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EFFECT OF MINERAL IONS ON THE FUNCTIONAL PROPERTIES OF STARCH
FILMS

ALIZA SIGDEL

A thesis submitted in partial fulfillment of the requirements for the degree
Master of Science
Major in Biological Sciences (Specialization in Food Science)
South Dakota State University
2019

EFFECT OF MINERAL IONS ON THE FUNCTIONAL PROPERTIES OF STARCH
FILMS

ALIZA SIGDEL

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Srinivas Janaswamy, PhD
Major Professor and Thesis Advisor

Date

Vikram Mistry, Ph.D.
Department Head, Dairy and Food Science
Department

Date

Dean, Graduate School

Date

I dedicate this thesis to my Late Grandfather Mr. Rewati Raman Sigdel

Your love, affection and blessings are constant source of my energy and my accomplishments my dear Grandpa. I still cherish your last words “I TRUST YOU AND HAVE FULL FAITH IN YOU”; promise I will live up to these words.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS.....	viii
LIST OF FIGURES	xi
LIST OF TABLES	xiii
ABSTRACT.....	xiv
CHAPTER 1. INTRODUCTION	1
1.1 Research hypothesis	2
1.2 Specific objectives.....	2
CHAPTER 2. LITERATURE REVIEW	3
2.1 Essential micronutrients	3
2.1.1 Minerals	3
2.1.2 Iron	7
2.1.3 Manganese	7
2.1.4 Copper.....	7
2.1.5 Zinc	7
2.2 Carbohydrates.....	12
2.2.1 Starch	14
2.2.2 Potato starch and Corn starch	16
2.2.3 Thermal properties of the starch granules.....	19
2.2.4 Non-starch polysaccharides	20
2.2.5 Sodium alginate	21
2.3 Metal-starch complexes.....	22
2.4 Starch films	23
CHAPTER 3. MATERIAL AND METHODS.....	28
3.1 Materials.....	28
3.2 Preparation of starch-mineral complexes.....	28

3.2.1	X-ray powder diffraction (XRD)	31
3.2.2	Fourier-transform infrared spectroscopy (FTIR)	31
3.2.3	Mineral load	31
3.3	Films preparation and characterization	31
3.3.1	Film color.....	35
3.3.2	Film transparency.....	35
3.3.3	Film thickness	36
3.3.4	Water solubility.....	36
3.3.5	Moisture absorption	36
3.3.6	Moisture content	37
3.3.7	Water vapor permeability	37
3.3.8	Tensile strength of films	38
3.3.9	Melting properties of films	38
3.3.10	X-ray powder diffraction of films.....	38
3.3.11	FTIR of films	38
3.3.12	Starch digestibility	39
3.4	Statistical analysis	39
4.1	Starch-mineral complexes	40
4.1.1	X-ray powder diffraction	40
4.1.2	FTIR characterization	43
4.1.3	Mineral loading.....	46
4.2	Films preparation.....	48
4.2.1	Film color.....	50
4.2.2	Film transparency.....	53
4.2.3	Film thickness	56

4.2.4	Water solubility.....	58
4.2.5	Moisture absorption	61
4.2.6	Moisture content	64
4.2.7	Water vapor permeability	66
4.2.8	Tensile strength.....	68
4.2.9	Melting properties of films	70
4.2.10	X-ray powder diffraction of films.....	72
4.2.11	FTIR of films	74
4.2.12	<i>In vitro</i> Starch digestion.....	77
CHAPTER 5. SUMMARY AND CONCLUSIONS		80
5.1	Future work	81
REFERENCES.....		83
APPENDIX.....		99

LIST OF ABBREVIATIONS

Å	Angstrom
°	Degree
°C	Degree Centigrade
2θ	2 theta
ΔH	Enthalpy
ΔE	Total color difference
α-	Alpha-
β-	Beta-
>	Greater- than
<	Less-than
μg	Microgram
μm	Micrometer
~	Approximately
λ	Lambda
%	Percentage
AACC	American Association of Cereal Chemist
Abs	Absorbance
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
CA	Citric Acid
Ca	Calcium
CaCl ₂	Calcium Chloride
CaCO ₃	Calcium Carbonate
CMC	Carboxy Methyl Cellulose
cm	centimeter
Co	Cobalt
Comb	Combination of Iron, Manganese, Copper, Zinc
Cu	Copper

CuCl ₂	Copper chloride
C-2	Carbon-2
C-3	Carbon-3
C-C	Carbon-Carbon
C-O	Carbon-Oxygen
C (1) -H	Carbon (1) -Hydrogen
DSC	Differential Scanning Calorimetry
dw	Dry Weight Basis
Fe	Iron
FeCl ₂	Iron Chloride
FTIR	Fourier-transform infrared spectroscopy
g	Gram
GOPOD	Glucose Oxidase/Peroxidase
HDPE	High Density Polyethylene
hrs	Hours
in. Hg	Inch of Mercury
kg	Kilogram
K ₂ SO ₄	Potassium Sulfite
LDPE	Low Density Polyethylene
log	Logarithm
LSD	Least Significant Difference
MC	Methylcellulose
Mg	Magnesium
mg	Milligram
mins	Minutes
mL	Milliliter
mm	Millimeter
Mn	Manganese
MnCl ₂	Manganese Chloride
MPa	Mega Pascal

Nano-SiO ₂	Nano-Silicon Dioxide
Nano-ZnO	Nano-Zinc Oxide
nm	Nanometer
NSP	Non-starch Polysaccharide
OH	Hydroxy group
psi	Pounds Per Square Inch
p-value	Probability value
PVA	Poly Vinyl Alcohol
RDI	Recommended Daily Intake
RH	Relative Humidity
rpm	Revolution Per Minute
s	Second
SD	Standard Deviation
SEM	Scanning Electron Microscope
SPI	Soy Protein Isolate
T ₆₀₀	% Transmittance at 600nm
TS	Tensile Strength
T _o	Onset transition temperature
T _p	Peak transition temperature
T _c	End set transition temperature
v/v	Volume Basis
WI	Whiteness Index
WVP	Water Vapor Permeability
WHO	World Health Organization
w/w	Weight basis
XRD	X-ray Powder Diffraction
YI	Yellowness Index
Zn	Zinc
ZnCl ₂	Zinc Chloride
ZnSe ATR	Zinc Selenide Attenuated Total Reflectance

LIST OF FIGURES

Figure 1. Classification of minerals based on the daily requirement.....	5
Figure 2. The worldwide deficiency of three common micronutrients vitamin A, iron and zinc	6
Figure 3. Classification of carbohydrates based on the number of sugar units	13
Figure 4. Starch granular structure.....	15
Figure 5. SEM images of native potato starch granules	18
Figure 6. Experimental design for the preparation of starch-mineral complex	30
Figure 7. Experimental design for the preparation of starch-mineral ions complexed films	34
Figure 8. The X-ray powder diffraction patterns of (a) corn starch and (b) potato starch complexes with mineral ions	42
Figure 9. The FTIR spectra of (a) corn starch and (b) potato starch and their complexes with mineral ions.....	45
Figure 10. The loading of mineral ions on (a) corn starch and (b) potato starch.....	47
Figure 11. The Hunter color values (L^* , a^* and b^*), total color difference (ΔE), yellowness index (YI) and whiteness index (WI) of films of (a) corn starch and (b) potato starch films in the presence of mineral ions.....	52
Figure 12. The transparency of (a) corn starch and (b) potato starch films in the presence of mineral ions	55
Figure 13. The thickness of (a) corn starch and (b) potato starch films in the presence of mineral ions.....	57
Figure 14. The water solubility of (a) corn starch and (b) potato starch films in the presence of mineral ions	60
Figure 15. The water absorption of (a) corn starch and (b) potato starch films in the presence of mineral ions	63
Figure 16. The moisture content of (a) corn starch and (b) potato starch films in the presence of mineral ions	65

Figure 17. The water vapor permeability of (a) corn starch and (b) potato starch films in the presence of mineral ions	67
Figure 18. The tensile strength comparison of (a) corn starch and (b) potato starch films in the presence of mineral ions	69
Figure 19. The X-ray powder diffraction patterns of (a) corn starch and (b) potato starch films in the presence of mineral ions	73

LIST OF TABLES

Table 1. Comparison of a few functions and deficiencies of micronutrients	9
Table 2. The recommended daily intake (RDI) of a few essential mineral ions and their food source.....	10
Table 3. A comparison of a few of starch films.....	25
Table 4. Optimization of starch and salt concentration for starch-mineral complex.....	29
Table 5. Optimization of sodium alginate and starch for film forming solution	33
Table 6. Visual observation of films at various starch alginate concentrations.....	49
Table 7. Comparison of onset (To), peak (Tp) and end (Tc) temperature and enthalpy of (a) corn starch and (b) potato starch films complexed with mineral ions.....	71
Table 8. Comparison of the FTIR wavenumbers in the range 4000-600 cm ⁻¹ from the (a) corn starch and (b) potato starch films in the presence of mineral ions	75
Table 9. The in vitro starch digestion at 20 mins and 120 mins of (a) corn starch and (b) potato starch films in the presence of mineral ions.....	79

ABSTRACT

EFFECT OF MINERAL IONS ON THE FUNCTIONAL PROPERTIES OF STARCH
FILMS

ALIZA SIGDEL

2019

Essential minerals are indispensable inorganic micronutrients that modulate vital physiological functions at the molecular level. Their deficiency, though required in small amounts, impairs health significantly. Their supplementation through diet is prudent and carbohydrates stand out as a favorable choice. Carbohydrates, starches and polysaccharides, exhibit unique chemical structures and functionalities, and interact with mineral ions in several ways. The aim of this research is to investigate the effect of mineral ions on the physicochemical properties, tensile strength and *in vitro* starch digestion of starch-films.

Corn starch and potato starch along with Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} ions have been chosen as model starch and mineral ions, respectively. The complexes have been prepared by treating 2% starches with 10% mineral ions. The X-ray powder diffraction and FTIR analyses confirm the starch-mineral complex formation. The maximum mineral loading is 56.5, 68.5, 44 and 15.7 mg of Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} ions, respectively, per gram of starch. The starch-mineral films have been prepared by mixing 1.4% (w/v) of starch, 0.1% (w/v) starch-mineral complex and 0.5% (w/v) sodium alginate in the presence of 1% (v/v) glycerol and casting films in a petri dish. The films are transparent, thin, flexible and homogeneous. The presence of Mn^{2+} and Fe^{2+} ions impart brownish to yellowish tint leading to higher color difference (ΔE) and yellowness index (YI) and in turn lower the films transparency. The water solubility and moisture absorption increase substantially compared to the control starch films. The potato starch films possess higher water vapor permeability of $1.8 \times 10^{-10} \text{ gm}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$ and tensile strength of 7.76 MPa and increase with mineral ions addition. The starch digestion increases with the presence of mineral ions. The outcome sets the stage for further research on mineral-carbohydrate

complexes to develop novel functional foods and to mitigate the micronutrient malnutrition and improve nutritious living.

Keywords: Essential mineral ions, Films, Starch digestibility

CHAPTER 1. INTRODUCTION

Currently, more than one-third of the world's population is affected by the malnutrition. The global population rise, 9.3 billion by 2050 (Lee, 2011), further poses challenges to meet the current and future demands on supplying safe, healthy and nutritious foods. A well-balanced proportion of macronutrients (proteins, lipids and carbohydrates) and micronutrients are critical for healthy living. Micronutrients – minerals and vitamins – though required in subtle amounts of micrograms to milligrams per day, play a vital role on human health. They stimulate cellular growth and metabolism by triggering a host of chemical reactions and aid in normal functioning of human body. Iron, zinc, magnesium, copper, calcium, phosphorus, vitamin A, vitamin B, vitamin C and folic acid are a few of the micronutrients essential for brain functioning, immune system and reproductive system along with physical development and energy metabolism (Kennedy, Nantel, & Shetty, 2003).

Malnutrition, also known as hidden hunger, is the deficiency or inadequate intake of essential micronutrients (Tontisirin, Nantel, & Bhattacharjee, 2002). It is prevalent in areas wherein the diet lacks variety, as is the case of many individuals in developing countries (Kennedy et al., 2003). It is also being noticed in developed countries predominantly due to increased reliance on processed foods that are high in saturated fat, sugar and salt but low in micronutrients (Annel, Dary, Benoist, Hurrel, 2006).

Malnutrition contributes, indeed, to the global burden of disease, physiological impairments, reduced resistance to infections, metabolic disorders, delayed or impaired physical and psychomotor development, increased risk of morbidity and mortality, poor growth and perinatal complications (Bailey, West Jr, & Black, 2015).

In recent years, micronutrients depletion in diet has gained widespread attention from food scientists, companies and lawmakers, and research efforts are directed at addressing this ailment. Renewable, abundant and staple foods such as starches, which offer unique morphological, rheological, thermal and textural properties to food products, could serve

as vehicles to mitigate the malnutrition. In this regard, starch complexation with micronutrients stand tall to develop food products (e.g. edible films, breads, yoghurt and cereals) with specific functionalities. More recently, concerns related to plastics biodegradability and associated health concerns are mounting, and in this regard, starch based products such as films that are biodegradable could serve as an economical and viable solution to combat plastics related issues without any environmental impact.

1.1 Research hypothesis

The proposed research is based on our hypothesis that mineral ions could be complexed with starches and such starch-mineral complexes could be used to prepare films. The following observations are the basis for the hypothesis.

1. The starch hydroxyl groups act as coordination sites for cations (Staroszczyk, Ciesielski and Tomasik, 2018; Luo et al., 2016) and such complexing modulates physicochemical properties of starches (Chuang, Panyoyai, Shanks and Kasapis, 2017; Moreau, Bindzus and Hill, 2011; Ahmad and Williams, 1999).
2. Starches are amenable for preparing films (Basiak, Lenart and Debeaufort; 2017; Vu and Lumdubwong, 2016). They synergistically interact with polysaccharides and yield films with improved functional properties (Borris, Fonseca and Mendez, 2019; Hornung et al., Ghanbarzadeh, Almasia, Entezamib 2010; Kim and BeMiller, 2012).

1.2 Specific objectives

The overall goal of this research is to investigate the effect of mineral ions on the physicochemical and mechanical properties of starch films. Toward this end, corn starch and potato starch along with Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} have been selected as model starches and minerals, respectively. The specific objectives are:

1. To prepare and characterize starch-mineral ion complexes.
2. To investigate physicochemical properties and tensile strengths of starch-mineral films.
3. To determine the *in vitro* starch digestibility of starch-mineral films.

CHAPTER 2. LITERATURE REVIEW

2.1 Essential micronutrients

The term micronutrient represents essential minerals and vitamins required from the diet to sustain cellular and molecular functions (Merson, Black, & Mills, 2006). Though required in minute quantities, their deficiency negates the optimal health substantially. There are around 51 nutrients needed for humans (Graham et al., 2007), however, there are chemical micronutrients called as trace elements. These are required in minute quantity, however, play a dynamic and indispensable role in maintaining integrity of various physiological and metabolic processes occurring within the living tissues. There are around 19 nutrients namely, calcium, phosphorous, magnesium, sulfur, zinc, copper, iron, manganese, chromium, iodine, fluorine, selenium, molybdenum and vitamins A, C, D, E, K and B that are considered essential for physical and mental development and immune system functioning along with a host of metabolic processes (Kennedy et al., 2003).

2.1.1 Minerals

These are the inorganic nutrients present in the human tissues and fluids. They are indispensable and modulate vital physiochemical processes at the molecular level. They are required in small amounts from less than 1 to 2500 mg per day (Soetan, Olaiya, & Oyewole, 2010). They could be broadly classified as macro (major) and micro (trace) minerals, depending on the daily requirement. As the name suggests, macro-minerals are required in greater amounts (> 100 mg/deciliter) than micro-minerals (<100 mg/deciliter).

Absence or insufficient intake of micronutrient-rich foods such as fruits, vegetables animal products and fortified foods results in micronutrient deficiency also known as hidden hunger. The signs of undernutrition are less visible and usually surface when the conditions are severe leading to serious health consequences (Burchi, Fanzo, & Frison, 2011). Throughout the world, the most widespread micronutrient deficiencies are iron, zinc, vitamin A, iodine and folate (Muthayya et al., 2013). At least two billion people are

affected by chronic micronutrients deficiency, predominantly children and women of reproductive age including pregnant and lactating mothers (Allen et al., 2010 ; Thompson & Amoroso, 2010).

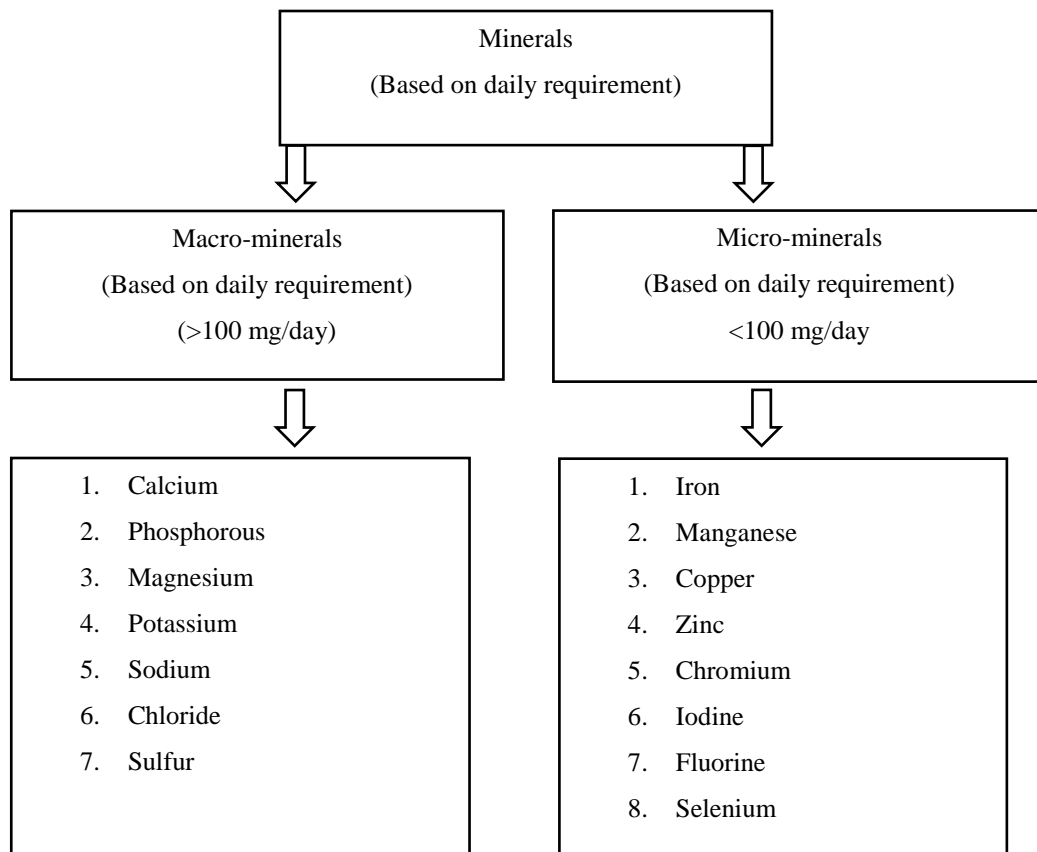


Figure 1. Classification of minerals based on the daily requirement

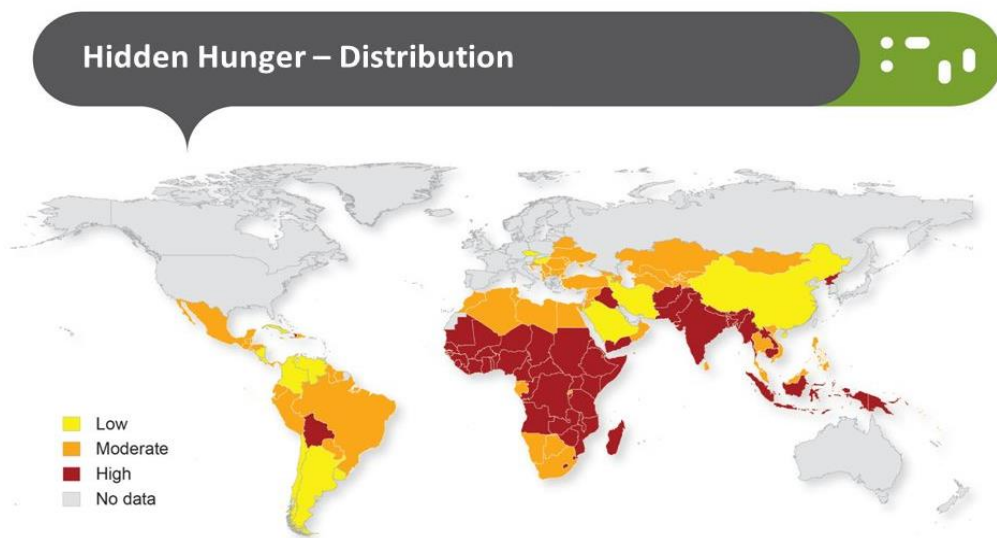


Figure 2. The worldwide deficiency of three common micronutrients vitamin A, iron and zinc (WHO, 2017)

2.1.2 Iron

Iron is a major component of the heme in the hemoglobin (functional iron) and facilitates the transfer of oxygen to tissues and vital organs (Haas & Brownlie IV, 2001). Iron deficiency is the most common nutritional problem in the world and causes anemia. It is estimated that around 24.8 % of the world's population is affected by anemia, which includes 42% of pregnant women, 30% of nonpregnant women and 47% of preschool children (Benoist, McLean, Egli, & Cogswell, 2008). In the US, approximately 10 million suffer from iron deficiency (Miller, 2013).

2.1.3 Manganese

Manganese is another essential mineral that is found in all tissues and is required for metabolizing amino acids, lipids, proteins and carbohydrates. It plays a key role in immune function, regulation of blood sugar and cellular energy, reproduction, digestion, bone growth, and aids in defense mechanisms against free radicals and in fetal bone formation during organogenesis (Hostetler, Kincaid, & Mirando, 2003). Its deficiency impairs growth and induces skeletal abnormalities, ataxia, and abnormal lipid and carbohydrate metabolism (Huskisson, Maggini, & Ruf, 2007).

