Efficacy studies of zinc fortified edible coated apricots

By

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HOLY PROPHET MUHAMMED

(Peace Be Upon Him)

DECLARATION

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LIST OF ABBREVIATIONS

Zn	Zinc
$ZnCl_2$	Zinc chloride
$ZnSO_4$	Zinc sulfate
RDA	Recommended Dietary Allowance
Fe	Iron
Se	Selenium
Cu	Copper
P:Zn	Phytates & zinc ratio
AIDS	Acquired immune deficiency syndrome
CNS	Central nervous system
WHO	World Health Organization
HDL	High density lipoproteins
LDL	Low density lipoproteins
EAR	Estimated Average Requirements
HSC	Hepatic stellate cells
DNA	Deoxyribo nucleic acid
RNA	Ribonucleic acid
ADHD	Attention deficit hyperactivity disorder
SOD	Superoxide dismutase
NK	Natural killer
NMDA	N-methyl-D-aspartate
ORS	Oral rehydration therapy
FDA	Food and Drug Administration
GRAS	Generally recommended as safe
ZnO	Zinc oxide
O_2	Oxygen
CO_2	Carbon dioxide
USDA	United State Department of Agriculture
TSS	Total soluble solids
RH	Relative humidity
WPC	Whey protein concentrate
K	Potassium
Ca	Calcium
Na	Sodium
Mn	Manganese
P	Phosphorus
Mg	Magnesium
TPC	Total phenolic contents
CVID	Common variable immunodeficient
TABRS	Thiobarbituric acid reactive substances
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
HCD	High cholesterol diet

WBC	White blood cells
RBC	Red blood cells
ALP	Alkaline phosphatase
LPS	Lipopolysaccharide
Cu-Zn SOD	Copper-zinc superoxide dismutase
NADPH	Nicotinamide adenine dinucleotide phosphate
IZiNCG	International Zn Nutrition Consultative Group
WIC	Women, infant & children
HPLC	High performance liquid chromatography
NFE	Nitrogen free extract
HNO_3	Nitric acid
$HClO_4$	Perchloric acid
H_2SO_4	Sulfuric acid
NaOH	Sodium hydroxide
TRBCs	Total red blood cells
hb	Hemoglobin
CRD	Completely randomized design
GLUTs	Glucose transporters

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ABSTRACT

In present investigation, apricots from locally grown 'Sufeda' variety were treated with edible coatings; chitosan and alginate @ 1 and 2% levels along with zinc salts fortification *i.e.* zinc sulfate and zinc chloride @ 30 and 50 ppm concentration. The compositional analysis showed that apricot is a good source of protein, fiber, calcium and potassium. The zinc fortified chitosan coated apricots showed better control over weight & moisture loss, TSS contents, pH & acidity, organic acids and sugars as compared to alginate based coatings. Similarly, chitosan coatings were efficient in the distribution and adsorption of zinc to the apricot surface as contrast to alginate ones. The edible coated zinc fortified apricots were affected significantly during storage as exhibited by their physico-chemical analyses. The results depicted that the maximum zinc contents of fortified coated apricot was noticed in T_{12} (2% chitosan containing with 50 ppm $ZnSO_4$) 4.83±0.24mg/100g followed by T_{16} (2% chitosan containing with 50 ppm $ZnCl_2$) 4.82±0.21mg/100g, T_4 (2% alginate containing with 50 ppm $ZnSO_4$) 4.78±0.23mg/100g and T_8 (2% alginate containing with 50 ppm $ZnCl_2$) 4.77±0.12mg/100g, respectively as contrast to T_0 (control) 0.26±0.02mg/100g. The sensory response of the fortified edible coated apricots was remained within range during storage. Afterwards, efficacy study was carried out in rabbits through two consecutive trials I & II for the validity of results. On the basis of *in vitro* analysis, HPLC characterization (citric & ascorbic acid and sugars) and zinc contents in edible coated zinc fortified apricots, four best treatments two from each coatings having different zinc salts T_4, T_8, T_{12} and T_{16} alongside control (unfortified apricots) were selected for the bio-evaluation trial. The consumption of zinc fortified chitosan and alginate coated apricots imparted substantial effect on feed & drink intake and body & organs weights during entire study. Likewise, serum and organs zinc contents of experimental rabbits were significantly improved by the provision of zinc fortified apricots with maximum serum zinc was observed in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$), G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) as 89.71±2.26, 87.43±2.14, 83.51±2.41 and 81.49±2.46µg/dL, respectively as comparison to G_0 as 72.56±2.85µg/dL. The percent increase in serum zinc was 24.63, 20.50, 15.09 and 12.31% in G_3, G_1, G_4 and G_2, respectively as comparison to G_0. Similarly, liver zinc was noticed as 23.97±1.41, 23.71±1.15, 23.53±1.28 and 23.36±1.45µg/g in G_3, G_1, G_4 and G_2 as contrast to G_0 by 22.42±1.36µg/g. Whilst in heart, maximum zinc was 17.59±0.55, 17.46±0.54, 17.22±0.42 and 17.18±0.46µg/g in G_3, G_1, G_4 and G_2 whereas, minimum in G_0 as 17.11±0.50µg/g. Whereas in kidney, zinc contents in G_3, G_1, G_4 and G_2 were noticed as 25.18±1.23, 24.97±1.56, 24.65±1.11 and 24.40±1.30µg/g in association to G_0 23.53±1.47µg/g. Results showed that percent increase in liver, heart and kidney zinc were 6.91, 2.84, 7.03 (G_3); 5.72, 2.08, 6.12 (G_1); 4.95, 0.66, 4.76 (G_4) and 4.19, 0.43, 3.68% (G_2), respectively compared to G_0. The attenuation in serum glucose of rabbits is an indicator for the positive impact of zinc salts fortification on this trait. The lowest serum glucose was 111.79±4.48mg/dL in G_3 nevertheless, maximum serum insulin 9.38±0.51µU/mL was observed in the same group. Similarly, the values for liver and kidney functions tests were within the normal range showing the safety of zinc fortified edible coated apricots. The hematological traits of rabbit's blood also demonstrated normal values for red & white blood cell indices. From instant exploration, it is deduced that zinc fortification through edible coating is a pragmatic choice to overcome hidden hunger with special reference to zinc.

CHAPTER 1

INTRODUCTION

Global nutritional crisis particularly in the underdeveloped and developing nations is an outcome of weakened supply of nutritious and wholesome food to the vulnerable segment for sustainable health. The micronutrient malnutrition is one of the serious issues of modern epoch leading to tragic consequences including impaired growth & development, physical disability and poor immune competency. Reliance on monotonous foods, inadequate micronutrient intake and an array of untold social factors are responsible for hidden hunger and allied health discrepancies (FAO, 2012). In the developing economies, daily edibles of the major population are deprived of multiple micronutrients. Amongst, zinc (Zn) is widely recognized as one of the deficient minerals in people with poor dietary habits. Pakistan is continuously facing the menace of Zn deficiency with special reference to pregnant women and children due to insufficient dietary intake. The researchers and nutritionists are focusing on economically affordable dietary strategies to address micronutrients deficiency for the individuals of middle to low income groups to meet their daily requirements (Yang *et al.,* 2005; NNS, 2011).

Recent era has witnessed the coinage of various dietary interventions aimed at combating malnutrition with special reference to hidden hunger. The micronutrients are regulating various metabolic pathways and their deficiencies lead to physiological abnormalities that may hamper the health stratum and life quality of the individuals (Prasad, 2012). In an effort to curtail the challenges by such diet linked maladies, various strategies have been devised. Amongst, fortification has proven as a vibrant, far reaching and effective choice to overcome the consequences associated with Zn deficiency. Additionally, this rationale is flexible and socially acceptable to improve the nutrients balance by incorporating certain deficient micronutrients in food (Wang *et al.,* 2002).

Among the various micronutrients deficiencies, hypozincemia is a pinching issue particularly in the developing economies. It has been estimated that about 95.4% of South Asian population is deficient in zinc contents due to poor dietary lifestyle (FAO, 2007). Zinc acts as an excellent anti-degenerative and anti-inflammatory agent (Plum *et al.,* 2010). It also participates in the dispensation, packing and consumption of insulin. It is vital for

gastrointestinal system owing to obligatory role in protein metabolism. Zinc involves in an array of metabolic path ways including carbohydrate, lipid, protein and nucleic acid synthesis or degradation. Zinc deficiency affects many organs functioning such as integumentary, gastrointestinal, central nervous, immune, skeletal and reproductive systems (Hotz and Brown, 2004; Rosado, 2003).

Instability of zinc homeostasis appears to be connected not only with diabetes, but also with several other diseases like tumors, liver cirrhosis, bowel disease and impaired immune system (Rink and Haase, 2007). Zinc metabolism appears to be changed in diabetic patients and its supplementation reveals positive effect, regarded as the future therapeutic intervention (Haase *et al*., 2008). The valuable effects of zinc supplementation are glycemic control in diabetics and zinc mediated defense of β-cells from T-cell mediated cytotoxicity and cytokines. Thus, zinc might be taken as a probable new aspirant molecule for diabetes prevention especially for type 2 diabetic patients (Jansen *et al.,* 2009).

Zinc deficiency in human body is linked with numerous malfunctions like diabetes mellitus, immune dysfunction and liver necrosis. Its application has proven effective to improve glycemic response in type 1 and 2 diabetes. Zinc put forth sound effect by sustaining the signal transduction of insulin and reducing the generation of cytokines, leads to β-cell death during the inflammatory process in the pancreas (Prasad, 2012). In avoidance to lower amount of zinc in the body, a mobile pool is mandatory for its distribution. Additionally, body zinc level is regulated by transporters and zinc binding proteins like metallothionein (Capdor *et al*., 2013).

Fruits are vital for balanced diet with plethora of minerals and vitamins, helpful to combat micronutrient insuffiencies. Pakistan is blessed with abundance of natural resources and favorable environment for the growth of array of fruits and vegetables around the year. The economic status of the country is improved through export of horticultural commodities especially fruits by earning foreign exchange (GOP, 2008). Nonetheless, a large amount of fruits goes as waste due to improper handling, mismanagement and inadequate processing conditions. Moreover, this situation is further worsened due to lack of proper storage and transportation facilities. Purposely, numerous innovative techniques are developed that provide nutritious food with extended shelf life (Ali *et al.,* 2011a; Betoret *et al*., 2011).

Among various preservation techniques, edible coatings and biodegradable films are considered as an effective tool for conserving the hedonic and rheological properties of the product. These coatings have numerous advantages over other type of packaging materials. Edible coatings/films applied to the product surface are responsible for better oxygen permeability, solute movement and provide moisture barrier. They tend to delay senescence and ripening by creating modified atmospheric conditions inside the fruit thus limiting interaction with surrounding environment thereby extend the shelf life (Falguera *et al*., 2011). The advantages of edible films over conventional packaging materials are improved structural properties, ability to incorporate pigments, flavorings and food additives alongside maintenance of quality during shipping & storage (Alandes *et al.,* 2009; Artharn *et al.,* 2009).

Apricot (*Prunus armeniaca*) is a perishable fruit with high sensory and nutritional profile. It may be eaten as fresh, pitted and dried, frozen or canned. In Pakistan, apricot is in abundance with total annual production about 0.325 million tons, mainly cultivated in Chitral, Gilgit and Baltistan (Ali *et al*., 2011b). More than 60 varieties of apricot are grown and graded according to their sugar content including sufeda, karfochuli, sharippa, halman hawalappa, yarqand, marghulam, stachu, badami, karthusa, castle bright and shatra karfu. Apricot is a good source of antioxidants, minerals like iron, potassium, calcium & manganese, vitamins and dietary fiber (Lou *et al.,* 2011).

Edible coating is one of the economical methods of preservation that provides value addition to the apricot. These are biodegradable and environment friendly films & coatings innocuous for the health. Accordingly, fruits especially apricots are frequently treated with edible coatings like chitosan, alginate, casinate, soy and whey proteins due to their improved antimicrobial activity (Mitrakas *et al*., 2008). In this perspective, edible coating is one of the promising techniques of preservation with high market potential and acceptability. It involves an intact transparent edible film that acts as a barrier to oxygen absorbency and solute movement, used as host for additives in preserving the properties of product and overall appearance (Falguera *et al.,* 2011). Owing to the consumer's demand for safe and wholesome food, biofilms have received immense attention of the researchers. To improve the overall appearance and retard senescence, edible coatings are applied directly on the food surface by dipping, spraying or brushing to create a modified atmosphere with a range of materials. It has been observed that edible coatings extend the shelf life of fruits through prevention of

gas, water and solutes migration using food grade emulsifying and wetting agents. These coatings have attained wide recognition as being environment friendly, non-toxic, biodegradable and relatively safe for food applications (Rojas-Grau *et al*., 2009).

Currently, nutritionists are mainly focusing on the agenda of food security & safety, malnutrition and to explore diet-health linkages. Nonetheless, many segments of the world population especially people in the developing countries are consuming food that is deficient in micronutrients, resulting in severe health issues (Shrestha *et al*., 2003). Accordingly, various measures like strong political assurance, nutritional education programs & implementation, supervisory control and investment are taken to control micronutrients deprivation for reducing morbidity and mortality in the vulnerable segment (Gibbs *et al*., 2011). According to an estimate, about 30% of the world's population is suffered from vitamin A, iron, zinc and iodine deficiencies leading to various physiological ailments (Muller and Krawinkel, 2005)

It is envisaged that edible and biodegradable coatings may act as a vehicle for various micronutrients, additives and serve as tool for fortification. In this reference, one of the safer and economical methods to meet the daily Zn requirement is zinc based edible coatings and films. It is a way for the implication of micronutrients and additives on different edible commodities. Furthermore, these novel coatings are perceived as an efficient medium for additives to meet the daily dietary requirements of individuals living in the developing world (Falguera *et al.,* 2011; Altamirano-Fortoul *et al.,* 2012). It is therefore encouraged to fortify the staple foods with zinc to improve its dietary intake in the vulnerable population (Gibbs *et al*., 2011). Among various zinc fortificants, zinc chloride ($ZnCl_2$) and zinc sulfate ($ZnSO_4$) are generally in practice (Poletti *et al*., 2004).

Development of novel coatings with improved functionality and performance for fresh and minimally processed fruits is one of the major challenges being faced by the fruit processing industry. In toto, the trend towards edible coatings is becoming popular considering the demand for healthier and safe foods. Edible coatings may act good oxygen and lipid barriers at low to intermediate relative humidity as the polymers can effectively make hydrogen bonds. An edible coating must have good sensory profile, acceptable color, flavor, taste and texture with shiny look. Edible coatings are generally biopolymers of proteins and

polysaccharides like soy, whey proteins, starch, chitosan and alginate (Valero *et al*., 2013). Biopolymers are usually hydrophilic thereby act as a good barrier against hydrophobic compounds like lipids, oxygen and certain flavors. The commodities with edible coating lose water slowly that have led its application in fruits like apple, mango strawberry, apricot, melon etc. (Dhall, 2013).

The daily requirement of zinc for men and women is 11 and 8 mg, respectively. However, it is reported as 11 and 12 mg in case of pregnant and lactating women. The recommended dietary allowance (RDA) for 6 months old infants or younger is 2 mg whilst, 3 mg for 7 months to 3 years old child. The adolescent boys and girls should consume 11 and 9 mg of zinc/day, respectively (DRI, 2001). Zinc deficiency symptoms include anemia, growth retardation, rough & dry skin, hypogonadism, mental lethargy, hepatosplenomegaly and geophagia. The persons with moderate zinc deficiency show non-specific indications as vulnerability towards infections and delayed wound healing. The zinc deficiency is usually due to inborn defects of zinc absorption or secondary factors like chronic renal & liver and sickle cell diseases (Raine, 2010; Prasad, 2012).

Unhealthy life style and prevalence of infectious diseases lead to vicious circle of hidden hunger in communities of poor nations. Besides, micronutrient deficiencies lead to various risk factors and ultimate death in pregnant women and the neonates. The discovery of different zinc salts motivated the researchers to probe their varied role in human health with special reference to metabolism and deficiency syndromes. The micronutrient deficiencies can be eliminated by fortification of deficient nutrients in the food and such dietary program ought to be launched in masses to combat the threat of hidden hunger. The fortification is an economical and easier way of improving health and survival of the mother and child (Poletti *et al*., 2004; FAO, 2010).

Holistically, in Pakistan little efforts have been carried out to combat micronutrients deficiency with special reference to zinc through dietary modifications. Realizing the mentioned facts, the current agenda was an endeavor to develop and optimize zinc fortified, chitosan and alginate based edible coatings that were further applied on locally available apricot of "Sufeda variety" to improve its nutritional quality and zinc status. Besides, application of selected coatings on apricots containing various levels of zinc chloride and

zinc sulfate to combat zinc deficiency through bioefficacy trial was the limelight of present research. The core objectives of instant investigation are herein;

- Development and optimization of biodegradable edible coatings
- Assess the storage behavior, stability and retention of zinc fortificants in edible coated apricots
- Evaluate the bioavailability of zinc using rabbits experimental model

CHAPTER 2

REVIEW OF LITERATURE

Sustainable food paradigm and adequate nutrient supply are the key components of novel nutritional guidelines to ensure optimum health. Nevertheless, hidden hunger has evolved as a serious public health issue of under developed economies, rising at an alarming pace. Currently, the developing world is also facing the challenge of food and nutrients insecurity. In this milieu, micronutrients conservation and improvement are the priority foci of nutritional framework in the developing world. Prolonged mineral deficits have led to various physiological malfunctions and disabilities affecting health, survival and economic development of poor nations. In Pakistan, researchers are trying to develop micronutrient based dietary strategies to overcome the menace of nutrients deficiencies notably zinc, iron and iodine. Various interventional approaches are applicable to elevate Zn content of edibles nonetheless, food fortification with appropriate fortificant and vehicle is the pragmatic strategy. Pakistan has anchored promising position worldwide for the production of quality fruits and vegetables. However, huge horticultural produces are wasted annually due to poor postharvest practices. Purposely, application of Zn fortified edible coatings is a promising tool to enhance the nutritional status of malnourished population. The increased consumers demand for nutritionally fortified fruits has fabricated the idea of edible coatings and films as carrier for mineral, vitamin and other nutraceutical bioactive moieties. Keeping in view the dietary needs of Zn, the current project was aimed to improve the Zn consumption by using apricot as a vehicle. Besides, the highlights of literature available regarding Zn deficiency and its dietary measures are discussed under the following subsets.

2.1. Micronutrients and allied deficiencies
2.2. Zinc; requirements and physiological benefits
2.3. Hypozincemia and interventional strategies
2.4. Edible coatings formulations
2.5. Apricot and nutrition facts
2.6. Efficacy studies
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2.1. Micronutrients and allied deficiencies

Micronutrients are crucial to perform normal body functions and homeostatic metabolic activities to ensure optimum health and nutrition. These components mainly target on the metabolic & biochemical pathways for maximal health thus prevent from certain ailments. The micronutrients like Zn, Fe, Se and Cu play a pivotal role in antioxidative enzyme as superoxide dismutase & glutathione peroxidase thus involved in defensive mechanism. Likewise, vitamin B_{12} & riboflavin act as cofactors in the metabolic functions of human system whilst, iodine in the synthesis of thyroxin (Ommen *et al*., 2008).

The malnutrition is compounded by two factors encompassing protein-energy malnourishment & micronutrient deficiency (Muller and Krawinkel, 2005). The micronutrient deficiency is primarily related to poor dietary preferences, food insecurity, neglected infant & mother care and lack of nutritional awareness programs (WHO, 2002; Hotz and Brown, 2004; Muller and Krawinkel, 2005). The persuasive disparities of health in developing world are generally interlinked with micronutrient deficiencies predominantly zinc, iron, iodine & vitamin A (WHO, 2002; Muller and Krawinkel, 2005) responsible for higher death rate especially in Afro-Asian communities. It has been reported that two billion people are directly or indirectly associated with Zn deficiency and one billion population with iron deficiency whilst, 740 & 250 million linked with iodine & vitamin A deficiencies, correspondingly (Muller and Krawinkel, 2005). Numerous studies inferred the micronutrient deficit as a serious problem, targeting 815 million people throughout the world (Poletti *et al.,* 2004). In this context, Zn deficiency has affected one third of world population *i.e.* 2 billion people all across the globe (Scrimgeour and Lukaski, 2008; Szewczyk *et al.* 2011; Grattan and Hadley, 2012; Sandstead, 2012).

Cumulative evidences have documented zinc deficiency in developing countries 33.5% as compared to developed states 9.5% (Borwankar *et al.,* 2007; Ozden *et al.,* 2012). Recently, Vardatsikos *et al*. (2013) mentioned WHO survey with 30% of entire population facing Zn paucity. The poor dietary habits and higher dependence on cereals & legumes have worsened the situation in developing communities. Phytates, being the major element of bread beans and some vegetables, have been considered as inhibitors of zinc and iron absorption in human body (FAO, 2001; Szewczyk *et al.,* 2011). The food products with phytates & zinc

(P:Zn) molar ratio >15 are directly related with Zn deficiency (FAO, 2001; Gibson, 2006). The reported data of P:Zn molar ratio for high fiber, milk, meat & vegetable based diets enumerated as 6, 8, 15 & 18, respectively (Hotz and Brown, 2004). The developed nations are frequent consumer of animal meat thus overwhelm the symptoms of micronutrient deficiencies (Hunt, 2003). Red meat is one of the rich sources of Zn (25% retention) as well as Se (Stehbens, 2003). Contrarily, the low income economies are facing nutrient malabsorption due to their high dependence on unrefined cereal based products. According to an experimental trial, P:Zn for flat bread (roti) and bun (refined baked product with less phytic acid) as 17 and 4, correspondingly (Karunaratne *et al*., 2008).

It is reported that insufficient food consumption, poor absorptive behavior, excessive Zn depletion through urination or presence of higher amounts of phytates in diet are the major causes of zinc scarcity. It has been observed that mild to moderate Zn deficiency is more prevalent (WHO, 2002; Bao *et al*., 2010). Zn deficit has been related to 1.4% of deaths throughout the world predominately killing neonates~ 0.8 million, indicating 18% malarial attacks, 16% infections of lower respiratory tract & 10% diarrheal incidences among infants of perinatal stages (WHO, 2002).

Zinc is an effective antioxidant in the prophylaxis of various persistent ailments such as diabetes, anemia, AIDS, liver & CNS dysfunctions, malarial attacks, cancer and other discrepancies linked with pre- & postnatal development (Hambidge, 2000; Ibs and Rink, 2003; Ho *et al*., 2003; Caufield *et al*., 2004; Ho, 2004; Rangan and Samir, 2012). Later, Hambidge and Krebs (2007) and Bao *et al.* (2010) estimated 800,000 additional deaths of children <5 years due to Zn deficiency, accounting 176,000 deaths owing to diarrhea, malaria 207,000 & pneumonia 406,000.

2.2. Zinc; Requirements and Physiological Benefits

Zinc is a cofactor for more than 300 enzymes, synthesizing and metabolizing macro- & other micronutrients. It is also found in various transcription factors and plays crucial role in genetic makeup (Blewett and Carla, 2012; Grattan and Hadley, 2012; Vardatsikos *et al*., 2013). The Zn is ubiquitous in plants & animals in metalloenzyme form and second most imperative metal found in body after iron (Hotz and Brown, 2004; Faa *et al.,* 2008; Hambidge and Krebs, 2007; Vardatsikos *et al*., 2013). Zn is divalent heavy metal with

amphoteric nature, discovered during Egyptian era (Vardatsikos *et al*., 2013). Various zinc based salts include $ZnSO_4$, $ZnCl_2$, Zn gluconate & Zn acetate are effectively used owing to their soluble and absorbable nature (Hotz and Brown, 2004; Scrimgeour and Lukaski, 2008).

The non-toxic nature of Zn is due to its inertness towards oxidation-reduction reactions within the biological systems. During reactions, it serves as an electron acceptor ***i.e.*** responsible in the formation of three-dimensional structure of protein. Zn forms finger like structure by folding with amino acids (Hotz and Brown, 2004). It has been observed that about 60-70% of total Zn in human serum is present in the form of albumin-zinc complex (Hotz and Brown, 2004; Gupta and Gupta, 2014) and acts as carrier for the transportation of plasma Zn across the cell membrane (Faa *et al.,* 2008). Therefore, hypoalbuminemia during pregnancy, viral hepatitis, cirrhosis and protein-energy malnutrition is a major determinant for Zn deficiency (Hotz and Brown, 2004; Faa *et al.,* 2008; Gupta and Gupta, 2014).

The Recommended Dietary Allowance (RDA) for Zn from 19 years to elderly people is 11 mg/day in males and 8 mg/day in females (Haase *et al*., 2006; Grattan and Hadley, 2012; Vardatsikos *et al.,* 2013). It has also been confirmed by WHO that its requirement for males is 9.4 to 9.5 mg and for females 6.5 to 7.1 mg (Maret and Sanstead, 2006). Later, Sandstead (2012) elucidated the negative impact of Zn deficiency on health with inadequate Zn intake proliferated among 50% of the whole population (Brown and Wuehler, 2000; Kristensen *et al.,* 2006).

The higher incidences of Zn deficiency have been reported in infants, children, adolescents, pregnant & lactating women thus their daily requirements are of critical concern (West Jr, 2004; Hotz and Brown, 2004). Zn deficiency impairs the enzymatic activity associated in the synthesis of protein & nucleic acid that are involved in the activities of growth, cell division and genetic expression (FAO, 2001; Stehbens, 2003). Later, it has been evaluated that only 18% of the pregnant women meeting their RDA in developed nations whilst, 10% of the overall population is fulfilling < 50% of the RDA (Mareta and Sandstead, 2006).

The moderately permitted dietary intake of zinc in pregnant women is 5.5, 7 & 10 mg/day during first, second and third trimester of pregnancy, correspondingly (FAO, 2001). However, in the 3rd trimester must maintain 2.6 mg of absorbed Zn/day for the improvement in pregnancy outcomes (Swanson and King, 1987). The Zn concentration in the milk of

lactating mothers is estimated as 2-3 mg/L that reduces after 3 months to 0.9 mg/L. The dietary requirement of Zn is reported as 9.5 mg/day during first 3 months of lactation and suppressed to 8.8 & 7.2 mg/day during the next 3-6 & 6-12 months of lactation period, respectively. Likewise, the dietary Zn recommended for infants are 2.8-5.6 mg/day however, the daily intake of 7.2-8.6 & 4.9-7 mg is crucial for adolescent & adults, respectively (FAO, 2001).

The upper threshold levels for Zn are proposed as 45 mg/day for adults and 23-28 mg/day premeditated for children (FAO, 2001). Whilst, long term or higher intake of 225-450 mg Zn/day has been correlated with hyperzincemia or Zn toxicity *i.e.* characterized by vomiting, nausea, diarrhea, cramps, fatigue and lethargy (Stehbens, 2003; Hotz and Brown, 2004). It has also been elucidated that 50 mg Zn/day reduces the Cu-Zn superoxide dismutase activity hence compromises immune function. Likewise, 50-160 mg Zn/day has been reported to reduce high density lipoproteins (HDL) and raised oxidized-LDL. Although no hazardous effects has been observed @ 25-35 mg Zn/day (Hotz and Brown, 2004).

Later, Rangan and Samir (2012) stated that children between 4-16 years should fulfill 68% of Zn intake from diet, constituting from meat & poultry 26-33%, milk 15-20%, cereal 13-15% & cereal based products 9-13%. Moreover, they reported that 29% of children are not consuming their Estimated Average Requirements (EAR). Likewise, 39-43% of adolescent girls between 12-18 year were failed to follow their RDA.

