0.73 MPa) of IONPs. Yet no significant difference has observed between the 0.25mM of IONPs (3.02±0.10 MPa), and WPC and WPC-0mM. Thereby, TS value depends on the concentration of the IONPs in the nanocomposite films.

The E% on the other hand has decreased significantly (P<0.05) in IONPs contained films compared to the films without IONPs. The E% has shown to be independent on the concentration of IONPs. Similar results were reported on IONPs loaded gelatin films. The workers have shown linearity of TS increase and significant decrease of E% with the increase of IONPs concentration due to the increase of rigidity [113]. The indications that have reported on cellulose films announced that small amount of IONPs increase the TS rapidly whereas the higher amounts of IONPs would lead to a decrease of the TS due to the inhomogeneous dispersion of Fe_3O_4 in the regenerated cellulose matrix [114].

Table 4.1. Mechanical properties of the prepared films.

Film	Thickness (mm)	Tensile strength (MPa)	E%
WPC	0.12±0.001[a]	2.42±0.69 [a]	13.55±1.34[a]
WPC-0 mM	0.12±0.002[a]	2.50±0.34 [a]	12.35±1.91[a]
WPC-0.25 mM	0.12±0.005[a]	3.02±0.10[a]	7.35±1.27[b]
WPC-0.5 mM	0.12±0.005[a]	4.40±0.19[b]	7.11±1.49[b]
WPC-0.75 mM	0.12±0.001[a]	5.82±0.73[c]	7.54±2.13[b]

Different letters indicate significant differences between each two groups (P≤0.05).

4.3.5. Water solubility of the nanocomposite WPC films

The percentage of WS for WPB$_F$ without the presence of IONPs (WPC and WPC-0 mM) and in the presence of IONPs (WPC-0.25mM, WPC-0.5mM and WPC-0.75mM) are documented in Table 4.2. There were no significant (P>0.05) differences between the WS% of WPC films of zero IONPs (25.92±10.40 and 17.60±7.23 for WPC and WPC-0mM, respectively) and the WPC films contained IONPs (19.96±1.91, 28.25±13.09 and 26.45±12.68 for WPC-0.25mM, WPC-0.5mM and WPC-0.75mM, respectively). Whey protein is a hydrophilic in nature and this reflected by its ability on dissolving in water [17]. The hydrogen bonding between NPs and protein molecules results in the modifications of water solubility [115].

Table 4.2. Water Solubility of the prepared films.

Film	WS%
WPC	17.60±7.23[a]
WPC-0 mM	25.92±10.40[a]
WPC-0.25 mM	19.96±1.91[a]
WPC-0.5 mM	28.25±13.09[a]
WPC-0.75 mM	26.45±12.68[a]

Different letters indicate significant differences between each two groups (P≤0.05).

4.3.6. Water vapour permeability of the nanocomposite WPC films

The WVP of the films are demonstrated in Table 4.3. The values of WVP are 29.98±2.82, 28.91±1.96, 25.87±0.68, 22.93±1.77, 22.93±1.77, and 21.77±1.04 g.mm/m^2.day.kPa for control film, control, 0.25mM, 0.5mM and 0.75mM IONPs contained films respectively. Significant (P<0.05) decrease of WVP has shown at 0.5mM and 0.75mM IONPs contained films compared to controls.

The films contained 0.25mM has shown non-significant differences in the WVP neither with controls nor with 0.5mM and 0.75mM contained films. Several intrinsic factors responsible for the permeability of the polymeric film such as the chemical nature of the polymer that determine its interactions with the gas molecules, and the

size of fabric bore within the polymeric film. Moreover, the thickness of the film and the environmental conditions such as heat and pressure have an influence of the film's permeability [4]. Increasing addition of IONP added to the WPC films might contribute more hydrogen bonds and covalent bonds between polymer chains which may have reduced the permeability of water molecules through the film matrix. Observation of an increase in the interactions between the protein chains in the FTIR spectrum of the film containing IONP also confirmed this fact. Similarly, the studies have reported that the increase of IONPs or ZnONPs concentration in gelatin or pectin/alginate films decreases their WVP, respectively [100, 115].