2.1.4 Copper

Copper regulates functioning of essential enzymes. It stabilizes the walls of blood vessels, strengthen the skin, blood vessels, epithelial and connective tissues of the body. Production of color components such as melanin, myelin, hemoglobin and thyroid gland functioning are the important aspect of copper. Deficiency results blood vessel breakage, iron deficiency anemia, osteoporosis and joint problems, brain disturbances, loss of pigment, weakness, fatigue, skin sores and poor thyroid function (Osredkar & Sustar, 2011).

2.1.5 Zinc

Zinc is an important mineral with catalytic activity for more than 200 enzymes (Sandstead, 1994). It plays a critical role in immune functionality, cell division, protein

and DNA synthesis, wound healing, normal growth and development during pregnancy, childhood and adolescence (Osredkar & Sustar, 2011). Low zinc intake appears to be a major public health problem especially in adults and results in oxidative damage to DNA. It upturns infection and diarrhea leading to death of about 800,000 children worldwide per year (Prasad, 2004).

Table 1. Comparison of a few functions and deficiencies of micronutrients

Micronutrient	Function	Deficiency	References
Iron (Fe^{2+})	<ul style="list-style-type: none"> • Facilitates transfer of electrons in the respiratory chain and ATP synthesis • Helps in red blood formation 	<ul style="list-style-type: none"> • Results in loss of mitochondrial complex in selected regions of the brain • Causes change in function, morphology, and physiology of the brain • Diminishes immune function and neuro muscular abnormalities 	Huskisson et al. (2007); Ames (2006)
Manganese (Mn^{2+})	<ul style="list-style-type: none"> • Involves in the metabolism of carbohydrates and gluconeogenesis • Forms the matrices of bones and eggshells 	<ul style="list-style-type: none"> • Impairs reproductive function in females and testicular degeneration in males • Decreases levels of clotting proteins and subtle reddening of hair 	Huskisson et al. (2007); Soetan et al. (2010)
Copper (Cu^{2+})	<ul style="list-style-type: none"> • Involves in iron metabolism 	<ul style="list-style-type: none"> • Alters the movement of nutrients through cell walls, irregular heartbeat and lowers body temperature 	Huskisson et al. (2007); Nath (1997)
Zinc (Zn^{2+})	<ul style="list-style-type: none"> • Plays structural role in cellular metabolism • Protects against accelerating aging and promotes faster recovery from injury 	<ul style="list-style-type: none"> • Depresses immune functionality • Impairs macrophage and neutrophil function • Inactivates zinc-containing proteins and induces genetic damage 	Shankar & Prasad (1998); Wintergerst, Maggini, & Horing (2007)

Table 2. The recommended daily intake (RDI) of a few essential mineral ions and their food source

Essential Minerals	RDI	Major source	Natural food	Market food	References
Iron (Fe ²⁺)	15 mg	<ul style="list-style-type: none"> Meat, legumes, nuts and certain vegetables 	Cow pea: 5 mg/kg, Dates: 20 mg/kg, Berries: 55 mg/100g, Wheat bran: 66 mg/kg, Lentils: 4-18 mg/kg, Broccoli: 7 mg/g, Almonds: 34mg/g	Fruit-enriched breakfast cereals: 10.7-13.3 mg/100g, Cornflake: 16-63 mg/100g, Chocolate donuts: 2.82 mg/100 g, Pizza: 1.63 mg/100 g, Apple sauce: 23.2 mg/kg dw Milk powder: 1.80 mg/kg dw	Moll and Moll (2000); Mesias, Seiquer, Nauarro (2013); Bhavya et al. (2012); Millikan (2012); Eldaim & Elnadi (2014); Bruggraber et al. (2012); Lenntech (n.d)
Manganese (Mn ²⁺)	5 mg	<ul style="list-style-type: none"> Cereals, vegetables, tea, coffee and red wine 	Cow pea: 27 mg/kg, Banana: 3.3 mg/kg, Legumes: 10.6 mg/kg, Potato: 1.2 mg/kg	Sausages: 1.2 mg/kg, Hamburger: 1.09 µg/g, Chicken noodle soup: 1.95 µg/g, Ketchup: 2.25 µg/g, Corn flask: 1.0 mg/kg and Multigrain bread: 17 mg/kg	Millikan (2012); Vique, Meca, & Seiquer (2014); Tinggi, Reilly, & Patterson (1997); Lenntech (n.d).

Essential Minerals	RDI	Major source	Natural food	Market food	References
Copper (Cu ²⁺)	2 mg	<ul style="list-style-type: none"> Cereals, fish, meat, poultry, eggs, vegetables and beverages 	Millet: 3.83 µg/g dw, White maize: 2.22 µg/g dw, Beef liver: 157 mg/kg Potato: 0.48-16.0 mg/kg	Sandwich: 0.9 mg/100 g, Chocolate: 0.64 mg/100 g, Ice-cream: 0.17 mg/100g Apple sauce: 0.184 mg/kg	Moll & Moll (2000); Vique et al. (2014); Jaryum, Okoye & Stoecker (2013); Marles (2017); Bost et al. (2016); Lenntech (n.d)
Zinc (Zn ²⁺)	15 mg	<ul style="list-style-type: none"> Meat, poultry, eggs, cereals and dairy products 	Wheat: 1.5-10.2 mg/100 g dw Ripe papaya: 0.39-2.80 mg/100g dw	Breakfast cereals: 0.28 mg/100g, Muffins: 1.29 mg/100g Chocolates: 0.12 mg/100g	Moll & Moll (2000); Vique et al. (2014); Marles (2017); Lenntech. (n.d)

2.2 Carbohydrates

Carbohydrates are polyhydroxy alcohols with active carbonyl group, which may be aldehyde or ketonic group or substances and yield alcohols on hydrolysis. These are the main source of energy for humans. They could be classified as monosaccharides, oligosaccharides and polysaccharides based on the number of sugar molecules present. Monosaccharides are the simple molecules with single sugar unit that are easily digestible. Oligosaccharides consist of 2-10 monomeric units bound through glycosidic bonds. On the other hand, polysaccharides contain around 10^7 simple molecules through repeating units (either mono- or disaccharides) joined together by glycosidic bonds.

Polysaccharides are relatively complex molecules. They provide good mechanical properties for applications as fibers, films, adhesives, rheology modifiers, hydrogels, emulsifiers and delivery agents of bioactive compounds. Some of them are linear while rest are branched; the degree of branching reflects on the physical properties such as water solubility, viscosity and gelling behavior (Liu, Zhang, & Laskowski, 2000). They are widely used in the food industry as functional ingredients. Starch, alginate, carrageenan and pectin are examples of most used polysaccharides in the food industry.

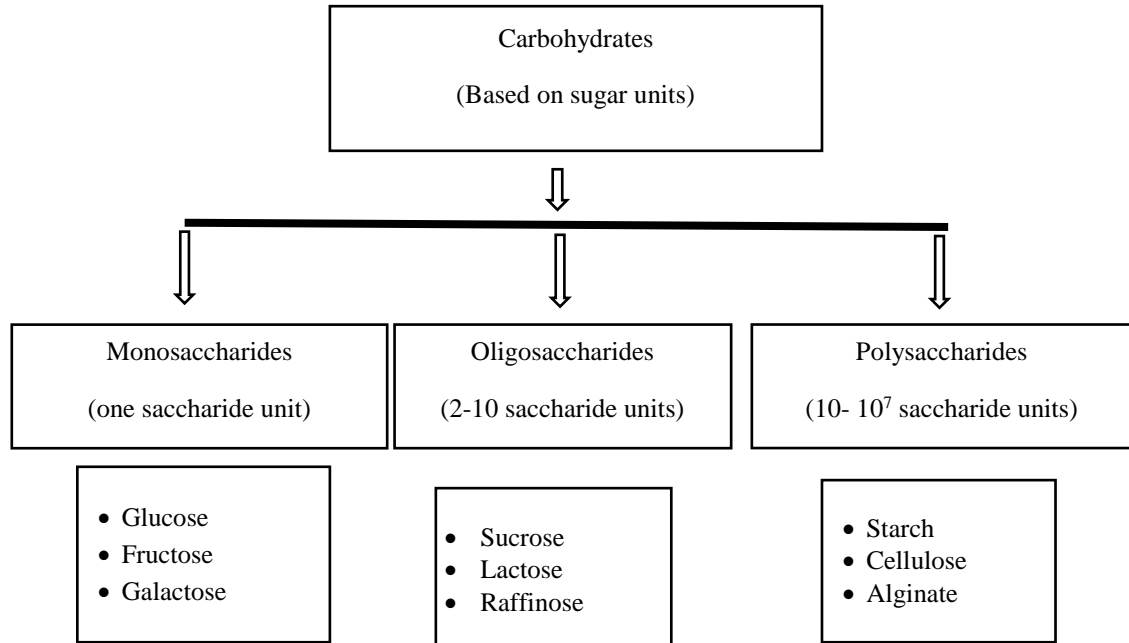


Figure 3. Classification of carbohydrates based on the number of sugar units

2.2.1 Starch

Starch exists naturally in plant tissues such as fruits, seeds, roots and tubers and without a doubt it is world's inexpensive source of food carbohydrate that provides bulk of nutrients and energy to humans. It acts as a thickener, stabilizer, texturizer and filler and used extensively in the production of variety of food products such as puddings, soups, sauces, snacks, breakfast cereals, biscuit, pasta and ready-to-eat foods.

Starch is made up of two structurally distinct molecules namely amylose and amylopectin (Stephen & Phillips, 2016). Amylose is a linear polymer composed of glucose units linked together through α -1,4 glycosidic bonds. Its molecular weight (MW) varies from ~500 to over 6000 anhydroglucose units depending on the source and starch type. Unlike amylose, amylopectin is a branched polymer with glucose units linked through α -1,4 and α -1,6 bonds. Most starches contain approximately 20–30% amylose and remaining 70–80% as the amylopectin. Starch granule is semi-crystalline in nature with alternating crystalline and amorphous regions. The crystalline regions are associated with amylopectin while the amorphous regions composed of amylose. Thus, higher proportion of amylopectin results in increased starch crystallinity.

Starch occurs naturally as discrete granules. Starch granules exhibit hydrophilic properties and strong inter-molecular association via hydrogen bonding interactions formed through the hydroxyl groups on the granule surface. They are composed of a number of concentric rings of crystalline layers and the space in between them is filled with amorphous layers. The amorphous layers are made up of entangled amylose and amylopectin chains (Tomasik, 2009). Amylopectin chains are clustered in double helix, medium and long chains amylopectin forms a very strong and stable crystalline structure (Shewry et al., 2009) whilst those with short chain are unable to form stable double helix structures, making them susceptible to heat (Singh, Singh, Isono, and Noda, 2009). Starches with amylose of high molecular weight yield higher paste clarity compared to amylose with low molecular weight (Mali, Karam, Ramos, and Grossmann, 2004).

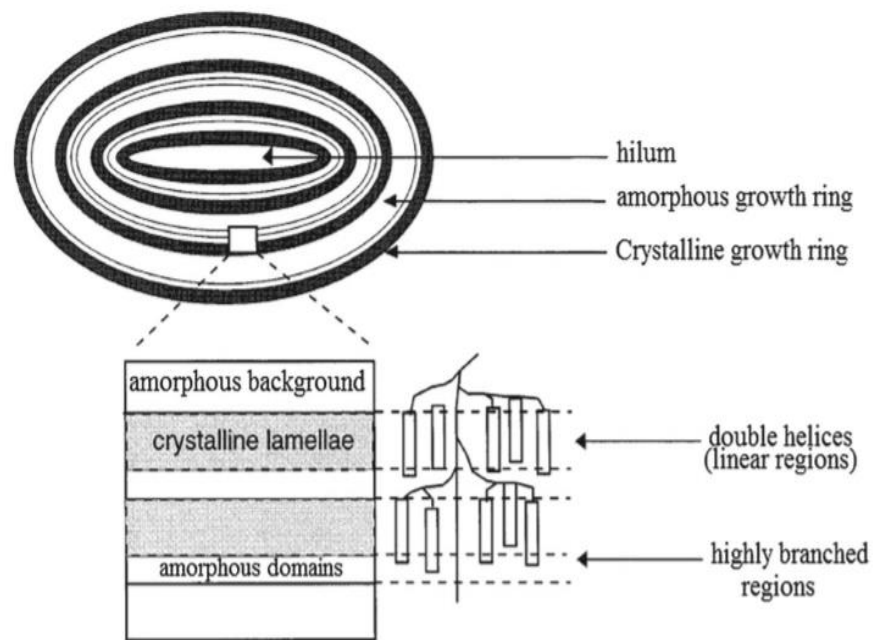


Figure 4. Starch granular structure: (a) the whole granule, (b) the lamellae, and (c) the polymer chains (Fasahat, Rahman, & Ratnam, 2014)

2.2.2 Potato starch and Corn starch

The granule size distribution, molecular characterization of amylose and amylopectin influence functional properties such as gelatinization onset temperature, paste viscosity and paste stickiness. Thus, end-use applications of starch depend on the botanical origin. Starch granules from tuber sources such as potatoes are functionally and morphologically different from cereal grains such as corn (Morrison et al., 2001).

Potato starch is unique from other starches with the presence of phosphate ester groups that are covalently bound to the amylopectin fraction. The average number of esters is one per every 8-12 glucose units in the outer branches. The ionic repulsion produced by the negatively charged phosphate monoesters weakens the association between the molecules increasing water-binding capacity and swelling power of granules (Zhou et al., 2014). Potato starch also offers high starch paste clarity and viscosity, high water binding capacity and swelling power with low tendency to retrograde (Singh, Chawla, & Singh, 2004). Nutritionally, potatoes are considered as a favorable alternative to fish and legumes due to availability of readily metabolized phosphorus. In aqueous solutions, native potato starch serves as ion-exchange in the presence of salts of metals such as sodium, potassium, magnesium and calcium. In addition, presence of higher phosphate groups and lower concentration of lipids in potato compared to corn contributes to highest solubility and granular swelling (Singh, Singh, Kaur, Sodhi, & Gill, 2003).

Morphology of granules can be studied from optical birefringence, X-ray diffraction and electron microscopy. Potato starch granules are the largest among all starches with a granule size range of 1-110 μm . They are oval and irregular or cuboidal in shape with rough surface that swell up to 100 times without disintegration (Stasiak et al., 2013). Typically, native potato starch contains 17- 22% amylose (Tomasik, 2009) and remaining being amylopectin. On the other hand, corn starch granules are angular-shaped with the size range from 1 to 20 μm and are less smooth than the potato starch. Their swelling capacity is up to 30 times (Stasiak et al., 2013).

Cereal starches such as corn starch display A- type X-ray diffraction patterns, and tuber starches such as potato starch exhibit B-type patterns. The A- and B-starch structures are composed of starch double helices but with different water molecules in the network. There are 4 water molecules in A-type starches and 36 water molecules in B-type structures (Lebail, Buleon, Shiftan, and Marchessault, 2000). The differences at the atomic level orchestrate variations in macroscopic functional properties. Apart from A- and B-type starches there are two more varieties known as C-type and V-type starches. The C-type structure is a mixture of the A- and B-type structures and is predominantly exists in legume starches for example in peas (Wang, Bogracheva, & Hedley, 1998). The V-type crystalline structure corresponds to single helical structure that is found in amylose complexed with lipids (Lebail et al., 2000).

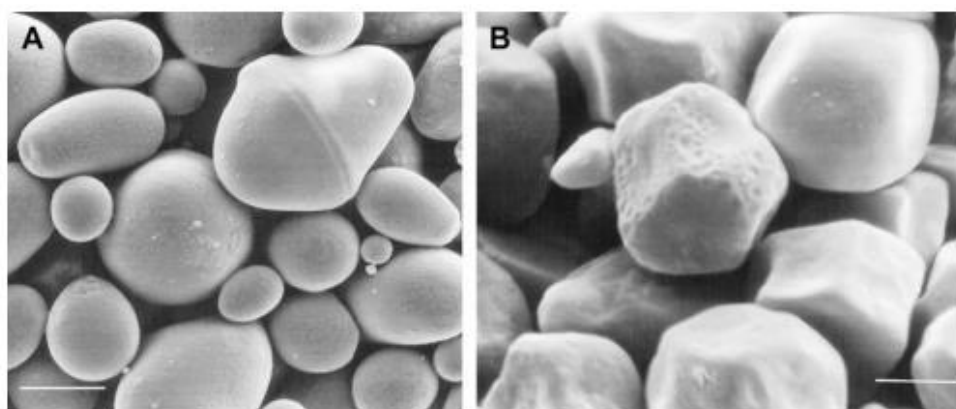


Figure 5. SEM images of native potato (A) (scale = 50 μm) and corn (B) (scale= 10 μm) starch granules

2.2.3 Thermal properties of the starch granules

2.2.3.1 Gelatinization

Starch granules are insoluble in cold water due to strong hydrogen bonds between amylose and amylopectin chains. However, when starch is heated in excess water, granules absorb water and swell followed by partial rupture of granules. During this process, water would be absorbed by the amorphous regions and later destabilizes granule's crystalline structure leading to the loss of birefringence (Ratnayake, & Jackson, 2006). The phenomenon of disordering of crystalline structure, that is related to swelling of granules and leaching of soluble polysaccharides (amylose) is commonly termed as gelatinization. Further heating causes granules to swell to greater extent where crystallites melt and increases molecular motion that eventually leads to complete separation of amylose and amylopectin (Keetels, Van, & Walstra, 1996). In general, the swollen granules are enriched in amylopectin, while the amylose diffuses out of the swollen granules and makes up the continuous phase outside the granules (Hermansson, & Svegmarm, 1996). The temperature where granules lose their birefringence is referred as gelatinization temperature. Generally, gelatinization occurs for most types of starches at a temperature between 60 to 70 °C. The amylose and amylopectin content, shear rate, time-temperature profile, pH and the presence of other constituents are factors that influence the gelatinization process (Hornung, 2018).

The thermal behavior of starches during heating is studied by DSC. Thermal transitions are defined in terms of onset (T_o), peak (T_p) and end set (T_c) transition temperature. The heat of transition or enthalpy (ΔH) is measured in Joules per weight (g) of sample, and it is calculated from the thermogram peak area (Brites, Dos Santos, Bagulho, & Beirao-da-Costa, 2008). The T_o is related to the beginning of the starch gelatinization and represents the melting of the weakest crystallites. The T_p is reached with a complete loss of birefringence. The T_c is related to the final temperature needed to complete the gelatinization process. The ΔH reflects changes during melting of the crystallites and provides a measure of the degree of crystallinity or a damage in the starch structure before the gelatinization occurs. The amylose content, small granules, and damaged

starch structure are inversely related to ΔH (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998).

2.2.3.2 Retrogradation

Starch retrogradation is a process observed during storage or cooling of gelatinized starch. The process occurs when the molecular chains in gelatinized starches begin to reassociate in an ordered manner (Atwell, Hood, Lineback, Varriano Marston, & Zobel, 1988) i.e., recrystallization of the amylose and amylopectin. Retrogradation directly interferes with the sensory quality (loss of moisture and consequent hardening of the final product) and nutritional (making the carbohydrate resistant to the digestive enzymes) of the product. Although both amylose and amylopectin are capable of retrograding, the amylopectin component appears to be more responsible for long-term quality changes in foods. During retrogradation, water molecules are expelled from the gel network (synereses) and highly stable hydrogen bonds are formed between the re-associated amylose molecules (Koksel, Masatcioglu, Kahraman, Ozturk, & Basman, 2008).

2.2.4 Non-starch polysaccharides

Non-starch polysaccharides (NSP) are the complex polymeric carbohydrate comprising up to 90% of the cell wall material of the plant (Selvendran & Du Pont, 1980). Cellulose, hemicellulose and pectin, fructans, glucomannans, galactomannans are commonly known NSP. Likewise, mucilages, alginates, exudate gums, β -glucans and various modified polysaccharides are other constituents of the NPs family (Schweizer & Edwards, 2013).

Type of monomeric units, their number and nature of shared linkage between molecules, are the most important parameters that make non-starch polysaccharides different from starches. Different monomeric units of NSPs are predominately linked through β -glycosidic bond while, starch being entirely glucose molecule shares α -glycosidic bond. The difference in bonding has reflective effects on digestibility due to need of different classes of enzymes for hydrolysis. Enzymes, namely α -amylase, α -glucosidase and oligo-1-6-glucosidase, hydrolyses the α -glycoside bonds of starch to yield glucose while

NSP, requires enzymes such as β -glucanase and β -xylanases (Sinha, Kumar, Makkar, Boeck, & Becker, 2011).

2.2.5 Sodium alginate

Sodium alginate is an anionic polysaccharide derived from brown algae, and is composed of linear polymers of β -(1-4)-D-mannuronic (M), and α -L-gluronic (G) acid residues arranged in homogenous (poly-G, polyM) or heterogenous (MG) block-like patterns (Torres et al., 2007). Alginates are available in various grades in terms of molecular weight, composition, and distribution pattern of M-blocks and G-blocks and thus affect physicochemical properties such as viscosity, sol/gel transition, and water-uptake ability (Szekalska, Pucilowska, Szymanska, Ciosek, & Winnicka, 2016). The conversion of alginic acid to sodium alginate allows its solubility in cold water and is widely used in food and pharmaceutical industries to increase viscosity and as a thickener, binder, suspension, emulsifier. In the presence of divalent ions (especially calcium), ion exchange reaction with monovalent ions (e.g., sodium in sodium alginate) occurs instantly, changing from a low viscosity solution to gel structure (Santos, 2017) without the need of heat.