The human body encompasses 2 to 3 g of Zn without any storage system (FAO, 2001; Haase *et al.,* 2006; Hambidge and Krebs, 2007; Plum *et al.,* 2010). Of the total zinc content in human body, the skeletal muscles contain 30% whilst, 0.1% is found in plasma. It has been reported that zinc accounts 300-500 mg/L in prostatic fluid, 270 µg/g in choroid and 100-200 µg/g in skeletal muscles (FAO, 2001). The average plasma zinc level has been reported as 96 & 89 µg/L in healthy adults & children (Halsted and J.Cecil, 1970). Aqueous supplementation of Zn has shown more absorptive behavior in small intestine as compared to solid foods. The natural Zn losses occur through intestine ranging from 0.5-3 mg/day followed by urine, skin & kidney however, extra losses are expected during hectic exercise and starvation (FAO, 2001). It has been studied that plasma Zn concentration reduced by 50% during 24 h fasting (Stehbens, 2003). The gastrointestinal system has been observed to

play a key role in Zn homeostasis where most of the absorption & excretion of Zn occurs. The excretion of zinc through this path has been noticed as 2.5-5.5 mg/day (Faa *et al*., 2008).

Zn regulates the homeostatic mechanism of human body by performing various physiological functions. The Zn-metalloproteinases are involved in immunity, defending against reactive oxygen species and excess collagen fiber, produced during liver cell death. It is also associated with homeostatic balance of pro-apoptotic proteins (Bcl-2) and antiapoptotic proteins thus regulate apoptosis in the body system. However, Zn deficiency has been linked with the activation of caspases- 3 & 8 hence induces apoptosis within the healthy liver cells therefore a linear correlation lies between Zn and liver health. Contrarily, Zn-metalloenzyme (collagenase) also activates nerve growth factor that triggers the apoptic cell death of hepatic stellate cells (HSC), accumulating collagen & extracellular matrix hence damages liver cells (Faa *et al.,* 2008).

Zinc and copper react together to form an antioxidative enzyme naming Cu-Zn-superoxide dismutase that dislocates the transition metals thus prevents lipid peroxidation in cell membranes, serving as an anti-inflammatory agent (Evans and Halliwell, 2001; Faa *et al*., 2008). It controls the signaling pathway of various transcription factors subsequently regulates the cell proliferation, apoptosis & immune functions. Zn increases the antioxidant potential of liver thus prevents from hepatic cirrhosis. It maintains the metabolic activities by conserving the role of several proteins, DNA transcription & RNA translocation. Metallothionein has a role in zinc transport from cytoplasm to the liver. The defensive role of metallothionein in human cells, predominantly in the hepatocytes and enterocytes has been similar to that of glutathione. Oxidation of metallothionein by oxidized glutathione releases zinc to particular ligands in the body. The extent of zinc dispatch from metallothionein depends on the quantity of glutathione in hepatocyte. Zinc reduces the toxic effects of free radicals producing superoxide dismutase enzymes and also decreases the glutathione production, which is pivotal in antioxidant defence in humans. It has been reported to decline the malondialdehyde concentration during ulcerative colitis (Faa *et al*., 2008).

The therapeutic potential of zinc against attention deficit hyperactivity disorder (ADHD) has been studied through numerous experimental trials. Zinc serves as a cofactor in the metabolism of neurotransmitter that is essential for proper brain functioning (Arnold *et al.,*

2008 and 2011). The zinc dependent enzymes are involved in brain growth and memory functions. It has also been associated with proper immune and T-cells working as a co-factor of thymus hormone (Nriagu, 2007). Furthermore, zinc based diet is proved beneficial to reduce oxidative damage associated with inflammatory cytokines such as tumor necrosis factor TNF-α & IL-1B (Prasad, 2009). Zinc has also been found to mitigate various virus-induced infections when administered for 4 to 8 weeks (Ferencik & Ebringer, 2003). It also plays a key role in immune system whilst, its deficiency causes chronic inflammations (Santos-Valente *et al.,* 2012).

Zinc synthesizes carbonic anhydrase in the body that releases defensive bicarbonate ions, protecting the gastrointestinal system (Haase *et al.,* 2008; Faa *et al.,* 2008). Likewise, it is involved in the formation of alcohol dehydrogenase that metabolizes alcohol and down regulates ethanol-induced apoptosis within the liver cells by repressing Fas/Fas ligand pathway. Zn deficiency deactivates the T lymphocytes thus reduces the phagocytic action of macrophages, weakening the immunity. It is also involved in the formation of superoxide dismutase and catalase in liver that plays disease modulatory role (Faa *et al.,* 2008). Moreover, zinc being the cofactor of superoxide dismutase (SOD) suppresses the mishaps during pregnancy. Therefore, maternal Zn level is one of the basic requirements for the normal functioning of conceptus and trophoblast (Nriagu, 2007; Mistry & Williams, 2011).

2.3. Hypozincemia and interventional strategies

Globally, Zn deficiency is listed among the top causes of abnormal health in developing world (Blewett and Carla, 2012). Zn acts as potent endogenous antioxidant that regulates disease modulatory pathway. In numerous studies, Zn-associated malabsorption syndromes have been reported including stress, inflammation, diabetes, gastrointestinal diseases, sickle cell disease, liver cirrhosis, (Faa *et al.,* 2008; Vardatsikos *et al*., 2013) abnormal fetus, low birth weight, nervous disorders, immunocompetence, (Laity and Glen, 2007) sickle cell, renal disease, poor wound recovery, (Stehbens, 2003) diarrhea, atherosclerosis and cancer (Sandstead, 2012).

Zn deficiency is considered as a global issue due to its altered immune response. The inadequacy of Zn results in impaired functions of NK (natural killer) cells, T & B cells, neutrophils and macrophages (phagocytosis). This situation induces lymphopenia (less

lymphocyte production) hence attacked by chronic pathogens, compromising immunity (Scrimgeour and Lukaski, 2008; Blewett and Carla, 2012).

Earlier, it has been studied that Zn deficiency impairs the innate immune response by over-expressing various pro-inflammatory cytokines such as IL-1β, ICAM-1 and TNF-α thus increases injury at tissue and organ levels (Bao *et al*., 2010). The positive response of Zn has been assessed on lymphocytes against antigen attack. The intensity of immune senescence is related with Zn supplementation, enhancing the defense response of elderly people. It has been documented that zinc helps in the formation & maturation of T-cells subsequently synthesizes IgA & IgG. Similarly, Zn supplementation was also found effective in restoring the function of T helper cells, if treated three weeks before immunization whilst, side effects were detected if consumed after vaccination (Shankar and Ananda, 1998; Hasse *et al.,* 2008).

During a trial Ho *et al*. (2001) strengthened the fact that zinc implementation down regulates the NF-*k*B expression hence reduced the inducible NO synthase that was activated by alloxan or streptozotocin based free radical mechanism. It represses the oxidative damage associated with diabetes due to its insulin sensitizing effects. It targets tyrosine phosphatase ***i.e.*** a negative regulator of insulin release, by dephosphorylating insulin receptors associated with insulin signaling (Haase *et al*., 2008). Earlier, Sondergaard *et al*. (2006) mentioned that pancreatic Zn level of hyperglycemic patients is lesser as compared to normal person. However, in β-cells, Zn plays a crucial role in the formation of hexameric crystalline structure by combining zinc and insulin. On the other hand, numerous mechanisms of insulin resistance are associated with Zn deficiency like hindrance in binding with insulin receptor, aberrant structure of glucose carriers and increased lipid peroxidation.

Vardatsikos ***et al.*** (2013) exhibited various mechanisms to illustrate the insulin mimetic effect of Zn. It was noticed that Zn triggers insulin signaling pathways by activating the tyrosine phosphorylation that further target other signaling expressions like extracellular signal regulated kinase, protein kinase B/Akt and phosphotidylinositol-3-kinase to enhance glucose uptake by muscle & fat cell leading to the formation of glycogen and lipids. Nonetheless, modulatory effects were observed on gluconeogenesis & lipolysis.

Scrimgeour and Lukaski (2008) explicated that Zn supplementation has potential to reduce pathogenic diarrhea by 40% during 48 h treatment in experimental animals. It has been

documented that 10-30 mg Zn/day effectively manages watery stools in infants & children < 5 years. Numerous diarrheal pathogens like *E. coli, Shigella* and *Campylobacter* are prevalent in low income countries. The mechanism of Zn against diarrheal pathogens has shown the reduced adherence of *E. coli* with epithelial cells of intestine in rabbit model. Zn also downregulates ecto-5'-nucleotidase enzyme, converting 5'-AMP to adenosine that not only promotes the growth of *E. coli* but also triggers the watery release from host intestinal cells.

Zn plays a key role in the genetic expression and signaling pathways whilst its depletion has been found to limit DNA, RNA and alkaline phosphatase content in rat models (Pfaffl and Wilhelm, 2003). The zinc finger domains have been detected around 3% of human genome (Hambidge and Krebs, 2007) and expected to identify by 10% (Grattan and Hadley, 2012). It has been studied that stress reduces Zn level in plasma therefore, additional intake of zinc substantially improves the CNS to alleviate depression (Szewczyk *et al.,* 2001). Later, Szewczyk *et al.* (2011) explicated that Zn deficiency is directly linked with neurodegeneration that impairs brain functioning by activating inflammatory pathways. Besides, Zn based diet has been characterized by reduced glutamate receptors especially N-methyl-D-aspartate (NMDA), regulating p53 and cell proliferation. The anti-depressent effect of Zn therapy normalizes hypozincemia hence protects against anxiety, fatigue and anorexia. Among various important micronutrients deficiency, zinc has been found as primary etiology of tumorigenesis (Ames, 1998).

Zn acts as anti-atherogenic agent, reducing oxidized-LDL and oxidative damage (Evan and Halliwell, 2001). The prophylactic role of Zn has been probed against oxidative damage induced by CCl_4 through its interaction with glutathione & superoxide dismutase thus reduces malondialdehyde level, hydrogen peroxide and lipid peroxidation in membranous structure (Stehbens, 2003). Zn supplementation modulates negative impact associated with rheumatoid arthritis by expressing its counter effect on cytokines like IL-1β and TNF-α (Haase *et al*., 2008).

Zn deficiency is rising at a faster pace predominantly in various developing countries thus dietary modification and counseling are imperative to overcome the prevailing issue (Karunaratne *et al*., 2008). The oral rehydration therapy (ORS) has reduced the mortality rate

from 4.6 to 2 million since 1980 (Scrimgeour and Lukaski, 2008). The nutrition focused interventional channel of Zn includes supplementation, fortification, dietary modification and biofortification. The objective of such strategies is to improve nutritional status of vulnerable population (Gibson and Ferguson, 1999; Hotz and Brown, 2004; Gibson, 2006).

The supplementation is a dietary strategy that defines the inclusion of particular micronutrient to target population with specific deficiency like addition of folate to increase the maternal and pre- & post-natal health. To ensure the success of supplementation strategies, a proper delivery channel should be managed to cope with poverty, lack of awareness and poor communication & transportation facilities. The proper knowledge about micronutrient including stability & compatibility in food systems are the major determinants of supplementation programs. In case of Zn, water soluble forms like Zn sulfate, gluconate and acetate are preferred (Hotz and Brown, 2004) however, Zn sulfate is not only cost effective but also approved by FDA as GRAS and therefore preferentially recommended for mother & child health (Gibson, 2006). The advocacy of Zn usage is recommended either alone after fasting to avoid its antagonistic interactions with phytates in food or between meals as multinutrient (Hotz and Brown, 2004).

Fortification is a strategy of adding micronutrients to food in which they are absent in order to secure the normative micronutrient status of target population (Hovdenak and Haram, 2012; Berner *et al*., 2014). The success of fortification program depends on the stability of active substance during cooking to avoid objectionable color, flavor and overall appearance. The absorptive behavior & organoleptic properties of Zn in the end product ensure the success of Zn fortification (Hotz and Brown, 2004). It is one of the cost effective strategies specifically $ZnSO_4$ and ZnO with comparable bioavailability. The taste and color compatibility of fortificant with particular food commodity is also of major public concern (Gibson, 2006). The third long term strategy to improve Zn status includes modifications in dietary habits. Awareness of households regarding consumption of red meat and methods to reduce phytate content in food through enzymatic hydrolysis or soaking of legumes & cereals also serve the purpose (Gibson and Ferguson, 1999; Gibson *et al*., 1998; Salgueiro *et al.,* 2002; Hotz and Brown, 2004; Gibson, 2006). Likewise, biofortification or modifications in genetic expressions and enrichment of soil with Zn based fertilizers enhance the Zn content in the respective crop (Hotz and Brown, 2004; Gibson, 2006). Later, Qin *et al.* (2012)

confirmed the efficiency of biofortification by producing Zn biofortified rice to combat zinc deficiency which is attaining popularity among diet related interventions in China.

Previously, Folwaczny (1997) explicated that Zn supplemented food stimulates the secretion of brush border enzymes in the mucosal cells of GI-tract, antibodies and insulin like growth factor-1 to enhance the retarded growth, immunity response and defense against diarrheal pathogens in children. It has also been observed that Zn therapy in the adjunct form may prove better such as with vitamin A that potentiates Zn sensitivity for Th1 (killer of diarrheal pathogens). Later, West Jr (2004) reported reduced risks associated with maternal health due to micronutrient supplementation in Nepal. Conclusively, fortification is a pragmatic solution to tackle zinc deficiency (Hambidge and Krebs, 2007).

2.4. Edible Coatings Formulations

Growing awareness regarding health enhancing aspects of fresh and minimally processed fruits and vegetables consumption has paved the foundation for environment friendly and efficient packaging techniques. Recently, edible coatings have gained immense importance in the field of food packaging as compared to traditional techniques that impart huge burden to the environment in the form of waste materials. Purposely, various novel techniques have been developed to reduce the bio-waste as well as to improve packaging of edible products. Numerous mechanisms have been studied regarding edible coatings with respect to their antibacterial, antifungal and mechanical properties. Although, blending of coating formulations with natural polymers and antioxidants have been found appropriate for preservation of fruits and vegetables (Elsabee and Entsar, 2013). An edible film is a semi permeable barrier or a seal that serves as a modified atmosphere around fruit, securing from gaseous exchange (Lima *et al*., 2010).

The nature of edible coatings varies on the basis of types of biological macromolecules involving polysaccharides, fats and proteins (Lima *et al.,* 2010; Al-Hassan and Norziah, 2012). Various biodegradable edible coatings including chitosan, alginate, casein, zein & soy protein have been proposed for fruits on the basis of their odorless, colorless and tasteless nature (Elsabee and Entsar, 2013). The easy application of chitosan based coating in the form of dipping and spraying is widely acceptable throughout the food industry (Dutta *et al*., 2009). The polycations of chitosan based coatings interfere with the negative charge of

microbial cell membrane or react with microbial proteins. Thus, interaction of amino group with phospholipids of bacterial cell membrane or electronegative charge on fungi surface suppresses the degrading features of microbes (Devlieghere *et al*., 2004; Elsabee and Entsar, 2013). Further, chitosan films have shown wide applications in food preservation (Dutta *et al.,* 2009).

The combination of chitosan with hydrocolloids like alginate enhances their mechanical properties as well. Chitosan is a copolymer of chitin (Dutta *et al*., 2009; Elsabee and Entsar, 2013) produced as a byproduct from shellfish industry (Xu *et al.,* 2005) that was permitted by FDA in 1983 for coating purpose (Zhao and Mc Daniel, 2005). Conversely, alginate is a polysaccharide originated from seaweeds and well known for transparency, uniformity and water solubility, creating semi-permeable environment for gaseous exchange thus enhances the quality attributes of fruits & vegetables. Moreover, it also provides clinging properties to respective food due to cationic characteristics (Elsabee and Entsar, 2013).

The degree of polymerization and pH are the important factors to assess the antimicrobial nature of chitosan. Generally, lower pH and high molecular weight strongly stresses the microbes. Chitosan contributes biocidic properties at pH < 6 by altering amino group (Dutta *et al*., 2009) whilst, losses its properties at pH 7 (Devlieghere *et al*., 2004). It has shown appreciable metal chelating an activity thereby protects the outer coating of fruit from pathogenic attack. It has also been reported that chitosan interacts with the formation of DNA, RNA and proteins in microbial body. The bacteriostatic action of chitosan is based on its molecular weight that is higher in case of *S. aureus*, preventing nutrients availability and lower for *E. coli*, restraining the entrance of microbes. The antimicrobial properties of chitosan can further be enhanced through the addition of ferulic acid and garlic oil (Dutta *et al.,* 2009). Earlier, Devlieghere *et al*. (2004) ascribed more sensitivity of chitosan in gram negative as compared to gram positive bacteria.

Besides, composite coatings are also gaining popularity among researchers owing to their affirmative role in various biopolymers formulation. Numerous studies have combined the lipid based coatings with protein or polysaccharides to exhibit less water vapor release and low lipid oxidation properties. Extensive research has been conducted to determine the effect of plasticizers on mechanical properties of coatings and films. In this context, coating

formulations like sago starch and fish gelatin along with glycerol have shown better plasticizing effect than that of sorbitol due to hydrophilic nature of glycerol. It has been expounded that water vapor permeability increases with the addition of sorbitol however, modulatory effect was noticed with glycerol (Al-Hassan and Norziah, 2012).

Earlier, Lima *et al.* (2010) studied the gas transfer properties of 0.5% galactomannans and 1.5% collagen based coating on mango (1.5% glycerol) and apple (without glycerol). The results showed 28 & 11% reduction of O_2 & CO_2 permeability in mango and 50% decline for gas transfer in case of apples. Likewise, the efficiency of three types of coatings was analyzed with respect to water and gas permeability. The resultant data showed higher reduction in gas permeability through chitosan+whey protein concentrates & chitosan+glycomacropeptide based coatings nevertheless, chitosan+lactoferrin proved better to overcome water and gas diffusion, owing to dual nature *i.e.* hydrophilic and hydrophobic prospects (Bourbon *et al*., 2011). The effect of chitosan addition was studied on the color attributes of starch based edible coating. It has also been found that inclusion of glycerol increases the interaction of chitosan-starch based coating thus retards water transmission (Chillo *et al.,* 2008).

Earlier, edible coatings were used as barrier against gas and water transfer however, now the functionality of edible coating has been improved due to addition of micronutrients (Vargas *et al*., 2008). The edible coatings have now become an efficient delivery system for nutraceuticals, minerals and vitamins thus improve allied health benefits. Moreover, incorporation of flavoring substances enhances the hedonic response of the product (Martin-Belloso *et al.,* 2006). The addition of functional ingredients to edible coatings depends on their dispersion properties and interactive behavior with polymer structure however, concentration & chemical features of active compounds are also valuable for functionality (Zhao and Mc Daniel, 2005). Addition of minerals and vitamins enhances the delivery of active components by increasing the nutritional & functional compatibility. Earlier, Park and Zhao (2004) noticed that 10-200% of calcium and 5-20% of zinc & vitamin E improve the water vapor resistance as well as nutritional status of the respective edibles.

One of the researchers groups, Rojas-Grau *et al.* (2007) assessed the preserving role of edible films or coatings on fruits. They incorporated various fortificants like zinc & calcium,

essential fatty acids (sunflower oil), antibrowning agents (N-acetylcystein), antioxidants (glutathione) and vitamin E in coating formulation to address malnutrition. According to their findings, addition of bioactive fortificants improved nutritional profile as well as keeping quality of food.

There is a growing interest in the development of edible coatings with different formulations using several ingredients like plasticizers, additives, extracts and antioxidants. As a result, various countries have adopted a general trend of using such components that are permitted safe by USDA thereby edible coatings have attained GRAS status (Rojas-Grau *et al*., 2009). The success of edible coatings application in fruit & vegetable depends on permeability of coatings with respect to the target commodity. It is deduced that edible coatings are compatible in various food products to minimize the postharvest losses (Park, 1999).

2.4.1. Storage Behavior of Edible Coatings

During the last few decades, various edible coatings have attained core attention of the researchers owing to their diversified application on food commodities to protect from ecological factors such as moisture loss, light exposure, oxygen, microorganisms and mechanical stress (Al-Hassan and Norziah, 2012). In a case study, Devlieghere *et al*. (2004) compared the antimicrobial nature of chitosan lactic acid formulation on strawberry and cabbage. The bactericidal effect was more prominent in strawberry as compared to cabbage, retaining their functional properties for 12 days. However, the weight loss was 17.97±9.47% (0.5% chitosan) as compared to control 49.38±10.47% at 12^{th} day of storage. The chitosan based coatings were relatively effective against *Brochotrix thermorphacta* & *Bacillus Cerus* as compared to *Listeria monocytogens* & Lactic acid bacteria whilst, minimal effect was observed in case of *Candida lambia* & *Cryptococcus humicolus*. The mechanism highlights the interaction of chitosan with electronegative charges of microbes as well as chitosan has humectants like properties that limits the enzymatic activity.

In a meta-analysis, various treatments of coating were used on fruit surfaces and noticed that 1.5% chitosan and 1.5% chitosan+0.75% calcium gluconate are an effective antifungal agents compared to control during 6 days storage. It has also been recorded that alginate coating protects against oxidative damage in fruits & vegetables. Thus, maintains tissue firmness and reduces microbial decay for long time after harvest (Elsabee and Entsar, 2013). During an *in*

vitro analysis, bacteriosidic activity of water- & acid soluble chitosan solution was assessed against apricot rot, caused by *Burkholderia Seminalis*. It was inferred that 2 mg/mL of acid soluble chitosan coatings express better antibacterial activity as compared its water soluble form. The mechanism defines the disruption or breakdown of bacterial cell walls by active chitosan hence protects the apricot from contamination (Lou *et al.,* 2011).

The effect of various chitosan based concentrations (1, 2 & 3%) was determined on fresh cut litchi fruit at -1 ºC. The results proved that 3% chitosan based coating is effective in improving sensory quality attributes whilst, decline in weight up to 31 & 50% at 3rd & 6th day of storage was reported, respectively. The data has also elicited a percent increase in TSS, ascorbic acid and total titratable acidity by 3.47, 20.5 & 10.86% at 6th day. The reduction in polyphenol peroxidase and polyphenol oxidase was measured by 20 & 30% and 40 & 28% at 3rd & 6th day, correspondingly thus improves shelf life (Dong *et al.*, 2004). In another study, chitosan+lauric acid based coating formulations were found more effective as compared to chitosan+stearic acid thereby reduced the water transfer up to 49% (Dutta *et al.,* 2009).

In a trial, Ali *et al*. (2011b) judged the sensory characteristics of whole papaya fruit coated with chitosan using 0.5, 1, 1.5 & 2% concentration at 12 ºC & 80-90% RH. The resultant data elucidated minimal water loss (< 6%) through 1.5 & 2% of chitosan as it covers the stoma cells of fruit cuticle. They also noticed that firmness of papaya depends on chitosan concentration and maximal firmness was achieved as 81 & 84.4 N @ 1.5 & 2% of chitosan during 3 weeks study thereby reduces the activity of pectinases. The peel color was also assessed during storage with L*, hue & chroma values by 64.46, 117.14 & 48.05 for control, indicating yellowness with shriveled, softened and fungal infected product. The reduced respiration & ripening achieved at 1.5 & 2% chitosan concentrations resulted in slow formation of soluble solids (carbohydrate hydrolysis). However, 2% chitosan coated fruit presented internal modified atmosphere with minimal ethylene production along with 2% change in color (olive brown). In toto, 1.5% concentration proved better to regulate ascorbic acid as well as scored higher due to glossy appearance without wrinkles at 5th week of storage.

During a study, the chitosan coating on sliced mangoes has resulted in slower water loss & microbial growth thus ensures optimal eating quality attributes and shelf life at 6 ºC

throughout the storage (Chien *et al*., 2007). Earlier, Pen and Jiang (2003) explored the storage behavior of 0.5, 1 & 2% chitosan coated fresh cut Chinese water chestnut at 4 ºC. The outcomes showed minimum alteration in the total polyphenols & sensory characteristics by retarding the activities of polyphenol oxidase, polyphenol peroxidase and microbes that subsequently slowed down the changes associated with TSS, acidity and ascorbic acid. During a storage study, the effect of chitosan (1 & 2%) was measured on longan fruit. The resultant data showed higher postharvest quality with maximum chitosan level that ensures reduction in color loss, weight loss and respiratory activity associated with polyphenol oxidase during 30 days storage (Jiang and Li, 2001).

Jiang *et al.* (2005) measured the impact of chitosan on commercial, sensory & shelf life enhancing features of litchi fruit during storage at 2 ºC. It was inferred that chitosan solution @ 2 g/100g is suitable to control outer peel discoloration thus delays the activity of polyphenol peroxidase, saving anthocyanins along with slower reduction in TSS, titratable acidity, color index and other deteriorative changes.

Rojas-Grau *et al*. (2009) studied the additional benefits of active molecules like antibrowning, nutraceutical, texture enhancers & antimicrobials through various coatings to improve the quality of fresh cut fruits. The results depicted that fortified alginate coating on apple decreases the growth of psychrophilic aerobes, mold and yeast. Likewise, oregano (0.5%) and lemongrass (1.0-1.5%) reduced >4 log CFU/g of inoculated *Listeria innocua*. In a research trial, the incorporation of essential oil & malic acid to alginate based coating was estimated to improve the shelf life of fresh cut melon up to 21 days nevertheless, 0.3% palmarosa oil was found effective to reduce the growth of *Salmonella Enteritidis* (Raybaudi-Massilia *et al.,* 2008). Later, Wu *et al.* (2010) studied the efficacy of zinc and cerium in chitosan coating for the preservation of Chinese jujube fruit. The results demonstrated a linear relationship of mineral and coating solutions with reduction of 11.72% moisture loss, 31.51% gaseous exchange and 7.07% polyphenol peroxidase activity. Whilst, enhanced polyphenols, TSS & ascorbic acid levels by 13.93, 15.45 & 14.55% were recorded as compared to control.

Han *et al*. (2004) confirmed chitosan coatings to preserve the nutrition and shelf life of strawberry especially in the form of chitosan+calcium & chitosan+vitamin E, as carriers of

calcium (78.9-180%) and vitamin E (85%). Accordingly, shelf life increased up to 3 weeks & 6 months at 2 & -23 ºC by reducing weight loss, discoloration, alteration in pH & acidity and drip loss after thawing.

Ayranci and Sibel (2003) determined the protective role of edible coating solutions including methyl cellulose, polyethylene & glycol and antioxidants *i.e.* stearic, ascorbic & citric acid on apricot. The results elucidated maximal reduction in weight loss with the inclusion of stearic acid alone 60.5% trailed by stearic acid+ascorbic acid 56%, stearic acid+citric acid 53% and coating alone 35%. Contrarily, vitamin C loss was minimized by stearic acid+ascorbic acid up to 53% followed by stearic acid+citric acid 46.9%, stearic acid 23% and coating alone 5.6%.

Edible coating is a contemporary approach to curtail deteriorative effect of environmental hazards thereby improves the quality, sensory and functional attributes of fresh cut fruit. During two week trial, the impact of alginate (2%), pectin (2%) & gellan (0.5%) based edible coatings were evaluated with the addition of 0.75% of each glutathione & N-acetyl cysteine on fresh cut pears at 4 ºC. The results inferred lower moisture diffusion, ethylene production, browning reaction & microbial activity with maximum antioxidant and sensory status. Conclusively, no adverse effect was noticed regarding firmness, color and appearance of pear wedges (Oms-Oliu *et al.,* 2008a).

Tapia *et al.* (2008) tested alginate and gellan based coatings plus functional ingredients on fresh cut papaya fruit. It was deduced that addition of 1-2% glycerol+1% ascorbic acid to alginate/gellan solutions enhanced water vapor resistance however, 0.025% sunflower reduced the water transfer rate up to 16 & 66% in alginate & gellan, correspondingly. It was further noticed that addition of ascorbic acid preserves the original ascorbic acid of papaya fruit thus ensures firmness as well as nutritional quality. Earlier, Rojas-Grau *et al.* (2007) documented the synergistic effect of sunflower oil 0.025-0.125% and alginate that retarded water vapor evaporation by 22%. Likewise, the addition of sodium-alginate @1-5% was resulted in enhanced shelf life of sweet cherries from 8 to 16 days at 2 ºC (Diaz-Mula *et al*., 2012).