Table 4.3. Water vapour permeability of the prepared films.

Film	WVP (g.mm/m^2.day.kPa)
WPC	29.98±2.82[a]
WPC-0 mM	28.91±1.96[a]
WPC-0.25 mM	25.87±0.68[a,b]
WPC-0.5 mM	22.93±1.77[b]
WPC-0.75 mM	21.77±1.04[b]

Different letters indicate significant differences between each two groups (P≤0.05).

4.3.7. Colour properties of the nanocomposite WPC films

Table 4.4 contains the colour properties parameters of the films. The presence of IONPs in the nanocomposite films has significantly (P<0.05) increased the ΔE compared to control films. The colour of the film became darker in the presence of IONPs. The film of 0.25mM IONPs has shown significant (P<0.05) increase in the ΔE (14.72±0.86), WI (14.13±0.66), and YI (22.40±1.24) compared to control films, but the differences were non-significant with the 0.5mM IONPs film in the three colour parameters (ΔE=15.85±0.39, WI=13.19±0.77, and YI=23.64±0.69). The film of 0.75mM exhibited significant higher ΔE (16.66±1.02), but non-significant differences in WI (14.08±0.60) and YI (24.95±1.53) compared to the films of 0.25mM and 0.5mM IONPs. Similar results have reported on IONPs loaded gelatin films, as they suggested the colour change indicated the successful incorporation of IONPs with gelatin matrix [113].

Table 4.4. Colour properties of WPC films.

Film	ΔE	WI	YI
WPC	9.74 ± 0.50^a	10.79 ± 0.80^a	13.66 ± 0.82^a
WPC-0 mM	9.23 ± 0.48^a	11.12 ± 0.26^a	13.58 ± 0.80^a
WPC-0.25 mM	14.72 ± 0.86^b	14.13 ± 0.66^b	22.40 ± 1.24^b
WPC-0.5 mM	15.85 ± 0.39^b	13.19 ± 0.77^b	23.64 ± 0.69^b
WPC-0.75 mM	16.66 ± 1.02^c	14.08 ± 0.60^b	24.95 ± 1.53^b

Different letters indicate significant differences between each two groups ($P\leq0.05$).

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

The yeast *S. cerevisiae* has shown to be a good agent in the green synthesis of IONPs. According to the conditions that used in the procedure of IONPs synthesis, hematite (α-Fe_2O_3) nanoparticles were obtained, as shown in the XRD analysis. Although, the crystal average size obtained for IONPs was 67.43 nm.

The addition of IONPs to the whey protein concentrate matrix does not empower the antimicrobial characteristics of these films at the three different concentrations of IONPs within the films (0.25, 0.5 or 0.75 mM). This may result from the interactions of IONPs with cellular components of *S. cerevisiae* or/and the whey protein, which probably arises a mesh that constrain the toxicity of IONPs against microbial strains.

Edible films that contained IONPs (0.5 and 0.75 mM) were exhibited significantly ($P<0.05$) higher TS, but significantly ($P<0.05$) lower E% compared to films with no-IONPs. Thus IONPs make a good choice for the improvement of mechanical properties of whey protein edible films.

Water solubility of edible films did not affected by the addition of IONPs, in which non-significant ($P>0.05$) differences of WS% were obtained among 0.25, 0.5 or 0.75 mM IONPs and no-IONPs films. On the contrary, water vapour permeability of the edible films was significantly ($P<0.05$) reduced with the presence of 0.5 or 0.75 mM of IONPs. The total colour change, WI and YI were significantly ($P<0.05$) increased in edible films contained 0.25, 0.5 or 0.75 mM of IONPs. The dark colour that edible films were gained after the addition of IONPs suggests the presence of protection effects of these films against light.

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RESUME

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EDUCATION

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