Sodium alginate is a soluble fiber that helps to increase the viscosity and delays the gastric emptying, eventually lowering postprandial rises in blood glucose (Torsdottir, Alpsten, Holm, Sandberg, & Tolli, 1991). Viscosity generated by soluble fibers restrict the enzyme hydrolysis and digestion of nutrients trapped in viscous solution/gel and thus, have glycemic control capacity on starches and sugar (Qi, Ghazzewi, & Tester, 2018). Starch in native or gelatinized could form hydrogen bonds with non-starch polysaccharides such as alginate, xanthan gum and guar gum (Sasaki, Sotome, & Okadome, 2015; Yoo, Kim, & Yoo, 2005; Singh, Sharma, Kumar, & Gupta, 2010). The blend of structurally different polymers, starch and non-starch polysaccharides do co-exist extensively in food and pharmaceuticals applications.

2.3 Metal-starch complexes

It is a well-known fact that functional groups of carbohydrates mainly hydroxyl groups on C-2 and C-3 atoms having cis axial-equatorial conformation or trans di-equatorial conformation bears an ability to participate in the complexation reactions. Thus, natural starch, maltodextrin, cellulose and guar gum, to name a few, potentially have the intrinsic ability to form complexes with guest atoms and molecules (Liu, Zhang, & Laskowski, 2000). Studies on metal ions interactions with native and modified starches have proven the binding of ions (Labanowska et al., 2013). Starch-metal complexes are prepared by solubilizing starch into paste and then reacting with higher concentration of mineral ions. Metal-starch complexes prepared with higher concentration of copper salt help to heal topical wounds and act as hair growth stimulants (Woo et al., 2006).

It is assumed that interactions between alkali metal ions (except lithium) and starch occurs with ions bonding with the protons of hydroxyl groups on the starch (Lii, Tomasik, Hung, & Lai, 2002). Herein, cations are held through the electrostatic forces. On the other hand, it is postulated that the alkali-earth metal ions such as beryllium (Ba), magnesium (Mg), calcium (Ca) can form complexes with starches through electrostatic interactions between the cations and anions interacting with the OH groups (Lai, Tomasik, Yen, Hung, & Lii, 2001). In the case of transition metals, it was found that complexes are formed in which glucose units of starch or its fractions (amylose and amylopectin) are coordinated to metal ions via free electron pairs of the oxygen atoms of OH groups (Labanowska et al., 2013).

Mineral ion bound starch can be prepared through chemical crosslinking of starch interaction with single mineral, mixture of minerals or mineral containing residues (Woo et al., 2006). The biologically useful nutrient minerals include, aluminum, calcium, sodium, potassium, iron, iodine, zinc, magnesium, manganese, copper, chromium and nickel. Binding takes place via Van der Waals interactions, electrostatic attraction, ionic bonding, hydrogen bonding, covalent bonding. For minerals to be effectively released

and available for biological needs after ingestion, the mineral-bound starch must be digested more than about 80%, more typically about 90%.

Mineral composition is an important characteristic of foods, for safety and nutritional reasons. The amount of mineral attached to the molecules confirmed the possibilities of binding and practical application in functional food development. Addition of mineral ion complexed starch over pure starch, in food processing fosters better overall health and promote consumption of micronutrients to meet the nutritional requirements.

2.4 Starch films

Films constitute thin layers of material that are suitable for consumption and have potential barrier against different agents (water vapor, oxygen, and moisture) and if intended to use as packaging materials (Eca, Sartori, & Menegalli, 2014). Starch being inexpensive and naturally available, it has been used in film making along with other polysaccharides such as cellulose, chitosan, methylcellulose, alginate, carrageenan and pectin (Chen, Liu, & Zhang, 2014).

A thin, transparent and resistant film could be formed when gelatinized starch is allowed to cool (Pauli, Quast, Demiate, & Sakanaka, 2011). Films can be prepared by casting, extrusion or lamination but in the lab-scale they are prepared through casting technique. It involves preparation of film forming solution by solubilization the starch in a suitable solvent (heating starch at 95 °C with continuous stirring) followed by casting in a plate, and then drying in a ventilated oven (60 °C) to a constant weight (Garcia et al., 2009). Starch films from solution occurs in three major steps; helical formation (caused by cooling) followed by aggregation or gelation and finally reorganization of the aggregates (And & Han, 2005). The latter two steps takes place during drying. Drying conditions such as time, temperatures and air humidity allow controlling film characteristics of water content, barrier property, mechanical properties and crystallinity (Garcia, Pinotti, Martino, & Zaritzky, 2009).

Most of the biopolymer's films are water sensitive and there are ample examples with nanoparticles (such as silicon dioxide, halloysite nanoclay) addition to increase their physical and mechanical properties. Nanoparticles suppresses the diffusion of water into starch matrix and lowers film solubility (Hassani, & Nafchi, 2014), increases tensile strength due to possible bonding of nanoparticles with OH groups (Muller, Laurindo, & Yamashita, 2011). Nanoparticles fill the interspace of matrix and reduce the diffusion of water through the films (Torabi, 2018), lower water absorption due to formation of more hydrogen bonds between nanoparticles and matrix thus limits strong interaction of free water with nanocomposite films (Nafchi, Nassiri, Sheibani, Ariffin, & Karim, 2013). They also enhance films ability to protect from light due to lower transmission (Nafchi, Alias, Mahmud, & Robal, 2012).

Presence of plenty of hydroxy groups in the film increases water penetration (Mollah, Akter, Quader, Sultana, & Khan, 2016). Use of plasticizers such as glycerol, sorbitol, polyethylene glycol reduces inter-molecular forces and increases the mobility of polymer chains by reducing the hydrogen bonds between the polymer molecules and make films more flexible and therefore less brittle (Donhowe, & Fennema, 1993). For example, increased concentration of glycerol in alginate/pectin films crosslinked via Ca^{2+} ions decrease tensile strength, increase water uptake, solubility, moisture content, flexibility and elongation at break (Silva, Bierhalz, & Kieckbusch, 2009).

Table 3. A comparison of a few of starch films

Raw material	Additives	Characteristics	Result	References
<ul style="list-style-type: none"> • Corn starch (3%) 	<ul style="list-style-type: none"> • Nutmeg (1, 2, 3%) • Glycerol (10, 20, 30%) 	<ul style="list-style-type: none"> • Tensile strength & elongation • Antibacterial 	<ul style="list-style-type: none"> • Glycerol decreased tensile strength, improved flexibility and lessen intermolecular forces • Nutmeg exhibited antimicrobial activity on <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> 	Aisyah, Irwanda, Haryani, & Safriani, (2018)
<ul style="list-style-type: none"> • Corn starch (5%), • Glycerol (40% v/w) 	<ul style="list-style-type: none"> • Carboxymethyl cellulose (CMC) (0, 5, 10, 15 and 20% w/w) 	<ul style="list-style-type: none"> • WVP • Moisture absorption, • Solubility • Tensile strength • Color 	<ul style="list-style-type: none"> • Highly crystalline and hydrophobic character of cellulose decreased WVP and water solubility • Increased tensile strength due to interfacial interaction between the matrix and filler of similar chemistry • CMC increased lightness (L*) and decreased b* values of the starch film 	Ghanbarzadeh et al. (2010)
<ul style="list-style-type: none"> • Potato starch (5%) • Glycerin (5% w/v) 	<ul style="list-style-type: none"> • Nano-SiO₂ (15, 30, 80, 100 nm) 	<ul style="list-style-type: none"> • WVTR, • Solubility • Moisture absorption • Tensile strength • Antibacterial activity 	<ul style="list-style-type: none"> • 100 nm nano-SiO₂ was well dispersed in the starch matrix with stronger hydrogen bond • Improved water and mechanical properties • Nano-SiO₂ destroyed the structure of bacterial cell (<i>Staphylococcus aureus</i> and <i>Escherichia coli</i>) 	Zhang, Wang, & Cheng (2018)

Raw material	Additives	Characteristics	Result	References
<ul style="list-style-type: none"> • Corn starch (5%), • Glycerol (40% v/w) 	<ul style="list-style-type: none"> • Citric acid (CA) (0, 5, 10, 15 and 20% w/w) 	<ul style="list-style-type: none"> • WVP • Moisture absorption, • Solubility • Tensile strength 	<ul style="list-style-type: none"> • CA (<10%) improved WVP, mechanical properties due to substitution of hydrophilic OH group by hydrophobic ester group • CA (>10%) exhibited plasticization effect and residual free CA increases interchain and spaces mobility lowering WVP, reducing mechanical property • Carboxy group of CA formed strong hydrogen bond with OH group of starch improved interaction and decreased water sensitivity, thus water absorption and solubility of film 	Ghanbarzadeh, Almasia, Entezamib (2011)
<ul style="list-style-type: none"> • Potato starch (2%) 	<ul style="list-style-type: none"> • Glycerol (10, 20, 30, 40 %) 	<ul style="list-style-type: none"> • Moisture content • Mechanical property, • Oxidative rancidity and peroxide value 	<ul style="list-style-type: none"> • Higher glycerol concentration increased moisture content and diminished protective capability • Hygroscopic character of glycerol resulted less strong film with higher flexibility 	Oses, Niza, Ziani, & Mate (2009)
<ul style="list-style-type: none"> • Sago starch 	<ul style="list-style-type: none"> • Nano-ZnO (1, 2, 3, and 5%) 	<ul style="list-style-type: none"> • Water solubility • WVP • Color 	<ul style="list-style-type: none"> • Decreased solubility • Lower water absorption • Decreased Lightness (L^*) and increased a^* and b^* with film color in between light yellow and red • Nanoparticles decreased the film permeability 	Nafchi et al. (2012)

Raw material	Additives	Characteristics	Result	References
<ul style="list-style-type: none"> • Cassava starch (5%) • Normal Rice starch (5%) • Waxy rice starch (4%) • Arabinoxylan (16%) • Agar (3%) 	<ul style="list-style-type: none"> • 15% glycerin 	<ul style="list-style-type: none"> • Solubility • WVP, • Contact angle • Water adsorption, • Microstructure, • Mechanical property 	<ul style="list-style-type: none"> • Starch films showed higher water solubility and lower TS compared to agar • High WVP values and hygroscopicity • Agar and cassava starch-based films were flexible, transparent, clear and homogeneous 	Phan, Debeaufort, Luu, & Voilley (2005)
<ul style="list-style-type: none"> • Cellulose (3 -4% w/w), • Glycerol (2.5 or 5% w/w) 	<ul style="list-style-type: none"> • Poly-vinyl alcohol (PVA) (2, 5 and 10 % w/w) 	<ul style="list-style-type: none"> • Mechanical properties • Water adsorption, • FTIR, • DSC 	<ul style="list-style-type: none"> • Toughness of cellulose-PVA film decreased with increased glycerol concentration • Water adsorption increased with PVA • FTIR and DSC showed an interaction of films components 	Cazon, Vazquez, & Velazquez (2018)
<ul style="list-style-type: none"> • Cellulose (3%) 	<ul style="list-style-type: none"> • ZnCl₂ (50-78%) 	<ul style="list-style-type: none"> • Solubility 	<ul style="list-style-type: none"> • Zn²⁺ ions paired to hydroxyl groups (O2H, O3H, and O6H) tampered the intrachain hydrogen bonding • Increased flexibility • Enhanced water penetration and aid in film solubilization 	Xu, Chen, Rosswurm, Yao, & Janaswamy (2016)

CHAPTER 3. MATERIAL AND METHODS

3.1 Materials

Corn starch and potato starch were purchased from Sigma Aldrich. Essential minerals iron, manganese, copper and zinc in the chloride salt form were procured from VWR international. Isopropanol and acetone for precipitation of starch complex was of analytical grade and purchased from Fisher Scientific.

3.2 Preparation of starch-mineral complexes

Predetermined concentration of 2% of potato and corn starches was dissolved separately in distilled water by heating the solution at 95 °C for an hour with continuous stirring. Initially, 10, 20, 30, 40, 50 and 65% of salt was added to starch solution with continued heating and stirring for 90 minutes so as to optimize the concentration for mineral for complexing. The hot mineral-starch mixture was then precipitated in double volume of 80% cold isopropanol (0 °C) followed by washing with 100% isopropanol and later with 100% acetone to remove any unbound cations and water. The complex was then dried overnight in a vacuum drier at 55 ± 5 °C. The dried complexes were ground into fine powder with motor pestle for further characterization.

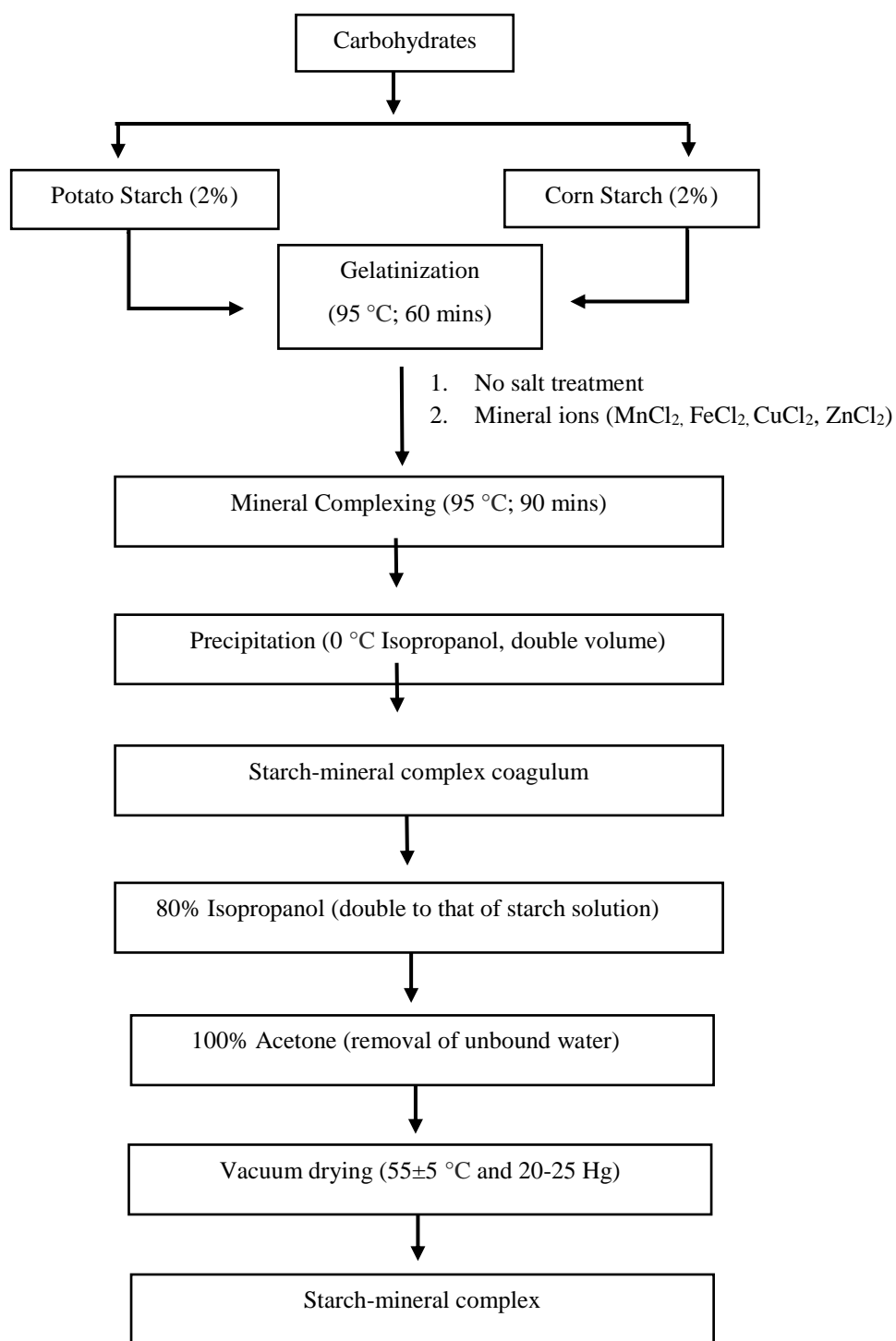


Figure 6. Experimental design for the preparation of starch-mineral complex

3.2.1 X-ray powder diffraction (XRD)

Starch-mineral complex was packed in an aluminum holder and mounted on a Rigaku SmartLab diffractometer interfaced to a computer equipped with SmartLab Studio II software. The intensity data were collected using the literature protocol (Janaswamy, 2014) with some modification. The sample was analyzed at room temperature using a graphite monochromator with CuK α -radiation ($\lambda = 1.5418 \text{ \AA}$). X-ray tube was operated at 40 kV and 30 mA in the scan range 3-40 ° (2 θ) in a step scan mode with 0.1 step width and 5 s at each step. The patterns were smoothed for further analysis.

3.2.2 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectroscopy was performed to establish the presence or absence of the various vibrational modes of carbohydrate molecules in the presence of mineral ions. Spectra were recorded using the Thermo Scientific Nicolet 380 FT-IR Spectrometer equipped with ZnSe ATR Crystal (Thermo Fisher Scientific Inc., USA) in monoreflexion at an angle of incidence of 45°. The powdered sample was placed in ATR crystal and pressed to collect spectra by using established procedure (Rao & Rao, 2015) with some modification, each spectra of the sample was recorded employing resolution of 8 cm⁻¹ and 256 scans with frequency array of 650 – 4000 cm⁻¹ against an empty cell as a background. Data was analyzed using the OMNIC software.

3.2.3 Mineral load

Dry oxidative ashing technique (AACC, Method 08-01.01) was used to estimate the amount of mineral load on starch. The powdered starch-mineral complex was incinerated at 525 °C for 12 hrs. in a muffle furnace and the resulting weight was measured. Average values from duplicate measurements was reported.

3.3 Films preparation and characterization

Sodium alginate powder was added at varying concentrations to 50 mL distilled water maintained at 90 °C with continuous stirring at 500 rpm. Once a clear solution of alginate was obtained, gelatinized starch was added with continued heating and stirring at

previously set condition till starch was dissolved completely. During this process, water was continuously added to maintain the final volume of 50 mL. Parallely, predetermined amounts of starch-mineral complex were dissolved in a separate beaker and once complete dissolution was obtained they were poured back into starch solution and mixed thoroughly. During this process the final volume was kept at 50 mL. In the end, 1% (v/v) of glycerol was added as a plasticizer. Then solution was subjected to vacuum drier to remove bubbles, if any, and films were casted in a circular (10 cm diameter) petri-plate. Films were dried in an oven at 50 ± 5 °C for 24 hrs. and were separated from the glass plate. A few of them were powdered using the liquid nitrogen.

Table 5. Optimization of sodium alginate and starch for film forming solution

Sodium Alginate	Starch		2% Film forming solution (solution for 50 mL)
	Complexed Starch without mineral treatment	Starch-mineral complex	
250 mg	700 mg	50 mg	1000 mg
500 mg	450 mg	50 mg	1000 mg
750 mg	200 mg	50 mg	1000 mg
-	950 mg	50 mg	1000 mg

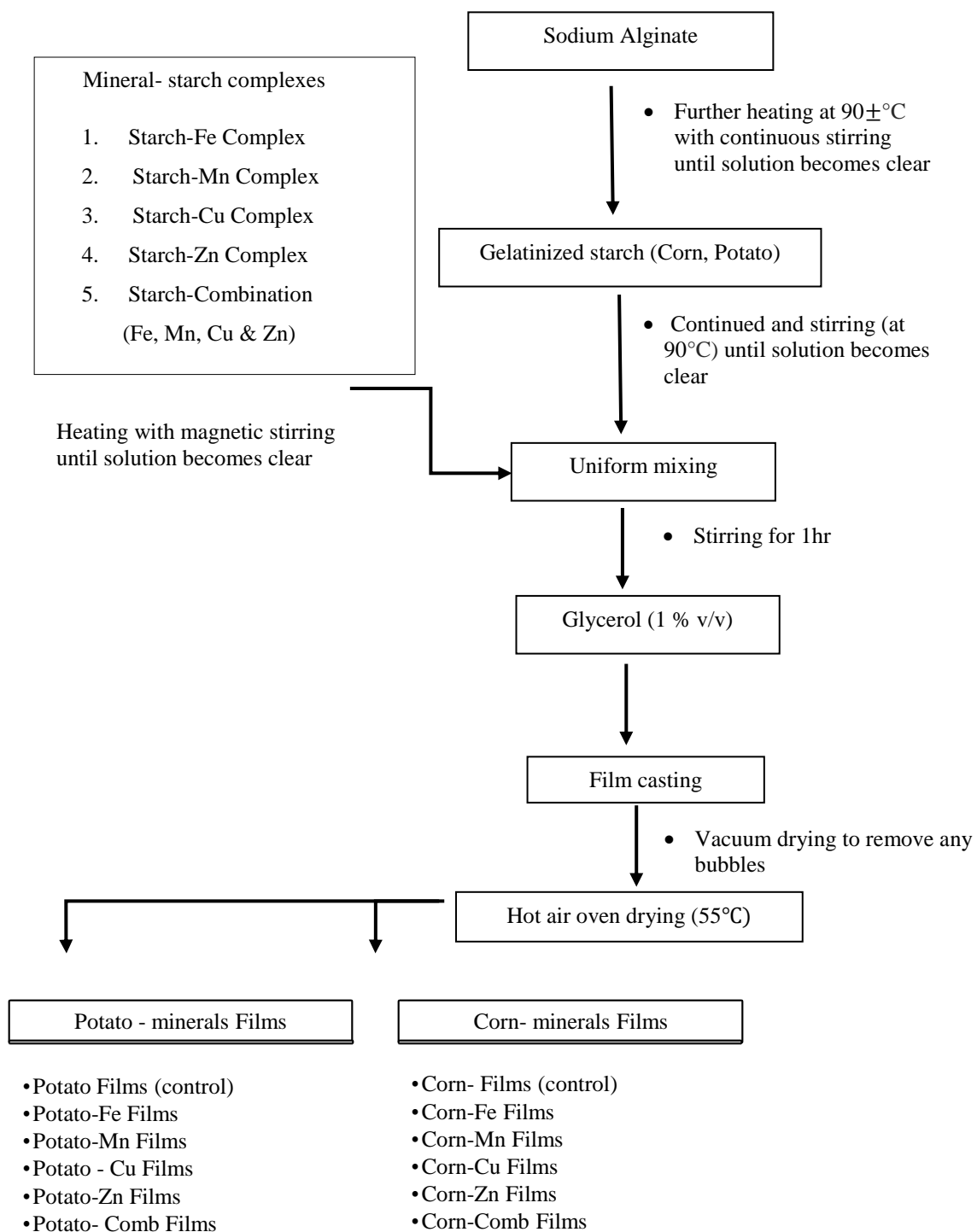


Figure 7. Experimental design for the preparation of starch-mineral ions complexed films

3.3.1 Film color

Color was measured to understand the effect of starch types on its interaction with salts, using colorimeter. A Konica Minolta colorimeter was used to evaluate the color profile using Hunter L*a* b* scale. Films were placed on a white standard plate and the L, a and b color values were measured. L values ranges from 0 (black) to 100 (white), a value ranges from -80 (greenness) to 100 (redness), and b values ranges from -80 (blueness) to 70 (yellowness). All measurements were performed in three replicates. The total color difference (ΔE), yellowness index (YI), and whiteness index (WI) were calculated using the following equations (Ghanbarzadeh et al., 2010);

$$\Delta E = [(L_{\text{standard}}^* - L_{\text{sample}}^*)^2 + (a_{\text{standard}}^* - a_{\text{sample}}^*)^2 + (b_{\text{standard}}^* - b_{\text{sample}}^*)^2]^{0.5} \dots (1)$$

$$YI = 142.86 \frac{b^*}{L^*} \dots \dots (2)$$

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5} \dots (3)$$

3.3.2 Film transparency

A smooth and undamaged film was selected and cut into ten strips of size 3 × 1 cm. Each strip was adhered on one side of a dry glass cuvette. Transparency of the films was calculated from the percent transmittance of light by exposing the films in the optical pathway at a wavelength of 600 nm on a spectrophotometer (Genesys 20, ThermoSpectronic) (Lan, He, & Liu, 2018; Czon et al., 2018). A dry, clean, and clear glass cuvette was used as the control and the transmittance was set to be 100% (Abs = 0) and the relative transmittance of the films was measured. Transparency was calculated using following equation;

$$\text{Transparency} = \frac{\log \% T_{600}}{X}$$

Where, % T_{600} is the transmittance at 600 nm and X is the film thickness measured in mm.