In a comparative analysis, alginate-acetylated monoglycerides-linoleic acid and alginate-butter-linoleic acid based coatings were applied on fresh cut apples. The results showed

comparatively better storage characteristics of alginate-acetylated monoglycerides-linoleic acid based coatings on fresh apple wedges during 10 days storage at 5 ºC. However, other coatings were found comparatively less effective (Olivas *et al.,* 2007). Later, Rojas-Grau *et al*. (2008) determined the effect of alginate and gellan based coatings to prolong the shelf life of apples from 4 days (control) to 2 weeks by reducing fermentative alterations *i.e.* production of ethylene (< 5 µl/L) and acetaldehyde.

One of their peers, Kristo *et al.* (2008) estimated the efficacy of various antimicrobials *i.e.* potassium sorbate, sodium lactate and nisin in sodium caseinate coatings to control the activity of *Listeria monocytogenes*. The results explicated maximum antimicrobial activity by nisin whilst minimum for sodium lactate. Later, Lee *et al.* (2003) found that whey protein concentrate (WPC) reduces respiration rate by 20% whilst, carrageenan controls by 5% only. They also observed the efficiency of various antibrowning agents at different concentrations. Amongst, 5% WPC+1% ascorbic acid+1% calcium chloride proved as one of the effective formulations to retard discoloration of apple.

In a study, application of folate fortified edible coating on milled rice has proved as a source of folic acid resulting minimal reduction in ethyl cellulose during washing & cooking trailed by pectin and locust bean composite formulations. Addition of these fortificants to other nutrient deficient sources not only improves the nutritional status but also serves as a preventive strategy to overcome anemia, neural tube defects, atherosclerosis & other associated disparities in developing economies (Shrestha *et al.,* 2003).

2.5. Nutritional facts of apricot

Apricot (*Prunus armeniaca*) is an important fruit of Pakistan enriched with numerous bioactive molecules. In an experimental trial, Ali *et al.* (2011b) examined six varieties of apricot including Shai, Neeli, Mirmalik, Khakhas, Habi and Alman for their chemical composition and found moisture content 78.8-85.3% on fresh weight whilst, crude fiber 11.85-13.6%, ash 9.45-12.1%, crude protein 6.18-8.7% and crude fat 2.1-3% on dry weight basis. Nonetheless, the antioxidant potential was determined as 56.84-82.33%, total phenolic components 4590-7310 mg GAE/100g, total carotenoids 10.09-18.13 mg/100g and ascorbic acid 67.39-90.94 mg/100g. The mineral analysis showed potassium (K) at higher concentration *i.e.* 2040±43.72-3000±61.48 mg/100g trailed by calcium (Ca) 102.5±1.72-

124.8±1.6 mg/100g, sodium (Na) 15.89±0.25-22.49±0.39 mg/100g, iron (Fe) 5.14±0.58-12.20±0.93 mg/100g, zinc 0.82-3.53 mg/100g and manganese (Mn) 0.51–1.18 mg/100g. According to Munzuroglu *et al.* (2003) apricot fruit comprised of carbohydrate & protein by 66.5 & 5 g/100g whilst, potassium (K), phosphorus (P) & vitamin C as 979, 108 &12 mg/100g on dry weight basis.

Likewise, the nutritional profile of mature apricot fruit accounting for 2.8-4.29% crude protein, 0.77-2.41% crude fiber, 0.55-3.12% crude oil, 2.72-5.34% ash, 48.3-74.7% & 19.9-25.9% water soluble as well as alcohol soluble components. The recorded pH and acidity (malic acid) were 4.16-5.23 & 0.17-0.79% on dry weight basis. Additionally, the predominant minerals like K, P, Ca, Na & magnesium (Mg) were ranged from 20791-33364, 1436.49-2643.42, 843.28-1896.53, 773.95-1129.74 & 402.82-765.62 ppm, respectively (Hacıseferogullar *et al.,* 2007). Additionally, mineral profiling of various apricot varieties revealed Na 8±0.4 to 17.8±4.6, Ca 87±2.6 to 240.5±10.5, K 1227±57 to 3455±63, Fe 2.34±0.52 to 11.3±0.73 and Zn 1.38±0.12 to 4.24±0.31 with ascorbic acid 28.5±0.7 to 96.8±9 and total sugar 68.61±1.61 to 93.88±4.49 mg/100g on dry weight basis (Akin *et al.*, 2008).

Later, Hussain *et al.* (2010) documented apricot as an energy source due to its compositional profile and therapeutic potential. Its composition was recorded as moisture 11.09±0.8-15.1±0.65, total sugar 6.74±0.3-13.94±0.72, crude fiber 2.27±0.6-3.26±0.65, total ash 2.62±0.11-4.86±0.13, crude fat 1.47±0.1-1.99±0.2, total acidity 1.44±0.11-2.83±0.11 and crude protein 0.8±0.12-1.2±0.1 g/100g of dried apricot. Its mineral profile indicated Na 14.2±0.2-20.8±0.3, Ca 95.5±2.2-110±3, K 490±5-520±5.5, Fe 1.4±0.04-2.4±0.05, Co 0.05±0.01-0.09±0.02 & Zn 0.9±0.02-2±0.05 mg/100g on dry weight basis.

One of the researchers groups, Leccese *et al*. (2007) recorded total phenolic contents (TPC) of apricot fruit from 20.78 to 75.76 mgGAE/100g. The TSS values for different apricot cultivars were fall between 9.9±0.4 to 16.3±0.6 ºB. In another analysis, dried apricots were assessed for TPC 4233.70-8180.49 mgGAE/100g and β-carotene 5.74-48.69 mg/100g out of total carotenoids 14.83-91.89 mg/100g (Akin *et al.,* 2008). The ripening process of apricot fruit has resulted in various enzymatic changes with the development of aromatic and polyphenolic compounds predominantly β-carotene *i.e.* 70-85% total carotenoids, imparting yellow to red color (Dragovic-Uzelac *et al.,* 2007). Afterwards, Saracoglua *et al.* (2009)

elucidated the minerals of apricot as 4.76-28.9 µg/kg chromium, 0.32-0.64 µg/g selenium, 0.92-6.49 µg/g copper, 0.97-8.27 µg/g manganese, 2.96-12.0 µg/g zinc and 10.4-80.1 µg/g iron. In a study, Asma *et al.* (2007) tested 17 genotypes of apricot with total acidity 0.35-1.8% and total soluble solid 12.7-26.5%. The dried apricots constituted 56.8-64.9% of total sugars mainly glucose, sucrose and fructose (Ali *et al.*, 2011b) with higher sorbitol content *i.e.* 16.91–26.84 mg/100 g in Malatya variety (Erdogan-Orhan and Murat, 2011).

Numerous studies have indicated that chemical and physiological reactions in climacteric fruits generate affirmative changes in quality thus improve palatability alongside bioactive molecules (Ambrosio *et al.,* 2013). During a storage study, the physicochemical characteristics of Bergeron apricot were recorded at two different ripening stages. At initial phase, reduced carbohydrate level was noticed in apricot batch kept in cold storage (1^{o}C) for three weeks due to their transformation into various sugar derivatives conversely, lactones, esters and terpenic compounds were increased momentously during subsequent ripening at 20^{o}C for 7 days. It has been observed that ripening rate significantly affected storage behavior of fruits irrespective of harvest stage (Aubert *et al.,* 2010).

The nutritional status of fruit depends on various attributes including varietal and geographical variations. The processing conditions are also been reported as an important criteria influencing the compositional features of apricot primarily vitamin A, C, E as well as minerals like selenium. In an experiment, sulfur treated dried apricots showed two folds higher vitamin C content than that of fresh fruit (Munzuroglu *et al.,* 2003). The apricots contain appreciable quantity of minerals especially K, P, Mg & Ca with lesser concentration of Na, Zn & Fe (Gezer *et al.,* 2000).

In the Northern regions of Pakistan almost 60 varieties of apricots are grown with 1.8 million productive trees (MFC, 2005; DOA, 2008) and 0.5 million metric tons production annually (FAO, 2008; DOA, 2008). Pakistan is ranked 3rd among apricot producing countries with maximum production in Gilgit and Baltistan, followed by NWFP (Malakand division) and higher areas of Balochistan (Jasra and Rafi, 2002). Nevertheless, the postharvest losses of apricot have been investigated around 44% due to fewer processing approaches and higher perishability rate (FAO and DOA, 2007).

2.6. Efficacy studies

Various bioefficacy trials have proved that hypozincemia increases the incidence of bacterial attack due to over-expression of NF-*k*B and targeted genes like IL-1β, ICAM-1 and TNF-α in Zn deficit mice. It has been noticed that Zn deficiency increases the inflammatory response resulting in reduced activity of vital organs including lung and liver. During a bioevaluation trial, Zn supplementation was found effective to mediate innate immunity by downregulating NF-*k*B & TNF-α signaling pathway. It has also been observed that Zn provision reversed the dysregulation of immune expression thus reduces the rate of morbidity (Bao *et al.,* 2010). In a biological study, comparison was made between healthy and common variable immunodeficient (CVID) patients to assess their serum Zn concentration. The lower zinc concentration was noticed in immunodeficient patients as compared to control group. It was further highlighted that lower zinc status worsens the disease condition thereby triggering the inflammatory response. Subsequently, Zn deficiency induces apoptosis in β cells and impairs antibodies & lymphocytes action in the body (Santos-Valente *et al.,* 2012).

Evans and Halliwell (2001) noticed lower concentration of Zn in serum, erythrocytes and glutathione in zinc deprived rats. Later, Zn deficiency was found to reduce body & organ weights in mice and observed reductions by 30, 50 & 70% in body, spleen & thymus weight, respectively (Blewett and Carla, 2012). It has been documented that zinc deficiency reduces thymus mass hence affects thymulin hormone. In a study trial, 32 malnourished children were tested for Zn response. It was observed that supplemented malnourished children recovered their immune system during 9 weeks with thymic mass 387.7 & 453 mm^2 in control & Zn-treated groups. It was revealed that Zn rehabilitation improves the immune function (Chevalier *et al.,* 1996).

Zn is found as one of the minerals that down regulates various signaling pathways that modulate cancer progression however, excessive consumption leads to deposition in lungs (Costello *et al.,* 2004). According to Prasad *et al.* (2004), Zn serves as an antioxidant @ 45 mg/day to healthy volunteers showing reduction in oxidative stress owing to the control of TNF-α that down regulates the expression of NF-*k*B and IL-1B.

The preventive role of zinc against lipid peroxidation was estimated during 10 weeks study based on its three different doses *i.e.* 38, 19 & 3.8 mg/kg body weight. The dose dependant

effect of zinc was measured through decreased values for total cholesterol, triglyceride, oxidized LDL, thiobarbituric acid reactive substances (TABRS) and liver enzymes such as liver aspartate aminotransferase (AST), alanine aminotransferase (ALT) whilst raised HDL level (Yousef *et al*., 2002). This fact was further supported by the findings of another study that depicted higher diseased rate by lowering Zn intake. The study elucidated direct relation of zinc deficiency with altered cholesterol, triglyceride and LDL levels. Furthermore, hematological parameters like total erythrocytes, hemoglobin & packed cell volume were also followed a declining trend (El Hendy *et al.,* 2001).

The anti-atherogenic effect of Zn supplementation was assessed on New Zealand White rabbits that divided in to three groups on the basis of diet *i.e.* control, high cholesterol diet (HCD) and Zn+HCD. The data showed reduction by 20.9, 56.9, 18.89 and 2.2% in total cholesterol, triglyceride, LDL and WBC of Zn treated group as compared to hypercholesterolemic group, respectively. Contrarily, an increasing trend in RBC, hemoglobin & platelets by 33.88, 25.5 & 11.4% was observed in Zn+HCD treated groups, correspondingly. The Zn supplementation also enhanced the serum Zn level up to 11.76% at the termination of 8 weeks study (Ren *et al*., 2006).

Duzguner and Kaya (2007) elucidated the modulatory role of Zn supplementation in diabetic New Zealand rabbits. The animals were distributed in three groups; control, diabetic and diabetic+zinc fed. After the completion of three months study, it was revealed that Zn supplementation 150 mg/L improves the antioxidative status of rabbits by enhancing glutathione, superoxide dismutase and catalase alongside reduction in malondialdehyde content. The higher body weight gain was observed in control animal 3.1% conversely, body weight gain was decreased 5.75% in diabetic animals followed by 1.53% in Zn supplemented diabetic rabbits. Likewise, glucose reduction up to 6.9% was noticed in Zn administered diabetic group (16.25±2.37 mmol/L) as compared to diabetic group (17.46±2.08 mmol/L) of rabbits. Previously, Thompson and Godin (1995) reported 50% reduction in blood glucose level by supplementing $ZnCl_2$ in the diet and serum Zn level reached to normal level.

Numerous studies have shown marked effect of Zn deficiency on animal health. Accordingly, a trial was conducted on young New Zealand White rabbits provided with three types of diets; *i.e.* Zn deficient diet (2 ppm), low Zn diet (7 ppm) and control diet (80-85 ppm). The

reported values for serum and liver Zn levels were 0.34±0.03, 0.79±0.05 & 1.47±0.05 µg/mL and 20.3±0.81, 28.0±1.0 & 24.6±0.75 µg/g for respective diet groups at the termination of 2 weeks trial. The results indicated a substantial reduction of Zn level in various organs & serum due to Zn deficit diet. It has also been observed that Zn deficient diet retards animal growth and impairs immune function within 5 week however, its specific body requirement is lesser due to high absorptive nature (Bentley and Grubb, 1991). Earlier, Keeling *et al.* (1980) analyzed Zn status in 27 patients with liver disease, showing an inverse correlation between serum zinc and liver disease. Thus, Zn levels were found as 0.9±0.03 µg/L (control), 0.64±0.06 µg/L (alcoholic cirrhosis), 0.7±0.03 µg/L (primary biliary cirrhosis) and 0.78±0.07 µg/L (active chronic hepatitis).

Biochemical analysis of normal rabbits was conducted by noting the glucose (7.92 mmol/L), urea (5.23 mmol/L), creatinine (62.66 µmol/L), AST (0.27 µkat/L) and ALT (1.01 µkat/L). Moreover, body weight gain (2620 g) and weight of liver, kidneys & heart as 79, 17.7 & 79.00 g, respectively (Süvegová *et al*., 2004). Later, efficacy study was carried out in order to assess the serum profile of normal rabbits and recorded glucose level by 142 mg/dL, ALT 0.18 µmol, AST 0.14 µmol, ALP 2.41nmol/L, urea 5.9 mmol/L, 92.9 µmol/L, T lymphocyte 60.6%, eosinophils 2.2%, monocytes 2.4% and neutrophils 34.8% (Ewuola *et al*., 2012). Previously, serum Zn level in normal rabbits was estimated as 0.82±0.07 mg/L whilst, a reduction was observed in case of diabetic group 0.54±0.03 mg/L that modulated in diabetic+Zn group 0.91±0.05 mg/L by supplementing Zn @ 150 mg/L (Duzguner and Kaya, 2007). Similarly, zinc in the blood samples of six month old rabbits was measured as 11.2, 1.2 & 0.17 mg/L for serum, red blood cells and whole blood (Jenner *et al.,* 2007; Rashtchizadeh *et al.,* 2008). In a bioevaluation trial, urea & creatinine levels in rats were increased from 14.58 & 3.38 to 15.91 & 3.82 mg/100mL by Zn supplementation (El Hendy *et al.,* 2001).

The Zn has been reported as a therapeutic agent during pregnancy complications and its deficiency reduces milk secretion in lactating mothers (Summersa *et al.,* 2008; Coylea *et al.,* 2009; Da Costa *et al.,* 2013). During experimentation, toxicity of lipopolysaccharide (LPS) mediated hypozincemia was studied in the embryonic growth of New Zealand White rabbits during gestation period. The results demonstrated reduction in serum Zn level from 1.74±0.067 µg/mL (control) to 0.53±0.01 µg/mL (24 hr after LPS treatment) and replenished

by 1.33±0.117 µg/mL after Zn supplementation (Pitt *et al.,* 1997). In a study, impact of hypozincemia was assessed in female rabbits. The results elucidated lesser feed intake, lower body weight, reproductive failure due to pale uteri, hair loss and dermatitis in Zn deprived rabbits (Shaw *et al*., 1974).

Later, Scheplyagina (2005) has deduced that maternal Zn deficiency in blood & milk is directly related with infant health. In case of neonates, Zn concentration < 13 µmol/L in umbilical blood is found to retard growth. It has also been reported that 77% mothers are deficit in Zn. It was further confirmed that a linear association exists between Zn intake and infant growth as well as weight gain (Brown *et al.,* 2002). One of the researchers groups, Salgueiro *et al.* (2002) delineated that Zn based diets are effective against neural tube defects. They further reported that 25 mg/day of Zn during last trimester may improve growth & increase the head circumference of infants.

During an experiment, Zn therapy was given to children < 5 years with persistent diarrhea. It has been noticed that Zn dose @ 10-40 mg/day is effective to lower the mortality rate up to 23% by controlling diarrheal episodes (Walker *et al.,* 2008). In India, a survey pertaining to Zn consumption showed reduction in severity and duration of diarrhea by 39 & 21%, respectively in the children < 3 years (Sazawal *et al.,* 1995). During clinical trial, efficacy of $ZnSO_4$ was assessed against attention deficit hyperactive disorder (ADHD). The non-toxic nature of $ZnSO_4$ supplementation was noticed with therapeutic potential against respective disease up to 28.7% (Bilici *et al.,* 2004).

Ranjan *et al.* (2011) documented competition between free radicals and Cu-Zn SOD (copper-zinc superoxide dismutase) during fluoride toxicity. Purposely, New Zealand White rabbits were given fluoride @ 50 µg/mL resulting in Zn reduction from 14.58±0.66 to 7.06±0.26, 11.32±0.38 to 7.43±0.36 and 10.36±0.51 to 6.27±0.17 µg/mL in liver, kidney and heart tissues, respectively. The fluoride toxicity was reduced from 178.5±59.41 & 50.3±35.99 µg/g (control) to 149.13±54.12 & 35.33±12.79 µg/g in liver and kidney, respectively by the provision of 30 g zinc chloride+35 g of nickel chloride to the experimental rabbits. Therefore, no substantial adverse effect was noticed on Zn concentration however, 30 g of Zn to the diet resulted increased Cd concentrations in kidney & liver (Kalafova *et al.,* 2012).

In an experimental trial, Karademir *et al*. (2011) assessed the synergistic effect of various acids with $ZnSO_4$ in young male New Zealand rabbits by determining serum Zn level. The data of three supplementation categories revealed higher serum Zn level in $ZnSO_4$+ascorbic acid group (272.04±11.4 µg/dL) followed by $ZnSO_4$+grapes vinegar (243.86±4.82 µg/dL) and $ZnSO_4$+distilled water (171.79±8.82 µg/dL) during 2.5 hr exposure. The results indicated higher Zn absorption in the presence of ascorbic acid.

During rodent modeling, the bioavailability of Zn was analyzed in Sprague Dawley rats rely on $ZnSO_4$ fortified whole wheat flour. The results depicted Zn absorption in plasma, liver and kidney by 1.83±0.08, 44.14±0.94 and 22.39±0.9 µg/g, respectively (Akhtar *et al*., 2010). In another study, efficacy of Zn was measured against renal disease in weaned male Wistar rats. The results presented reduced glomerular filtration, activity of nitric oxide synthase and NADPH diaphorase in renal system hence protects against oxidative stress (Tomat *et al.,* 2007). During a population based trial, antidepressant functioning of Zn supplementation was assessed in 60 individuals. The recorded data explicated enhanced serum zinc concentration that reduced depression up to 22% (Siwek *et al.,* 2010).

2.7. Nutrition Education

In the current situation, it is important to create awareness regarding the significance of balanced diet among the vulnerable segments to tackle the menace of hidden hunger. Purposely, well intended promotional activities including nutrition education, social marketing and mass media campaigns are the appropriate strategies to disseminate information among the general public. All the integrated therapies are well implemented and updated by International Zn Nutrition Consultative Group (IZiNCG) to alleviate Zn deficiency & resultant infections throughout the world. The IZiNCG has promoted various programs at community level to educate regarding Zn related nutrition and immunity problems in infants & mothers specifically (Hess and King, 2009). The intake of Zn has been improved up to 10.6 & 9.2% by women, infant & children (WIC) & Food Stamp programs, correspondingly (Rose, 1999).

One of the objectives of dietary interventional approache is to provide nutritional education at domestic as well as industrial level in order to correct the deficiency symptoms. In this context, public should be educated about the phytates:Zn ratio as well as strategies for

phytates ratio reduction through soaking, fermentation, thermal processing and enzymatic hydrolysis (Hotz and Brown, 2004; Gibbson, 2006). The consumption of red meat & organs especially liver should be encouraged among the masses to address their nutritional deficiencies (Hotz and Brown, 2004). Likewise, addition of fish powder in cereal based products is another pragmatic approach to enhance Zn consumption in the target population (Gibbson, 2006). Mothers should be educated about the breastfeeding to protect neonates from diarrheal infections or impaired immune system. The Zn enriched complementary foods should be introduced among infants to compensate their increasing Zn demand (Salgueiro *et al*., 2002).

The mass media campaign ought to be launched for bridging the gap between the stake holders to achieve desirable health status nationwide (Srinivasan, 2003). Biofortification will also encourage within the developing economies to introduce novel breeding & farming practices that enhance Zn content in various food commodities. Likewise, food industry should adopt fortification based approaches to overcome the disease symptoms cost effectively (Hotz and Brown, 2004; Gibbson, 2006). In this context, edible coatings serve as a vehicle to transfer functional ingredients including micronutrient, nutraceuticals and additives to improve the nutritional value of product as well as protect from environmental hazards (Muranyi, 2013).

Considering the poor nutritional status of Pakistani population, the instant research agenda was planned to address the socio-economic indicators of Pakistan where bulk of fresh fruit & vegetable are going as waste due to poor postharvest practices. Accordingly, several carbohydrate & protein based coatings were developed and fortified with zinc to improve the nutritional profile of apricot. The findings of the investigation will encourage the growers, processors and exporters to divert towards the novel techniques of edible coating for extending the shelf life of fruits that in turn generate foreign exchange.

Chapter 3

MATERIALS AND METHODS

The current research work was carried out at the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (UAF), Pakistan. In the present research, locally available apricot variety (Sufeda) was used for zinc fortification through alginate and chitosan based edible coatings. Furthermore, the storage behavior of coated apricots was assessed followed by bioefficacy trial. The materials and protocols followed are elaborated herein.

3.1. Procurement of raw materials

Selection of fresh apricots was made on the basis of uniformity in size, shape, color and absence of physical damage. The analytical and HPLC grade reagents and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). For bioevaluation trials, rabbits were acquired and reared in the Animal Room of NIFSAT, UAF. For bioeffecacy assessment, diagnostic kits were purchased from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemical (Cayman Euroupe, Estonia).

3.2. Preparation of whole fruit

The collected apricots were washed with water to loosen the dirt and grits adhered to the surface of fruit in the Canning Hall of the NIFSAT, UAF. Afterwards, the apricots were randomly assorted into lots and stored at 4-6 ^{o}C to avoid browning and undesirable biochemical changes before further treatment.

3.3. Proximate analyses

Apricot samples were analyzed for moisture, crude protein, crude fat, crude fiber, ash and nitrogen free extract (NFE) according to their respective method.

3.3.1. Moisture content

The moisture content of apricots was determined following the procedure mentioned in AOAC (2006) Method No. 934-01. Accordingly, 10 g sample was dried in Hot Air Oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105±5 °C till constant weight. Afterwards, the dried sample weight was assessed and percent moisture content was calculated using following equation.

$$\% \text{ Moisture content} = \frac{(\text{Initial weight of sample} - \text{Final weight of sample})}{\text{Initial weight of sample}} \times 100$$

3.3.2. Crude protein

The crude protein of apricot samples was determined using Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, Gmbh-Germany) by adopting the protocol of AOAC (2006) Method No. 984-13. For the purpose, apricot samples were digested with concentrated H_2SO_4 and digestion mixture (K_2SO_4:$FeSO_4$:$CuSO_4$) until the color turned to light greenish. The digested material was diluted up to 250 mL in volumetric flask. The diluted sample (10 mL) was treated with equal volume of 40% NaOH in distillation apparatus. The liberated ammonia was captured in 4% boric acid solution using methyl red as an indicator that turned to golden. Thus, percentage of nitrogen in the sample was estimated by titration of distillate against 0.1 N H_2SO_4 solution. Finally, % nitrogen was calculated by the following expression.

$$\% \text{ Nitrogen} = \frac{\text{Volume of 0.1N H2SO4 used} \times 0.0014 \times \text{Dilution volume}}{\text{Weight of sample} \times \text{Volume of dilluted sample taken}} \times 100$$

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

3.3.3. Crude fat

Apricot samples were subjected to crude fat determination by following Method No. 920-39 of AOAC (2006). The dried sample (3g) was refluxed in Soxhlet System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) using n-hexane as solvent. Five continuous siphon washings were given to the samples for maximum extraction of crude fat. Afterwards, the

sample was taken out and dried till constant weight; loss in weight was expressed for crude fat content.

$$\text{Crude Fat (\%)} = \frac{\text{Initial weight of sample} - \text{Final weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.3.4. Crude fiber

The crude fiber was calculated by adopting the guidelines of AOAC (2006) Method No. 978-10. Fat free sample was digested with 1.25% H_2SO_4 followed by 1.25% NaOH solution in Labconco Fibertech (Labconco Corporation Kansas, USA). After filtration and washing with distilled water remaining residues were dried, weighed and ignited in Muffle Furnace at 550 °C till grayish white ash. The crude fiber was estimated according to the expression given below.

$$\text{Crude Fiber (\%)} = \frac{\text{Loss in weight of sample after ignition}}{\text{Initial weight of sample}} \times 100$$

3.3.5. Total ash

The ash content of apricots was estimated according to the procedure outlined in AOAC (2006) Method No. 942-05. Purposely, 5 g dried sample was directly charred on flame in crucible until fumeless. Afterwards, sample was ignited in Muffle Furnace (MF-1/02, PCSIR, Pakistan) @ 550 ºC until grayish white residue was obtained.

$$\text{Total ash (\%)} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

3.3.6. Nitrogen free extracts (NFE)

The nitrogen free extract (NFE) of apricot samples was calculated by following equation;

$$\text{NFE} = 100 - \%(\text{ moisture} + \text{ crude fat} + \text{crude protein} + \text{crude fiber} + \text{ ash})$$

3.4. Mineral contents

The apricot samples were subjected to mineral profiling by adopting the guidelines of AOAC (2006). Dried apricot sample (0.5 g) was digested with nitric acid (HNO_3) and perchloric acid ($HClO_4$) on hot plate till 1 to 2 mL solution remained, followed by

dilution up to 100 mL. The minerals including sodium, potassium and calcium were estimated through Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) whilst zinc, iron, cobalt and manganese were determined through Atomic Absorption Spectrophotometer (Varian AA240, Australia).

3.5. Development of fortified edible coatings

Zinc fortified carbohydrate based coatings (chitosan and alginate @ 1 and 2% each) were developed using various levels of zinc sulfate ($ZnSO_4$) and zinc chloride ($ZnCl_2$) as fortificants mentioned in Table 1.