3.3.3 Film thickness

The film thickness was determined using the literature report (Silva et al., 2009) with some modification. Each film was cut into 4 equal parts and measurements were taken at 5 different positions of each half with a hand-held digital micrometer with a precision of 0.01 mm. The average reading was used for further analysis.

3.3.4 Water solubility

The water solubility of films is defined as the percentage of the dry matter solubilized in water after 24 hrs immersion (Gontard, Duchez, Cuq, & Guilbert, 1994). The water solubility of films was determined based on a literature report (Silva et al., 2009) but with some modification. The 3 × 3 cm cut films were dried at 100 °C overnight and weighed. Later they were immersed in 50 mL water container. The container was then placed in a water bath maintained at 175 rpm and 25 °C for 24 hrs. The final insolubilized dry matter was determined gravimetrically in the vacuum oven (105 °C /24 hrs). The percentage of total soluble matter (TSM) in the film was calculated as a function of the initial dry matter. The test was carried out in duplicate and average values are reported.

$$\% \text{ TSM} = \frac{W_1 - W_2}{W_1} \%$$

W_1 is the initial dry weight of films (g) and W_2 is the Films weight after drying.

3.3.5 Moisture absorption

The moisture absorption of the films is the amount of water it can uptake or hold. It was determined at room temperature using the procedure outlined by Ghanbarzadeh et al., 2010 but with a slight modification. The films were cut into patches of size 3 × 3 cm and dried at 105 °C overnight. The initial mass (W_1) of the dried films were quantified and were immersed in a beaker containing 100 mL of distilled water for time intervals of 5, 10, 15, 20, 30, 60, 90 and 120 mins. The wet samples were carefully blotted using a paper towel to remove excess water from surface and reweighed (W_2). The water uptake of each sample was calculated as follows:

$$\text{Water uptake \%} = \frac{W_2 - W_1}{W_1} \times 100 \%$$

Wherein W_1 and W_2 are the weights of the sample after desired interval time and the initial weight of the dried sample, respectively. All measurements were performed in duplicate and average values reported.

3.3.6 Moisture content

Films were cut into 3×3 cm size and placed in a forced air convection oven for 2 hrs. at 135°C . The loss of water was used to calculate the moisture content (AOAC, 2005). Duplicate analysis was carried out for an average reading.

3.3.7 Water vapor permeability

The water vapor permeability (WVP) was measured using the reported method but with some modification (Ghanbarzadeh et al., 2010). Special glass cups with an average diameter of 1.8 cm was used. Films were cut into discs with a diameter slightly larger than the diameter of the cup. Anhydrous CaSO_4 of 4 g was placed at the bottom of the cup to maintain the relative humidity (RH) of 0 and then films were fixed at the top with the glue. The cups were then placed in a desiccator containing saturated K_2SO_4 solution at the bottom desiccator that provided a RH of 97% at room temperature. The cups were weighed at 8 hrs intervals and water vapor transport was determined by the weight gain of the cups. Changes in the weight of the cups were recorded as a function of time and slopes was determined by linear regression (weight change vs. time). The water vapor transmission rate (WVTR) was calculated as the slope (g/h) divided by the transfer area (m^2). The WVP ($\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) was calculated using the following equation:

$$\text{WVP} = \frac{\text{WVTR}}{P(R_1 - R_2)} X$$

wherein P is the saturation vapor pressure of water (Pa) at the test temperature (25°C), R_1 is the RH in the desiccator, R_2 , the RH in the cup and X is the film thickness (m). Under these conditions, the driving force [$P(R_1 - R_2)$] is 3073.93 Pa. All measurements were performed in triplicate and average values reported.

3.3.8 Tensile strength of films

The tensile strength of the films was determined using the established protocol (Xu, Chen, Rosswurm, Yao, & Janaswamy, 2016) but with some modification. Briefly, the films were cut into rectangular strips of 8 cm long and 1 cm wide, and the tensile strengths was measured using the MTS EM Tensile with mechanical grip at room temperature. The installation parameters followed the initial grip spacing of 60 mm with extension rate 15 mm/s, data collection frequency of 50Hz, and a trigger force of 50 N. Average values from triplicate measurements was used to calculate the film strength as follow:

$$TS = \frac{F}{S}$$

Where TS is the tensile strength (Pa), F is the maximum force when the Films breaks (N), and S is the cross-sectional area of the films (width × thickness; m²).

3.3.9 Melting properties of films

The melting behavior of powdered films was evaluated using the DSC instrument Q₁₀₀ TA instruments equipped with the universal software. Sample weight of 3 mg was placed in TA Tzero pan and sealed with TA Tzero lid. The DSC head was pre-cooled to 0 °C using liquid nitrogen (20 psi). The sample pan was placed on the sample holder with an empty pan as the reference. The pans were heated at a rate of 5 °C per min in the range 20 to 150 °C at nitrogen flow rate of 20 mL/min. The enthalpy, onset temperature, peak temperature and end temperature were calculated. Average values from duplicate measurements are reported.

3.3.10 X-ray powder diffraction of films

X-ray diffraction patterns of the powdered films were collected using the protocol outlined in section 3.2.1.3

3.3.11 FTIR of films

FTIR spectra of ground films was collected using the protocol outlined in section 3.2.2.

3.3.12 Starch digestibility

The *in vitro* digestibility of gelatinized starch and films was measured according to the established protocol (Englyst, Kingman, & Cummings, 1992) with some modifications. Initially, starch content of the films was determined using total starch assay method (Megazyme). Samples of 550 mg (dry weight basis) were suspended in 50 mL centrifuge tube containing 10 mL distilled water and vortexed for 2 mins for universal mixing followed by addition of 10 mL sodium acetate, 50 mg of guar gum and 15 small glass beads (5 mm). Tubes were then added with 5 mL enzyme, prepared by adding 4.5 g of pancreatin in 30 mL water along with 3.9 mL of amyl glucosidase. Immediately, tubes were placed in a water bath and stabilized at 37 °C. During digestion, 500 µL aliquots were removed at 20 and 120 mins and mixed with 2 volumes of 80% ethanol and centrifuged for 15 mins. The supernatant was collected and the GOPOD (Glucose Oxidase/Peroxidase) procedure was conducted using an assay kit (Megazyme) to measure the glucose concentration. Average value from duplicate measurement was reported.

3.4 Statistical analysis

The data were analyzed using the Minitab 18 software programming and Microsoft Excel (version 2016). Least significant difference (LSD) was determined through one-way analysis of variance, ANOVA at confidence level 95% with two-sided confidence level interval. Further Post Hoc comparison was done using Tukey test assuming equal variance. A p value of 0.05 was utilized for defining the implication of the results.

CHAPTER 4. RESULT AND DISCUSSION

4.1 Starch-mineral complexes

There are several reports on films with 2-5% of starch concentration and with variable amounts of plasticizers (Oses et al., 2009), nanoparticles (Nafchi et al., 2012; Zhang et al., 2018) and antimicrobials (Aisyah et al., 2018), to name few. However, there is no documented evidence on the addition of mineral ions to starch films. In this regard, the present study focuses on complexing of essential mineral ions namely, Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} ions with corn starch and potato starches and their effect on physicochemical properties of films. Herein, 2% starch is taken as a test concentration and complexed with 10, 20, 30, 40, 50 and 65% mineral ions. As the potato starch is rich in amylose it has greater ability to form paste, and thus optimization of mineral concentration was performed using potato starch. Heating of starch in the presence of mineral ions, mainly manganese results in increased viscosity with increasing the ions concentration. This eventually affected drying of complexes. Increased viscosity could be due to strong interactions between starch and mineral ions (Samutsri, & Suphantharika, 2012) and inter-or intra-molecular crosslinking with starch chains (Wyatt, & Liberatore, 2010). Also, with increasing concentration of salt, solution become more difficult to handle during precipitation. Thus, 10 % has been selected as suitable salt concentration for complexing mineral ions with starches.

4.1.1 X-ray powder diffraction

Depending on the botanical origin and composition (amylose/amylopectin ratio, amylopectin branch length), starches are classified as A-type, B-type and C-type corresponding cereal, tuber/root and legumes respectively. In addition, another starch V-type is also known that is primarily amylose-lipid or amylose small molecule complexes (Manek, Builders, Kolling, Emeje, & Kunle, 2012, Morrison, Law, & Snape, 1993). The X-ray powder diffraction patterns of non-gelatinized corn starch and potato starch along with their complexes with mineral ions complexes are shown in Figure 8. The native corn starch displays a typical A-starch type diffraction with strong intense peaks at $2\theta \sim 11^\circ$,

15, 17 and 23° along with weak peaks at $2\theta \sim 13, 18, 20$ and 27°. Similarly, native potato starch displays typical B-starch type diffraction with a strong intense peak at $2\theta \sim 17^\circ$ followed by relatively medium peaks at $2\theta \sim 6, 15, 22$ and 24°. The diffraction pattern of gelatinized starch followed by precipitation is composed of two strong intense peaks at $2\theta \sim 13$ and 20° followed by a weak peak at 7° , portraying the V-type starch diffraction pattern (Shogren, Fanta, & Felker, 2006, Lopez, Flanagan, Gilbert, & Gidley, 2008). Generally, amylose adopts a double helical structure, however, after starch gelatinization, it forms a single helical structure yielding crystalline V_{hydrate} amylose that forms $V_{\text{Anhydrous}}$ amylose upon dehydration (Buleon, Delage, Brisson, & Chanzy, 1990). The V-type patterns also refers to starch single helices co-crystallized with inclusion substances such as iodine, DMSO, fatty acids, flavor compounds and alcohols, to name a few.

The V-type diffraction pattern continues even after complexing the mineral ions with the starches but with intensity variations. In addition to V-type a few new peaks have been observed. For example; corn starch contain peaks at $2\theta \sim 7^\circ$ (Zn^{2+}), 10° (Cu^{2+}), 12° (Fe^{2+} , Mn^{2+} , Cu^{2+}), 16° (Mn^{2+} , Cu^{2+}), 19° (Mn^{2+} , Cu^{2+} , Zn^{2+}), and $\sim 22^\circ$ (Zn^{2+}), 38° (Mn^{2+}). In the case of potato starch, complexing with Mn^{2+} ions results with broader 13 and 19° peaks suggesting the loss of order as observed in slowly cooled jet-cooked corn starch (Shogren, Fanta, & Felker, 2006). Overall, complexing mineral ions with the starch chains yields V-type starch structure. It is not clear about the location of the mineral ion on the starch helix either on the exterior or interior of the amylose helix. Further research is needed to establish the mineral ion location.

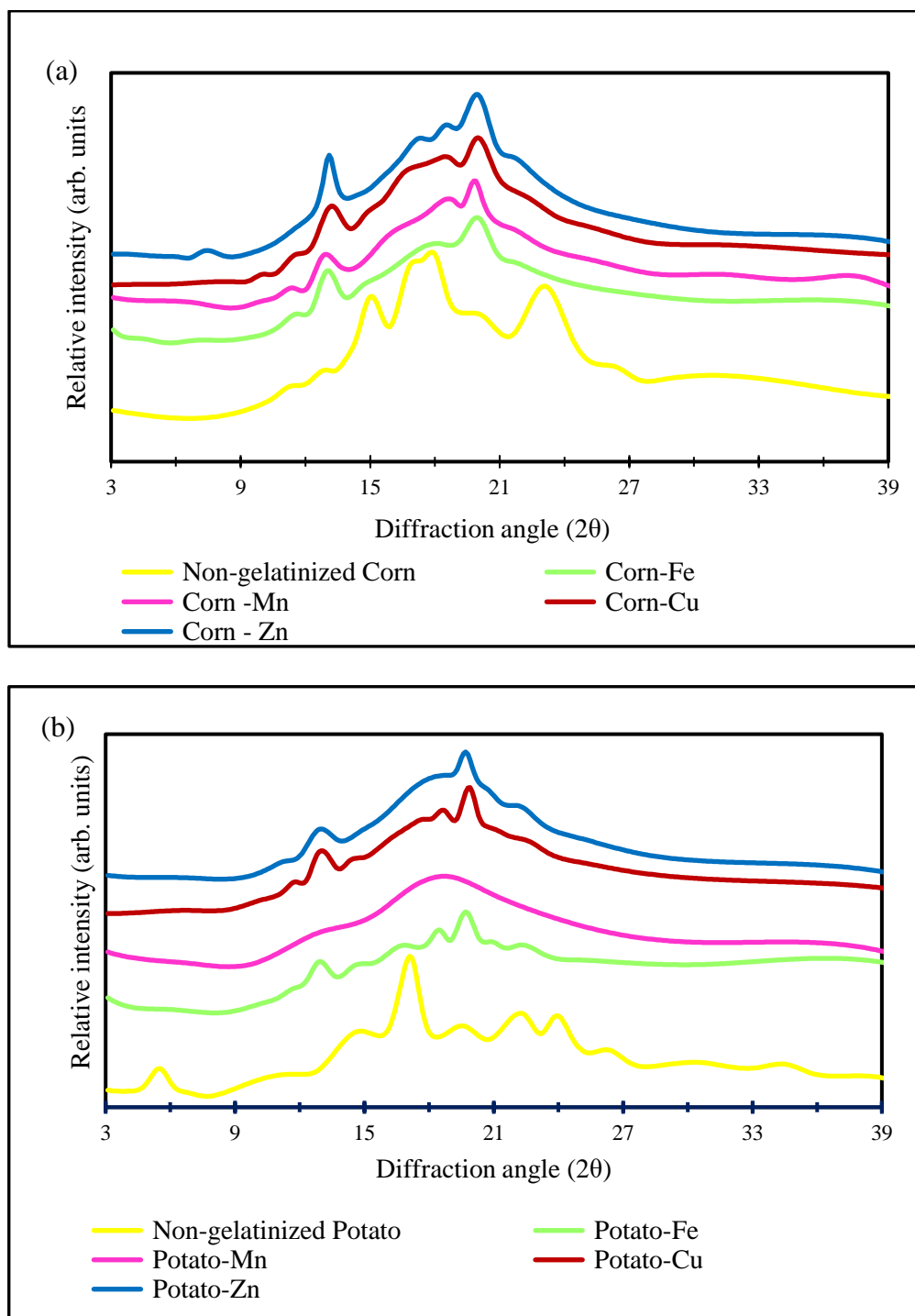


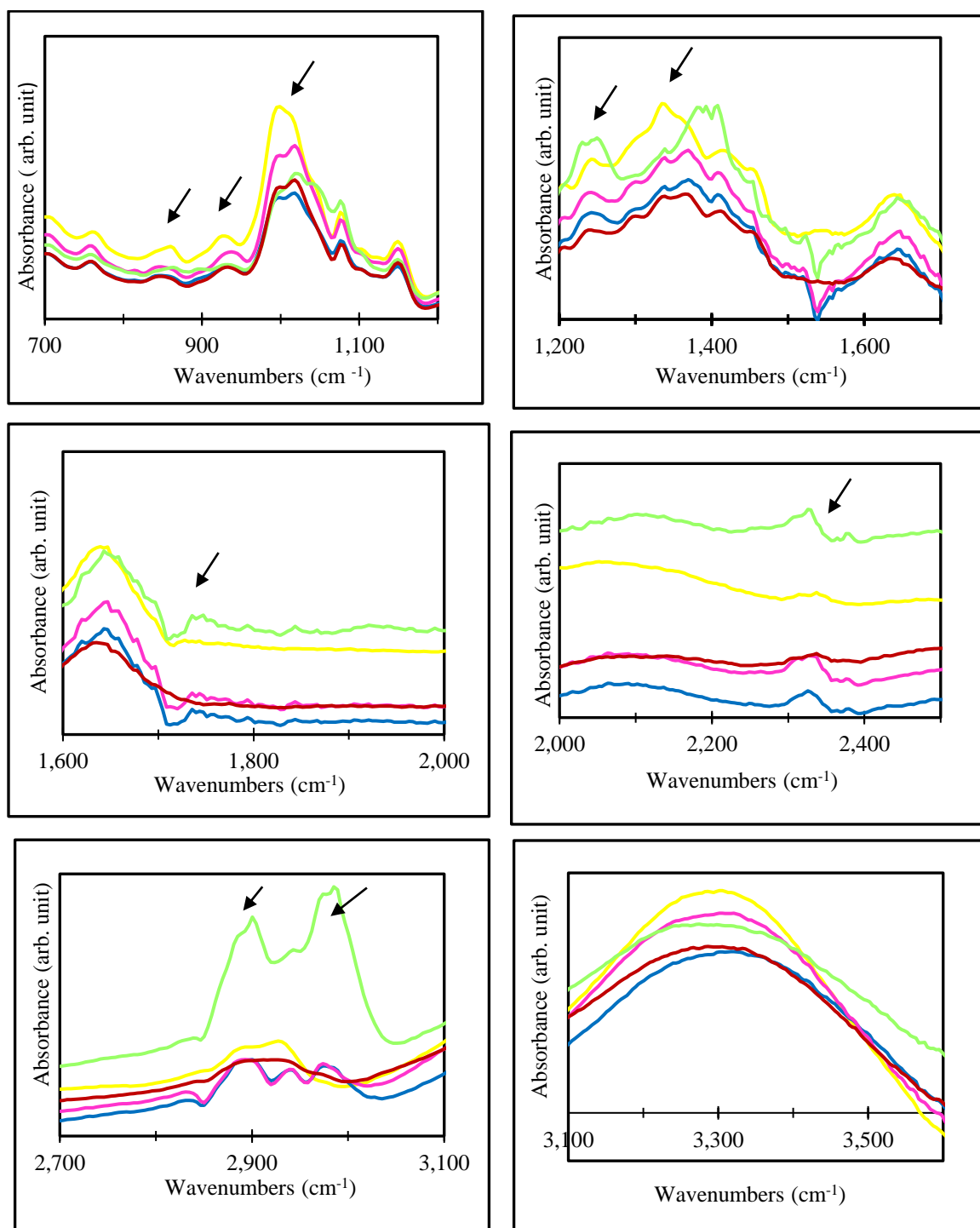
Figure 8. The X-ray powder diffraction patterns of (a) corn starch and (b) potato starch complexes with mineral ions

4.1.2 FTIR characterization

Figure 9 highlights the FTIR spectra of (a) corn starch and (b) potato starch in the wavenumber range 600- 3600 cm^{-1} and their complexes with four mineral ions Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} .

The vibrational bands at 998, 1022, 1049, 1079 and 1153 cm^{-1} signify the C-C and C-O bond stretching as well as COH bending vibration (Smrckova et al., 2013, Chi et.al., 2008). The bands in the region of 1400-1650 are due to CH_2 and O-H bending (Cabado, Ruiz, Casado, & Roman, 2016). In the presence of mineral ions, new peaks at 1396, 1758, 1770, 2329, 2379, 2973 and 2985 cm^{-1} have been observed. In addition, peak shifts are noticed at 852, 941, 1018, 1369, 1407 and 2938 cm^{-1} . These changes in advertently confirm the binding of mineral ions to starch chains.

(a)



(b)

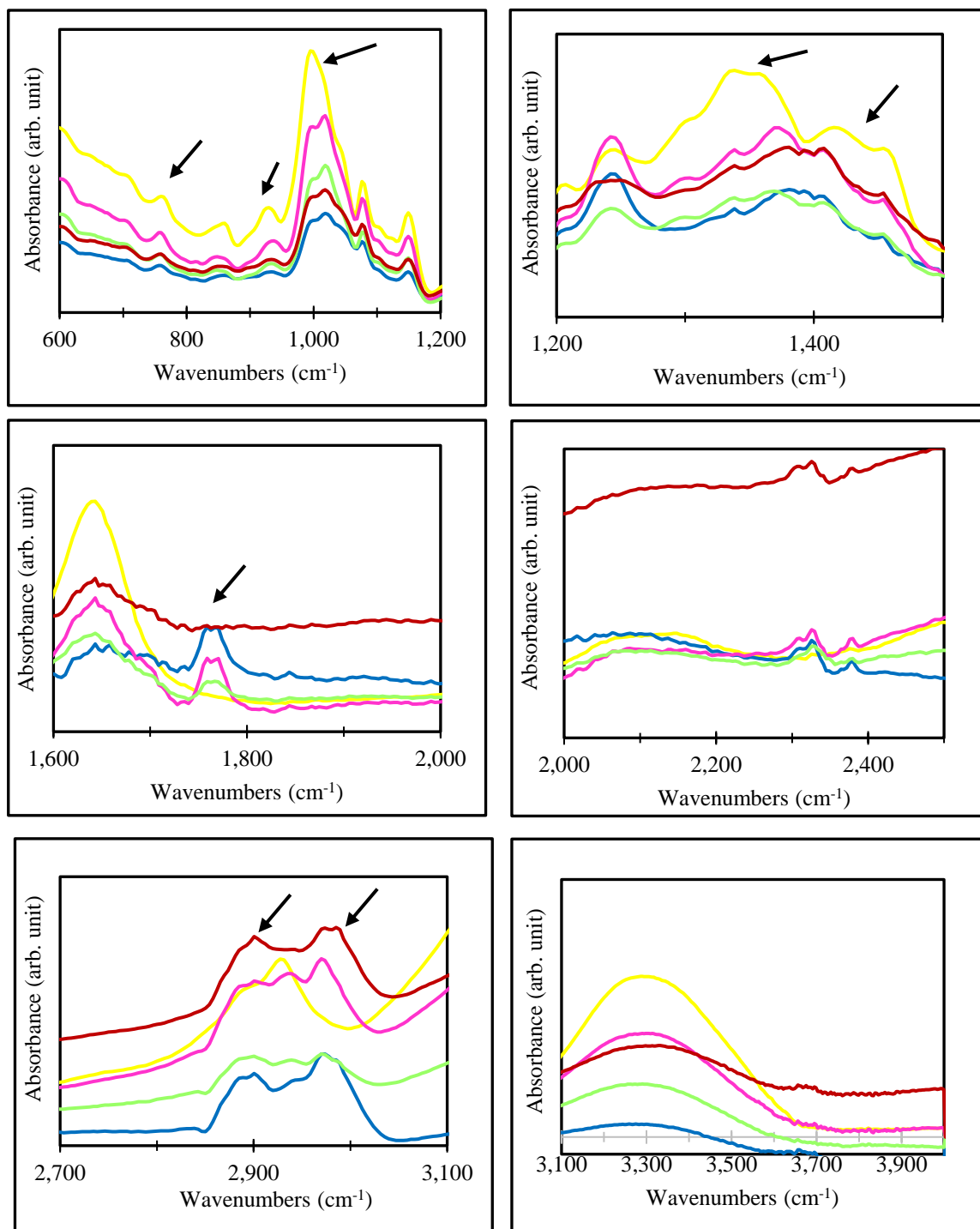


Figure 9. The FTIR spectra of (a) corn starch and (b) potato starch and their complexes with mineral ions (Arrows indicate the change in position or formation of new peaks)

4.1.3 Mineral loading

The dry ashing technique has been used to determine the amount of mineral ion complexed to starch. In the case of corn starch, the Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} ions loading (Figure 10) has been found to be 52.4 ± 5 , 60.3 ± 5 , 39.7 ± 5 and 15.2 ± 5 mg per gram of starch, respectively. Slightly elevated values of 56.5 ± 5 , 68.5 ± 5 , 44 ± 5 and 15.7 ± 5 , in the same mineral ion order, have been observed for the potato starch. The increased values could be due to the presence of phosphate groups in the potato starch granules. Overall, the order of mineral loading follows the pattern of $\text{Mn}^{2+} > \text{Fe}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+}$ for both the starches.

As there is no reference material for mineral binding on to the starch molecules, recovery of the mineral ions has been estimated in this research. The initial amount of mineral added to starch was 5000 mg/g, and upon precipitation followed by washing and drying, the final recovery of complexed potato starch was found to be 1.1% for Fe, 1.4% for Mn, 0.9% for Cu and 0.3% for Zn. Thus, the unbound mineral ions appear to have washed out along with water and organic solvents used in the starch-mineral complex precipitation.

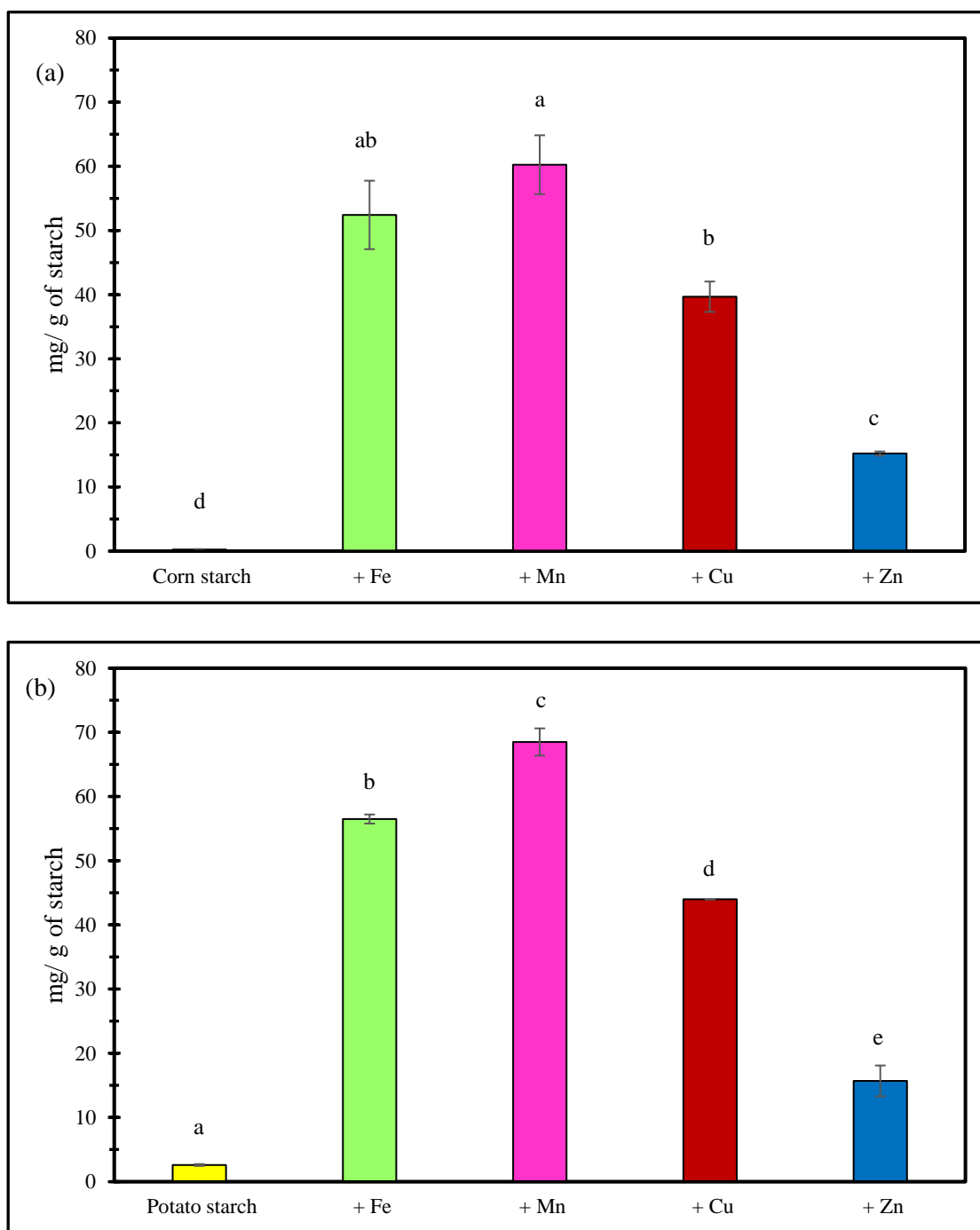


Figure 10. The loading of mineral ions on (a) corn starch and (b) potato starch
Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2 Films preparation

Corn starch and potato starch along with sodium alginate have been used at varying concentrations to prepare films. Alginate enhances the viscosity and is useful in film preparation (Parreidt, Muller, & Schmid, 2018). Film making solution consists of 2% starch and alginate in the ratio of 1:3, 1:1, 3:1 and 1:0.1 (w/v). Starch used in film preparation represents the starch that has been complexed with and without mineral ions. Based on the Recommended Daily Intake (RDI) of mineral ions, the amount of starch-mineral complex is fixed to 100 mg per 100 mL of solution. During film preparation, 100 mL of solution is poured in two different petri-plates each having 50 mL of 2% solution. Taking the example of Cu^{2+} , starch-mineral complex has a mineral load of 44 mg, while addition of 100 mg of complex results in 4.4 mg of Cu^{2+} in the solution, thus each film consists of 2.2 mg of Cu^{2+} equivalent to the RDI. On this note, each starch film prepared in this research will deliver 2.9, 3.4 and 0.75 mg of Fe^{2+} , Mn^{2+} and Zn^{2+} ions. The films based on 3:1 (starch: alginate) ratio yield homogeneous, flexible, thin and strong and thus this combination has been selected for further research.

Table 6. Visual observation of films at various starch alginate concentrations

S. N.	Film forming concentration			Overall appearance	Final selection
	Gelatinized starch (mg)	Starch mineral complex (mg)	Sodium Alginate (mg)		
1)	200	50	750	Dense	×
2)	450	50	500	In between films 1) and 3)	×
3)	700	50	250	Flexible and very thin with easy to handle	√
4)	850	50	100	Too soft and difficult to handle	×

4.2.1 Film color

The color parameters, Hunter lab color scale ($L^*a^*b^*$), total color difference (ΔE), yellowness index (YI) and whiteness index (WI) of corn starch and potato starch films in the presence of four essential mineral ions and their combination are presented in Figure 11.

Corn starch and potato starch films are transparent. The control film with no mineral ions displays significantly higher L^* (lighter) and lower a^* & b^* values. This trend continues in the presence of Zn^{2+} and Cu^{2+} ions and values are not significantly different ($p < 0.05$). However, addition of Mn^{2+} and Fe^{2+} ions results in variations. The films containing Fe^{2+} and combination of ions are not significantly different ($p < 0.05$) from each other in contributing lowest L^* value. The impact of different L^* value readily reflects on the whiteness index (WI). Significantly higher WI has been observed for the control, Zn^{2+} and Cu^{2+} films compared to those with Fe^{2+} , Mn^{2+} and combination of ions.

The Cu^{2+} starch films display greenish tint with negative a^* value (greenish). In contrast, films with Mn^{2+} and Fe^{2+} ions show brownish to yellowish tint with lower L^* values (darker) coupled with positive a^* (redness) and higher b^* values (yellowness). As b^* transitions from negative to positive range, blueness (-80) increases to yellowness (70) hence, increase b^* values in the films result in yellow color and ultimately with increasing yellowness index. Films with all four minerals yield a maximum b^* value of 28.04 ± 0.38 and 26.83 ± 0.43 for corn starch and potato starch, respectively. The Least Significant Difference (LSD) does not show significant difference among the Fe^{2+} and combination of ions films, however, in terms of b^* there appears some significant difference in the Mn-starch films. This difference manifests in brownish to yellowish tint as YI increases to 48.25 ± 0.39 (combination of ions) from 19.86 ± 0.69 (Mn^{2+}) and 46.05 ± 0.10 (Comb-) from 17.26 ± 0.34 (Mn^{2+}) for corn starch and potato starch films, respectively. The greenish, brownish and yellowish tints are predominantly due to the incorporation of mineral ions in the starch films. This observation is congruence with the

retention of pale color from the transition metal ions such as Mn^{2+} , Cu^{2+} and Co^{2+} on metal-starch complexes (Ciesielski, Lii, Yen and Tomasik, 2003).

The preservation of color by the films could be further described using another color function namely ΔE , which indicates the degree of total color difference against the standard white plate. Films with lower b^* and higher L^* values show relatively lower ΔE than those with higher b^* and lower L^* . The lower ΔE of control films as well those of Zn^{2+} and Cu^{2+} corn starch and potato starch films correlates to the minimal color difference from the standard white plate. It further attributes to enhanced clearness and in other words to higher L^* with increased lightness. On the other hand, films with Mn^{2+} , Fe^{2+} and ions combination exhibit significantly higher ΔE due to lower L^* and hence noticeable color change is evidenced. These observations agree with the decreased L^* as a function of increased solute concentration of tea extracts (Jourbat, 1995).

Overall, changes in the surface color of films reflect the effect of complexation of mineral ions on to the starch alginate chains along with the ions mediated cross-linking between the alginate and starch chains. Color has been proven to be a good indicator of objective evaluation of food products. In this regard, film color could as well serve as a factor in gaining consumer acceptance.

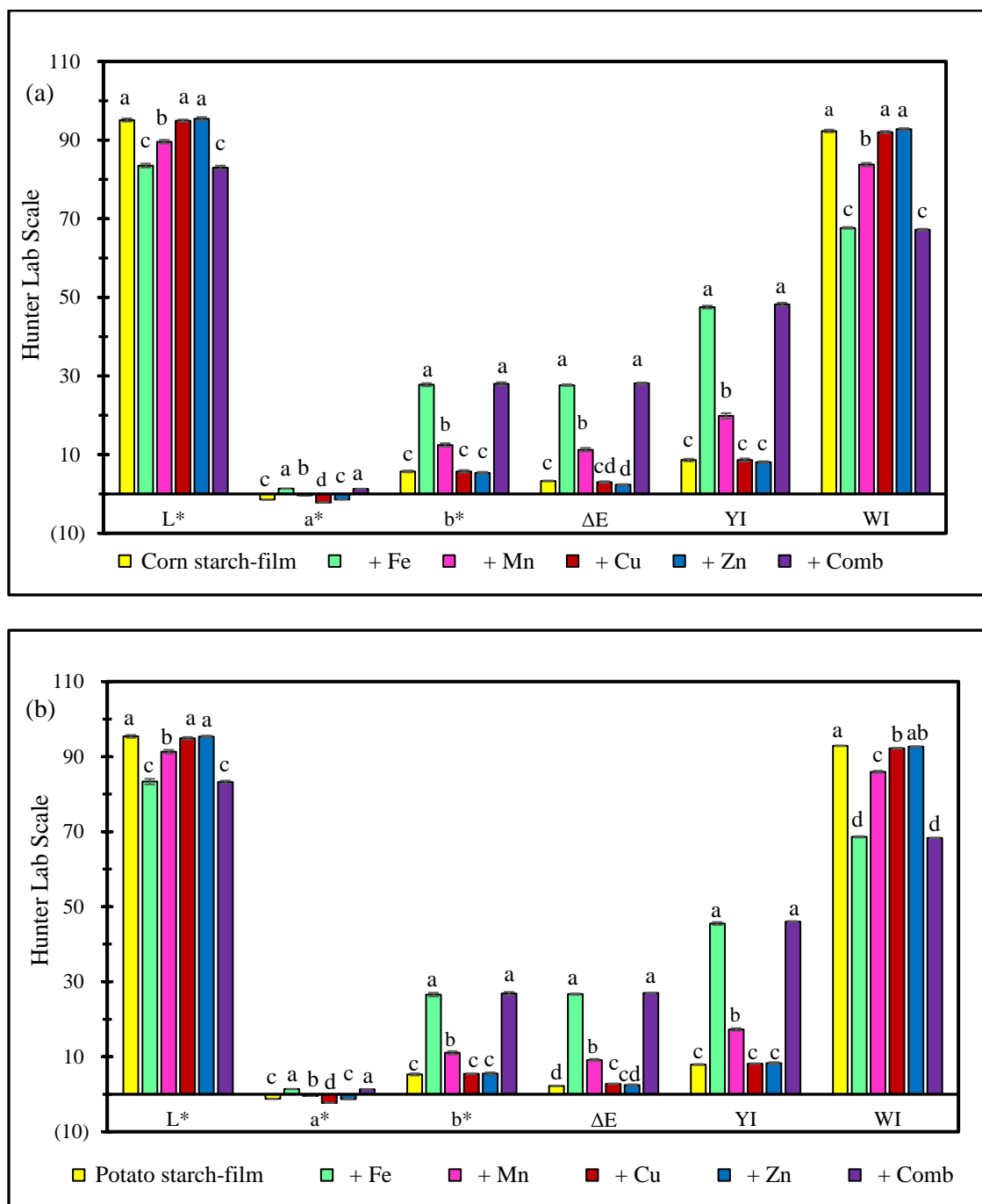


Figure 11. The Hunter color values (L^* , a^* and b^*), total color difference (ΔE), yellowness index (YI) and whiteness index (WI) of films of (a) corn starch and (b) potato starch films in the presence of mineral ions

* Mean sharing same letters for each parameter in each image, are not significantly different from each other ($p < 0.05$)

4.2.2 Film transparency

Figure 12 highlights the transparency of corn starch and potato starch films in the presence of mineral ions. The transparency of corn films is 14.68, 13.47, 13.51, 14.9, 14.64 and 13.57 for the control, Fe^{2+} , Mn^{2+} , Cu^{2+} , Zn^{2+} and combination of ions, respectively. Though, the Cu^{2+} -corn film has higher transparency it is statistically ($p < 0.05$) not different from that of control and Zn^{2+} -films. Interestingly, the presence of Mn^{2+} and Fe^{2+} ions influence the transparency compared to the rest. Brownish to yellowish tint in the films has been observed in the presence of Mn^{2+} and Fe^{2+} ions (see color analysis, section 4.2.1) and thus, it appears that due to the chromophoric nature of these ions lower amount of light is being transmitted through the films leading to decreased transparency. It has been observed that the presence of nanoparticles results in lower transparency in corn starch films (Sun, Xi, Li, & Xiong, 2014). In the similar lines, formation of starch-mineral nanoparticles could reduce the transparency but more research is needed to establish this concept.

Starch is composed of amylose and amylopectin and a recent study clearly demonstrates the binding of Zn^{2+} ions to the amylose molecules (Liu, Li, Shang, & Xie, 2019). Thus, minerals could complex with amylose and also amylopectin and in turn impart changes on the quality attributes of films. In addition, film thickness and particle size larger than the visible wavelength could as well impact the film properties (Kampeeraappun, Ahtong, Pentrakoon, & Srikulkit, 2007).

In the case of potato starch films, the presence of Zn^{2+} -ions raises the transparency value significantly to 15.72 from that of control and Cu^{2+} -films. The increase could be attributed to variations in the film thickness. For example, Zn^{2+} -films are thinner by about 0.01 mm compared to the rest. The role of film thickness on the transparency has been established in cellulose-glycerol-polyvinyl alcohol composite films (Cazon et al., 2018).

Overall, the transparency values of starch films and their complexes with mineral ions agree well with those of polyethylene films (Lee, Son, & Hong, 2008). Films with higher

transparency are desirable for food packaging and coating as consumers prefer to see foods that are being packaged. However, colored films with lower transparency could as well be preferred for food packaging as they could serve as barriers to prevent lipid oxidation.

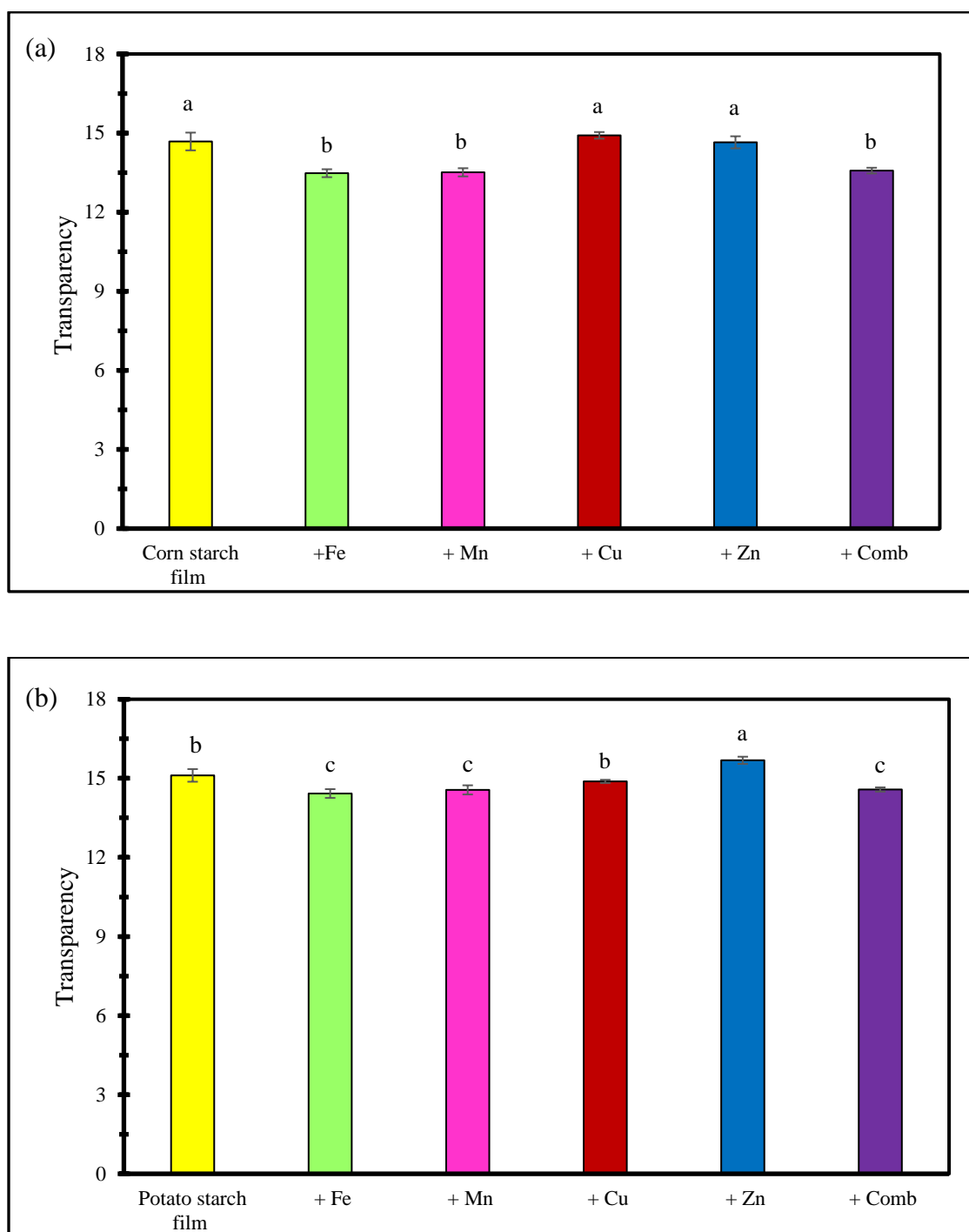


Figure 12. The transparency of (a) corn starch and (b) potato starch films in the presence of mineral ions

Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2.3 Film thickness

Thickness is one of the important parameters that influences the mechanical and physical properties of films. The film thickness ranges from 0.09 to 0.16 mm (Figure 13) with an average of 0.13 mm and 0.14 mm for potato starch and corn starch films, respectively. These values match well with those of potato starch films loaded with nano sized SiO₂ (Zhang et al., 2018). Herein, no significant difference ($p < 0.05$) has been observed between the control and mineral complexed films. Film thickness also varies by amount of plasticizer (e.g. glycerol) added during film preparation. For example, film thickness enhances to 0.0938 mm from 0.0699 mm as the glycerol concentration raises to 30% from the initial 10% (Aisyah et al., 2018). This is due to glycerol binding to water, thereby raising the solution viscosity and affecting the water evaporation rate during drying leading to thicker films.