Table 1: Study plan for the development of zinc fortified edible coatings

Coating Type	Fortificant	Treatments	Coating (%)	Fortificant level (ppm)
Control	-	T_0	-	-
Alginate	**$ZnSO_4$**	T_1	1	30
		T_2	1	50
		T_3	2	30
		T_4	2	50
	$ZnCl_2$	T_5	1	30
		T_6	1	50
		T_7	2	30
		T_8	2	50
Chitosan	**$ZnSO_4$**	T_9	1	30
		T_{10}	1	50
		T_{11}	2	30
		T_{12}	2	50
	$ZnCl_2$	T_{13}	1	30
		T_{14}	1	50
		T_{15}	2	30
		T_{16}	2	50

3.5.1. Zinc fortified alginate based coatings

Alginate based coatings were prepared by following the protocol of Rojas-Grau *et al.* (2008). The film forming solution was prepared by dissolving alginate powder (2 g) in 100 mL of distilled water and heated at 70 °C with continuous stirring until the clear solution was formed. Citric acid (1 g/100mL) was added followed by continuous stirring for 30 min to prevent enzymatic browning. Glycerol was added as plasticizer (1.5 g/100mL) in alginate solution. Film forming solution was emulsified with sunflower oil (0.025 g/100mL) followed by the addition of N-acetyl L-cysteine (1 g/100mL) and calcium chloride (2 g/100mL water) required for cross linking of carbohydrate polymers. The concentrations of ingredients used in these formulations are shown in Table 2. The fortificants were added as per study plan (Table 1).

Table 2: Alginate based coatings formulation

Ingredients	**Alginate based coating**	
	1%	**2%**
Sodium alginate	1 g	2 g
N-acetyl L-cysteine	1 g	1 g
Calcium chloride	2 g	2 g
Glycerol	1.5 g	1.5 g
Citric acid	1g	1 g
Sunflower oil	0.025 g	0.025 g
Distilled water	100 mL	100 mL
Fortificants	As per study plan	

3.5.2. Zinc fortified chitosan based coatings

Chitosan based coatings were prepared according to the procedure of Simoes *et al.* (2009). Coating formulation was prepared by dissolving chitosan (crab shell chitosan, Sigma Chemicals) in distilled water (100 mL) with the addition of glacial acetic acid (1 g) to dissolve chitosan (Table 3). To achieve complete dispersion, solution was heated at 25 °C with continuous stirring for 1 hr. Ascorbic acid (2 g/100mL) and citric acid (1 g/100mL)

were added to prevent enzymatic browning followed by continuous stirring for 30 min. Glycerol was added as plasticizer at 1.5 g/100mL to reduce brittleness caused by extensive intermolecular bonding. Film forming solution was emulsified with sunflower oil (0.025 g/100mL) to improve the water vapor barrier properties. For addition of fortificants, Table 1 was followed.

Table 3: Chitosan based coatings formulation

Ingredients	**Chitosan based coating**	
	1%	**2%**
Chitosan	1 g	2 g
Acetic acid	1 g	1 g
Ascorbic acid	2 g	2 g
Citric acid	1 g	1 g
Glycerol	1.5 g	1.5 g
Sunflower oil	0.025 g	0.025 g
Distilled water	100 mL	100 mL
Fortificants	As per study plan	

3.6. Application of edible coatings

After the development of zinc fortified edible coatings *i.e.* alginate and chitosan with their two levels 1 and 2% containing fortificants *i.e*. $ZnSO_4$ & $ZnCl_2$ @ 30 & 50 ppm of each were applied on different lots of apricots through dipping. Later, the coated apricots were allowed to dry for 15-20 min.

3.7. Storage of treated apricots

The treated apricots were placed in Controlled Climate Chamber at 4±1 $^{\circ}$C temperature and 85±5% relative humidity for eight weeks. Fortnightly analyses regarding physicochemical traits, total zinc content and sensory evaluation were performed during two month storage.

3.8. Physicochemical analyses

The resultant treatments were subjected to various physicochemical analyses to examine the storage behavior of coated fruit.

3.8.1. Weight loss

Weight loss of all treatments during the entire storage was determined by following the protocol of AOAC (2006). Over the storage, weight was regularly monitored and the loss calculated by observing the initial and final weights.

$$\textbf{Weight loss} = \textbf{Initial weight} - \textbf{Final weight}$$

3.8.2. Moisture loss

Moisture loss of varyingly treated apricots was recorded by adopting the method of AOAC (2006). The moisture loss percentage relative to initial weight was calculated by weighing the samples at regular intervals during storage.

3.8.3. Color

Color of coated apricots was estimated according to the method of Rocha and Morais (2003) with the help of hand held Tristimulus Colorimeter (Neuhaus Neotec, Germany, Color meter, Colortest II, Serial No. 95808). The color of fruit was recorded by placing it under the photocell. Individual reading was noted in color test number (ctn) and compared with the standard.

3.8.4. Texture

Texture analysis was carried out by using (20 mm) needle probe according to the method of Mizarch (2008). The coated apricot was placed under the needle of Texture Analyzer (TA-XT2, Stable Microsystems, Surrey, UK) and punctured, force required was noted and expressed as g.

3.8.5. Extraction of juice

For juice extraction, 100 g fruit was blended in 200 mL distilled water followed by filtration to remove insoluble contents. The extracted juice was subjected to various tests like pH, titratable acidity and total soluble solids.

3.8.5.1. pH

The pH of all samples was determined using digital pH meter following the guidelines of AOAC (2006).

3.8.5.2. Total soluble solids

Total soluble solids of treated apricots were recorded by Refractometer (ABBE'S Refractometer, Bellingham Stanley, BS eclipse, UK, 45-03) according to standard procedure of AOAC (2006) on fortnightly basis up to two months. Purposely, a drop of juice was placed on refractometer and reading was noted. The results were expressed as °Brix.

3.8.5.3. Titratable acidity

The acidity of each sample was determined using digital Acidity Meter (QA supplies LLC, USA). Accordingly, 300 µL of juice was taken followed by the addition of 30 mL distilled water. The resultant solution was poured on electronic detector of the digital acidity meter and expressed as % acidity on citric acid basis.

3.8.5.4. Total sugars

Total sugars in prepared treatments were estimated through HPLC by adopting the protocol of Kelebek *et al.* (2009). The freshly extracted juice samples were centrifuged (Eppendorf 5805 R, Hamburg, Germany) at 4000 rpm for 20 min. Afterwards, the resultant supernatants were collected and filtered through 0.45 µm membrane filter followed by storage at -18 ^{o}C. Total sugars were estimated by injecting 20 µL sample of prefiltered juice into HPLC system (PerkinElmer, Series 200a, USA) equipped with pump and refractive index detector operated at 80 ^{o}C while maintaining flow rate of 0.6 mL/min of DDH_2O in Rezex RCM-

Monosaccharide Ca^{+2} Phenomenex column. The eluted peak area was measured and compared with the standard.

3.8.5.5. Ascorbic acid

The treatments were subjected to ascorbic acid determination according to the guidelines of Kelebek *et al.* (2009) using HPLC (PerkinElmer, Series 200a, USA) at the mentioned intervals during two months storage. Aliquots of apricot juice were filtered through 0.45 µm membrane filter and stored at -18 ºC till further analysis. The apricot juice (20 µL) from each treatment was loaded to HPLC system using 0.25% acetic acid as mobile phase at a flow rate of 1mL/min using C_{18} column (Shim-Pack CLC-ODS, 25cm × 4.6mm, 5µm). For detection, UV-visible detector operating at 254 nm was used at room temperature. The peak area of the eluted samples was measured.

3.8.5.6. Citric acid

The HPLC system (PerkinElmer, Series 200a, USA) equipped with pump system and UV-visible detector monitored at 254 nm was employed at room temperature for detection of the elution coming out from the C_{18} column (Shim-Pack CLC-ODS, 25 cm × 4.6 mm, 5 µm) with 0.25% acetic acid mobile phase at a flow rate of 1 mL/min (Kelebek *et al.,* 2009).

3.9. Total zinc contents

The zinc concentration in different treatments of apricot was evaluated on fortnightly basis upto two months following the prescribed guidelines of AOAC (2006). For the purpose, 0.5 g dried sample of the apricot was digested in 100 mL conical flask by adding 10 mL HNO_3 followed by heating at 60 to 70°C for 20 min and then digested with 5 mL $HClO_4$ under same conditions. Subsequently, temperature was raised to 195 °C till the sample was transparent. The digested samples were diluted up to 100 mL in a volumetric flask with deionized water. The samples were loaded in air acetylene flame equipped Atomic Absorption Spectrophotometer containing standard atomizer and zinc lamp at 213.9 nm with slit width 1.30 nm at an oxidant pressure 160 kPa and 15 L/min flow rate.

3.10. Mold count

Mold count was carried out by adopting the protocol described in AACC (2000) on prescribed intervals during storage. Media was prepared by using potato dextrose agar (Jenway 3510-UK). The sample (1 g) was taken followed by the addition of 9 mL sterile buffer phosphate diluent on low speed for 1-2 min. All dilutions were well agitated. Each dilution bottle was shaken to reuse the suspended substance and then transferred 1 mL from each dilution to appropriately marked petri dishes. Potato dextrose agar (cooled to 45°) @ 12-15 mL was poured in plates within 15 min of original dilution, mixed well and allowed to solidify. Dilutions were poured in control plates for each series of samples. The sample dilutions and agar medium were mixed by rotating plates on the surface. Petri dishes were allowed to solidify before inverting plates and incubated at 22-25 °C for 3 days. All colonies were counted on the plates and multiplied by dilution factor.

3.11. Sensory evaluation

Sensory evaluation regarding color, flavor, taste, firmness and overall acceptability was carried out using 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) according to the procedure of Meilgaard *et al.* (2007). The detail is given in Appendix I. A panel of 25 trained judges were asked to express their opinion about the sensory attributes of zinc fortified coated apricots by assigning scores. Sensory response of the fortified apricots for various traits was carried out at 0, 15, 30, 45 and 60 days. This part of study was conducted in the Sensory Evaluation Laboratory of the NIFSAT, University of Agriculture, Faisalabad. On the evaluation day, panelists were provided separate booths equipped with white fluorescent light. For enhancing the accuracy, panelists were provided distilled water along with unsalted crackers to neutralize their mouth feel during testing. To remove any biased from the experiment, samples were presented to the judges randomly and requested to assign scores for selected characteristic.

3.12. Selection of best treatment

On the basis of physicochemical characteristics, total zinc contents and sensory response, four best treatments, two from each type of coating with different fortificants were selected for the bioefficacy trial.

3.13. Efficacy studies

To evaluate the bioavailability of zinc fortificants, a model feed trial was performed. For the purpose, 115 normal, healthy rabbits having weight and age ranged between 0.5-0.65kg and 7-8 months, respectively were procured and housed in the Animal Room of National Institute of Food Science and Technology. The rabbits were acclimatized on basal diet (fodder) for the period of seven days under controlled conditions. The temperature (23±2 ºC) and relative humidity (55±5%) was maintained throughout the experiment with 12 hr light-dark period. Before the initiation of trial, baseline values for selected parameters were established. During 56 days study span, the rabbits were randomly divided into five groups, ten in each and provided with selected uncoated (control) and zinc fortified (fresh & whole) apricots (150 g/day/rabbit) along with normal diet (Table 4). After consumption of entire apricots and basal diet, the feed intake data was recorded. For the collection of blood samples in rabbits, arterial blood sampling was done by removing the hairs from the ear and blood was taken from the marginal ear vein. The blood samples were collected from the overnight fasted rabbits at 0, 15^{th}, 30^{th}, 45^{th} and 60^{th} day of modeling. For serum collection, blood samples were subjected to centrifugation. The sera samples were examined for total zinc contents, glucose & insulin levels and serum biochemistry (liver & renal function tests) using respective protocols. The collected organs including liver, kidneys and heart were used for the determination of zinc contents and weighed to calculate organ to body weight ratio. Earlier, collected blood samples were analyzed for hematological parameters with special reference to red and white blood cells indices. The entire biological trial was repeated sequentially to draw a conclusive inference and the data from the trial was lumped together.

Table 4: Diet plan used in the *in vivo* studies

Groups	Diet plan
G_0	Control (unfortified apricots)
G_1	Apricots coated with 2% alginate containing 50 ppm $ZnSO_4$
G_2	Apricots coated with 2% alginate containing 50 ppm $ZnCl_2$
G_3	Apricots coated with 2% chitosan containing 50 ppm $ZnSO_4$
G_4	Apricots coated with 2% chitosan containing 50 ppm $ZnCl_2$

Group I:

In this group, rabbits rely on unfortified apricots along with normal diet.

Group II:

The group II was comprised of rabbits fed on alginate coated apricots containing 50 ppm $ZnSO_4$ with simultaneous intake of normal diet for a period of 8 weeks.

Group III:

In this group, rabbits were provided alginate coated apricots carrying $ZnCl_2$ @ 50 ppm along with basal diet.

Group IV:

Group IV comprised of rabbits that administered chitosan coated apricots having 50 ppm of $ZnSO_4$ with simultaneous provision of normal feed during the experimental period.

Group V:

In case of group V, chitosan coated apricots containing 50 ppm $ZnCl_2$ were given to the rabbits during the entire study to assess the significance of fortificants in physiological system.

During the animal housing period following parameters were taken into consideration.

3.13.1. Feed and drink intake

Net diet and drink intakes of each group were measured on daily basis (Wolf and Weisbrode, 2003).

3.13.2. Body weight gain

Gain in body weight of experimental groups was recorded weekly throughout the study period (Wolfgor *et al*., 2002).

3.13.3. Organs weight

Organs *i.e.* liver, heart and kidneys were collected after dissection to determine the effect of diets on organ weights of rabbits. The organs were properly cleaned and weighed on electronic balance for organ to body weight ratio (Dyer *et al*., 2008).

3.13.4. Determination of zinc in sera

Zinc content in serum was determined by following the method of Doretto *et al.* (2002) and Karademir (2011). The sera were collected after centrifugation of blood @ 3500 rpm for 15 min. Afterwards, deproteinization of serum was done with trichloroacetic acid (20% w/v). Accordingly, serum sample (1 mL) was mixed with trichloroacetic acid followed by heating at 80^{o}C for 15 min and centrifuged followed by the separation of supernatant. The collected supernatant was used to determine the zinc content through Atomic Absorption Spectrophotometer (Varian AA240, Australia). An air-acetylene flame was used at an oxidant pressure of 160 kPa and 15 L/min flow rate equipped with Atomic Absorption Spectrophotometer along with standard atomizer and zinc lamp at a wavelength of 213.9 nm.

3.13.5. Estimation of zinc deposition in rabbit's organs

The organs including liver, heart and kidneys were tested for zinc status using Atomic Absorption Spectrophotometer (AOAC, 2006). The rabbits were dissected and the organs were removed from the carcass and kept frozen before further analyses. About 0.5 g sample of respective organ was digested in 100 mL conical flask adding 10 mL HNO_3. During subsequent steps, heating was carried out at 60 to 70 °C for 20 min followed by digestion with 5 mL $HClO_4$. The temperature was raised by 195 °C till the sample turned transparent and reduced to 1 to 2 mL. Afterwards, the digested samples were diluted in a volumetric

flask with deionized water up to 100 mL. The resultant samples were loaded to Atomic Absorption Spectrophotometer and concentration of zinc was measured.

3.13.6. Serum glucose and insulin levels

The collected serum samples were evaluated for glucose concentration by GOD-PAP method as described by Kim *et al.* (2011). Further, insulin level was assessed by the guidelines of Ahn *et al*. (2011).

3.13.7. Liver function tests

Liver function tests including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin were assessed according to the method of Basuny *et al.* (2009). Levels of AST and ALT were measured by the dinitrophenylhydrazene (DNPH) method using Sigma Kits 59-50 and 58-50, respectively and ALP by Alkaline Phosphates–DGKC method.

3.13.8. Renal function tests

The renal functionality parameters like serum urea (GLDH-method) and creatinine (Jaffe-method) were recorded through their respective commercial kits (Jacobs *et al*., 1996; Thomas, 1998).

3.13.9. Hematological analyses

Red blood cells indices with special reference to total red blood cells (TRBCs) and hemoglobin (hb) were estimated. Likewise, white blood cell indices including monocytes, T-lymphocytes, b-lymphocytes, eosinophils and neutrophils were measured by using Automatic Blood Analyzer (Nihon Kohden, Japan) (Al Haj *et al.,* 2011).

3.14. Statistical analysis

The collected data were analyzed statistically by applying two factor factorial under completely randomized design (CRD) using Cohort version 6.1 (Costat-2003). The level of significance was also determined (ANOVA) according to the guideline of Steel *et al*. (1997).

CHAPTER 4

RESULTS AND DISCUSSION

Micronutrients provide protection against various physiological disorders and dysfunctions thereby enhancing the overall human health. To overcome micronutrients deficiency, fortification is a rational and cost effective approach. Accordingly, edible coated apricots were fortified by using different salts of zinc holding potential to alleviate hypozincemia. In the present research, the locally grown apricot variety was analyzed for its proximate composition, mineral profile and then fortified with zinc salts *i.e.* $ZnSO_4$ and $ZnCl_2$ through different edible coatings. Lastly, on the basis of physico-chemical profiling, HPLC characterization (organic acids and sugars), hedonic response and zinc content in the edible coated fortified apricots, four best treatments (two from each type of coatings with different salt) were selected and subjected to bioefficacy trial through rabbit's experimental modeling. The results with discussion of examined attributes are conferred herein:

4.1. Quality analyses

4.1.1. Proximate and minerals profile of apricot

Proximate composition is a key factor for assessing the nutritional quality of raw material. Apricot was subjected to different quality traits and revealed crude fat, crude protein, crude fiber, ash and NFE as 2.17±0.16, 6.91±0.29, 11.19±0.74, 9.15±0.35 and 70.57±2.35%, respectively (Table 5). Mineral profile in the current study (Table 5) comprised of sodium, calcium, potassium, iron, cobalt, zinc and manganese, and their respective values were 14.28±0.63, 112.58±3.19, 1322.06±35.21, 7.35±0.58, 0.17±0.01, 1.61±0.03 and 1.72±0.05mg/100g.

Previously, in an experimental trial, Ali *et al.* (2011b) examined six varieties of apricot including Shai, Neeli, Mirmalik, Khakhas, Habi and Alman for their chemical composition and found crude fiber 11.85-13.6%, ash 9.45-12.1%, crude protein 6.18-8.7% and crude fat 2.1-3% on dry weight basis. Moreover, mineral analysis showed potassium (K) at higher concentration *i.e.* 2040±43.72-3000±61.48mg/100g trailed by calcium (Ca) 102.5±1.72-124.8±1.6mg/100g, sodium (Na) 15.89±0.25-22.49±0.39mg/100g, iron (Fe) 5.14±0.58-12.20±0.93mg/100g, zinc 0.82-3.53mg/100g and manganese (Mn) 0.51-1.18mg/100g.

Table 5: Compositional profiling of apricot

Proximate assay	**(%)**
Crude Fat	2.17±0.16
Crude Protein	6.91±0.29
Crude Fiber	11.19±0.74
Ash	9.15±0.35
NFE	70.57±2.35
Minerals	**(mg/100g)**
Sodium	14.28±0.63
Calcium	112.58±3.19
Potassium	1322.06±35.21
Iron	7.35±0.58
Cobalt	0.17±0.01
Zinc	1.61±0.03
Manganese	1.72±0.05

Values are expressed as means ± standard deviation

The results of present investigation are in line with the earlier findings of Akin *et al.* (2008), narrated ash contents from 2.90 to 3.11%. Additionally, mineral profiling of various apricot varieties expounded Na 8±0.4 to 17.8±4.6, Ca 87±2.6 to 240.5±10.5, K 1227±57 to 3455±63, Fe 2.34±0.52 to 11.3±0.73 and Zn 1.38±0.12 to 4.24±0.31mg/100g.

Earlier, Hacıseferogullar *et al.* (2007) documented the nutritional profile of mature apricot fruit as 2.8-4.29% crude protein, 0.77-2.41% crude fiber, 0.55-3.12% crude oil, 2.72-5.34% ash, 48.3-74.7% & 19.9-25.9% of water and alcohol soluble components. The results for sodium, calcium, potassium and iron are also in accordance with the above findings; they tested different apricot verities and found variations in minerals profiling as 773.95-1129.74ppm, 843.28-1896.53ppm, 20791-33364ppm and 398-433mg/kg, respectively. Later, Hussain *et al.* (2010) narrated the proximate composition of apricot as crude fiber 2.27±0.6-3.26±0.65, total ash 2.62±0.11-4.86±0.13, crude fat 1.47±0.1-1.99±0.2 and crude protein 0.8±0.12-1.2±0.1g/100g. They also documented apricot as a good mineral source with various health prospective. Its mineral profile indicated Na 14.2±0.2-20.8±0.3, Ca 95.5±2.2-110±3, K 490±5-520±5.5, Fe 1.4±0.04-2.4±0.05, Co 0.05±0.01-0.09±0.02 & Zn 0.9±0.02-2±0.05mg/100g on dry weight basis.

According to Munzuroglu *et al.* (2003), apricot fruit comprised of carbohydrate & protein by 66.5 & 5g/100g whilst, potassium (K), phosphorus (P) & vitamin C by 979, 108 & 12mg/100g on dry weight basis. Afterwards, Saracoglua *et al.* (2009) elucidated the minerals profile of apricot as 4.76-28.9µg/kg chromium, 0.32-0.64µg/g selenium, 0.92-6.49µg/g copper, 0.97-8.27µg/g manganese, 2.96-12.0µg/g zinc and 10.4-80.1µg/g iron. However, the results for cobalt content are in line with the work of Duran *et al.* (2008), examined variations from 0.2-1.78 µg/g and ascribed as a function of climate, soil and agronomic practices.

The compositional differences in apricot regarding proximate and minerals profiling are due to varietals variations, climatic conditions, topographic locations and agronomic practices. Moreover, the maturity of the fruit and stage of picking are also the prime factors responsible for nutritional profiling.

4.1.2. Physico-chemical analyses of zinc fortified edible coated apricots

Mean squares regarding weight loss, pH, titratable acidity and total soluble solids of zinc fortified edible coated apricots showed significant differences due to treatments and storage (Table 6).

Amongst treatments, the maximum value of weight loss was reported in T_0 (control) 49.03±3.51g however, the minimum in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 55.05±2.53g followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 53.36±3.16g, T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 53.35±3.41g and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 53.35±3.18g, respectively (Table7). It is evident from means that there was a gradual decline in weight of fortified apricots ranged from 57.56±3.42 at initiation to 54.63±3.54, 52.12±3.45, 49.45±3.52 and 47.54±3.39g at 15th, 30th, 45th and 60th day, correspondingly. By the application of edible coatings in apricots, the weight loss was less as compared to uncoated ones.

Likewise, the maximum value for pH was observed in T_0 as 4.76±0.39 though, the minimum in T_{12}, T_{16}, T_4 and T_8 as 4.18±0.31, 4.22±0.34, 4.25±0.32 and 4.27±0.24, respectively (Table 8). The pH significantly increased during storage that varied from 4.18±0.13 at beginning to 4.58±0.37 in the end of storage.

Similarly amongst treatments, the maximum recorded titratable acidity was in T_0 (0.18±0.02) whilst the minimum 0.27±0.02, 0.26±0.02, 0.25±0.02 and 0.24±0.02 (% malic acid) in T_{12}, T_{16}, T_4 and T_8, respectively (Table 9). During storage, there was a momentous decline in titratable acidity that differed from 0.27±0.02 at start to 0.19±0.01 (% malic acid) at 60th day.

The results showed amongst treatments, the minimum value of total soluble solids was recorded in T_0 (11.95±0.56) whilst, the maximum in T_{12} (13.39±0.64), T_{16} (12.98±0.46), T_4 (13.94±0.32) and T_8 (12.89±0.26^{o}Brix), respectively (Table 11). It is obvious from the means that there was a gradual increase in total soluble solids of edible coated fortified apricots; 12.24±0.41, 12.55±0.26, 12.72±0.46, 12.83±0.59 and 13.02±0.66^{o}Brix at 0, 15, 30, 45 and 60 day of storage.

Table 6: Means squares for weight loss, pH, titratable acidity and total soluble solids

S.O.V	Df	Weight loss	pH	Acidity	Total soluble solids
Treatment (T)	16	28.961**	0.33603**	0.00727**	1.30482**
Storage (S)	4	813.925**	1.06636**	0.05888*	4.48803**
S x T	64	2.710^{NS}	0.08831^{NS}	0.00054^{NS}	0.26422^{NS}
Error	170	1.308	0.04292	0.00309	0.14696

($p<0.05$)
** = Highly significant
^{NS} = Non significant

Table 7: Effect of treatments and storage on weight loss of zinc fortified edible coated apricots (g)

Days	Treatments																Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	59.20 ±3.79	58.00 ±2.92	58.40 ±3.67	56.24 ±3.39	57.43 ±2.94	57.65 ±3.10	56.94 ±2.85	56.13 ±2.54	57.16 ±2.62	56.92 ±3.02	58.43 ±3.66	57.63 ±3.07	58.96 ±2.77	59.13 ±2.41	57.49 ±3.97	55.42 ±2.57	57.33 ±2.44	57.56± 3.42a
15	52.40 ±3.46	54.44 ±3.23	55.09 ±3.19	54.55 ±2.69	55.23 ±3.13	54.95 ±2.78	53.66 ±3.21	54.36 ±2.41	55.17 ±2.52	54.15 ±2.65	55.20 ±3.56	54.65 ±3.21	57.06 ±2.91	55.63 ±3.35	54.35 ±3.00	52.62 ±2.37	55.27 ±3.61	54.63± 3.54b
30	46.36 ±3.38	51.52 ±2.34	52.44 ±2.56	51.82 ±2.94	53.65 ±3.24	52.50 ±3.21	51.41 ±2.43	51.59 ±2.58	53.33 ±3.65	52.52 ±2.21	52.86 ±3.25	52.22 ±3.32	55.30 ±2.63	53.09 ±2.81	51.92 ±3.36	50.11 ±3.10	53.39 ±3.18	52.12± 3.45b
45	44.03 ±2.75	49.25 ±2.61	49.92 ±3.08	47.78 ±3.09	51.16 ±3.13	49.22 ±2.64	48.61 ±2.95	48.94 ±2.99	51.78 ±3.79	49.37 ±2.74	49.18 ±2.95	49.55 ±2.88	53.14 ±2.46	50.84 ±3.06	49.27 ±2.99	47.14 ±3.46	51.55 ±3.40	49.45± 3.52c
60	43.15 ±3.69	47.31 ±2.66	48.53 ±2.82	46.38 ±3.62	49.30 ±2.86	47.41 ±3.30	46.81 ±3.58	45.98 ±3.44	49.30 ±3.30	46.99 ±3.11	47.68 ±2.83	47.32 ±2.82	50.81 ±2.65	49.07 ±3.37	47.58 ±3.02	45.35 ±2.37	49.24 ±3.54	47.54± 3.39d
Means	49.03 ±3.51 d	52.10 ±2.78 bc	52.88 ±3.32 bc	51.35 ±3.37 c	53.35 ±3.41 b	52.35 ±3.29 bc	51.49 ±3.0c	51.40 ±2.8c	53.35 ±3.18 b	51.99 ±2.82 c	52.67 ±3.27 bc	52.28 ±3.21 bc	55.05 ±2.53 a	53.55 ±3.44 b	52.12 ±3.16 bc	50.13 ±3.18 cd	53.36 ±3.16 b	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 8: Effect of treatments and storage on pH of zinc fortified edible coated apricots