The films dried in a hot air oven 55 °C for a day had thin and transparent appearance and have been flexible and homogenous without any visible cracks and pores. Starch plasticized with 50 parts by weight of glycerol as in this study, could have increased the film flexibility, making them easier to handle with no any shrinkage. This could be due to faster evaporation of water from thinner films that limits the molecular movement in the films toward responding to shrinkage (Jansson and Thuvander, 2004). The amount of amylopectin could have influenced the film thickness (Basiak, Lenart and Frederic, 2017) as well, however, in this research no such effect has been observed and certainly warrants more research.

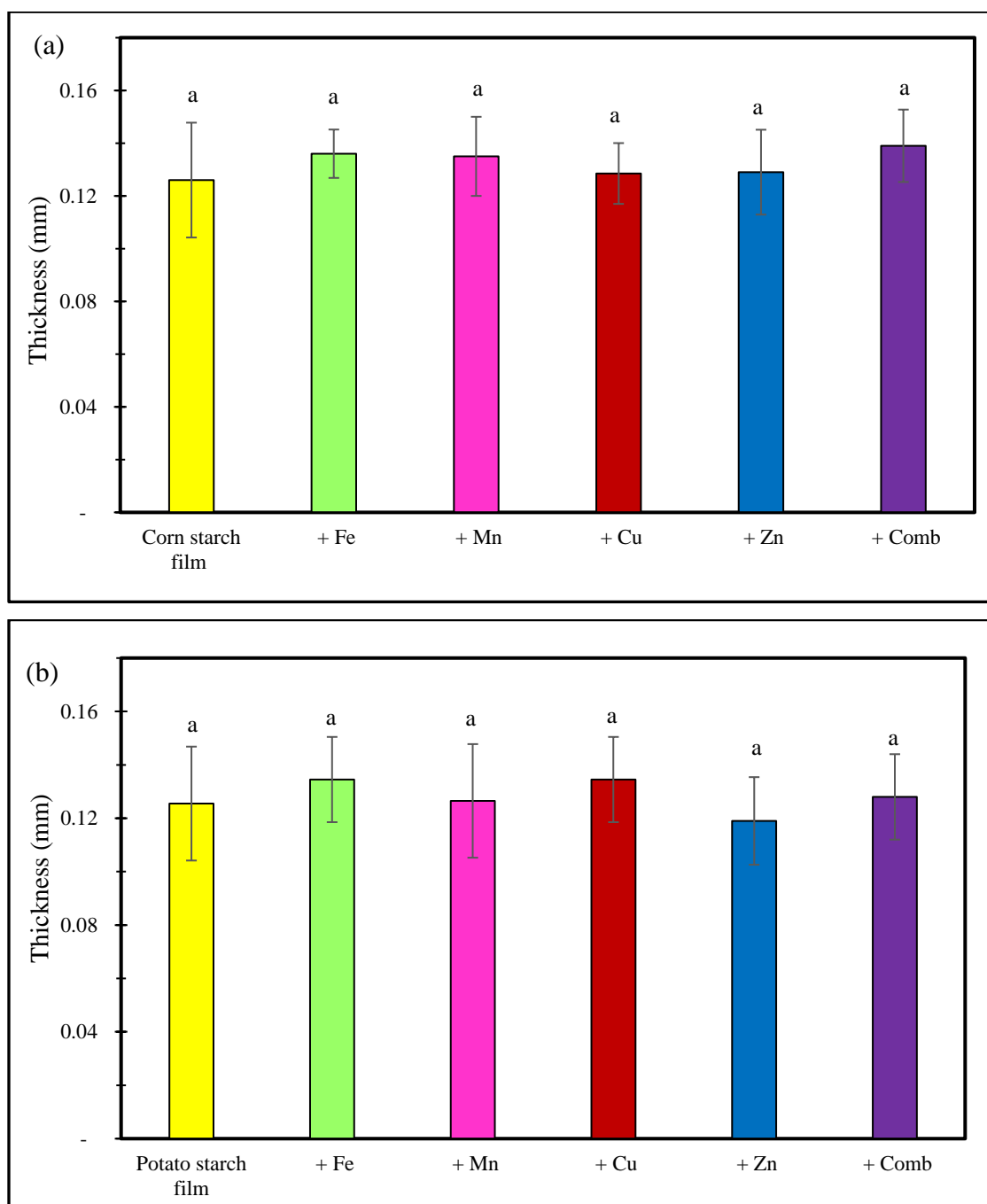


Figure 13. The thickness of (a) corn starch and (b) potato starch films in the presence of mineral ions

Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2.4 Water solubility

Water solubility is an important parameter of biodegradable films as it is a key indicator to the water resistance by the films. The percentage water solubility of all films is illustrated in Figure 14. The corn starch films possess a value of $29.2 \pm 3.5\%$. On the other hand, potato starch films display lower water solubility of $27.4 \pm 0.3\%$. These values agree well with the 27.5% from cassava starch films (Chiumarelli, & Hubinger, 2014), 20% from tapioca starch films (Kim, Choi, Kim, & Lim, 2015) and 27.3% from acetylated corn starch films (Lopez, Garcia, & Zaritzky, 2008). In the case of corn starch films, the water solubility increases to 35.1 and 38.2% with the presence of Fe^{2+} and Cu^{2+} ions, respectively. It further enhances to 45.1 ± 3.6 and $42.6 \pm 3.2\%$ with the presence of Zn^{2+} and Mn^{2+} ions, respectively. As with the corn starch films, presence of Fe^{2+} and Cu^{2+} ions increases the potato starch films water solubility to 38 ± 1.2 , $37.5 \pm 0.9\%$, respectively, and further to 47.0 ± 3.1 and $44.9 \pm 2.1\%$ with Zn^{2+} and Mn^{2+} ions, respectively. The ion combination results in around 37% water solubility in both the films. The water solubility is significantly higher ($p < 0.05$) in the presence of Zn^{2+} and Mn^{2+} ions, causing the films to disintegrate. The water solubility appears to be more driven by cation type but with subtle differences between the starch types. More research involving various starches with different amylose and amylopectin content needs to be studied, however.

The addition of hydrophilic molecules such as citric acid (CA) is found to enhance the water solubility of corn starch films (Ghanbarzadeh et al., 2011). Thus, it appears that presence of hydrophilic groups attracts more water and interrupts the existing hydrogen bonding network in the films and in-turn reduce the cohesiveness in the matrix leading to the increase solubility.

Incorporation of nanocomposites such as nano-silver and silver zeolite has been found to increase the water solubility of chitosan films (Rhim, 2006). On the other hand, addition of starch in the highly gelling collagen results in voids in the film matrix that are amenable for water penetration and increased solubility (Wang et al., 2017).

Interestingly, alginate films dissociate completely in water but crosslinking through Ca^{2+} ions promotes water resistance (Rhim 2004). These three instances could possibly occur in the present starch-mineral films. The alginate chains might preferably bind to divalent ions resulting in voids in the matrix and in-turn increasing the water uptake and solubility of films. More research is needed to establish these facts, however.

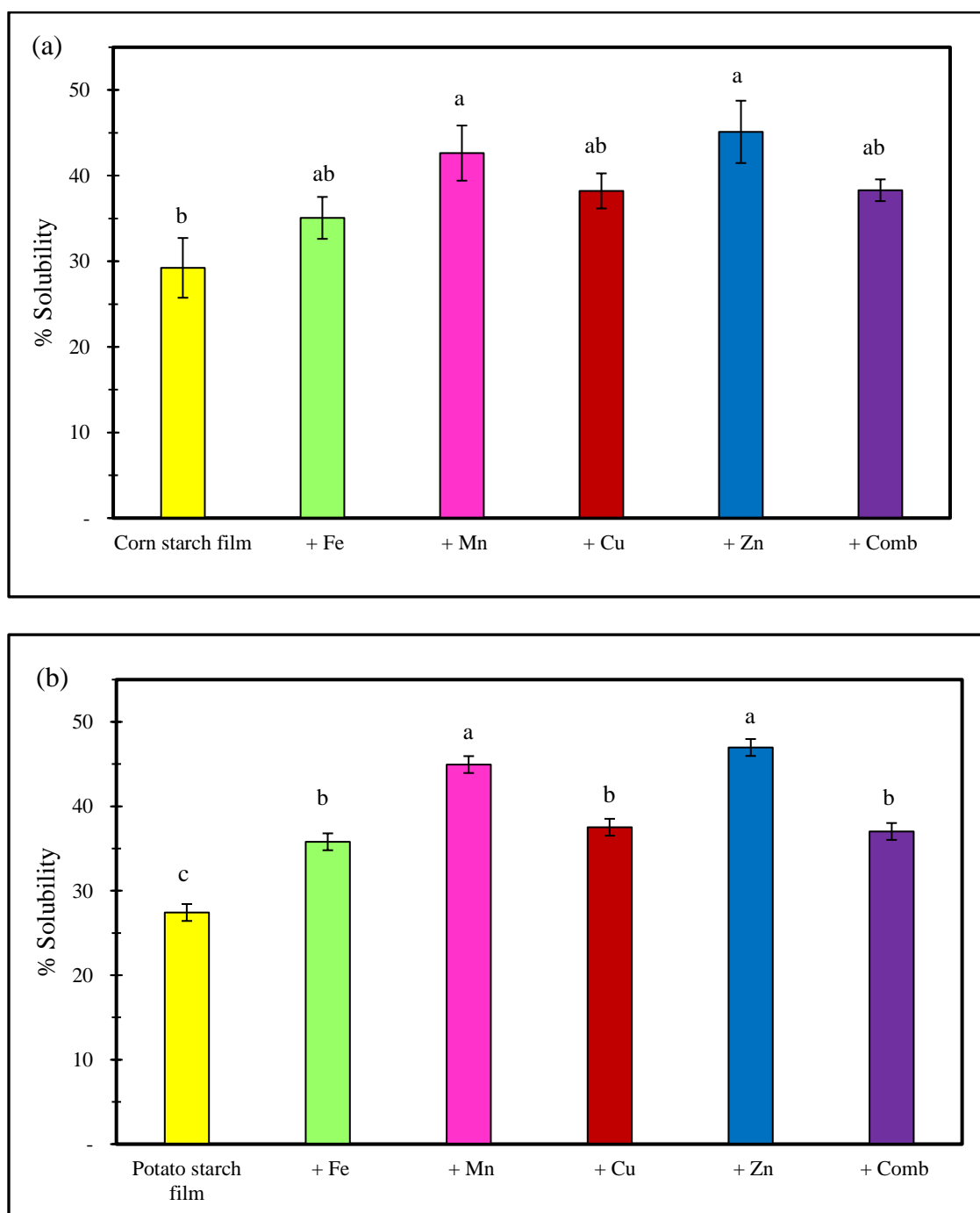


Figure 14. The water solubility of (a) corn starch and (b) potato starch films in the presence of mineral ions

Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2.5 Moisture absorption

The moisture absorption of corn starch and potato starch films as a function of time, for 120 mins, is shown in Figure 15. A maximum water absorbency of 696% by the corn starch film is observed at 90 mins. The presence of mineral ions increases the moisture absorbency significantly. The films absorb as high as 1231% in the presence of Mn^{2+} ions followed by 884 and 809% with Zn^{2+} and Fe^{2+} ions, respectively. Interestingly, in the presence of Cu^{2+} ions the absorbency is less than the corn starch with a value of 512%. In contrast to the corn starch films, potato starch films display a higher water uptake value of 3600%. The maximum swelling reported in the case of pure potato starch films is around 69.9% as compared to 34.7% from pure corn starch films (Basiak et al., 2017). The much-increased moisture absorbance observed in the present case could be due to the presence of hydrophilic alginate chains. Even in the potato starch films the Mn^{2+} ions yield maximum absorbency of 4166% followed by 3912% with the Zn^{2+} ions. The Cu^{2+} incorporated films show lowest absorption value of 248%. Overall, Mn^{2+} and Zn^{2+} ions significantly enhance the water uptake by the films.

Films with higher water absorptivity disintegrate completely in water. As shown in the Figure 15, there are three zones: in the first water absorption adapts a linear trend, during the first 30 mins, and in the second zone it increases gradually toward saturation and finally in the third zone decreases slightly. This decrease could be due to solubility of films in the water. This trend is also noticed in cassava starch films that display steady step of water absorption whilst rice and waxy rice starch films tend to have a linear but with high rate adsorption (Phan et al., 2005). Generally, swelling occurs due to water penetration into the film matrix. Once the polar, hydrophobic and ionic groups are saturated with the bound water, additional swelling takes place due to free or bulk water that fills the space in the network in addition to large pores and voids, if any (Ismail, Irani, and Ahmad, 2013). The intrinsic interactions between the amylose and alginate chains that further modified by the presence of mineral ions and amylopectin chains result in a complex network and could result in such changes. Overall, presence of hydrophilic groups as in starch, alginate and glycerol coupled with the coordination

nature of mineral ions and the number of pores created, to name a few, dictate the films swelling behavior. Further research is needed to establish in this observation.

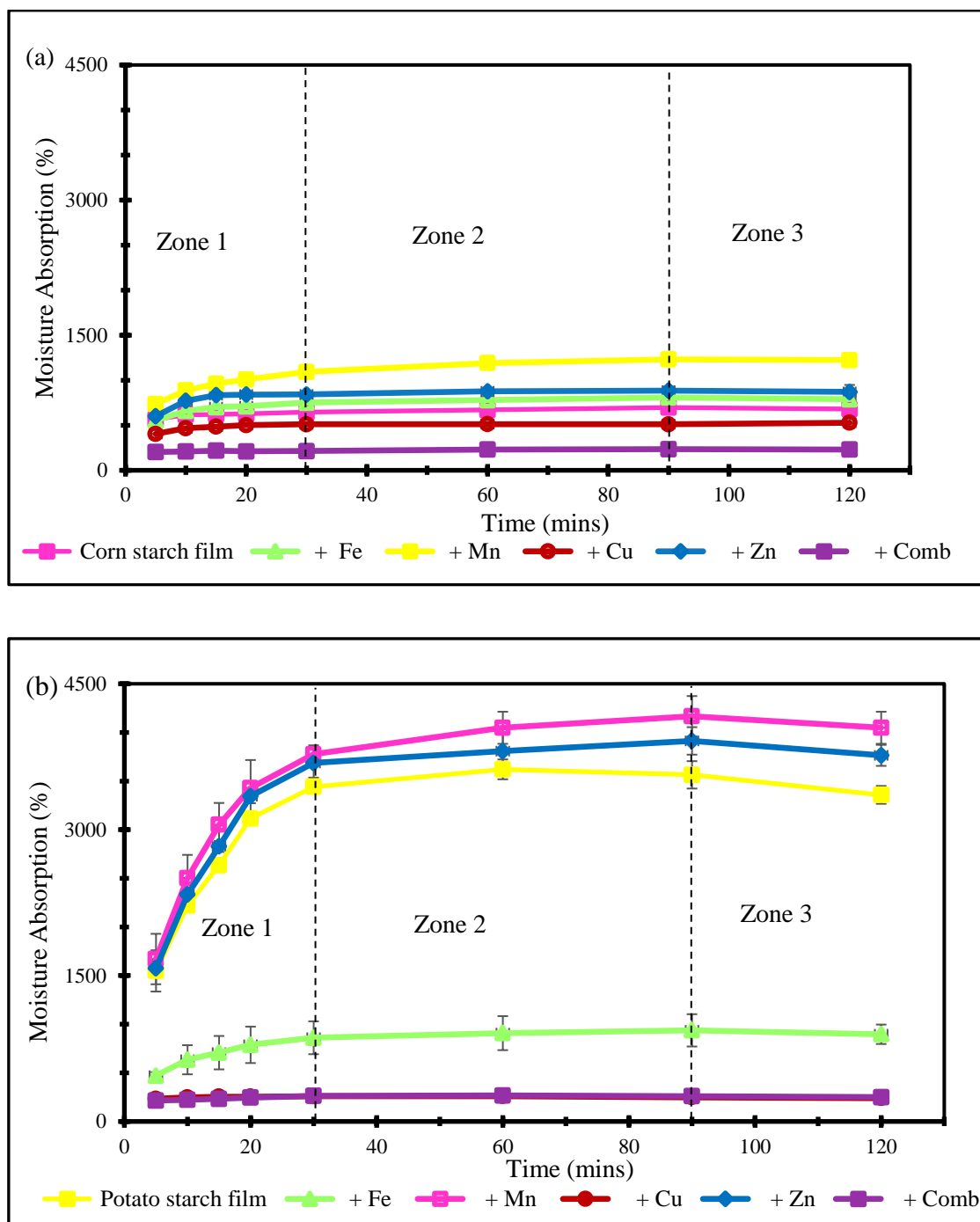


Figure 15. The water absorption of (a) corn starch and (b) potato starch films in the presence of mineral ions

4.2.6 Moisture content

The amount of moisture present in films indicates the hydrophilicity. Figure 16 highlights the effect of mineral ions on the moisture content in the corn starch and potato starch films. The films are not significantly different ($p < 0.05$) compared to the control films. As in moisture water solubility (section 4.2.4) and absorption (section 4.2.5), the highest experimental value of 33% for corn starch films and 30% for the potato starch films has been observed in the presence of Mn^{2+} and Zn^{2+} ions. Thus, it appears that films with Mn^{2+} and Zn^{2+} ions possess more hydrophilic characteristics compared to the rest. Indeed, such hydrophilic nature of starch-mineral complexes increases the water content in the films and in-turn affects the water solubility. These changes will also reflect on the water sorption behavior of the films.

The moisture content in the films appears to depend on several factors such as nature of the biopolymer and the presence or absence of hydrophobic and hydrophilic polymers. For example, potato starch films display 25.5% of moisture content (Oses et al., 2009). Similarly, 21.5% by the cassava starch films (Adjouman et al., 2017), 26.3% by the sodium alginate films (Syarifuddin, Dirpan, & Mahendradatta, 2017) and 16.8% by the whey protein isolate films (Ramos et al., 2012). In the chitosan films, moisture content decreases from 19.3 to 15.8% with the addition of 5% mango leaf extract, mainly due to hydrophobic components of the extract (Rambabu, Bharath, Banat, Show, & Cocolletzi, 2019). On the other hand, with the addition of chitoligossacharides to the whey protein isolate films water content increases to 23 from 16% (Ramos et al., 2012). The addition of hydro-alcohol extract and presence of polyvinyl alcohol increases the moisture content of chitosan films (Mujtaba et al., 2018; Srinivasa, Ramesh, Kumar, & Tharanathan, 2003).

The observation of enhanced moisture content in the starch films due to the presence of mineral ions, as in the present case, opens up new opportunities to develop films with the required moisture.

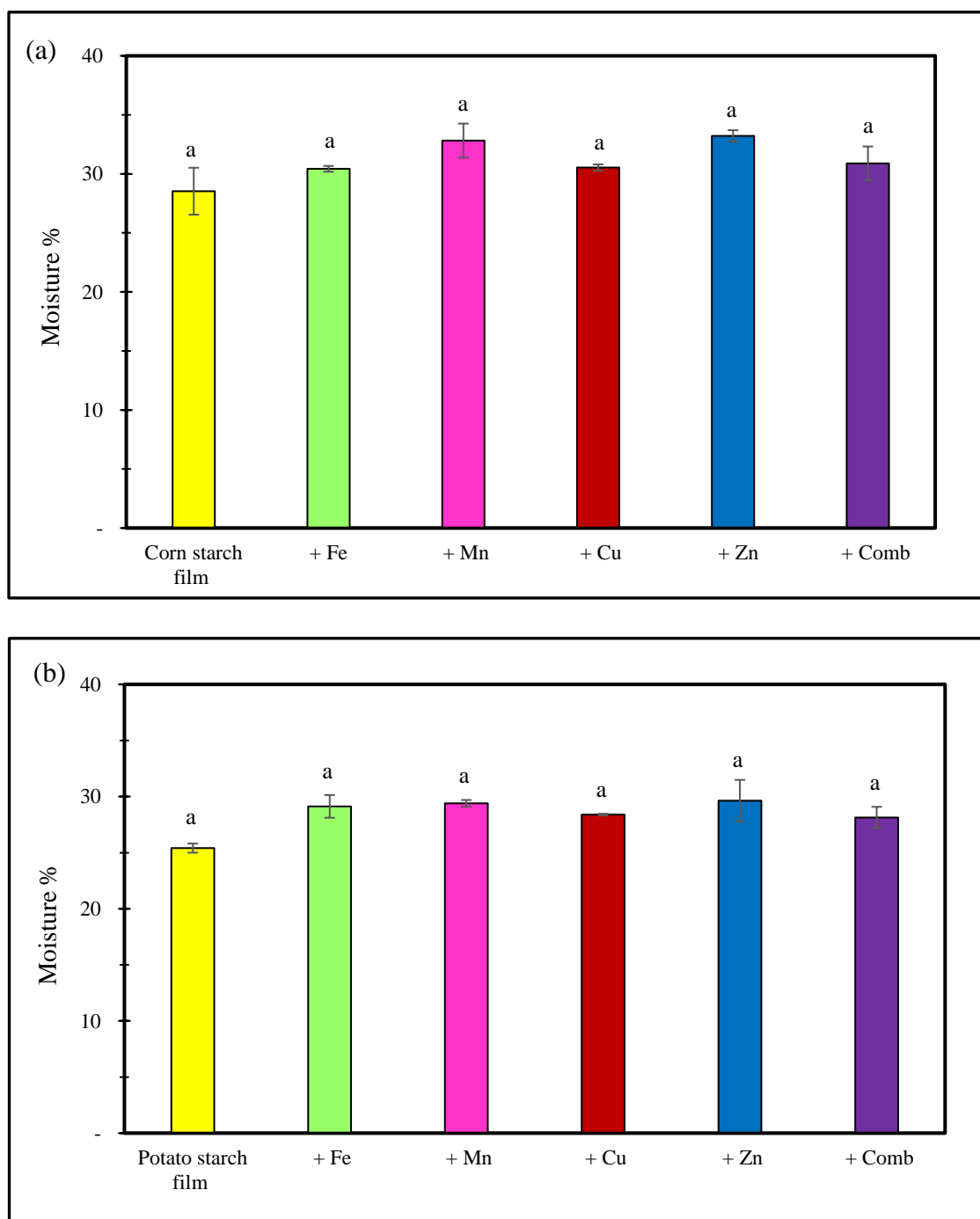


Figure 16. The moisture content of (a) corn starch and (b) potato starch films in the presence of mineral ions

Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2.7 Water vapor permeability

Moisture transfer between food and surroundings controls food perishability. Films stand out as viable alternatives for food packaging applications. Thus, developed films should completely avoid or retard the moisture transfer. Toward this end, Water Vapor Permeability (WVP) of films plays a significant role.

Figure 17 highlights the WVP of corn starch and potato starch films without and with the presence of mineral ions. The pure corn starch and potato starch films display the WVP of 1.4×10^{-10} and $1.8 \times 10^{-10} \text{ g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$, respectively. The addition of mineral ions tends to decrease the WVP. For example, in the presence of Fe^{2+} ions the WVP of corn starch films is found to be $1.0 \times 10^{-10} \text{ gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$ and $0.8 \times 10^{-10} \text{ gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$ for Mn^{2+} potato starch films. Overall, there are subtle variations in the WVP values but are not statistically different ($P > 0.05$). It has been reported that film thickness influences the moisture transfer rate and permeability (Phan et al., 2005). In the present case, film thickness does not show any dependence on the mineral type and thus the observed subtle changes in WVP are not surprising. The lower WVP values suggest reduced chain mobility in the films mainly due to crosslinking (Rhim, 2004) as noticed in pectin films with Ca^{2+} ions (Pavlath, Voisin, & Robertson, 1999) and corn starch films with citric acid (Ghanbarzadeh et al., 2011) and CaCO_3 nanoparticles (Sun et al., 2014).

The WPA values of mineral-starch films are lower compared to some known polysaccharides and commercial films. For example, cassava, cellophane and LDPE films have a WPA of 1.2, 0.8 and $0.02 \times 10^{-10} \text{ gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}$, respectively (Phan et al., 2005). In the present case, the presence of alginate would have resulted in increased moisture permeability.

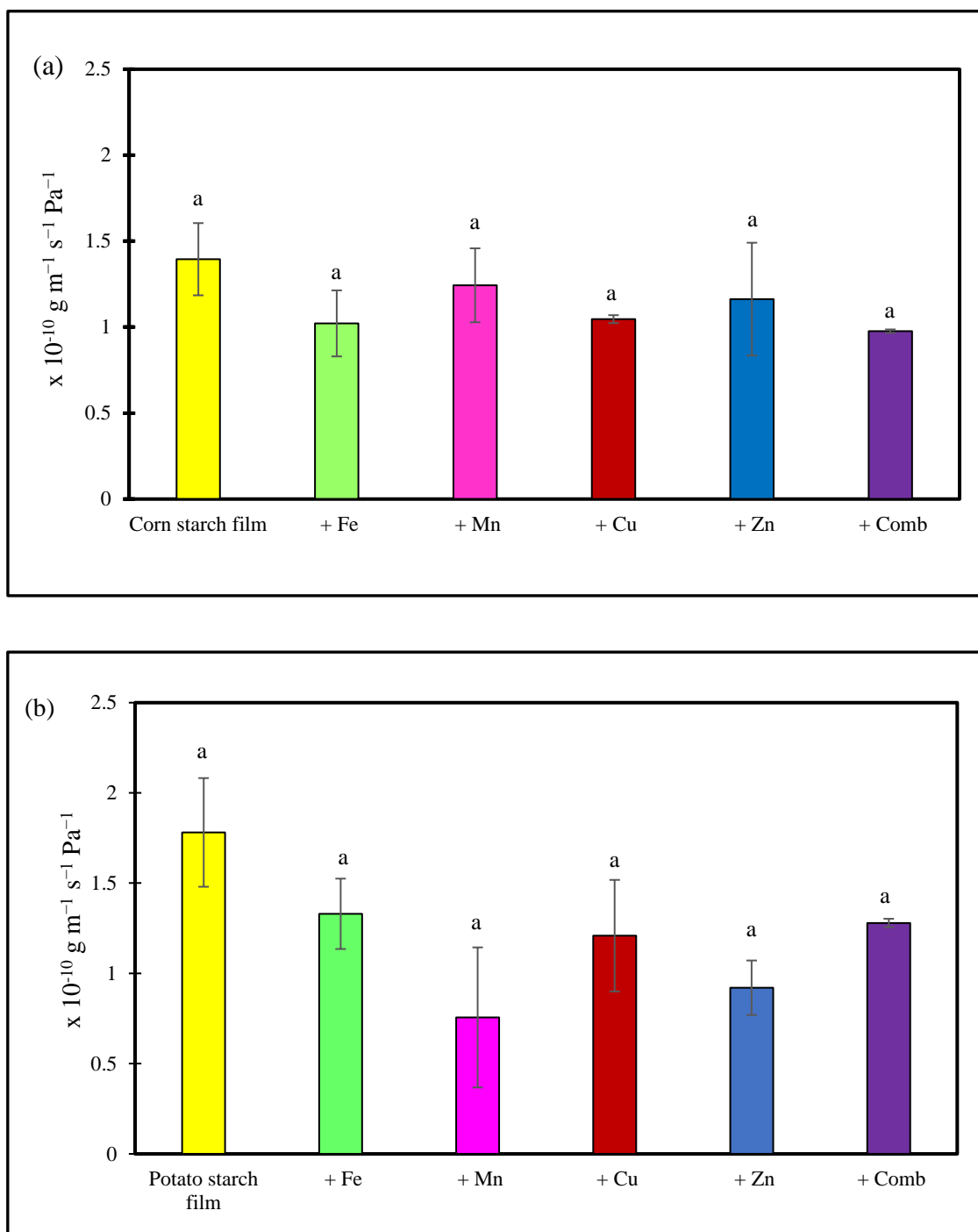


Figure 17. The water vapor permeability of (a) corn starch and (b) potato starch films in the presence of mineral ions

Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2.8 Tensile strength

Tensile strength is a mechanical property of a film that reflects the maximum stress that a film could sustain before it eventually breaks. It also reflects film's resistance to the mechanical damage during processing, handling and storage (Aisyah et al., 2018). The effect of mineral-ions on the tensile strength of corn starch and potato starch films are depicted in Figure 18. The mean value obtained for potato starch and corn starch films is 7.76 ± 0.83 MPa and 5.22 ± 1.40 MPa, respectively. The pure corn starch and potato starch films have value of 6.6 and 38.3 MPa, respectively (Ghanbarzadeh et al., 2010; Sun et al., 2014; Oses et al., 2009). The variations could be attributed to the presence of alginate chains in the present films.

In the case of potato starch films, presence of mineral ions increases the tensile strength. For example, in the presence of Fe^{2+} ions a maximum value of 10.42 ± 0.83 MPa has been observed. The Mn^{2+} , Cu^{2+} and Zn^{2+} yield a value of 9.8 ± 1.70 , 10.00 ± 0.98 and 8.00 ± 0.74 MPa, respectively. These values are relatively low in the case of corn starch films. The Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} ions result in a tensile strength of 4.5 ± 0.5 MPa, 7.5 ± 0.34 MPa, 4.4 ± 0.09 MPa, 5.6 ± 0.79 MPa, respectively.

For a particular starch, though variable tensile strength values are observed they are not statistically different ($p < 0.05$). Interestingly, films composed of potato starch display higher tensile strength values. It appears that the presence of higher amounts of amylose, as in potato starch compared to relatively none in corn starch, facilitates complexing with the alginate chains that further strengthens with the presence of mineral ions and translates to increased tensile strengths. Lack of amylose in corn starch might preclude starch alginate complexing and in-turn could result in lower tensile strength of the films.

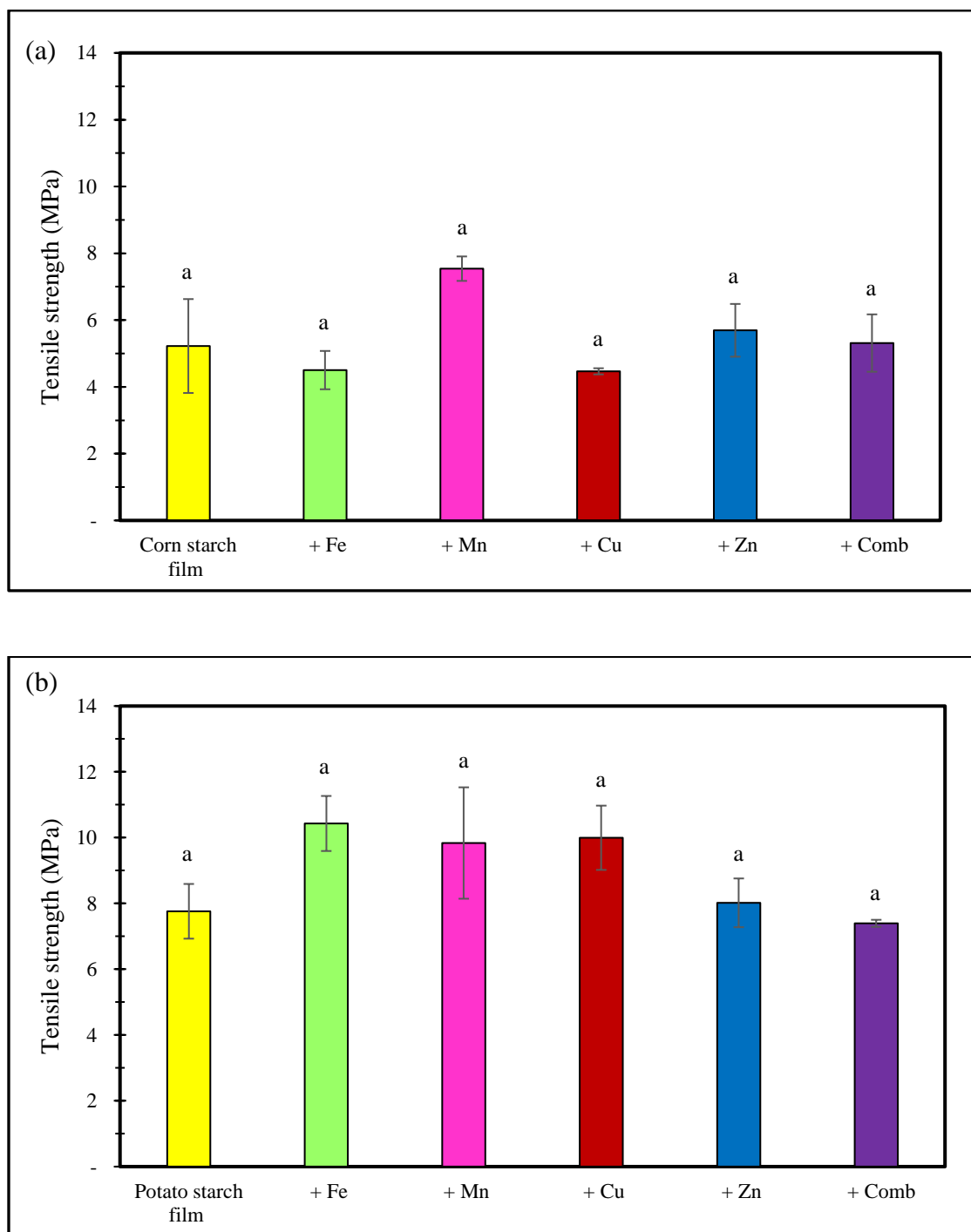


Figure 18. The tensile strength comparison of (a) corn starch and (b) potato starch films in the presence of mineral ions

Means sharing same letters, in each image, do not statistically differ from each other ($p < 0.05$)

4.2.9 Melting properties of films

A summary of DSC findings on the corn starch and potato starch films in the presence of mineral ions is shown in Table 7. The endothermic transitions for the corn starch and potato starch films occur in the temperature range 44 to 152 °C and 31 to 149 °C, respectively. The corn starch films display a well-defined endothermic peak at 93.6 ± 0.3 °C and an onset temperature of 51.6 ± 0.2 °C and end transition temperature of 141.2 ± 1.3 °C. The melting enthalpy is found to be 63.6 ± 4.2 J/g. The impact of mineral ions addition is subtle on the onset, peak and end-temperature but with an exception for the Mn^{2+} corn starch films that display highest peak temperature of 98.2 ± 2.7 °C and end temperature of 152.4 ± 2.3 °C. However, there have been significant changes on the total enthalpy. In the presence of Mn^{2+} ions the enthalpy is as high as 88.3 ± 3.5 J/g. The Fe^{2+} , Zn^{2+} and Cu^{2+} ions yield 84.7 ± 0.2 , 82.1 ± 3.0 and 70.8 ± 2.7 J/g, respectively. The presence of all mineral ions results in 73.5 ± 3.3 J/g. These changes highlight the synergistic interactions between starch and alginate chains that further strengthen by the presence of mineral ions.

In the case of potato starch films, the onset, peak and end temperatures are 34.4 ± 2.8 , 82.9 ± 0.5 and 138.7 ± 5.4 °C, respectively. Compared to the corn starch films, potato starch films display decreased onset, peak and end temperatures. These variations are due to differences in the amylose and amylopectin contents as the presence of increased amylose results in easy melting initiation coupled with lower melting energy. However, amylopectin demands more energy to melt due to highly ordered structure. Interestingly, higher melting enthalpy of 100.4 ± 4.2 J/g is observed in the potato starch-films. It could be due to preferential interactions between amylose and alginate chains leading to requirement of higher heat energy to melt. The interactions between the amylose and alginate chains further get strengthened with the presence of mineral ions. This manifests in significant changes in the peak and end temperatures but with subtle changes in the melting enthalpy.

Table 7. Comparison of onset (To), peak (Tp) and end (Tc) temperature and enthalpy of (a) corn starch and (b) potato starch films complexed with mineral ions

(a)

Film type	Onset Temperature (°C)	Peak Temperature (°C)	Enthalpy (J/g)	End Temperature (°C)
Corn starch film	51.6±0.2 ^a	93.6±0.3 ^a	63.6±4.2 ^d	141.2±1.3 ^b
+ Zn	45.7±0.1 ^a	93.8±3.3 ^a	82.1±3.0 ^{abc}	143.3±1.8 ^{ab}
+ Mn	48.5±1.6 ^a	98.2±2.7 ^a	88.3±3.4 ^a	152.4±2.3 ^a
+ Fe	49.2±2.4 ^a	94.9±0.9 ^a	84.7±0.2 ^{ab}	145.7±3.9 ^{ab}
+ Cu	44.1±5.4 ^a	91.9±0.1 ^a	70.8±2.7 ^{cd}	147.7±3.5 ^{ab}
+ Comb	50.6±1.6 ^a	92.2±1.01 ^a	73.5±3.3 ^{bcd}	143.2±1.3 ^{ab}

(b)

Film type	Onset Temperature (°C)	Peak Temperature (°C)	Enthalpy (ΔE)	End Temperature (°C)
Potato starch film	34.4±2.8 ^a	82.9±0.5 ^b	100.4±4.2 ^a	138.7±5.4 ^b
+ Zn	32.7±1.0 ^a	92.0±4.5 ^{ab}	104.9±4.2 ^a	155.2±6.0 ^a
+ Mn	33.0±0.5 ^a	90.5±4.8 ^{ab}	101.3±0.3 ^a	147.9±3.4 ^{ab}
+ Fe	33.0±0.6 ^a	91.3±2.0 ^{ab}	99.9±0.9 ^a	148.6±1.7 ^{ab}
+ Cu	31.8±1.6 ^a	94.4±0.6 ^a	97.4±8.7 ^a	148.3±0.6 ^{ab}
+ Comb	33.1±0.1 ^a	93.9±0.8 ^{ab}	113.7±1.3 ^a	149.6±2.4 ^{ab}

Means sharing same letters, in each column of the table, do not statistically differ from each other (p<0.05)

4.2.10 X-ray powder diffraction of films

The control films made of gelatinized starch and alginate along with the plasticizer glycerol yield amorphous patterns with a large hallow peak at around 20° . This observation agrees well with the reported loss of starch crystallinity when complexed with alginate (Fontes, Calado, Rossi, & Rocha, 2013).

The films with mineral ions also display similar amorphous diffraction patterns. However, additional but feeble crystalline peaks at 17° and 22° have been observed in the case of potato films. These peaks are characteristics of E_H -type and V_H -type structures, E_H type signifies the recrystallization of amylopectin and V_H -type amylose crystallization (Ren, Dang, Pollet, & Averous, L. 2018). The peaks at 17° and 22° suggest the recrystallization of amylopectin in potato starch films in the presence of Zn^{2+} and Fe^{2+} ions and for the films that contain all mineral ions.

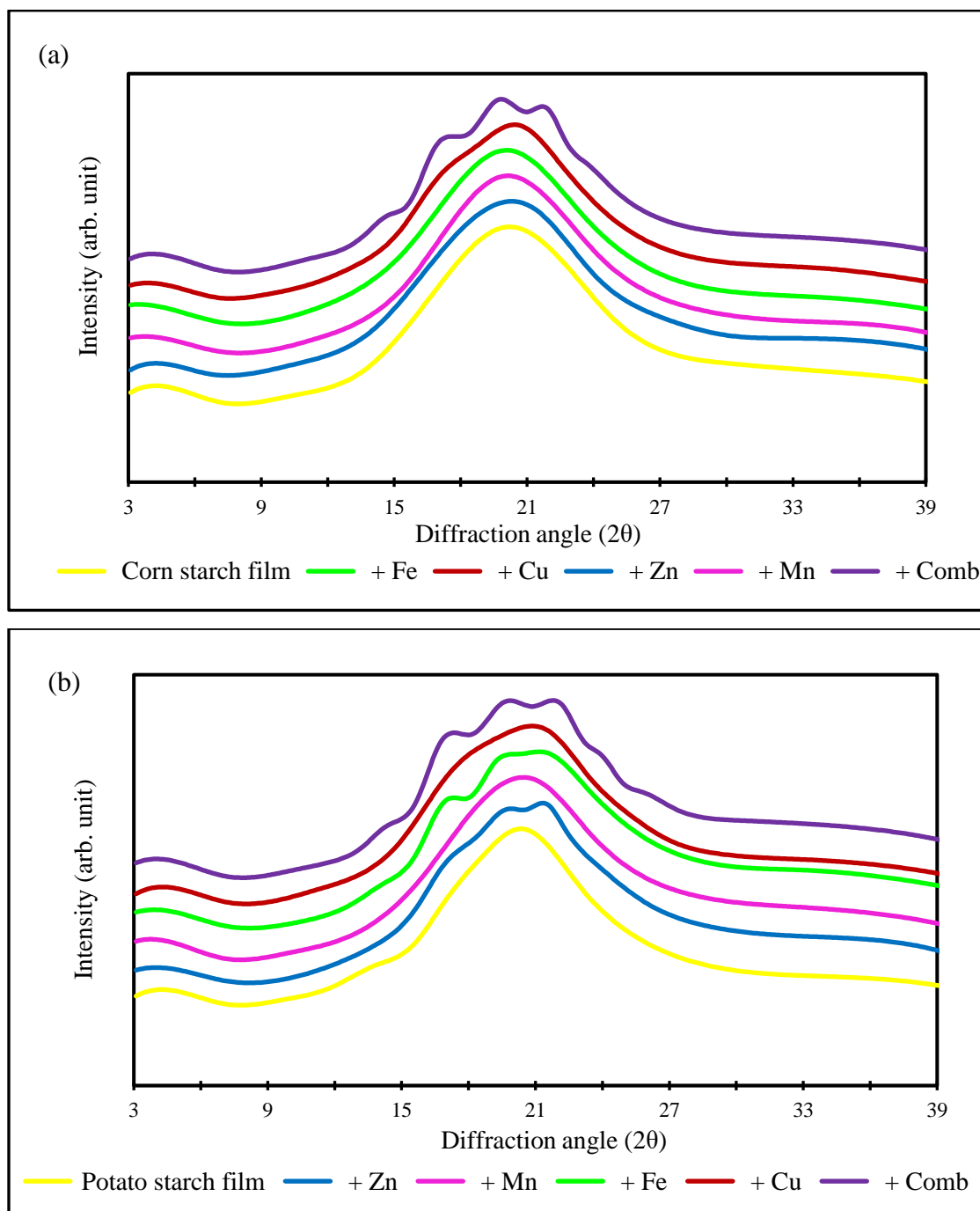


Figure 19. The X-ray powder diffraction patterns of (a) corn starch and (b) potato starch films in the presence of mineral ions

4.2.11 FTIR of films

The major functional vibrations from the FTIR spectra have been listed in the Table 8. The vibrations 863, 1025, 1103, 1338, 1396, 1407, 1454 cm^{-1} signify the skeletal vibrations of anhydroglucose ring, C-C and C-O stretching, C-H bending, O-H stretching (Chi et.al., 2008; Fan, Dai, & Huang, 2012; Giacomo, Cesaro, & Quaroni, 2008; Staroszczyk, & Janas, 2010). In the presence of mineral ions, peak shifting to 852, 1018, 1122, 1284-1342, 1388, 1400, 1423 and 1465 cm^{-1} has been noticed. In addition, bands at 1041 cm^{-1} (C (1)-H bending) and 1076 cm^{-1} (C-O stretching) are absent among all the mineral treated films excepting the corn-Cu²⁺ films. Similarly, the broad peaks 1230-1253 cm^{-1} , and 2969 -2985 cm^{-1} , are also absent. The peaks at 1238 and 2935 cm^{-1} are new and present only in the mineral treated films. The absence or shifting of characteristic peaks in these films clearly indicate binding of mineral ions to the starch and alginate matrix.