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	4.26± 0.13	4.24± 0.15	4.14± 0.11	4.15± 0.10	4.10± 0.14	4.15± 0.09	4.26± 0.16	4.25± 0.15	4.17± 0.12	4.12± 0.08	4.18± 0.07	4.17± 0.09	4.18± 0.06	4.20± 0.14	4.22± 0.17	4.10± 0.10	4.17± 0.08	4.18±0. 13c
15	4.56± 0.18	4.35± 0.16	4.35± 0.21	4.31± 0.22	4.22± 0.23	4.26± 0.16	4.35± 0.18	4.35± 0.21	4.28± 0.24	4.25± 0.20	4.34± 0.19	4.29± 0.15	4.26± 0.22	4.31± 0.25	4.33± 0.27	5.29± 0.24	4.28± 0.28	4.37±0. 26b
30	4.71± 0.31	4.40± 0.26	4.35± 0.18	4.33± 0.27	4.29± 0.21	4.31± 0.16	4.40± 0.31	4.38± 0.24	4.34± 0.28	4.30± 0.19	4.39± 0.11	4.43± 0.28	4.22± 0.22	4.44± 0.36	3.53± 0.29	4.53± 0.24	4.29± 0.27	4.39±0. 35b
45	5.05± 0.42	4.34± 0.39	4.26± 0.26	4.22± 0.37	4.24± 0.29	4.35± 0.21	4.34± 0.28	4.46± 0.31	4.20± 0.24	4.51± 0.28	4.70± 0.41	4.63± 0.43	4.06± 0.44	4.94± 0.46	4.60± 0.41	4.34± 0.42	4.12± 0.21	4.43±0. 41a
60	5.20± 0.41	4.49± 0.34	4.41± 0.21	4.37± 0.38	4.39± 0.27	4.50± 0.24	4.49± 0.21	4.61± 0.23	4.35± 0.37	4.66± 0.19	4.85± 0.21	4.78± 0.36	4.21± 0.32	5.09± 0.20	4.75± 0.18	4.49± 0.15	4.27± 0.33	4.58±0. 37a
Means	4.76± 0.39a	4.36± 0.21a b	4.30± 0.33b	4.28± 0.21b	4.25± 0.32b	4.31± 0.36a b	4.37± 0.24a b	4.41± 0.23a b	4.27± 0.24b	4.37± 0.35a b	4.49± 0.28a b	4.46± 0.27a b	4.18± 0.31b	4.59± 0.37a b	4.48± 0.22a b	4.55± 0.21a b	4.22± 0.34b	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 9: Effect of treatments and storage on acidity of zinc fortified edible coated apricots (% Malic acid)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	0.24± 0.03	0.26± 0.02	0.29± 0.03	0.28± 0.01	0.29± 0.03	0.26± 0.02	0.22± 0.01	0.27± 0.02	0.29± 0.03	0.23± 0.01	0.29± 0.03	0.28± 0.02	0.32± 0.03	0.27± 0.01	0.26± 0.01	0.27± 0.02	0.34± 0.03	0.27±0. 02a
15	0.21± 0.02	0.23± 0.01	0.26± 0.02	0.25± 0.01	0.26± 0.02	0.23± 0.02	0.19± 0.01	0.24± 0.02	0.26± 0.03	0.20± 0.01	0.26± 0.03	0.25± 0.02	0.28± 0.03	0.24± 0.02	0.23± 0.01	0.24± 0.01	0.31± 0.02	0.24±0. 01ab
30	0.19± 0.02	0.22± 0.02	0.24± 0.01	0.24± 0.01	0.25± 0.02	0.23± 0.02	0.20± 0.02	0.24± 0.01	0.24± 0.02	0.20± 0.01	0.25± 0.02	0.24± 0.01	0.27± 0.02	0.23± 0.02	0.22± 0.02	0.23± 0.01	0.24± 0.01	0.23±0. 02ab
45	0.15± 0.01	0.19± 0.02	0.21± 0.02	0.21± 0.02	0.22± 0.01	0.20± 0.01	0.17± 0.02	0.21± 0.02	0.21± 0.02	0.17± 0.01	0.22± 0.02	0.21± 0.01	0.24± 0.02	0.20± 0.01	0.19± 0.02	0.20± 0.01	0.21± 0.01	0.20±0. 02b
60	0.12± 0.01	0.18± 0.02	0.18± 0.01	0.17± 0.02	0.23± 0.02	0.19± 0.01	0.19± 0.01	0.18± 0.02	0.19± 0.01	0.17± 0.02	0.23± 0.01	0.16± 0.02	0.22± 0.01	0.20± 0.02	0.21± 0.01	0.19± 0.02	0.18± 0.02	0.19±0. 01b
Means	0.18± 0.02b	0.22± 0.02a b	0.23± 0.02a b	0.23± 0.01a b	0.25± 0.02a	0.22± 0.01a b	0.19± 0.01b	0.23± 0.02a b	0.24± 0.02a b	0.19± 0.02b	0.25± 0.01a b	0.23± 0.01b	0.27± 0.02a	0.23± 0.02a	0.22± 0.01a b	0.23± 0.01a b	0.26± 0.02a	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Mean squares for moisture loss in edible coated zinc fortified apricots delineated significant variations with respect to treatments and storage (Table 10). Furthermore, interaction between them was found non-significant.

Moisture loss was directly affected by the applications of edible coatings/film. It is apparent from results the highest value of moisture loss was noticed in T_0 (control) 21.48±1.88g whilst, the lowest in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) trailed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$), T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) by 8.27±0.74, 8.67±0.62, 8.87±0.57 and 8.34±0.39g, respectively (Table 12). There was a gradual increase in moisture loss of edible coated apricots as the storage proceed that differed from 5.05±0.47 to 9.40±0.83, 14.05±0.78 and 17.39±1.57g at 15th, 30th, 45th and 60th day, respectively.

Earlier, Lima *et al.* (2010) observed the gas transfer properties of 0.5% galactomannans and 1.5% collagen based coating on mango (1.5% glycerol) and apple (without glycerol). The results indicated 28 & 11% reduction of O_2 & CO_2 permeability in mango and 50% decline for gas transfer in case of apples. They documented that an edible film is a semi permeable barrier or a seal that serves as a modified atmosphere around fruit, securing from gaseous exchange.

In this context, Bourbon *et al.* (2011) analyzed the efficiency of three types of coatings with respect to water and gas permeability. The resultant data showed higher reduction in gas permeability through chitosan with whey protein concentrates & chitosan with glycomacropeptide based coatings nevertheless, chitosan with lactoferrin proved better to overcome water and gas diffusion owing to dual nature *i.e.* hydrophilic and hydrophobic properties.

Earlier, Park and Zhao (2004) found that coating applications assure minimum weight loss of apricots during storage. They incorporated the minerals and vitamin E into chitosan based coatings *i.e.* 10-200% Gluconal Cal (mixture of calcium gluconate and lactate), 5-20% zinc lactate and 5-20% alpha-tocopheryl acetate with acetylated monoglyceride. They recorded that 10-200% of calcium and 5-20% of zinc & vitamin E improve water vapor resistance as well as nutritional status of the respective edibles.

Table 10: Means squares for moisture loss

S.O.V	df	MS
Treatment (T)	16	104.58**
Storage (S)	3	1483.26**
S x T	48	3.29^{NS}
Error	136	2.02

($p<0.05$)
** = Highly significant
^{NS} = Non significant

Table 11: Effect of treatments and storage on total soluble solids of zinc fortified edible coated apricots (°Brix)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	12.13 ±0.13	12.22 ±0.16	12.18 ±0.18	12.14 ±0.11	12.25 ±0.26	12.21 ±0.27	12.21 ±0.15	12.34 ±0.24	12.25 ±0.29	12.24 ±0.24	12.16 ±0.14	12.30 ±0.31	12.30 ±0.35	12.28 ±0.26	12.30 ±0.34	12.29 ±0.42	12.31 ±0.33	12.24± 0.41c
15	12.15 ±0.23	12.35 ±0.35	12.31 ±0.38	12.57 ±0.27	12.49 ±0.38	12.47 ±0.29	12.66 ±0.29	12.41 ±0.28	12.74 ±0.32	12.56 ±0.27	12.61 ±0.22	12.47 ±0.17	12.87 ±0.36	12.49 ±0.23	12.56 ±0.25	12.66 ±0.13	12.90 ±0.38	12.55± 0.26b
30	12.19 ±0.11	12.67 ±0.29	12.69 ±0.35	12.77 ±0.34	12.91 ±0.39	12.66 ±0.26	12.72 ±0.21	12.75 ±0.37	12.87 ±0.24	12.68 ±0.30	12.64 ±0.19	12.74 ±0.22	12.92 ±0.31	12.69 ±0.35	12.69 ±0.28	12.74 ±0.37	12.91 ±0.27	12.72± 0.46ab
45	11.61 ±0.29	12.70 ±0.18	12.79 ±0.36	12.70 ±0.27	13.45 ±0.46	12.69 ±0.24	12.73 ±0.48	12.74 ±0.47	13.06 ±0.50	12.64 ±0.41	12.75 ±0.35	12.74 ±0.27	14.21 ±0.24	12.70 ±0.43	12.79 ±0.53	12.66 ±0.34	13.18 ±0.51	12.83± 0.59a
60	11.68 ±0.45	12.64 ±0.37	12.62 ±0.53	12.70 ±0.50	13.59 ±0.29	12.65 ±0.31	12.87 ±0.47	13.22 ±0.39	13.55 ±0.47	13.80 ±0.45	12.37 ±0.31	13.04 ±0.58	14.66 ±0.84	12.60 ±0.26	12.68 ±0.55	13.10 ±0.58	13.60 ±0.40	13.02± 0.66a
Means	11.95 ±0.56 c	12.51 ±0.45 bc	12.52 ±0.36 bc	12.58 ±0.58 bc	13.94 ±0.32 ab	12.54 ±0.52 bc	12.64 ±0.36 bc	12.69 ±0.54 bc	12.89 ±0.26 ab	12.78 ±0.58 b	12.51 ±0.43 bc	12.66 ±0.55 bc	13.39 ±0.64 a	12.55 ±0.59 bc	12.60 ±0.47 bc	12.69 ±0.58 bc	12.98 ±0.46 ab	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 12: Effect of treatments and storage on moisture loss of zinc fortified edible coated apricots (%)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
15	11.48 ±0.62	6.12± 0.46	5.66± 0.17	2.97± 0.19	3.82± 0.14	4.64± 0.28	5.68± 0.24	3.16± 0.21	3.47± 0.12	4.85± 0.30	5.51± 0.40	5.18± 0.43	3.22± 0.22	5.92± 0.45	5.47± 0.13	5.05± 0.33	3.59± 0.33	5.05±0. 47d
30	21.70 ±1.61	11.15 ±0.87	10.20 ±0.28	7.83± 0.34	6.57± 0.35	8.89± 0.52	9.63± 0.60	8.06± 0.48	6.70± 0.27	7.69± 0.29	9.51± 0.73	9.39± 0.61	6.20± 0.55	10.21 ±0.82	9.69± 0.79	9.56± 0.64	6.87± 0.67	9.40±0. 83c
45	25.61 ±2.14	15.08 ±0.92	14.51 ±0.94	15.03 ±0.82	10.92 ±0.25	14.62 ±0.61	14.57 ±0.71	12.80 ±0.29	9.42± 0.98	13.22 ±0.81	15.83 ±0.73	14.02 ±0.78	9.86± 0.43	14.05 ±0.68	14.31 ±0.30	14.95 ±0.26	10.08 ±0.45	14.05± 0.78b
60	27.12 ±2.56	18.43 ±0.77	16.89 ±1.23	17.55 ±0.91	14.17 ±0.66	17.76 ±0.83	17.79 ±1.06	18.09 ±1.32	13.75 ±1.01	17.46 ±0.82	18.39 ±0.63	17.89 ±1.14	13.82 ±1.17	17.02 ±1.42	17.25 ±1.37	18.17 ±1.20	14.12 ±0.57	17.39± 1.57a
Means	21.48 ±1.88 a	12.70 ±0.88 b	11.82 ±0.75 bc	10.85 ±0.90 c	8.87± 0.57d	11.48 ±0.92 bc	11.92 ±0.47 bc	10.53 ±0.52 c	8.34± 0.39d	10.81 ±0.58 c	12.31 ±0.80 b	11.62 ±0.98 bc	8.27± 0.74d	11.80 ±0.78 bc	11.68 ±0.51 bc	11.93 ±0.99 bc	8.67± 0.62d	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

One of the researchers groups, Rojas-Graü *et al.* (2007) assessed the preserving role of edible films or coatings on fruits. They incorporated various fortificants like zinc & calcium, essential fatty acids (sunflower oil), antibrowning agents (N-acetylcystein), antioxidants (glutathione) and vitamin E in coating formulation to address malnutrition and infestation. They delineated that addition of bioactive fortificants improved the nutritional profile as well as keeping quality of the coated food. They also documented the synergistic effect of sunflower oil (0.025-0.125%) and alginate that retarded water vapor evaporation by 22%.

There is a growing interest in the development of edible coatings with different formulations using several ingredients like plasticizers, additives, extracts and antioxidants. Resultantly, various countries have adopted such components that are permitted safe by USDA referred as GRAS (Rojas-Grau *et al.,* 2009). The success of coatings application in fruit & vegetable depends on their permeability with respect to the target commodity. It is deduced that edible coatings are compatible for various food products to minimize the postharvest losses (Park, 1999). Later, Devlieghere *et al.* (2004) observed weight loss in coated apricots 17.97±9.47% (0.5% chitosan) as compared to control 49.38±10.47% at 12^{th} day of storage. They concluded that an edible coating on apricot reduces weight loss due to restriction in water evaporation.

The effect of various chitosan based concentrations (1, 2 & 3%) was determined on fresh cut litchi fruit at -1 ºC. The results demonstrated that 3% chitosan based coating is effective in improving sensory attributes whilst, decline in weight up to 31 & 50% at 3^{rd} & 6^{th} day of storage was reported, respectively. Furthermore, an increase in total titratable acidity by 10.86% at 6^{th} day of storage period was observed (Dong *et al.,* 2004). The recent findings regarding total soluble solids are also in accordance with their results that chitosan based coatings are effective in improving TSS by 3.47% during storage.

Jiang and Li (2001) investigated the effect of chitosan coatings (1 & 2%) on longan fruit. The resultant data showed improved postharvest quality with maximum chitosan level that ensures reduction in color, weight loss and respiratory activity associated with polyphenol oxidase.

The current results are in agreement with the earlier investigations of Han *et al.* (2004) who reported a significant decline in strawberries weight loss treated with various coatings. They expounded that chitosan coatings are effective to preserve the nutrition and shelf life of strawberry especially in the form of chitosan+calcium & chitosan+vitamin E, as carriers of

calcium (78.9-180%) and vitamin E (85%). They were of the view that coatings provide resistance to increase in pH during storage of apricots. Accordingly, chitosan coatings increase the shelf life up to 3 weeks & 6 months at 2 & -23 ºC by by reducing weight loss, discoloration, alteration in pH & acidity and drip loss after thawing.

Afterwards, Tapia *et al.* (2008) tested the effect of alginate and gellan based coatings alongside functional ingredients on fresh cut papaya fruit. They observed that addition of 1-2% glycerol with 1% ascorbic acid to alginate/gellan solutions enhanced water vapor resistance however, 0.025% sunflower reduced the water transfer rate up to 16 & 66% in alginate & gellan, respectively. Likewise, Tapia *et al.* (2007) reported similar results for alginate and gallan based coatings. Present results are in accordance to the finding of Hacıseferogullar *et al.* (2007), recorded pH and acidity (malic acid) 4.16-5.23 & 0.17-0.79% on dry weight basis.

Likewise, Ali *et al.* (2011a) has also attained similar conclusions regarding physico-chemical profiling of edible coatings. They were of the view that coatings help to achieve greater acceptability. They noticed pH of the apricot in the range of 3.8-5.2. Similarly, the behavior of coatings in maintaining titratable acidity was also assessed and reported a marked improvement for this trait during storage. They observed that titratable acidity varied from 0.45 to 0.86 (% Malic acid) and TSS contents 12.67-20ºBrix. Instant investigation regarding moisture loss is also correlated with their work; they judged the hedonic response of whole papaya fruit coated with chitosan 0.5, 1, 1.5 & 2% concentration at 12 ºC & 80-90% RH. The resultant data elucidated minimal water loss (< 6%) by the application of 1.5 & 2% of chitosan as it covers the stoma cells of fruit cuticle. One of the scientists groups Ali *et al.* (2011b), examined six varieties of apricot including Shai, Neeli, Mirmalik, Khakhas, Habi and Alman for their chemical composition and found moisture content 78.8-85.3% on fresh weight basis.

Earlier, Ghasemnezhad *et al.* (2010) reported that coatings have a significant role in the oxygen permeability, moisture, pH and acidity retention over the storage and observed variability in pH from 3.2 to 3.5 whilst, titratable acidity form 2.5 to 2.7%. They also demonstrated that edible coatings are important for consistent behavior in TSS and their values were in the range of 8.5-10.5ºBrix. The instant results are also in harmony with Akin

et al. (2008) that pH of apricot was in the range of 3.61-3.83 whereas, acidity 0.17-0.79 (% malic acid) and 0.62-2.5 (% citric) on dry weight basis.

In another exploration, Jiang *et al.* (2005) concluded that chitosan coating @ 2 g/100g causes slower reduction in titratable acidity. They also measured the impact of chitosan on physico-chemical characteristics of litchi fruit during storage at 2 ºC and noticed that coated fruit has fewer reductions in TSS. Afterwards, Hussain *et al.* (2010) documented apricot total acidity as 1.44±0.11-2.83±0.11 g/100g on dry weight basis. One of the peers, Aubert *et al.* (2010) recorded the total titratable acidity of apricot 28.8-29.5 mEq/100g and TSS 9.7-10.6ºBrix. The results of instant investigations are in accordance with the findings of Ambrosio *et al.* (2013), explicated that edible coatings are promising tool for retaining the acidity of fruit and noted the value for this trait 15.12-18.66g malic acid/L. They also revealed that coatings have a positive role in maintaining TSS during storage and observed total soluble solids contents in the range of 13.57.15.98ºBrix.

Previously, Wu *et al.* (2010) determined the efficacy of zinc and cerium in chitosan coating for the preservation of Chinese jujube fruit. The results demonstrated a linear relationship between mineral and coating solutions with enhanced 15.45% TSS and reduction of 11.72% moisture loss, 31.51% gaseous exchange & 7.07% polyphenol peroxidase activity as compared to control. One of the researchers groups, Leccese *et al.* (2007) recorded TSS values for different apricot cultivars that fall between 9.9±0.4 to 16.3±0.6ºBrix. In a study, Asma *et al.* (2007) tested 17 genotypes of apricot with total soluble solid contents 12.7-26.5%. Earlier finding showed that blending of coating formulations with natural polymers and antioxidants are appropriate for preservation of fruits and vegetables (Elsabee and Entsar, 2013).

In numerous studies lipid based coatings were combined with protein or polysaccharides for less water vapor release and low lipid oxidation. In this context, coating formulations like sago starch and fish gelatin along with glycerol have shown better plasticizing effect than that of sorbitol due to hydrophilic nature of glycerol. It has been expounded that water vapor permeability increased with the addition of sorbitol however, modulatory effect was noticed with glycerol (Al-Hassan and Norziah, 2012).

The effect of chitosan addition was checked on the color attributes of starch based edible coating. It has been found that inclusion of glycerol increases the interaction of chitosan-

starch based coating thus retards water transmission (Chillo *et al.,* 2008). Earlier, edible coatings were used as barrier against gas and water transfer however, the functionality of edible coating was improved due to addition of micronutrients (Vargas *et al.,* 2008). In another study, chitosan with lauric acid based coating formulations were found more effective as compared to chitosan with stearic acid and reduced the water transfer up to 49% (Dutta *et al.,* 2009).

During a study, the chitosan coating on sliced mangoes has resulted in slower water loss & microbial growth thus ensures optimal eating quality and shelf life at 6 ºC (Chien *et al.,* 2007). Accordingly, Oms-Olius *et al.* (2008a and b) explored that edible coating is a contemporary approach to curtail deteriorative effect of environmental hazards thereby improves the quality, sensory and functional attributes of fresh cut fruit. During two week trial, the impact of alginate (2%), pectin (2%) & gellan (0.5%) based edible coatings were evaluated with the addition of 0.75% of each glutathione & N-acetyl cysteine on fresh cut pears at 4 ºC. The results inferred lower moisture diffusion, ethylene production, browning reaction and microbial activity with maximum antioxidant.

Likewise, Diaz-Mula *et al.* (2012) observed that addition of sodium-alginate @1-5% resulted in enhanced shelf life of sweet cherries from 8 to 16 days at 2 ºC. One of the peers, Olivas *et al.* (2007) applied alginate-acetylated monoglycerides-linoleic acid and alginate-butter-linoleic acid based coatings on fresh cut apples. The results showed comparatively better storage characteristics of alginate-acetylated monoglycerides-linoleic acid based coatings on fresh apple wedges during 10 days storage at 5 ºC. Later, Rojas-Grau *et al.* (2008) determined the effect of alginate and gellan based coatings to prolong the shelf life of apples from 4 days (control) to 2 weeks by reducing fermentative alterations *i.e.* production of ethylene (< 5µl/L) and acetaldehyde.

The weight loss is primarily coupled with moisture evaporation and respiration through the outer covering of fruit. The rate at which water is evaporated depends on the water pressure gradient between the fruit tissue and surrounding environment. Chitosan coatings function as a barrier thereby restricting water transfer and protecting fruit from mechanical injuries, thus delaying dehydration (Ribeiro *et al.,* 2007).

The increase in pH of apricot during storage might be due to the semi-permeable chitosan film formed on the surface of the fruit that has customized the internal atmosphere,

endogenous O_2 and CO_2 concentration of the fruit thus retarding ripening (Lowings and Cutts, 1982; Bai *et al.,* 1988).

The titratable acidity is principally correlated to the concentration of organic acids found in the fruits. The decline in acidity during storage is probably due to the occurrence of metabolic changes in fruits or also because of the consumption of organic acid in respiratory process that is synchronized with the observation of Echeverria and Valich (1989).

One of the researchers groups, Ayranci and Tunc (1997) conducted trial on coated apricots with composition containing stearic acid and noticed lower water losses might be due to the hydrophobicity provided by the stearic acid.

Current study depicted that chitosan based coatings (T_4, T_8, T_{12} and T_{16}) performed better than that of alginate types might be due to good suspending, stabilizing, gel producing and film forming properties with minimum drop-off. Coatings developed from alginate exhibit poor water resistance because of their hydrophilic nature. Additionally, chitosan forms transparent film which enhances keeping quality and extends the storage life of apricots. Chitosan films are normally cohesive, compact and the coated surface has a smooth texture without pores. The chemical composition of chitosan and structure of their polymer affect film permeability. Another justification regarding better performance of chitosan based coatings is that their polymers bonded through hydrogen and other forces eventually develop strong crumb like structure which restrict moisture to evaporate from the surface of fruit. The ionic functional groups generate strong polymer chain interactions which limit chain motion. Cross-linking of polymers chain with ions lowers the permeability as well as transforms the pH and resultantly protects the fruit from dehydration.

4.1.3. Organic acids, total sugars, physical and mold analyses

The mean squares in Table 13 revealed momentous difference in ascorbic & citric acids and total sugars of zinc fortified edible coated apricots due to treatments and storage period. On the other hand, their interactions were affected non-significantly.

In treatments, the lowest value in ascorbic acid contents was noticed in T_0 (control) 40.35±2.73mg/100g whereas, the highest in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 53.71±3.51mg/100g followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 53.40±3.62mg/100g, T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 53.31±3.37mg/100g and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 53.29±3.79mg/100g, correspondingly (Table 14). It is obvious from the results that there was a gradual decline in ascorbic acid contents from 56.10±4.17 at start to 54.55±4.36, 51.45±4.54, 49.28±3.87 and 45.58±3.72mg/100g at 15th, 30th, 45th and 60th day of storage, respectively.

Likewise amongst treatments, the maximum turn down in citric acid contents was observed in T_0 as 422.28±11.60mg/100g though, the minimum as 468.83±13.59, 465.23±11.12, 461.51±12.00 and 457.81±12.62mg/100g in T_{12}, T_{16}, T_4 and T_8, respectively (Table 15). The results delineated a progressive reduction in citric acid contents of apricot ranged from 470.63±12.65 at initiation to 411.08±14.38mg/100g at the end of storage study.

The results regarding total sugars depicted that amongst treatments the minimum value of sugars in T_0 as 59.61±3.23mg/100g while, the maximum in T_{12}, T_{16}, T_4 and T_8 as 71.49±4.54, 71.06±3.71, 70.62±4.02 and 70.33±4.88mg/100g, respectively were recorded (Table16). During storage a consistent increase in sugars of zinc fortified coated apricots was noticed as 65.48±3.87 at beginning to 70.62±4.53mg/100g at 60th day.

Table 13: Means squares for ascorbic acid, citric acid and total sugars

S.O.V	df	Ascorbic acid	Citric acid	Total sugars
Treatment (T)	16	139.832**	2595.3**	98.407**
Storage (S)	4	897.229**	32416.3**	198.946**
S x T	64	12.441^{NS}	224.8^{NS}	1.194^{NS}
Error	170	5.062	12.6	1.687

($p<0.05$)
** = Highly significant
^{NS} = Non significant

Table 14: Effect of treatments and storage on ascorbic acid of zinc fortified edible coated apricots (mg/100g)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	53.20 ±2.89	54.64 ±3.65	54.19 ±3.66	54.94 ±3.12	59.07 ±2.95	54.23 ±3.67	55.56 ±3.76	56.78 ±4.00	59.42 ±3.77	52.78 ±3.72	56.70 ±4.50	59.05 ±4.31	55.91 ±4.38	54.90 ±3.49	56.23 ±4.14	57.75 ±3.51	58.40 ±3.85	56.10± 4.17a
15	44.77 ±2.48	53.94 ±4.39	53.18 ±3.06	54.25 ±3.81	57.61 ±3.98	53.37 ±4.12	55.03 ±3.78	55.14 ±4.28	57.24 ±3.97	51.86 ±4.16	55.78 ±4.20	56.80 ±4.11	55.05 ±3.91	54.13 ±4.32	55.57 ±4.45	56.41 ±4.43	57.17 ±3.60	54.55± 4.36ab
30	40.77 ±1.56	49.94 ±3.35	81.18 ±3.07	52.11 ±3.59	53.61 ±4.57	49.70 ±3.00	51.03 ±4.10	52.50 ±2.20	53.91 ±4.56	50.83 ±4.10	52.78 ±3.95	53.13 ±3.90	53.35 ±4.32	50.36 ±4.08	52.20 ±4.16	53.41 ±2.53	53.84 ±3.74	51.45± 4.54b
45	34.67 ±1.77	48.35 ±2.55	49.89 ±3.38	51.36 ±4.39	51.40 ±3.10	48.28 ±3.78	48.74 ±2.19	50.50 ±2.06	50.44 ±2.26	49.79 ±2.46	49.88 ±1.18	50.56 ±1.00	52.42 ±1.35	48.63 ±2.43	49.57 ±2.75	51.46 ±3.96	51.91 ±2.12	49.28± 3.87c
60	28.33 ±1.81	45.64 ±2.59	49.52 ±2.80	50.98 ±2.30	44.85 ±2.32	45.80 ±2.35	44.72 ±2.72	46.03 ±2.39	45.46 ±2.58	49.09 ±2.08	45.37 ±2.57	45.31 ±3.11	51.82 ±2.61	45.66 ±2.22	45.78 ±3.24	44.80 ±2.70	45.71 ±2.65	45.58± 3.72d
Means	40.35 ±2.73 c	50.50 ±3.42 b	51.59 ±3.28 ab	52.73 ±3.26 ab	53.31 ±3.37 a	50.28 ±3.54 b	51.01 ±2.52 ab	52.19 ±3.83 ab	53.29 ±3.79 a	50.87 ±3.63 b	52.10 ±3.64 ab	52.97 ±3.55 ab	53.71 ±3.51 a	50.74 ±3.91 b	51.87 ±3.51 ab	52.77 ±3.46 ab	53.40 ±3.62 a	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 15: Effect of treatments and storage on citric acid of zinc fortified edible coated apricots (mg/100g)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	459.92±15.81	466.57±18.45	468.32±12.86	470.03±13.67	477.06±12.82	468.61±12.22	467.66±13.48	466.76±12.95	467.24±13.59	470.87±14.40	471.86±13.12	467.65±13.77	486.95±14.11	469.18±12.05	468.15±14.45	470.50±12.69	483.44±11.01	470.63±12.65a
15	450.92±14.46	457.57±11.97	459.32±12.89	461.03±12.90	468.59±11.26	459.61±15.24	458.66±12.92	457.76±12.25	462.79±15.27	461.87±12.08	462.86±11 1.73	458.65±12.72	477.95±13.44	460.18±13.44	459.15±12.90	461.50±11.90	474.44±11.27	461.93±15.22a
30	423.67±11.57	435.17±14.93	432.35±11.94	435.35±13.77	460.25±13.80	433.27±13.61	434.96±12.44	433.61±13.43	452.89±13.72	434.38±12.61	434.30±13.50	433.23±13.22	471.76±14.09	434.79±13.11	432.79±12.63	434.74±12.72	468.37±13.76	440.35±13.83b
45	404.67±14.54	416.17±15.23	413.35±12.25	416.35±13.37	449.26±11.95	414.27±12.20	415.96±12.38	414.61±12.62	450.57±17.61	415.38±11.66	415.30±10.90	414.23±13.91	452.76±15.06	415.79±14.68	413.79±11.60	415.74±13.85	449.37±12.27	422.80±15.72b
60	372.22±14.96	401.17±11.36	398.94±12.27	399.90±12.01	452.41±13.15	402.94±10.98	397.73±12.66	402.16±15.89	455.54±15.26	400.86±12.25	398.05±12.68	400.52±12.55	454.74±13.94	399.82±11.94	399.59±11.69	401.27±12.65	450.53±12.76	411.08±14.38c
Means	422.28±11.60b	435.33 ±12.85ab	434.45 ±11.42ab	436.53 ±10.72ab	461.51 ±12.00a	435.74 ±11.63ab	434.99 ±10.45ab	434.98 ±11.44ab	457.81 ±12.62a	436.67.67 ±11.06ab	436.47.47 ±12.18ab	434.86 ±13.61ab	468.83 ±13.59a	435.95 ±13.12ab	434.69 ±12.16ab	436.75 ±12.70ab	465.23 ±11.12a	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Mean squares for color, texture and mold count of edible coated apricots expounded significant variations due to treatments and storage intervals (Table 17).

Edible coatings have impact on maintaining the color profile of apricot and noticed minimum value in T_0 as 98.61±5.45ctn whilst, maximum in T_{12} (118.42±5.28) followed by T_{16} (116.26±5.44), T_4 (115.12±5.26) and T_8 (115.07±5.33ctn) (Table 18). The results for color demonstrated a substantial decline that ranged from 119.86±6.69 at initiation to 117.56±5.92 (15^{th} day), 109.15±4.96 (30^{th} day), 105.46±5.05 (45^{th} day) and 101.34±4.37ctn (60^{th} day).

Likewise, the maximum texture drop down was observed in T_0 (control) 102.36±4.55g whilst, the minimum 120.11±5.29, 117.51±5.23, 115.87±4.88 and 114.59±5.40g in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$), T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$), T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$), correspondingly (Table 19). Similarly, there was a momentous decline in texture of coated apricots that varied from 121.46±6.68 at initiation to 118.42±5.81, 111.86±4.51, 109.60±4.75 and 103.61±3.82g at 15^{th}, 30^{th}, 45^{th} and 60^{th} day of storage trial, respectively.

Edible coatings put forth considerable effect for controlling the mold growth and it is demonstrated from the Figure 1 that there was a mild increase in mold count of edible coated apricots as comparison to control. Chitosan based coatings have better effect to suppress to mold growth as compared to alginate coated apricots. The treatments showed maximum growth in T_0 as 30.13±1.37cfu whereas, minimum in T_{12}, T_{16}, T_4 and T_8 as 8.00±0.21, 8.53±0.67, 8.87±0.53 and 9.27±0.49cfu, respectively. However, the mold count ranged from 0.29±0.01 at beginning to 27.12±0.71cfu at the end of study.

The results of present investigations are in agreement with Dong *et al.* (2004), noted the effect of chitosan coating on litchi. They observed that application of 3% chitosan coating is effective in improving the ascorbic acid by 20.5% at 6^{th} day of storage.

Table 16: Effect of treatments and storage on total sugars of zinc fortified edible coated apricots (mg/100g)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	58.29 ±2.95	66.27 ±2.10	65.97 ±2.94	66.01 ±2.77	67.95 ±2.65	65.60 ±2.30	65.90 ±2.69	64.76 ±2.74	66.98 ±1.97	65.25 ±3.09	64.97 ±3.25	65.22 ±3.41	68.04 ±3.11	64.58 ±2.52	64.42 ±2.34	65.11 ±3.32	67.90 ±3.26	65.48± 3.87b
15	59.79 ±2.39	67.77 ±3.80	67.47 ±3.79	67.51 ±3.34	69.45 ±2.41	67.10 ±3.68	67.40 ±2.60	66.26 ±2.76	68.48 ±3.54	66.75 ±2.97	66.47 ±2.60	66.72 ±2.89	69.54 ±3.40	66.08 ±2.44	65.92 ±2.54	66.61 ±3.08	69.40 ±3.40	66.98± 4.55b
30	58.37 ±3.78	68.73 ±4.86	69.52 ±4.98	68.05 ±4.02	71.62 ±4.01	68.36 ±4.37	67.93 ±4.94	68.13 ±4.13	71.08 ±3.86	68.76 ±4.30	67.89 ±4.31	67.76 ±4.35	72.63 ±3.25	68.60 ±4.45	67.95 ±3.86	68.83 ±4.00	72.28 ±3.90	68.62± 3.89ab
45	61.65 ±3.53	70.10 ±4.50	69.45 ±4.45	70.07 ±4.77	70.85 ±4.36	69.93 ±4.48	69.70 ±4.58	70.29 ±4.85	72.41 ±4.11	69.80 ±4.27	69.44 ±4.02	69.30 ±4.95	73.03 ±4.36	69.38 ±3.25	69.72 ±2.21	69.35 ±3.95	71.84 ±4.66	69.78± 3.53a
60	59.97 ±3.51	70.33 ±3.13	71.12 ±4.92	69.65 ±4.37	73.22 ±4.25	69.96 ±4.23	69.53 ±4.63	69.73 ±4.87	72.68 ±4.46	70.36 ±4.29	69.49 ±4.96	69.36 ±4.69	74.23 ±4.98	70.20 ±4.74	69.55 ±4.41	70.43 ±3.41	73.88 ±4.82	70.62± 4.53a
Means	59.61 ±3.23 c	68.64 ±4.39 ab	68.71 ±4.25 ab	68.26 ±4.38 ab	70.62 ±4.02 ab	68.19 ±4.71 ab	68.09 ±4.65 ab	67.83 ±4.01 ab	70.33 ±4.88 ab	68.19 ±4.83 ab	67.65 ±4.65 b	67.67 ±4.63 b	71.49 ±4.54 a	67.76 ±4.47 b	67.51 ±4.31 b	68.07 ±3.58 ab	71.06 ±3.71 a	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 17: Means squares for color, texture and mold count

S.O.V	df	Color	Texture	Mold count
Treatment (T)	16	276.58**	188.74**	363.59**
Storage (S)	4	3166.98**	2575.96**	6170.99**
S x T	64	20.34^{NS}	14.79^{NS}	54.03^{NS}
Error	170	5.61	4.42	2.21

($p<0.05$)
** = Highly significant
^{NS} = Non significant

Table 18: Effect of treatments and storage on color of zinc fortified edible coated apricots (ctn)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	110.17±6.61	117.27±6.90	118.57±6.34	120.42±6.49	119.75±5.19	119.51±6.72	121.07±5.49	120.95±6.30	120.13±5.46	121.20±6.17	120.06±6.03	119.98±5.93	122.87±6.55	120.65±6.23	121.96±6.88	121.18±6.90	121.86±6.47	119.86 ±6.69a
15	107.87±5.22	114.97±5.70	116.27±4.71	118.12±5.46	117.45±6.83	117.21±5.52	118.77±6.54	118.65±6.25	117.83±5.31	118.90±4.04	117.76±5.12	117.68±5.59	120.57±5.14	118.35±5.65	119.66±5.71	118.88±4.68	119.56±4.54	117.56 ±5.92ab
30	95.04 ±4.81	108.44±5.05	103.07±5.35	108.41±4.28	114.91±4.53	107.48±4.16	110.09±3.39	111.28±5.19	114.70±5.68	110.82±4.01	108.20±4.89	107.35±5.01	118.22±5.67	105.68±5.28	106.53±4.74	109.48±5.62	115.81±4.11	109.15 ±4.96b
45	90.97 ±4.27	104.36±4.24	100.50±5.90	103.85±4.66	112.90±5.72	103.65±4.66	103.06±4.12	106.31±4.20	112.67±5.11	103.73±5.87	104.51±4.01	104.60±5.27	116.28±4.87	103.80±5.55	103.75±3.03	104.63±3.32	113.28±3.13	105.46 ±5.05bc
60	89.01 ±3.37	97.06 ±3.91	99.86 ±4.20	99.89 ±3.92	110.60±4.02	98.35 ±4.41	99.17 ±4.68	99.01 ±4.57	110.03±4.93	96.43 ±4.29	98.87 ±3.78	100.64±3.56	114.18±4.11	100.16±4.15	99.45 ±3.72	99.33 ±4.50	110.77±3.11	101.34 ±4.37c
Means	98.61 ±5.45 c	108.427±5.63ab	107.66±5.60b	110.14±5.31ab	115.12±5.26ab	109.24±5.01ab	110.43±5.12ab	111.24±5.32ab	115.07±5.33ab	110.22±5.08ab	109.88±5.38ab	110.05±5.80ab	118.42±5.28a	109.73±5.60ab	110.27±5.64ab	110.70±5.67ab	116.26±5.44ab	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 19: Effect of treatments and storage on texture of zinc fortified edible coated apricots (g)

Days	Treatments																Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	114.52±6.01	124.34±6.66	123.18±5.65	125.08±5.60	123.10±4.73	124.58±5.00	120.71±5.67	121.30±5.59	119.74±4.72	120.17±4.71	119.78±4.32	122.38±4.79	125.84±6.15	119.60±4.32	118.49±5.29	118.77±4.65	123.26±5.24	121.46 ±6.68a
15	108.52±5.71	121.44±5.85	120.28±5.52	122.18±4.07	120.20±2.45	121.68±2.11	117.81±3.33	118.40±2.41	117.51±5.74	117.27±2.89	116.88±2.76	119.48±2.66	122.94±2.82	116.70±3.76	115.59±2.13	115.87±2.69	120.36±4.91	118.42 ±5.81a
30	101.89±4.78	108.74±4.40	109.34±4.68	108.75±5.97	115.95±2.81	110.95±5.27	110.97±4.41	111.19±2.32	115.01±5.71	112.31±2.28	111.23±2.10	111.96±2.79	120.11±3.91	110.50±2.27	112.45±3.93	112.99±3.56	117.32±2.72	111.86 ±4.51b
45	98.44 ±3.09	106.92±4.97	107.95±4.55	107.43±3.09	111.21±4.89	105.70±3.43	108.70±4.86	110.27±4.50	113.06±4.92	111.23±3.14	109.37±2.19	109.17±3.66	119.04±3.51	109.07±3.44	108.63±3.59	110.67±4.27	116.35±3.00	109.60 ±4.75bc
60	98.44 ±2.77	106.49±2.85	107.95±2.24	107.43±2.51	111.21±3.80	105.70±2.94	108.70±3.18	110.27±2.70	113.06±2.07	111.23±2.68	109.37±2.70	109.17±1.53	109.04±2.81	102.63±2.60	102.20±3.10	104.40±2.15	110.25±2.66	103.61 ±3.82c
Means	102.36±4.55c	112.39±5.36ab	112.39±4.76ab	112.82±4.39ab	115.87±4.88ab	113.57±4.61ab	112.16±4.48ab	112.87±4.61ab	114.59±5.40ab	113.21±4.89ab	112.28±4.63ab	113.01±4.59ab	120.11±5.29a	111.70±4.91b	111.47±4.55b	112.54±5.32ab	117.51±5.23ab	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Later, Ali *et al.* (2011a) checked the ascorbic acid content of whole papaya fruit coated with chitosan using 0.5, 1, 1.5 & 2% concentration. The resultant data elucidated that 1.5% concentration of chitosan proved better to minimize the loss of ascorbic acid at 5th week of storage. They noticed the peel color during storage with L*, hue & chroma values by 64.46, 117.14 & 48.05 for control, indicating yellowness with shriveled, softened and fungal infected product. They observed that firmness of papaya depends on chitosan concentration and maximal firmness was achieved as 81 & 84.4 N @ 1.5 & 2% of chitosan during 3 weeks study due to reduced activity of pectinases.

Earlier, Pen and Jiang (2003) explored the effect of 0.5, 1 & 2% chitosan coating on fresh cut Chinese water chestnut. The outcomes indicated minimum alteration in the ascorbic acid content. These results are also in agreement with Wu *et al.* (2010) who worked on the efficacy of zinc and cerium in chitosan coating for the preservation of Chinese jujube fruit. They demonstrated a linear relationship of mineral and coating solutions with enhanced ascorbic acid levels by 14.55%. Similarly, Ayranci and Sibel (2003) determined the protective role of edible coating solutions including methyl cellulose, polyethylene & glycol and antioxidants *i.e.* stearic, ascorbic & citric acid on apricot. The results elucidated that vitamin C loss was minimized by adding stearic with ascorbic acid up to 53% followed by stearic with citric acid 46.9%, stearic acid 23% and coating alone 5.6%.

It has been observed that addition of stearic acid alone or in combination with citric & ascorbic acids in coating formulations is effective to control vitamin C loss in apricot and green pepper. Additionally, it was noticed that coatings containing citric acid was more efficient to prevent the vitamin C loss of both commodities then the formulations having ascorbic acid. The reduction of vitamin C loss of both commodities with coatings containing antioxidants (citric acid or ascorbic acid) can be ascribed to the low oxygen permeability of these coatings (Ayranci & Tunc, 2003).

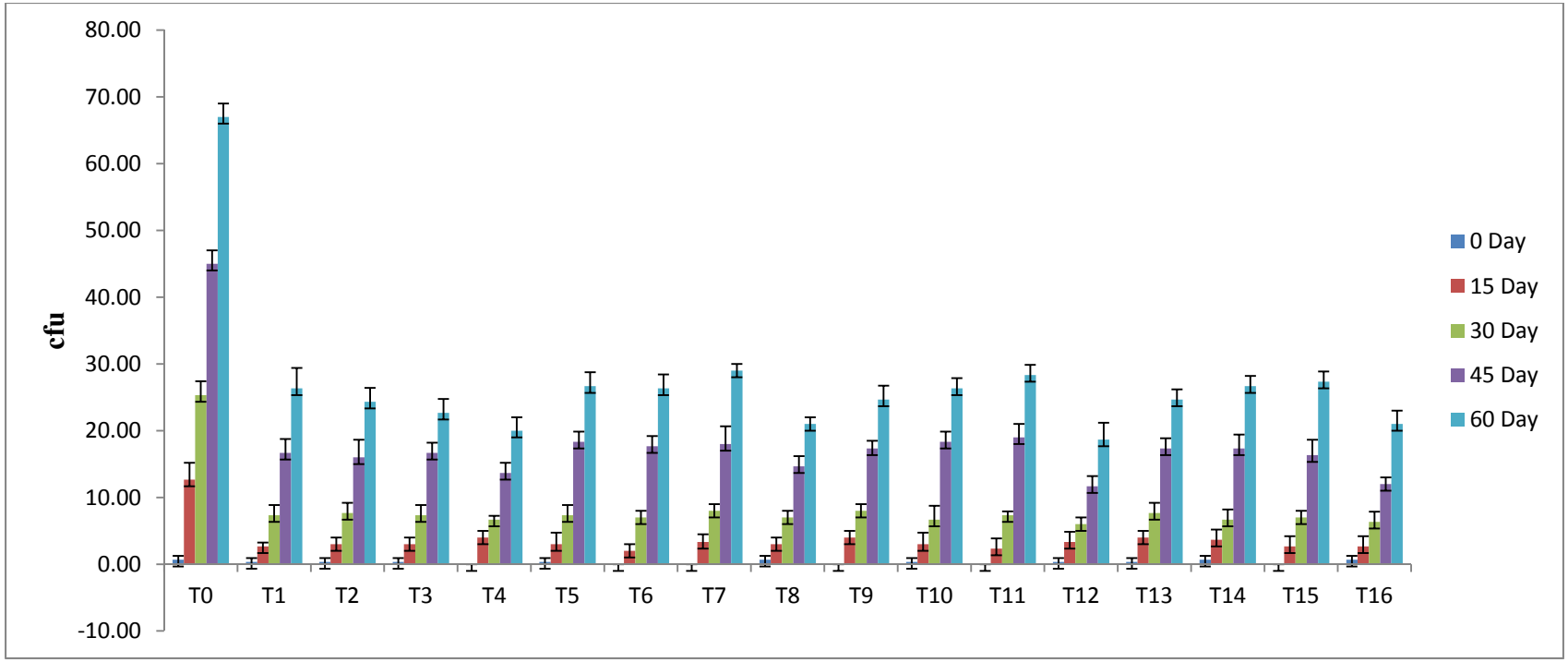

Figure 1: Effect of treatments and storage on mold count (cfu) of zinc fortified edible coated apricots

Keeping oxygen away from the commodities delays the deteriorative oxidation reaction of vitamin C. It was noticed that the oxidation of ascorbic acid to the dehydroascorbic acid does not correspond to the loss of vitamin C, as both forms have the vitamin C functionality. The loss of vitamin C, at this point, represents the conversion of dehydroascorbic acid to diketogulonic acid by additional oxidation (Rai and Saxena, 1988).

These results are in line with the previous findings of Ali *et al.* (2011b), examined six varieties of apricot including Shai, Neeli, Mirmalik, Khakhas, Habi and Alman for their chemical composition and found ascorbic acid in the range of 67.39-90.94mg/100g. Additionally, Akin *et al.,* 2008 concluded that apricot has 28.5±0.7 to 96.8±9mg/100g ascorbic acid contents, 431.8-9997.1(776.2)mg/100g of citric acid and 68.61±1.61 to 93.88±4.49mg/100g of total sugars on dry weight basis. The findings of instant investigation are in harmony with the work of Munzuroglu *et al.* (2003), they reported a conservation in ascorbic acid level by coating application on apricot. Furthermore, they were of the opinion that coatings have significant effect on the physicochemical profile of the fruit thereby ensured uniformity in the overall preference. They elucidated that ascorbic acid contents were in the range of 47.9-71.4µg/100g.

Afterwards, Hussain *et al.* (2010) demonstrated that apricot has 6.74±0.3-13.94±0.72g/100g of total sugar on dry weight basis. Previously, in a study, Erdogan-Orhan and Murat (2011) found that dried apricots constituted 56.8-64.9% of total sugars mainly glucose, sucrose and fructose, with higher sorbitol content *i.e.* 16.91–26.84mg/100g in Malatya variety.

In a study, Jiang and Li (2001) tested the chitosan with varying levels on longan fruit and observed reduction in color loss. Moreover, Jiang *et al.* (2005) measured the impact of chitosan on commercial, sensory and shelf life enhancing features of litchi fruit during storage at 2 ºC. It was inferred that chitosan solution @ 2 g/100g is suitable to control outer peel discoloration thus delays the activity of polyphenol peroxidase and saving anthocyanins along with slower color reduction.

Previously, Han *et al.* (2004) conducted a trial using chitosan coatings to preserve the nutrition and shelf life of strawberry especially in the form of chitosan with calcium & chitosan with vitamin E, as carriers of calcium (78.9-180%) and vitamin E (85%). Accordingly, shelf life increased up to 3 weeks & 6 months at 2 & -23 ºC by reducing

discoloration. Earlier, Lee *et al.* (2003) found that whey protein concentrate (WPC) reduces respiration rate by 20% whilst, carrageenan controls by 5% only. They also observed the efficiency of various antibrowning agents at different concentrations. Amongst, 5% WPC with 1% ascorbic acid & 1% calcium chloride proved as one of the effective formulations to retard apple discoloration.

Later, Oms-Oliu *et al.* (2008a) noted the impact of alginate (2%), pectin (2%) & gellan (0.5%) based edible coatings with the addition of 0.75% of each glutathione & N-acetyl cysteine on fresh cut pears at 4 ºC. The results inferred no adverse effect regarding firmness of pear wedges. They also documented that edible coating is a contemporary approach to improve the quality and functional attributes of fresh cut fruit.

One of the peers, Tapia *et al.* (2008) tested alginate and gellan based coatings containing functional ingredients on fresh cut papaya fruit that preserve the original ascorbic acid content thus ensure firmness as well as nutritional quality. The present results are in harmony with Dragovic-Uzelac *et al.* (2007), recorded the firmness of apricot was 6.75-11.25N during the storage trial. Recently, Ambrosio *et al.* (2013) determined the firmness of apricot was 2.13-4.23kg/0.5 cm^2.

One of the scientists groups, Rojas-Grau *et al.* (2009) explored the additional benefits of active molecules like antibrowning, nutraceutical, texture enhancers & antimicrobials through various coatings to improve the quality of fresh cut fruits. The results depicted that fortified alginate coating on apple decreases the growth of psychrophilic aerobes, mold and yeast. One of the researchers groups, Kristo *et al.* (2008) estimated the efficacy of various antimicrobials *i.e.* potassium sorbate, sodium lactate and nisin in sodium caseinate coatings to control the activity of *Listeria monocytogenes*. Their conclusions explicated maximum antimicrobial activity by nisin whilst minimum for sodium lactate. Accordingly, Mitrakas *et al.* (2008) suggested that fruits especially apricots are frequently treated with edible coatings like chitosan, alginate, casinate, soy and whey proteins to attain better antimicrobial activity. Afterwards, Elsabee and Entsar, (2013) documented that polycations of chitosan based coatings interfere with the negative charge of microbial cell membrane or react with microbial proteins. Thus, interaction of amino group with phospholipids of bacterial cell

membrane or electronegative charge on fungi surface suppresses the degrading features of microbes.

Earlier, Devlieghere *et al.* (2004) compared the antimicrobial nature of chitosan lactic acid formulation on strawberry and cabbage. The bactericidal effect was more prominent in strawberry as compared to cabbage, retaining functional properties for 12 days. The chitosan based coatings were relatively more effective against *Brochotrix thermorphacta* & *Bacillus Cerus* as compared to *Listeria monocytogens* & Lactic acid bacteria whilst, minimal effect was observed in case of *Candida lambia* & *Cryptococcus humicolus*. The mechanism highlights the interaction of chitosan with electronegative charges of microbes as well as chitosan acts like humectant that limit the enzymatic activity. During a trial, the chitosan coating on sliced mangoes has resulted in slower microbial growth thus ensures optimal eating quality attributes and shelf life (Chien *et al.,* 2007).

Mechanically, the ascorbic acid increased slightly at the start and thereafter declined with the progress of storage, might be due to reason that it is proficient to scavenge the hydroxyl radicals and superoxide, as well as redevelop a-tocopherol (Davey and O'Toole, 2000). The instant results depicted that T_{12}, T_{16}, T_8 and T_4 are best amongst all treatments, treated with chitosan coating to preserve the acid content and to improve the total sugars of coated apricots. The increase in the total sugars is possibly due to decomposition/breakdown of carbohydrates into simple sugars resulted increased sweetness level of the coated fruit.

It is concluded from the findings that T_{12}, T_{16}, T_8 and T_4 are effective to retain the color and texture of the coated fruits as comparison to control. The color and texture strength of the apricot were decreased because of fruit softening and shrinking skin due to prolonged storage. These treatments are equally effective to protect the apricot from the mold attack. Edible coatings provide protective layer on the upper surface of fruit, block the pore size and create ionic imbalance thereby moisture is unavailable on the outer covering resultantly, create unfavorable conditions for mold growth.

4.2. Total zinc contents

Mean squares regarding zinc contents of zinc fortified edible coated apricots elucidated significant variations due to treatments however, momentous behavior was observed during storage (Table 20).

Amongst treatments, the minimum value for zinc contents was reported in T_0 (control) 0.26±0.02mg/100g whilst, the maximum in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 4.83±0.24mg/100g trailed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 4.82±0.21mg/100g, T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 4.78±0.23mg/100g and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 4.77±0.12mg/100g, respectively (Table 21). The results depicted a non-momentous decrease in zinc contents during storage that varied from 3.53±0.21mg/100g at start to 3.51±0.18, 3.52±0.16, 3.49±0.18 and 3.50±0.19mg/100g at 15th, 30th, 45th and 60th day, respectively.

Accordingly, Figure 2 demonstrated that T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) showed maximum fold increase (18.24) of zinc contents while T_{11} (apricot containing 2% chitosan coating with 30 ppm $ZnSO_4$) had 10.63 fold incline as comparison to control.

The results are in accordance to Ali *et al.* (2011b), examined six varieties of apricot including Shai, Neeli, Mirmalik, Khakhas, Habi and Alman for their mineral profile and noticed 0.82-3.53mg/100g of zinc contents. The results are also in line with Saracoglua *et al.* (2009) that revealed 2.96-12.0µg/g zinc in the apricot.

One of their peers, Akhtar *et al.* (2011) reported that numerous zinc salts are available for food fortification or enrichment purpose that differed in their solubility. Previously, Chana *et al.* (2007) analyzed the cereals and legumes for zinc, iron, calcium and phytate contents. The results expounded that unfortified cereals have lower levels of zinc (1.5-3.2mg/100g), iron (0.3-5.4mg/100g), calcium (5-48mg/100g) and phytates (70-246mg/100g) as compared to legumes contain 3.2-5.8, 2.9-17.4, 41-926 and 177-1042mg/100g of zinc, iron, calcium and phytates, respectively.

Table 20: Means squares for total zinc contents

S.O.V	df	MS
Treatment (T)	16	26.3370**
Storage (S)	4	0.0101^{NS}
S x T	64	0.0001^{NS}
Error	170	0.0003

($p<0.05$)

** = Highly significant

^{NS} = Non significant

Later, Prom-u-thai *et al.* (2010) evaluated the effectiveness of zinc in parboiled rice. Their findings depicted that zinc concentration in parboiled-polished rice was increased and eventually its bioavailability was enhanced in the meals. Results showed that zinc fortification in whole paddy grain up to 50-400mg Zn/kg paddy rice during parboiling increased zinc concentration from 1.3 to 4.5 times as contrast to unfortified parboiled rice.
One of the researchers groups, Tripathi *et al.* (2010) carried out zinc fortification in sorghum and pearl millet flours by the application of different additives like zinc stearate and EDTA. The zinc stearate added in the formulation at a level that provided 5mg Zn/100g flour. The results indicated marked variations among the flours with reference to bioavailability of zinc. The total zinc content of unfortified sorghum and pearl millet were 1.68 and 4.04 mg/100g whilst, for fortified treatments 6.68 and 9.04 mg/100g, respectively. Additionally, zinc bioavailability in sorghum, sorghum+zinc stearate, sorghum+EDTA and sorgum+zinc stearate+EDTA were 0.37, 0.61, 0.87 and 1.45mg/100g, respectively whereas, in case of pearl millet 0.70mg/100g, pearl millet+zinc stearate 0.79mg/100g, pearl millet+EDTA 1.37mg/100g and pear millet+zinc stearate+EDTA 1.69mg/100g.

Similarly, mineral profiling of various apricots varieties revealed that they contain about 1.38±0.12 to 4.24±0.31mg/100g of zinc on dry weight basis (Akin *et al.,* 2008). Afterwards, Hussain *et al.* (2010) examined the mineral profile of apricot and noticed zinc content in the range of 0.9±0.02-2±0.05mg/100g.

From the above debate, it is concluded that zinc contents of the fortified apricots are increased depending upon the concentration of respective zinc salts. Additionally, the fruit fortified with 50 and 30ppm of zinc salts alongside chitosan coatings showed better performance to increase the zinc content as compared to alginate coatings.