Table 8. Comparison of the FTIR wavenumbers in the range 4000-600 cm⁻¹ from the (a) corn starch and (b) potato starch films in the presence of mineral ions

(a)

Corn starch film	+ Zn	+ Mn	+ Fe	+ Cu	+ Comb
863	848	848	848	863	863
929	929	929	929	929	929
998	998	998	998	998	998
1025	1018	1018	1018	1025	1025
1049	*	*	*	1049	1049
1076	*	*	*	1076	1076
1103	1122	1122	1122	1103	1103
1149	1149	1149	1149	1149	1149
1230 and 1253	*	*	*	1230 and 1253	1230 and 1253
	1238	1238	1238	*	*
1334	1288-1353	1288-1354	1288-1355	1334	1334
1380	1388	1388	1388	1380	1380
1396	1400	1400	1400	1396	1396
1407	1423	1423	1423	1407	1407
1454	1465	1465	1465	1454	1454
1608	1608	1608	1608	1608	1608
2900	*	*	*	2900	2900
	2935	2935	2935	*	*
2973 and 2989	*	*	*	2973 and 2985	2973 and 2989
3235-3400	3235-3400	3235-3400	3235-3400	3235-3400	3235-3400
3664	*	*	*	3664	3664

*Indicates the absence of characteristic peaks of corn starch film, in mineral ions complexed films

(b)

Potato starch film	+ Zn	+ Mn	+ Fe	+ Cu	+ Comb
763	763	763	763	763	763
863	852	852	853	854	855
929	929	929	929	929	929
998	998	998	998	998	998
1025	1025	1025	1025	1025	1025
1041	*	*	*	*	*
1076	*	*	*	*	*
1103	1122	1123	1124	1125	1126
1157	1157	1157	1157	1157	1157
1230 and 1253	*	*	*	*	*
	1238	1238	1238	1238	1238
1338	1284 -1342	1285 -1342	1286 -1342	1287 -1342	1288 -1342
1380	1388	1388	1388	1388	1338
1396	1400	1400	1400	1400	1400
1407	1423	1423	1423	1423	1423
1454	1465	1465	1465	1465	1465
1608	1608	1608	1608	1608	1608
2900	*	*	*	*	*
	2935	2935	2935	2935	2935
2969 and 2985	*	*	*	*	*
3212-3370	3212-3370	3212-3370	3212-3370	3212-3370	3212-3370
3660-3687	*	*	*	*	*

*Indicates the absence of characteristic peaks of potato starch film, in mineral ions complexed films

4.2.12 *In vitro* Starch digestion

The Table 9 highlights the percentage amount of starch digested at 20 and 120 mins. The gelatinized corn starch and potato starch digest fast and around 82.9 and 84.7%, respectively, of digestion is noticed at 20 mins. This further increases to 85.0 and 88.8%, in the same order, at 120 mins. These values and trend are in accord with the reported gelatinized starches (Sasaki et al., 2015). The presence of non-starch polysaccharides such as guar gum (Bordoloi, Singh, & Kaur, 2012), retards the starch digestion and this has been noticed in the present research wherein films have been prepared by complexing starch with alginate. In the case of corn starch films at 20 mins the starch digestion reduces to 67.2% and a similar value of 67.6% is noticed with potato starch films. This trend continues and even after 120 mins and only around 72.5 and 73.6%, in the same order, has been digested. The viscosity increase due to alginate in the starch slurry during digestion is responsible for the reduction in the starch digestion. Indeed, such a behavior is advantageous in developing food products with reduced glycemic response.

Interestingly, mineral ions presence enhances the starch digestion. For example, in the case of corn starch films, at 20 mins the amount of starch digested is 81.6, 88.4, 75.6 and 78.4 for Zn^{2+} , Mn^{2+} , Fe^{2+} and Cu^{2+} ions, respectively. The increase in the starch digestion is due to enhanced enzyme activity as ions could serve as electron donors and participate in the enzyme catalytic activity (Okwuenu, Agbo, Ezugwu, Eze, and Chilaka, 2017). For example, Mn^{2+} ions could activate a large number of enzymes such as hydrolases, kinases, decarboxylases, and transferases (Rucker, Fascetti, & Keen, 2008). Highest activity of aminoglucosidase has been noticed in the presence of Mn^{2+} ions (Adeoyo, Pletschke, & Dames, 2018). Similarly, enhanced enzyme activity of amylase is reported in the presence of Zn^{2+} and Fe^{2+} ions (Raza & Rehman, 2016). Thus, it appears that upon the addition of enzyme to mineral ion complexed starch films, mineral ions get detached from the starch matrix and get anchored to enzyme toward increasing the enzyme activity and in-turn digest more amount of starch. This effect, indeed, continues to 120 mins of digestion. However, presence of viscous alginate reduces the overall digested starch amount.

This trend further continues to potato starch films but the values are relatively lower such as 85.8, 78.5, 67.0 and 67.8%, for Zn^{2+} , Mn^{2+} , Fe^{2+} and Cu^{2+} ions, respectively. Thus, it appears the amount of starch available for digestion is reduced due to formation of starch alginate complexes. Based on these observations, it could be concluded that in the case of potato starch films the network could be composed of binary mixtures of amylose-alginate complex and amylopectin while corn starch films are filled with alginate and amylopectin chains. The mineral ions would further promote interactions between these chains. The resulting amylose-alginate chains could be the reason for the observed reduced starch digestion and increased tensile strength of the films. Further research is needed to establish in this phenomenon.

Table 9. The *in vitro* starch digestion at 20 mins and 120 mins of (a) corn starch and (b) potato starch films in the presence of mineral ions

(a)

Sample	20 mins	120 mins
Gelatinized corn	82.9±0.8 ^{ab}	85.0±0.3 ^a
Corn starch film	67.2±2.9 ^c	71.5±1.5 ^b
+ Zn	81.6±1.2 ^{ab}	80.9±3.1 ^{ab}
+ Mn	88.3±4.8 ^a	83.6±2.2 ^{ab}
+ Fe	75.6±1.9 ^{bc}	73.2±0.8 ^{ab}
+ Cu	78.4±1.5 ^b	80.2±3.0 ^{ab}
+ Comb	77.6±2.1 ^b	79.6±6.6 ^{ab}

(b)

Sample	20 mins	120 mins
Gelatinized potato	84.7±2.9 ^a	88.8±1.9 ^a
Potato starch-film	67.6±1.3 ^b	73.6±1.5 ^c
+ Zn	85.8±3.9 ^a	75.0±2.5 ^{bc}
+ Mn	78.5±1.1 ^a	84.7±2.3 ^a
+ Fe	67.0±0.7 ^b	72.3±2.5 ^c
+ Cu	67.9±0.5 ^b	81.3±1.6 ^{ab}
+ Comb	55.9±1.7 ^c	68.6±0.6 ^c

*Means within the individual picture, sharing same letters do not statistically differ from each other ($p < 0.05$); values are expressed as mean \pm standard deviation.

CHAPTER 5. SUMMARY AND CONCLUSIONS

Starches and polysaccharides are abundant and inexpensive and offer unique physicochemical properties with myriad food applications. They further stand out as a favorable carrier matrix to supplement mineral ions. This study involves the preparation and characterization of starch mineral complexes toward film development. Corn starch and potato starch have been selected as the model carrier matrix and Fe^{2+} , Mn^{2+} , Cu^{2+} and Zn^{2+} ions in their chloride salt form as the model mineral ions. The mineral ions have been complexed with starch and films have been prepared in the presence of a hydrocolloid sodium alginate and plasticizer glycerol.

The V-amylose X-ray diffraction patterns and additional peaks at $2\theta \sim 7, 10, 12, 16, 19, 22, 38^\circ$ to name few, have been observed for the starch-mineral complexes. The FTIR analysis of starch mineral complexes reveal new peaks in the region $1700\text{--}2400\text{ cm}^{-1}$, at $2938, 2973, 2985\text{ cm}^{-1}$ along with wavenumber shifts in the starch characteristics peaks of starch, to $852, 941, 1018, 1369$ and 1407 cm^{-1} confirming the starch-mineral complex formation. Dry ashing revealed the higher amount of mineral binding to potato starch than the corn starch presumably due to the presence of phosphate groups. The Mn^{2+} ions show highest loading of 68 mg per gram of potato starch and 60 mg for corn starch per gram. The starch films had higher L^* and lower a^* and b^* values yielding higher whiteness index (WI) while addition of mineral ions as such Mn^{2+} and Fe^{2+} ions changed the film color imparting brownish to yellowish tint giving films lower L^* value coupled with positive a^* (redness) and higher b^* (yellowness). The starch mineral films are thin, transparent, flexible and homogenous without any visible cracks and pores. Thus, has no significant difference in the film thickness.

The moisture content of the films did not depend on the film type, but the moisture absorption of potato starch films is higher than the corn starch films. The presence of mineral ions especially Mn^{2+} and Zn^{2+} ions accelerate the water holding capacity of the films. Increased water solubility of potato starch and corn starch films has been noted in

the presence of mineral ions owing to hydrophilic property, pH, pores and voids formation in the film matrix. The corn starch and potato starch films display high Water Vapor Permeability (WVP) of 1.4×10^{-10} and $1.8 \times 10^{-10} \text{ g m}^{-1}\text{s}^{-1}\text{Pa}^{-1}$, respectively. The addition of mineral ions decrease the WVP of the films.

The potato starch films display higher tensile strengths compared to corn starch films due to the presence of higher amount of amylose that facilitates complexing to the alginate chains and presence of mineral ions could further augment the tensile strength. Such a complexation in the potato starch films yields higher melting energy compared to the corn starch films. The X-ray and FTIR analyses on the films confirm the association among the starch, alginate and mineral ions. The gelatinized starches show higher digestibility. Alginate presence, however, reduces the starch digestion but increases with the addition of mineral ions. The interplay between increased viscosity as well as enhanced enzyme activity in the presence of mineral ions could have triggered such changes.

5.1 Future work

- 1) The V-type starch-amylose-mineral ion complexes have potential for encapsulating bioactive compounds and drug molecules toward novel food and drug applications.
- 2) The biodegradability of the films needs to be investigated at different environmental conditions so as to use thus prepared films in food packaging applications.
- 3) Effects of varied glycerol concentrations need to be examined for film moisture absorption, solubility and mechanical properties.
- 4) *In vivo* study of films and bioavailability of minerals need to be carried out for glycemic response and nutritional applications of the films.
- 5) Sensory analysis involving human subjects needs to be established for large-scale applications of starch mineral films.

- 6) Due to edible nature of the starch-mineral films, their safety against food microorganisms need to be proven toward improving the shelf life of food products.

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APPENDIX A

Corn starch complexes in the presence of mineral ions



Potato starch complexes in the presence of mineral ions

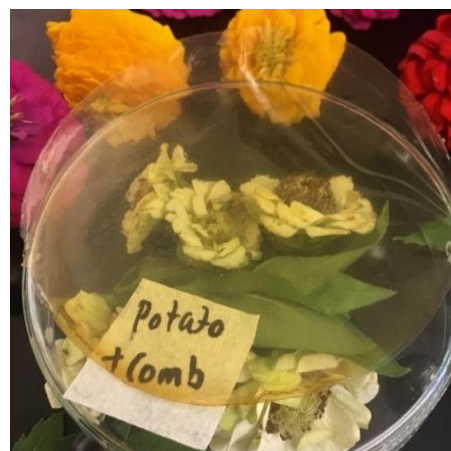
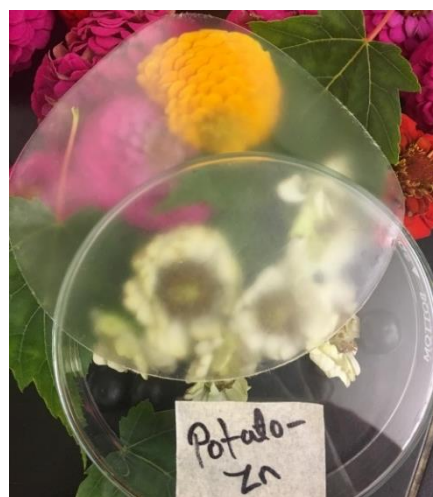
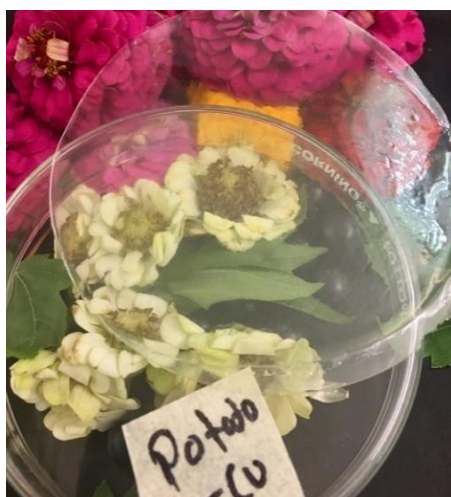
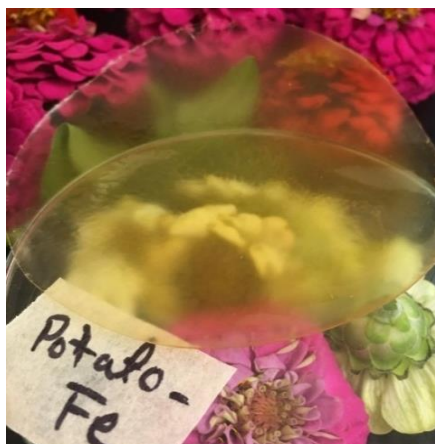


APPENDIX B

Visual observation of corn starch films in the presence of mineral ions and the Konica
Minolta colorimeter for measuring the color properties films



Visual observation of potato starch films in the presence of mineral ions and the Konica Minolta colorimeter for measuring the color properties films

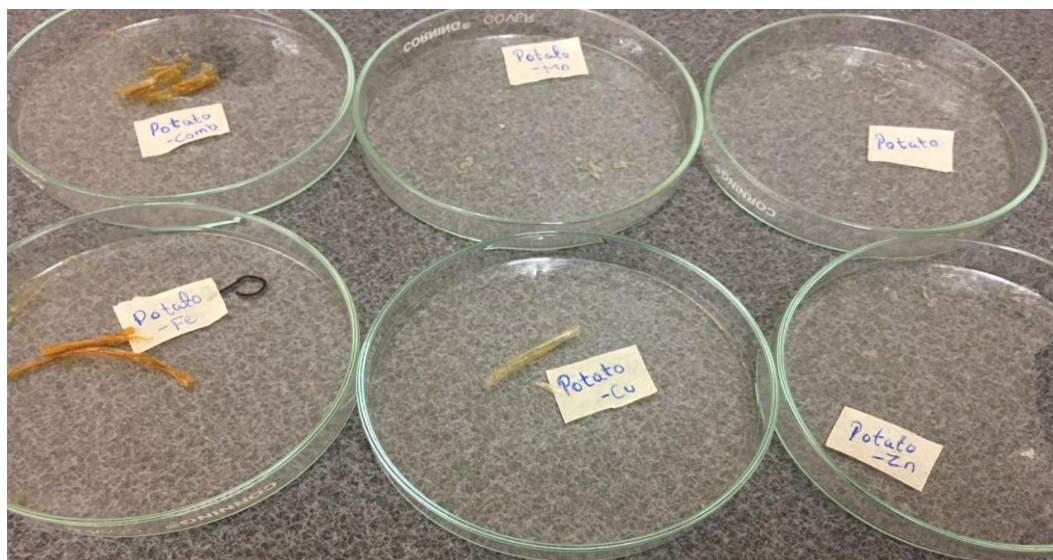


APPENDIX C

Insolubilized hot-air oven dried corn starch films in the presence of mineral ions,
obtained after the solubility test

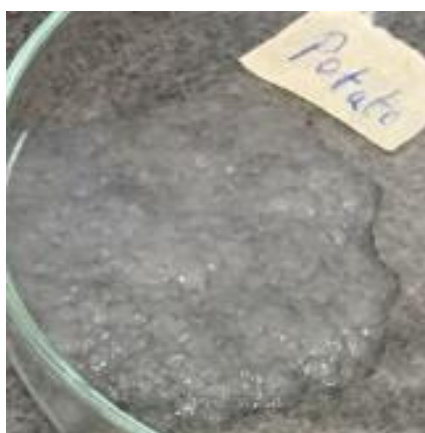
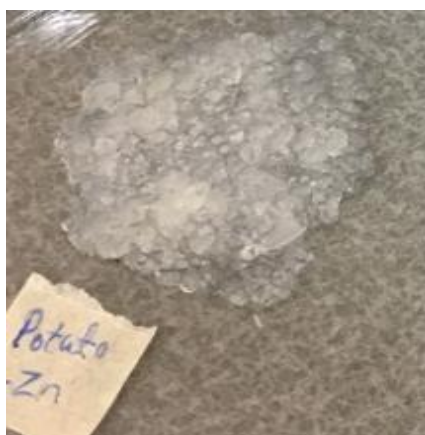
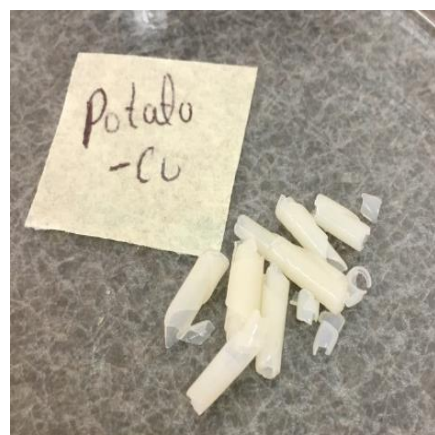
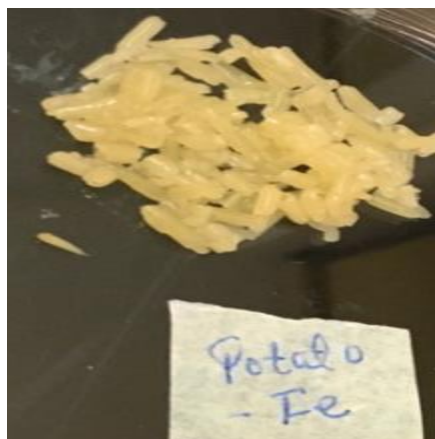


Insolubilized hot-air oven dried potato starch films in the presence of mineral ions,
obtained after the solubility test

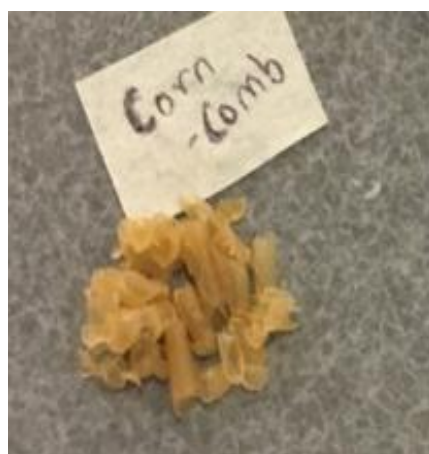
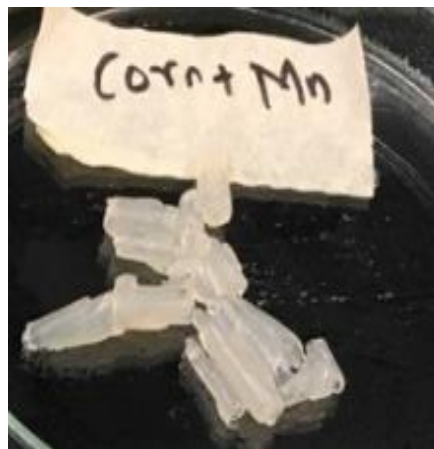


APPENDIX D

Potato starch films in the presence of mineral ions, obtained after the moisture absorption test



Corn starch films in the presence of mineral ion complexes, obtained after the moisture absorption test



APPENDIX E

Experimental setup for the WVP test on the corn starch and potato starch films in the presence of mineral ions



The color change in the silica beads from initial blue to pink at the end of the WVP test on the corn starch and potato starch films in the presence of mineral ions



APPENDIX F

The powdered potato starch films in the presence of mineral ions



The powdered corn starch films in the presence of mineral ions