Table 21: Effect of treatments and storage on total zinc contents of zinc fortified edible coated apricots (mg/100g)

Days	Treatments																	Means
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	
0	0.28± 0.02	2.68± 0.15	4.69± 0.16	2.73± 0.08	4.80± 0.12	2.67± 0.16	4.67± 0.14	2.71± 0.17	4.78± 0.22	2.70± 0.20	4.73± 0.23	2.75± 0.11	4.85± 0.18	2.69± 0.18	4.70± 0.15	2.73± 0.13	4.83± 0.21	3.53±0 .21
15	0.27± 0.02	2.66± 0.18	4.67± 0.22	2.71± 0.19	4.78± 0.12	2.65± 0.12	4.65± 0.13	2.69± 0.05	4.76± 0.22	2.68± 0.05	4.71± 0.18	2.73± 0.06	4.83± 0.21	2.67± 0.04	4.68± 0.06	2.71± 0.05	4.81± 0.17	3.51±0 .18
30	0.25± 0.01	2.67± 0.18	4.68± 0.23	2.72± 0.04	4.79± 0.13	2.66± 0.03	4.66± 0.20	2.70± 0.07	4.77± 0.11	2.69± 0.18	4.72± 0.22	2.74± 0.01	4.84± 0.22	2.68± 0.05	4.69± 0.16	2.72± 0.05	4.82± 0.21	3.52±0 .16
45	0.26± 0.01	2.64± 0.15	4.65± 0.18	2.70± 0.05	4.76± 0.23	2.64± 0.17	4.64± 0.21	2.68± 0.04	4.75± 0.21	2.66± 0.14	4.69± 0.15	2.71± 0.13	4.81± 0.23	2.65± 0.18	4.66± 0.22	2.69± 0.16	4.80± 0.13	3.49±0 .18
60	0.24± 0.01	2.65± 0.18	4.66± 0.23	2.69± 0.18	4.77± 0.14	2.65± 0.19	4.65± 0.20	2.69± 0.14	4.76± 0.23	2.67± 0.17	4.70± 0.21	2.72± 0.13	4.82± 0.16	2.66± 0.11	4.67± 0.20	2.70± 0.15	4.81± 0.12	3.50±0 .19
Means	0.26± 0.02d	2.66± 0.20c	4.67± 0.23b	2.71± 0.19b c	4.78± 0.23a b	2.66± 0.16c	4.65± 0.22b	2.69± 0.17c	4.77± 0.12a b	2.68± 0.20c	4.71± 0.17a b	2.73± 0.13b c	4.83± 0.24a	2.67± 0.19c	4.68± 0.22b	2.71± 0.11b c	4.82± 0.21a	

Means sharing the same letter in a row are not significantly different

T_0: Control (without fortificant)

T_1: Apricot containing 1% alginate coating having 30 ppm $ZnSO_4$

T_2: Apricot containing 1% alginate coating having 50 ppm $ZnSO_4$

T_3: Apricot containing 2% alginate coating having 30 ppm $ZnSO_4$

T_4: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$

T_5: Apricot containing 1% alginate coating having 30 ppm $ZnCl_2$

T_6: Apricot containing 1% alginate coating having 50 ppm $ZnCl_2$

T_7: Apricot containing 2% alginate coating having 30 ppm $ZnCl_2$

T_8: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$

T_9: Apricot containing 1% chitosan coating having 30 ppm $ZnSO_4$

T_{10}: Apricot containing 1% chitosan coating having 50 ppm $ZnSO_4$

T_{11}: Apricot containing 2% chitosan coating having 30 ppm $ZnSO_4$

T_{12}: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$

T_{13}: Apricot containing 1% chitosan coating having 30 ppm $ZnCl_2$

T_{14}: Apricot containing 1% chitosan coating having 50 ppm $ZnCl_2$

T_{15}: Apricot containing 2% chitosan coating having 30 ppm $ZnCl_2$

T_{16}: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

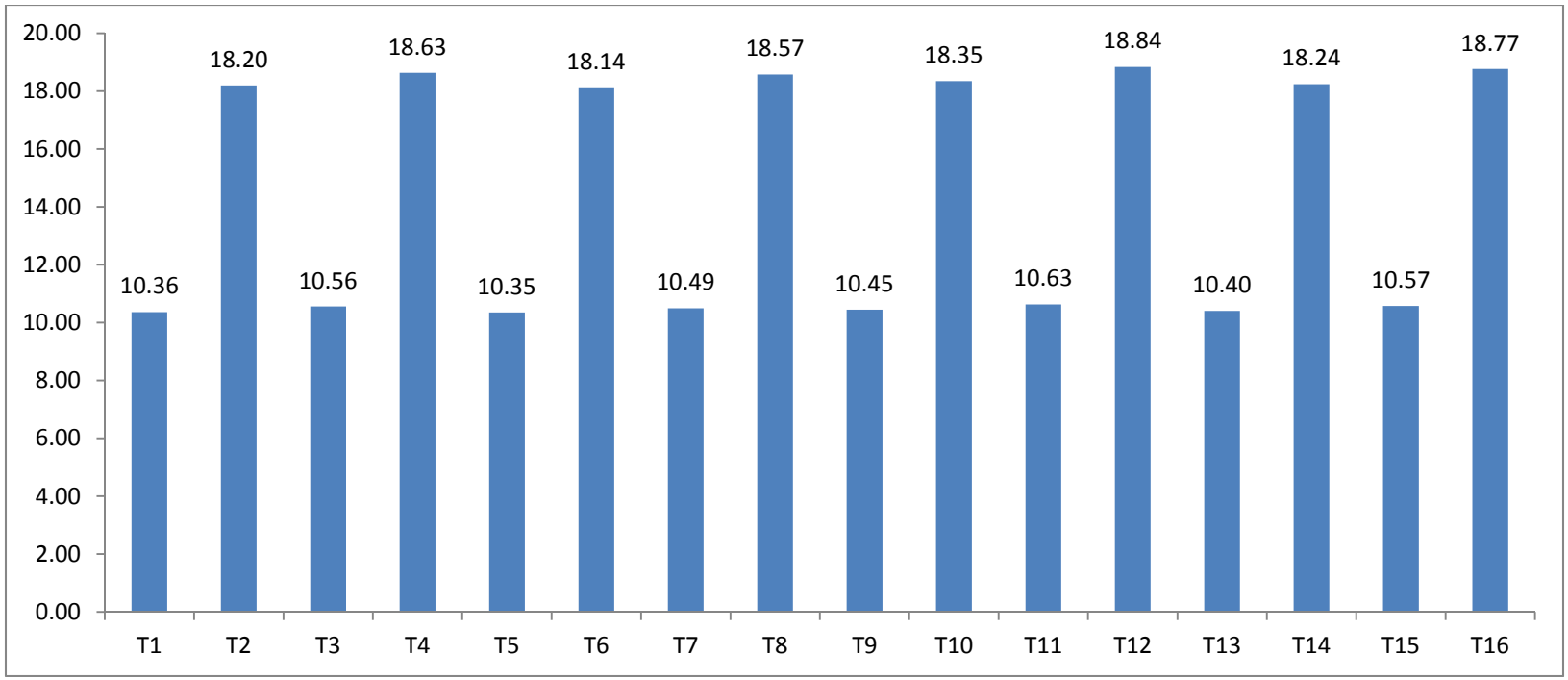

Figure 2: Total increase in zinc contents (fold) of zinc fortified coated apricots as compared to control

4.3. Sensory evaluation

Sensory perception of food containing novel health ingredients is the primary step to assess the consumer response and marketability. In the instant trial, apricots were treated with alginate & chitosan based coatings and ascertained for color, flavor, taste, firmness and overall acceptability at selected intervals.

Mean squares pertaining to color, flavor, taste, firmness and overall acceptability of edible coated apricots delineated significant variations due to treatments and storage intervals however, non-substantial differences were found due to their interaction (Table 22).

It is evident from Figure 3 that the maximum color scores were assigned to T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$), T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) as 7.34±0.37, 7.28±0.28, 7.17±0.31 and 7.10±0.22, accordingly whilst, minimum to T_0 (control) 5.26±0.14. Additionally, storage led a decline in color scores *i.e.* 7.47±0.36 at start to 7.15±0.39, 6.74±0.23, 6.10±0.14 and 5.54±0.19 at 15th, 30th, 45th and 60th day, respectively.

Similarly amongst treatments, the recorded values for flavor were 7.17±0.28, 7.13±0.31, 7.11±0.23, 7.01±0.16 and 5.01±0.11 in T_{12}, T_{16}, T_4, T_8 and T_0, respectively (Figure 4). Storage was resulted a slight decline in flavor rating from initiation to termination of study as 7.58±0.21 to 5.64±0.18. In treatments, the recorded scores for taste of T_{12}, T_{16}, T_4, T_8 and T_0 were 7.17±0.28, 7.12±0.17, 7.05±0.14, 7.04±0.32 and 4.66±0.09, respectively (Figure 5). During storage from zero to 60th day, observed scores were recorded between 7.38±0.21 to 5.83±0.11.

Likewise, the minimum value for firmness was noticed in T_0 as 4.93±0.08 whilst, maximum 7.05±0.18, 7.00±0.22, 6.92±0.26 and 6.87±0.29 in T_{12}, T_{16}, T_4 and T_8, respectively (Figure 6). The results demonstrated that firmness also differed during storage from 7.42±0.21 at beginning to 7.00±0.18 (15th day), 6.59±0.26 (30th day), 6.08±0.28 (45th day) and 5.43±0.13 (60th day). Finally, the scores for overall acceptability were reported as 7.33±0.21, 7.23±0.30, 7.20±0.15, 7.07±0.34 and 4.85±0.18 in T_{12}, T_{16}, T_4, T_8 and T_0, respectively (Figure 7).

Table 22: Means squares for sensory attributes

S.O.V	df	Color	Flavor	Taste	Firmness	Overall acceptability
Treatment (T)	16	3.9052**	3.4941**	4.7636**	3.1935**	4.4222**
Storage (S)	4	34.2052**	30.7807**	18.9499**	30.9431**	17.8511**
S x T	64	0.1601^{NS}	0.1303^{NS}	0.0991^{NS}	0.1100^{NS}	0.0907^{NS}
Error	170	0.3828	0.1450	0.2605	0.1971	0.1801

($p<0.05$)
** = Highly significant
^{NS} = Non significant

The overall acceptability indicated gradual decrease from 7.28±0.20 at initiation to 5.76±0.16 up to the end of study.

The results regarding sensory profile are in accordance with Chillo *et al.* (2008), noted the effect of chitosan on color attributes of starch based edible coating. The findings of instant investigation is in harmony with the work of Oms-Oliu *et al.* (2008a and b), elucidated that coatings have significant effect on color and other sensory parameters of fruits during storage. Furthermore, they were of the opinion that incorporation of antibrowning and antioxidants may help to assure uniformity in color during storage. Additionally, during two week trial the impact of alginate (2%), pectin (2%) & gellan (0.5%) based edible coatings were evaluated by the addition of 0.75% of each glutathione & N-acetyl cysteine on fresh cut pears. The results inferred lower ethylene production and browning reaction with no adverse effect on color, firmness and appearance of pear wedges. Conclusively, edible coating is an applicable approach to curtail deteriorative effect of environmental hazards thereby improves the nutritional quality and sensory attributes of fresh cut fruits.

The instant results regarding retention of flavor in apricot by the application of edible coatings are equally supported by the views of Olivas and Barbosa-Cánovas (2005 and 2009), declared coatings application as a tool to retain the natural flavor of fruits and vegetables during storage. One of the scientists groups, Yaman and Bayoındırh (2002) observed a better response in the keeping quality of fruits by the application of edible coatings. Moreover, Rojas Grau *et al.* (2009) were of the opinion that coating treatment has significant role in enhancing the nutritional quality of product.

These results are also in agreement with Elsabee and Entsar (2013), documented that 1.5% chitosan and 1.5% chitosan+0.75% calcium gluconate are effective to maintain tissue firmness of fruit for prolonged period after harvest. Previously, Tapia *et al.* (2008) narrated that coatings assure to attain a better hedonic response with special reference to firmness.

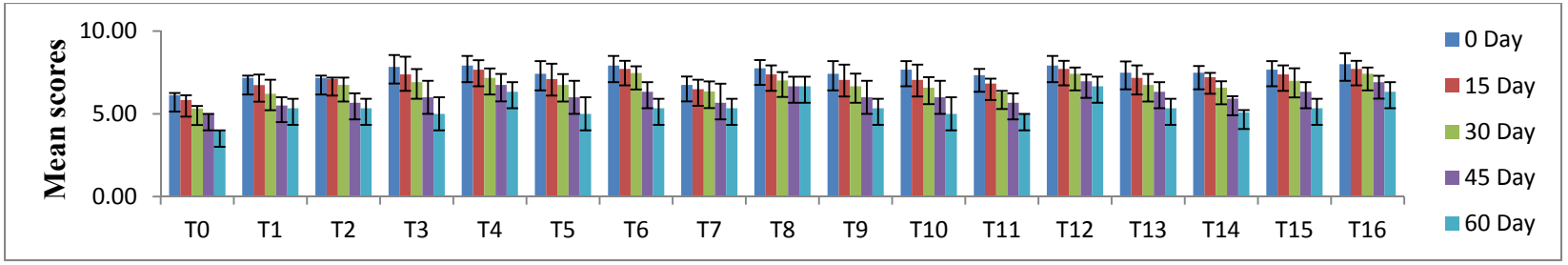

Figure 3: Effect of treatments and storage on color of zinc fortified edible coated apricots

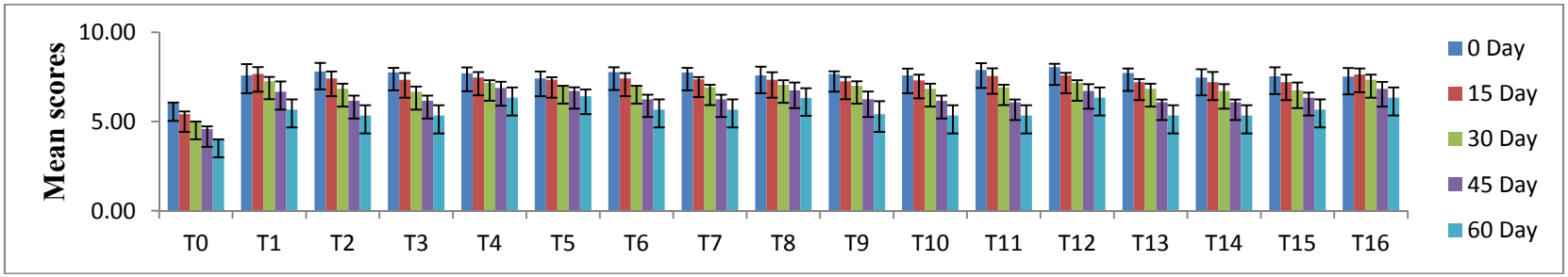

Figure 4: Effect of treatments and storage on flavor of zinc fortified edible coated apricots

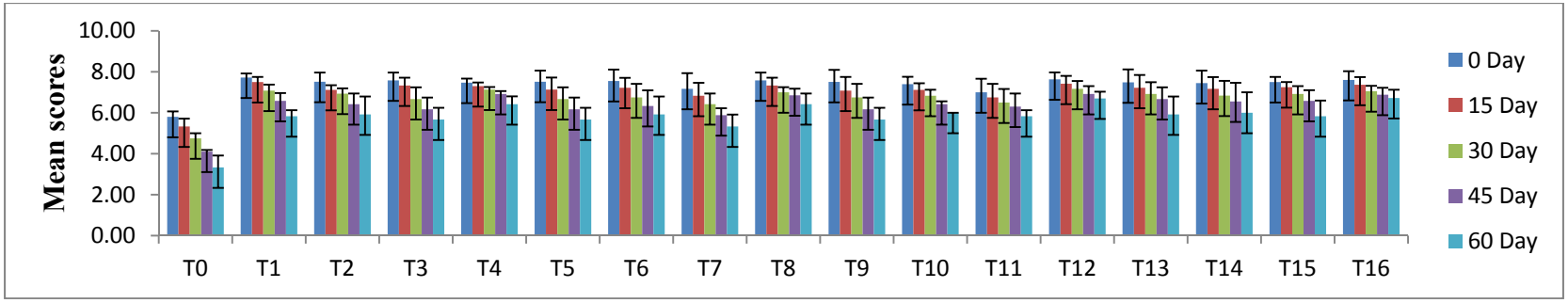

Figure 5: Effect of treatments and storage on taste of zinc fortified edible coated apricots

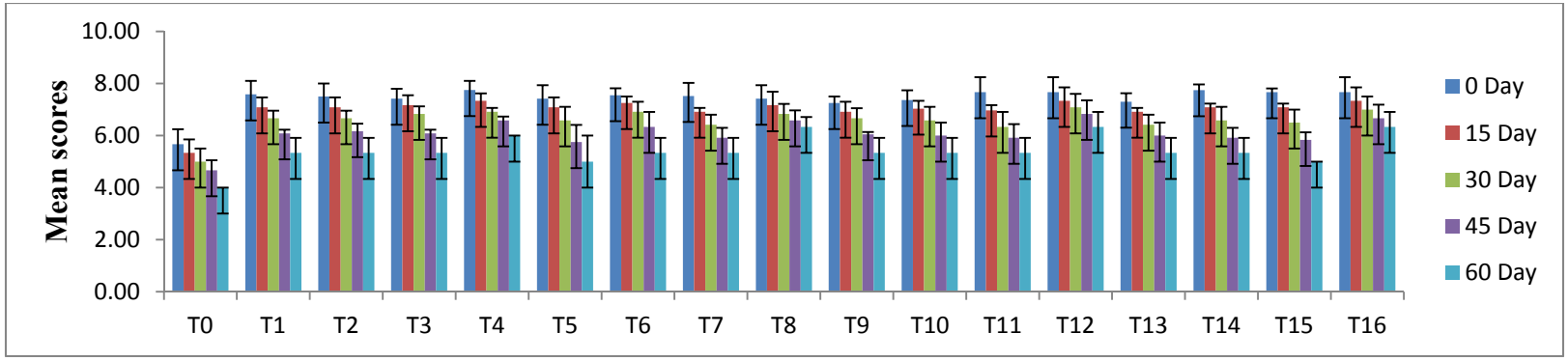

Figure 6: Effect of treatments and storage on firmness of zinc fortified edible coated apricots

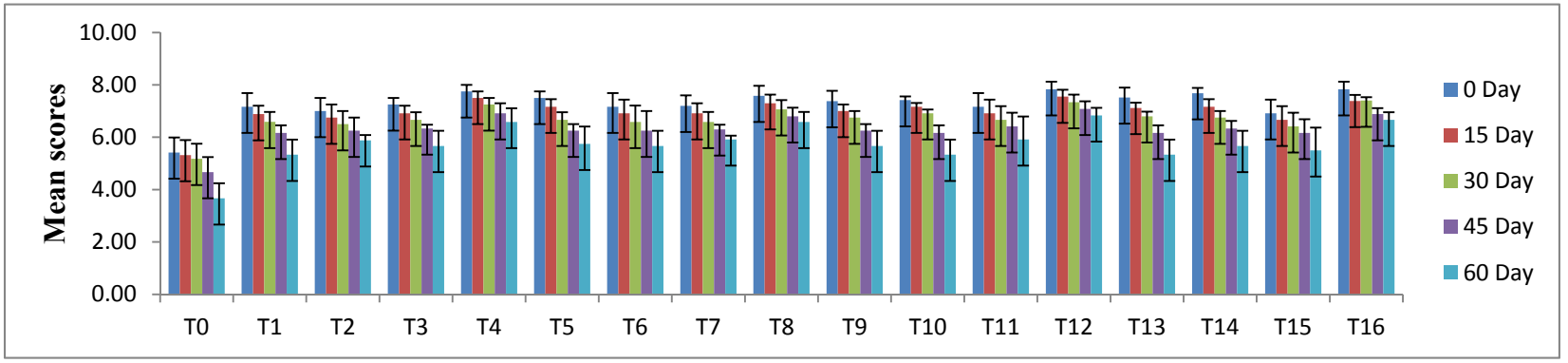

Figure 7: Effect of treatments and storage on overall acceptability of zinc fortified edible coated apricots

Instant findings are duly supported by the work of Ayranci and Tunc (2004), reported an improved sensory perception for coated apricots. Besides sensory profile, nutritional composition of the coated fruits is also improved as compared to uncoated ones.

One of their peers, Dong *et al.* (2004) expounded that chitosan based coatings are effective in improving the sensory quality. In a trial, Ali *et al.* (2011a) judged the sensory characteristics of whole papaya fruit coated with chitosan using its 0.5, 1, 1.5 & 2% level and noted a marked improvement in the hedonic response. Earlier, Pen and Jiang (2003) explored the storage behavior of 0.5, 1 & 2% chitosan coated fresh cut Chinese water chestnut. The outcomes showed improvement in sensory characteristics by retarding the activities of polyphenol oxidase and polyphenol peroxidase.

Similarly, Jiang *et al.* (2005) measured the impact of chitosan on the sensory and shelf life enhancing features of litchi fruit during storage and found that resultant coatings are effective in improving the sensory characteristics.

4.4. Selection of best treatment for efficacy study

On the basis of physico-chemical characteristics, zinc contents and sensory response; four best treatments, two from each type of coating with different zinc fortificants were selected for the bioefficacy trial (Table 23).

4.5. Bio-evaluation trials

To evaluate the bioavailability of zinc fortificants, a model animal trial was conducted to explore the potential of zinc fortified edible coated apricots to uplift the serum zinc level. For the purpose, 115 New Zealand white rabbits were procured and housed in the Animal Room of National Institute of Food Science and Technology. The rabbits were acclimatized on basal diet for the period of seven days under controlled conditions. The temperature (23±2 ºC) and relative humidity (55±5%) were maintained throughout the experiment with 12 hr light-dark period. During 56 days study span, the rabbits were randomly divided into five groups, ten in each and provided with selected uncoated (control) and zinc fortified apricots (150 g/day/rabbit) along with normal diet (Table 23). In the current exploration, the blood samples were collected from the overnight fasted rabbits at 0, 15^{th}, 30^{th}, 45^{th} and 60^{th} day of modeling trial. For serum collection, blood samples were subjected to centrifugation. The sera samples were examined for total zinc contents, glucose & insulin levels and serum biochemistry (liver & renal function tests) using respective protocols. The collected organs including liver, kidneys and heart were used for the determination of zinc contents and weighed to calculate organ to body weight ratio. Earlier, collected blood samples were analyzed for hematological parameters with special reference to red and white blood cells indices. For better understanding, the whole trial was repeated to attain conclusive approach.

Table 23: Efficacy study plan

Groups	Treatments
G_0	Control (unfortified apricots)
G_1	Apricots coated with 2% alginate containing 50 ppm $ZnSO_4$
G_2	Apricots coated with 2% alginate containing 50 ppm $ZnCl_2$
G_3	Apricots coated with 2% chitosan containing 50 ppm $ZnSO_4$
G_4	Apricots coated with 2% chitosan containing 50 ppm $ZnCl_2$

4.5.1. Feed & drink intakes and body weight gain

Mean squares (Table 24) elucidated that the treatments and time intervals affected the feed & drink intakes and body weight gain significantly in different groups. Means in Figure 8 depicted maximum feed intake 389.02±14.50g/rabbit/day in G_3 group (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$), G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) as 386.32±13.51, 383.65±13.21 and 382.58±14.51g/rabbit/day, respectively whereas, minimum 362.69±12.01g/rabbit/day in G_0 (control).

Feed and drink intake of rabbits were recorded on daily weight basis and the mean values regarding feed intake showed an increasing trend as 356.53±15.95, 362.73±17.76, 374.40±12.12, 383.33±11.30, 391.80±12.52, 401.13±12.89, 402.03±13.46 and 402.43±18.02g/rabbit/day at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8^{th} week, correspondingly.

Similarly, mean values in Figure 9 demonstrated highest drink intake as 66.32±2.08, 66.28±2.16, 64.33±3.01 and 64.18±3.06mL/rabbit/day in G_3, G_1, G_4 and G_2 groups, respectively whereas, lowest in G_0 as 58.66±2.53mL/rabbit/day. Rabbits modeling depicted an increase in drink intake at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8^{th} week by 60.58±1.56, 61.52±1.64, 62.92±2.40, 64.51±2.32, 65.36±2.61, 66.21±3.49, 66.91±2.97 and 67.41±2.84mL/rabbit/day, accordingly.

Table 24: Means squares for feed & drink intake and body weight gain

S.O.V	Df	Feed intake	Drink intake	Body weight gain
Groups (G)	4	2952.12*	264.551*	2380.96*
Weeks (W)	8	5951.28**	113.854*	6535.53**
W x G	32	154.30^{NS}	2.279^{NS}	148.60^{NS}
Error	90	3.69	0.109	1.39

($p<0.05$)

* = Significant

^{NS} = Non significant

Likewise, Figure 10 exposed values for body weight in G_3, G_1, G_4 and G_2 groups as 874.76±13.09, 873.89±14.87, 871.80±13.65 and 869.38±12.08g/rabbit, respectively while, minimum in G_0 (851.97±12.45g/rabbit). The incline in body weight gain was noticed as 844.83±13.23 at 1st week and 899.38±14.91g/rabbit at 8th week.

The results of instant study are supported by the findings of Wan *et al.* (2013), delineated improvement in feed efficiency ratio of rats by consuming active ingredients. They deduced that the satiety and improved feed efficiency are the possible reasons behind this trend. Conclusively, increase in drink consumption was noticed in efficacy studies with substantial improvements in body weight. Similar effect of fluid intake was reported by Alshatwi *et al.* (2011); Uchiyama *et al.* (2011) and Kuan *et al.* (2005).

Afterwards, Blewett and Carla (2012) observed that Zn deficiency was found to reduce body weight in mice by 30%. Previously, Sese and Berepubo (1996) conducted a study by the incorporation of 0, 5, 10, 15, 20 and 25% graded soybean to six formulated isocaloric and isonitrogenous diets and administered to rabbits for a period of 56 days. The results elucidated that as the concentration of soybean in diet was increased, the feed intake and body weight gain also improved.

Similarly, Brown *et al.* (1925 and 1926) expounded that body weights of rabbits were increased with the passage of time by the application of functional ingredient in the diet. One of the scientists groups, Süvegová *et al.* (2004) conducted a trial using experimental rabbits and noticed that body weight was increased by the addition of zinc in the diet.

The mechanism behind the increase in body weight due to zinc addition is its involvement in the development of protein, basic structural part for developing body. By the provision of zinc salts, a finger-like structure known as a zinc finger motif (zinc binding proteins) is developed which further stabilizes the protein (Zemel et al., 2002). Another possible justification regarding the significance of zinc salt on body weight is that its deficiency in serum resulted decreased activity of deoxythymidine kinase and reduced level of adenosine tetraphosphate. Resultantly, DNA synthesis & cell division through these systems are affected leading to decreased body weight (MacDonald, 2000).

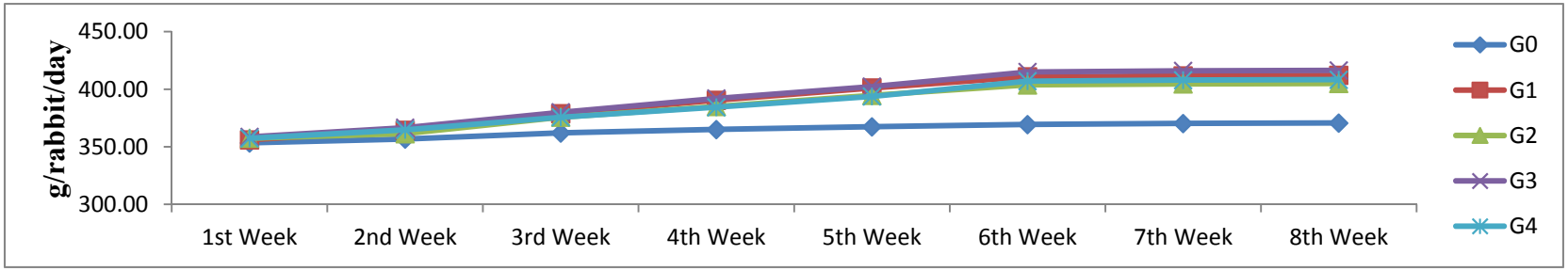

Figure 8: Effect of zinc fortified apricots on feed intake (g/rabbit/day) of different rabbits groups

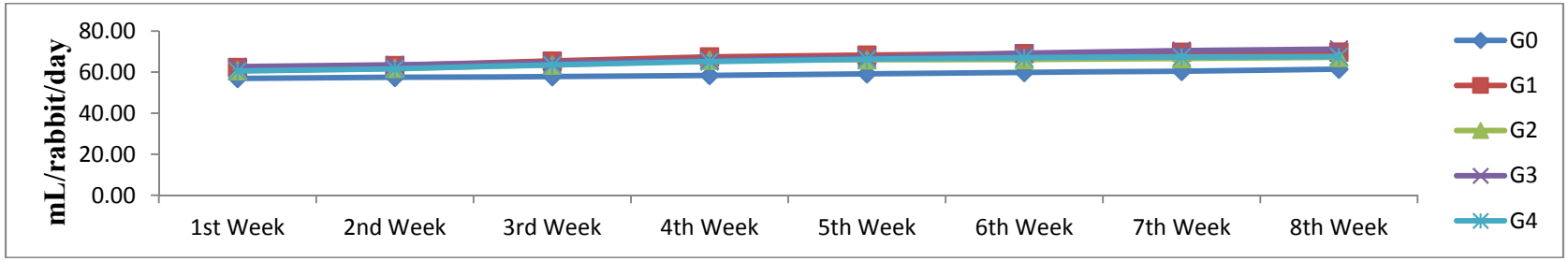

Figure 9: Effect of zinc fortified apricots on drink intake (mL/rabbit/day) of different rabbits groups

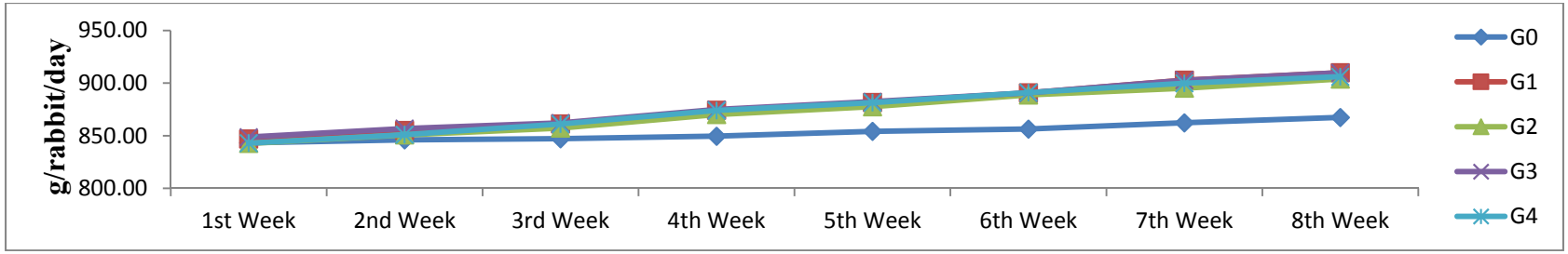

Figure 10: Effect of zinc fortified apricots on body weight gain (g/rabbit/day) of different rabbits groups

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

4.5.2. Organs weight

The mean squares explicated that liver & kidneys weight of experimental rabbits affected significantly due to treatments whilst non-significant variations were observed for heart weight (Table 25).

The results in Table 26 indicated that maximum liver weight was 48.60±3.37g/rabbit in group G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$), G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) as 48.01±3.03, 46.99±3.26 and 46.67±3.20g/rabbit, respectively whilst, minimum in G_0 (control) 45.63±2.55g/rabbit.

Likewise, mean values in Table 26 showed heart weight in G_3, G_1, G_4 and G_2 groups by 2.88±0.19, 2.87±0.22, 2.86±0.23 and 2.85±0.20g/rabbit, respectively whereas, the vale for this trait in G_0 was 2.82±0.16g/rabbit. Similarly, the kidney weight as 9.33±0.64, 9.28±0.72, 9.25±0.74 and 9.23±0.73g/rabbit in G_3, G_1, G_4 and G_2 groups, respectively while, lowest in G_0 by 8.96±0.64g/rabbit (Table 26).

The current results are in agreement with Blewett and Carla (2012), they demonstrated that Zn deficiency is one of the reasons to reduce organs weight in mice and observed reductions by 50 & 70% in spleen & thymus weight, respectively. One of the researchers groups, Süvegová *et al.* (2004) noticed that organs weights are significantly affected by respective diet and the functional ingredient. Accordingly, weights of liver, heart and kidneys were 79.00, 7.32 and 17.7g, respectively. Likewise, Sese and Berepubo (1996) expounded that liver and kidney weights are 2.66-3.50 and 0.56-0.72% on body weight basis, respectively whereas, the value for heart as 3.95-7.68g.

According to El Hendy *et al.* (2001), zinc has great potential to eradicate metabolic dysfunctions thus facilitates normal growth. They studied the effect of different zinc levels (38mg/kg diet, control) and the concentration that creates zinc discrepancy (19mg/kg diet, 1/2 of control & 3.8mg/kg diet, 1/10 of control) in male and female rats. During the study trial, they evaluated the effect of zinc on body weight gain, organs weight, blood and serum parameters. The results

Table 25: Means squares for organs weight

S.O.V	df	Liver weight	Heart weight	Kidney weight
Groups (G)	4	4.03308*	0.00133^{NS}	0.06343*
Error	10	0.09595	0.00069	0.00085

($p<0.05$)
* = Significant
NS = Non significant

depicted that rats fed on zinc-deficient diet gained less weight as compared to control. There was inclined in liver & spleen weights while testes weight was dropped. On the other hand, brain, heart, lung and kidneys weights were not affected significantly by the application of zinc.

Afterwards, Hilarious *et al.* (2012) observed that the organs weight are increased during the course of modeling and noticed values for liver, heart and kidney weights of rabbits as 2.94-3.07, 0.19-0.23 and 0.71-0.79% of body weight, respectively. One of their peers, Crile and Quiring (1940) recorded that rabbit's liver, heart and kidneys weights are varied from 2.57 to 2.63, 1.03 to 1.16 and 1.29 to 1.31% body weight, respectively.

Similarly, Bentley and Grubb (2001) conducted a trial using rabbits by the provision of different concentrations of zinc diets, 2 ppm of zinc (zinc-deficient diet), control diet (80-85 ppm zinc) and 7 ppm of zinc (low-zinc diet). It has been observed that zinc has a direct impact on the body and organs weight. Earlier, Shaw et al. (1974) induced Zn deficiency in female rabbits by feeding diet containing 0.2 ppm of Zn and strictly limiting their admittance from other sources. The finding showed that zinc deficiency reduces food intake, weight gain and haematocrit levels.

Conclusively amongst treatments, G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) and G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) found efficient regarding this traits in rabbits. This was probably due to better muscular activity and cell metabolism that were improved by the application of zinc and eventually there was an increase in feed & drink consumption, organs weight and overall growth.

Table 26: Effect of zinc fortified apricots on organs weight of rabbits (g)

Groups	Liver weight	Heart weight	Kidney weight
G_0	45.63±2.55c	2.82±0.16	8.96±0.64b
G_1	48.01±3.03ab	2.87±0.22	9.28±0.72ab
G_2	46.67±3.20b	2.85±0.20	9.23±0.73ab
G_3	48.60±3.37a	2.88±0.19	9.33±0.64a
G_4	46.99±3.26ab	2.86±0.23	9.25±0.74ab

Means sharing the same letter in a column are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

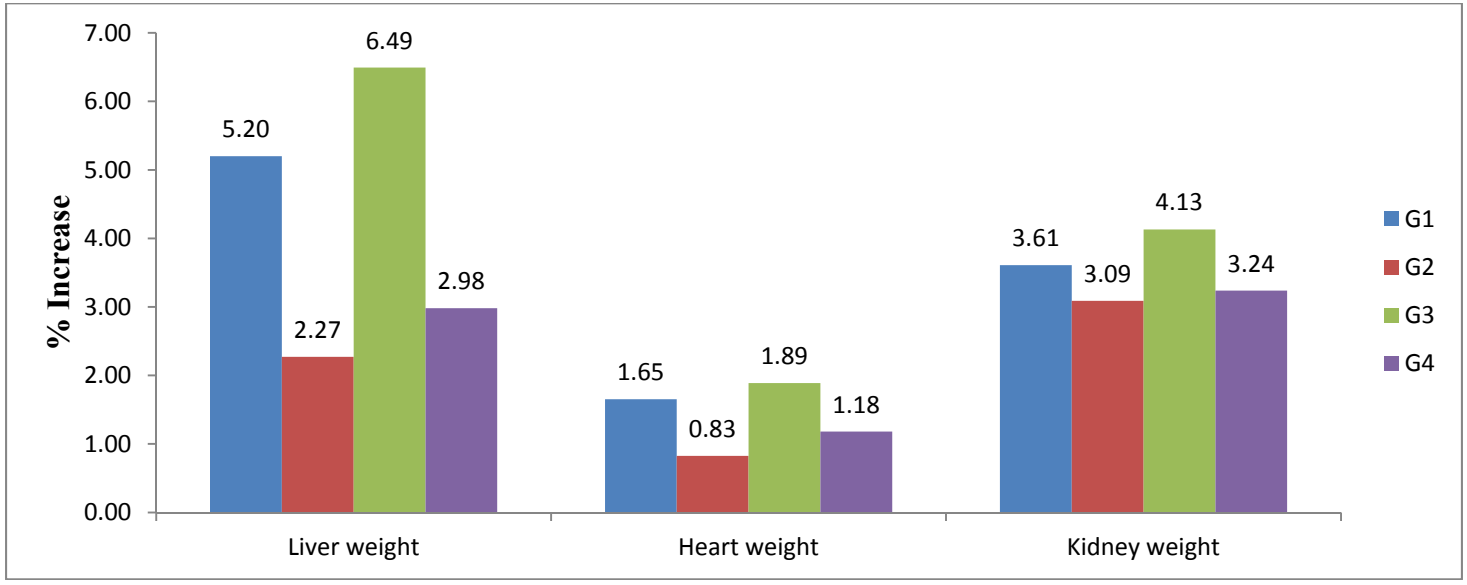

Figure 11: Percent increase in organs weight of different rabbits groups as compared to control

4.5.3. Sera zinc

Mean squares regarding serum zinc of experimental rabbits affected significantly due to treatments and time intervals while their interaction was found to be non-significant (Table 27).

The results in Table 28 indicated highest value of sera zinc in G_3 group (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 89.71±2.26µg/dL trailed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 87.43±2.14µg/dL, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 83.51±2.41µg/dL and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 81.49±2.46µg/dL, respectively whilst, lowest value for this trait 72.56±2.85µg/dL in G_0 (control). The percent increase in serum zinc was 24.63, 20.50, 15.09 and 12.31% in G_3, G_1, G_4 and G_2 groups, respectively as comparison to G_0 (Figure 12).

The sera zinc increased progressively with the passage time and at 0, 15, 30, 45 and 60 days the values for this trait were 73.39±1.74, 75.33±2.16, 82.74±3.33, 88.24±3.63 and 95.00±4.46µg/dL, respectively. The results proved that chitosan (2%) coatings along with $ZnSO_4$ (50 ppm) are effectual to improve the serum zinc status. The $ZnSO_4$ has better potential to uplift the sera zinc level as comparison to $ZnCl_2$, because $ZnSO_4$ has high bioavailability (28-32%).

The present results are in agreement with Evans and Halliwell (2001), noticed lower concentration of Zn in serum, erythrocytes and glutathione in zinc deprived rats. Afterwards, Jenner *et al.,* 2007; Rashtchizadeh *et al.,* 2008 conducted a trial and observed value for this trait in normal rabbits 0.82±0.07mg/L whilst, a reduction was observed in case of diabetic group 0.54±0.03mg/L that was modulated in diabetic+Zn fed group 0.91±0.05mg/L by supplementing Zn @ 150mg/L (Duzguner and Kaya, 2007). Similarly, zinc contents in the blood samples of six month old rabbits were measured by 11.2, 1.2 and 0.17mg/L for serum, red blood cells and whole blood.

Later, Akhtar *et al.,* 2010 analyzed the bioavailability of Zn in Sprague Dawley rats rely on $ZnSO_4$ fortified whole wheat flour. The results elucidated zinc absorption in plasma by 1.83±0.08µg/g. Numerous studies have shown marked effect of Zn deficiency on the animal health. Accordingly, a model feed trial was conducted on young New Zealand White rabbits

Table 27: Means squares for sera zinc

S.O.V	df	MS
Groups (G)	4	660.88**
Duration gap (D)	4	1209.34**
D x G	16	82.00^{NS}
Error	50	0.92

($p<0.05$)
** = Highly significant
^{NS} = Non significant

provided three types of diets; *i.e.* Zn deficient diet (2 ppm), low Zn diet (7 ppm) and control diet (80-85ppm). The reported values for serum and liver Zn levels were 0.34±0.03 & 0.79±0.05, 1.47±0.05µg/mL & 20.3±0.81 and 28.0±1.0 & 24.6±0.75µg/g for respective diet groups at the termination of 2 weeks trial. The results indicated a substantial reduction of Zn level in various organs & serum due to Zn deficit diet. It has also been observed that Zn deficient diet retards animal growth and impairs immune function within 5 week however, its specific body requirement is lesser due to high absorptive nature (Bentley and Grubb, 1991).

The Zn salt has been reported as a therapeutic agent during pregnancy complications and its deficiency reduces milk secretion in lactating mothers (Summersa *et al.,* 2008; Coylea *et al.,* 2009; Da Costa *et al.,* 2013). During experimentation, toxicity of lipopolysaccharide (LPS) mediated hypozincemia was studied in the embryonic growth of New Zealand white rabbits during gestation period. The results demonstrated reduction in serum Zn level from 1.74±0.067µg/mL (control) to 0.53±0.01µg/mL (24 hr after LPS treatment) and replenished by 1.33±0.117µg/mL after Zn supplementation (Pitt *et al.,* 1997). In a study, impact of hypozincemia was assessed in female rabbits. The findings elucidated lesser feed intake, lower body weight, reproductive failure due to pale uteri, hair loss and dermatitis in Zn deprived rabbits (Shaw *et al.,* 1974).

In an experimental, Karademir (2011) assessed the synergistic effect of various acids with $ZnSO_4$ in young male New Zealand rabbits by determining their serum Zn level. The data of the three supplementation categories revealed higher serum Zn level in $ZnSO_4$+ascorbic acid group (272.04±11.4µg/dL) followed by $ZnSO_4$+grapes vinegar (243.86±4.82µg/dL) and $ZnSO_4$+distilled water (171.79±8.82µg/dL) during 2.5 hr exposure. The results indicated higher Zn absorption in the presence of ascorbic acid.

The instant results are in accordance with Ren *et al.* (2006), they used rabbits as an experimental units in an efficacy study and noticed 11.83ppm serum zinc in rabbit. Current results are also supported with the findings of Paik *et al.* (1999), reported 0.66-0.74ppm of zinc in the rabbit sera. One of their peers, Tamura *et al.* (1994) recorded 0.89ppm of zinc in the experimental rabbits.

The findings are also in line with the previous work of Verrotti *et al.* (2002), recorded serum zinc level of rabbits from 3.47 to 3.49ppm. Similarly, Mafra and Silvia, (2004) found

Table 28: Effect of zinc fortified apricots on zinc in sera of rabbits (µg/dL)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	70.82±1.28	73.63±1.84	74.98±2.03	73.33±1.62	74.20±2.07	73.39±1.74e
15	71.61±2.50	77.30±1.94	76.37±1.75	77.31±2.74	74.09±2.65	75.33±2.16d
30	72.69±2.33	89.33±2.71	78.95±3.06	91.37±3.02	81.35±3.14	82.74±3.33c
45	73.23±3.29	95.03±3.56	83.64±3.66	98.86±3.71	90.44±3.92	88.24±3.63b
60	74.44±4.17	101.86±4.75	93.54±4.04	107.69±4.30	97.46±4.15	95.00±4.46a
Means	72.56±2.85e	87.43±2.14b	81.49±2.46d	89.71±2.26a	83.51±2.41c	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

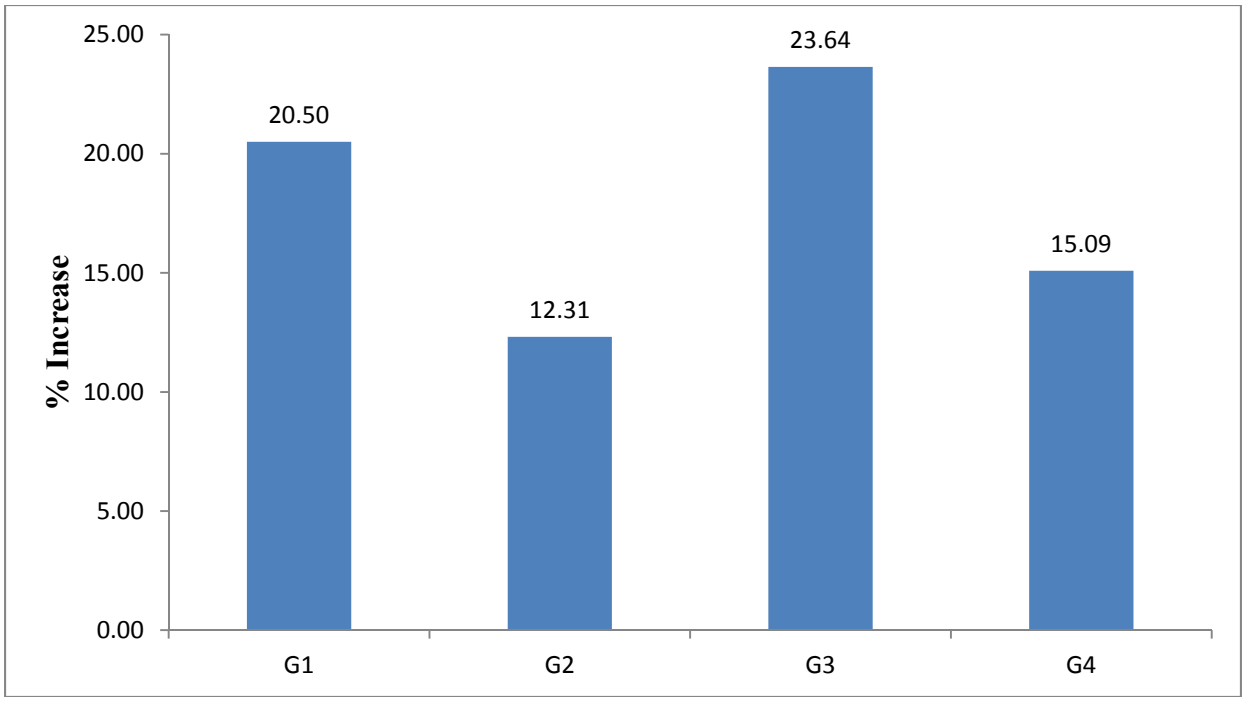

Figure 12: Percent increase in sera zinc of different rabbits groups as compared to control

0.764 ppm of zinc in the sera of experimental rabbits. The results are also supported by the rabbits modeling trials of Liu *et al.* (2005), indicated 0.517ppm of sera zinc.

One of the scientists groups, Piao *et al.* (2003) observed sera zinc of rabbit between 4.93 to 8.23ppm. Previously, Bentley and Grubb (2001) recorded serum zinc concentration in zinc-deficient rabbit's 0.35µg/ml as comparison with 0.8µg/ml for low-zinc diet and 1.4µg/ml for control diet.

It is concluded from the above debate that G_3 and G_1 groups containing $ZnSO_4$ are potential contributors to uplift the serum zinc concentration of experimental rabbits as compared to G_4 and G_2 groups possibly be due to the high bioavailability of zinc sulfate as compared to its chloride salt.

4.5.4. Organs zinc

Mean squares for liver, heart and kidneys zinc contents of rabbits were affected significantly due to treatments (Table 29).

There was momentous increase in the zinc contents of liver, heart and kidneys of experimental rabbits fed on zinc fortified edible coated apricots during eight weeks. Means in Table 30 showed highest liver zinc in group G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 23.97±1.41µg/g followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 23.71±1.15µg/g, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 23.53±1.28µg/g and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 23.36±1.45µg/g, respectively while, lowest 22.42±1.36µg/g in G_0 (control). The results exposed that liver zinc increased by 6.91, 5.72, 4.95 and 4.19% in G_3, G_1, G_4 and G_2, correspondingly as compared to control (Figure 13).

Similarly, the values for heart zinc were 17.59±0.55, 17.46±0.54, 17.22±0.42 and 17.18±0.46µg/g in G_3, G_1, G_4 and G_2 groups, respectively whereas, minimum in G_0 by 17.11±0.50µg/g (Table 30). In case of heart, percent increase in zinc was 2.84, 2.08, 0.66 and 0.43% in G_3, G_1, G_4 and G_2 groups, accordingly as compared to G_0 (Figure 13).

Likewise, mean values in Table 30 elucidated deposition of kidney zinc in G_3, G_1, G_4 and G_2 groups by 25.18±1.23, 24.97±1.56, 24.65±1.11 and 24.40±1.30µg/g, respectively whilst, minimum 23.53±1.47µg/g in G_0. The kidney zinc was also influenced by the fortification practice and noticed an increase by 7.03 (G_3), 6.12 (G_1), 4.76 (G_4) and 3.68% (G_2) as comparison to control (Figure 13).

The present results are supported with the work of Akhtar *et al.* (2010), analyzed the bioavailability of Zn in Sprague Dawley rats rely on $ZnSO_4$ fortified whole wheat flour. The results depicted liver and kidney zinc by 44.14±0.94 and 16.17ug/g, respectively. One of the researchers groups, Shaw *et al.* (1974) conducted rabbits modeling trial for the application of zinc diet and found that rabbit's liver & kidney contain 50.19 & 17.65ppm of zinc, respectively. Afterwards, Pitt *et al.* (1997) expounded that liver of rabbit contains 29.1ppm of zinc contents. Later, Kalafova *et al.* (2012) documented that zinc contents in the liver and kidney of rabbits varied from 119 to 178ppm and 15.30 to 50.30ppm, respectively.

Table 29: Means squares for organs zinc

S.O.V	df	Liver zinc	Heart zinc	Kidney zinc
Groups (G)	4	1.04682**	0.12757*	1.23851**
Error	10	0.09935	0.00156	0.09343

($p<0.05$)

* = Significant

** = Highly significant

One of the scientists groups, El Hendy *et al.* (2001) recorded rabbits liver, heart and kidney zinc contents 3.61, 0.5 and 1.0g/100g body weight, respectively. One of their peers, Ye *et al.* (2001) analyzed the rats for their organs zinc contents and observed that liver and heart contain 51-53ppm and 37-39ppm of zinc, accordingly. The instant results are in agreement with Ranjan *et al.* (2011), concluded that sera and organs zinc level improved by the application of zinc in the diet. Their results elucidated that rabbit liver, heart and kidneys had 14.58, 10.36 and 11.32ppm of zinc, correspondingly.

Earlier, Bentley and Grubb (2001) demonstrated that tissue zinc concentration gradually declined in low-zinc and zinc-deficient diet rabbits. The results showed that due to zinc-deficient diet, zinc in liver & testes decreased up to 20% each, skin by 35% and brain by 10%. Similarly, Piao *et al.* (2003) observed the effect of zinc on the functioning of different organs and tissues of experimental rats. Accordingly, rats were divided into four groups as control (saline), low zinc group (4mg/kg of zinc acetate), high zinc group (8mg/kg of zinc acetate) and cyclophosphamide group (50mg/kg); they observed that zinc concentrations in the blood and organs were noticeably affected by the amount of zinc fed to the rats.

Conclusively, zinc salts were absorbed in the tissues & organs of the animal body and the increased zinc contents are might be due to their deposition in respective organs. Additionally, the concentration of zinc in each organ is dependent on the amount of its salt added to the formulation. Moreover, bioavailability of zinc salts is also a cardinal factor for uplifting the zinc contents in various organs.

Table 30: Effect of zinc fortified apricots on organs zinc of rabbits (µg/g)

Groups	Liver zinc	Heart zinc	Kidney zinc
G_0	22.42±1.36c	17.11±0.50c	23.53±1.47c
G_1	23.71±1.15a	17.46±0.54a	24.97±1.56a
G_2	23.36±1.45b	17.18±0.46b	24.40±1.30b
G_3	23.97±1.41a	17.59±0.55a	25.18±1.23a
G_4	23.53±1.28ab	17.22±0.42ab	24.65±1.11b

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

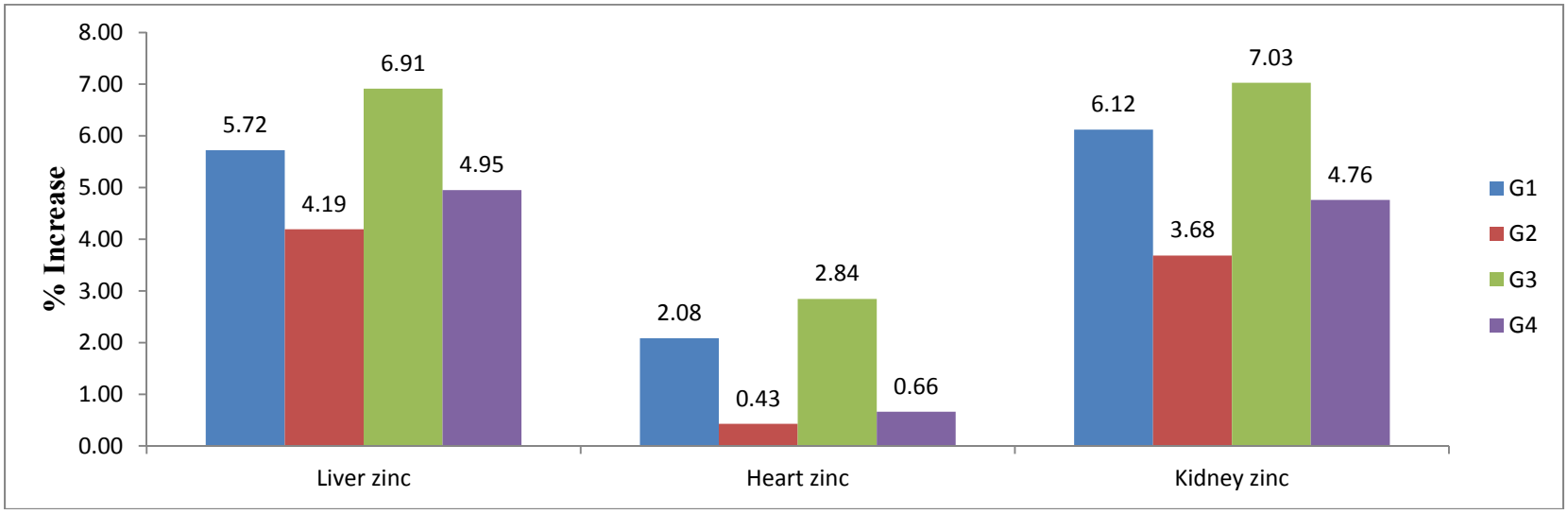

Figure 13: Percent increase in organs zinc of different rabbits groups as compared to control

4.5.5. Serum glucose and insulin

Mean squares in Table 31 indicated that serum glucose and insulin levels of experimental rabbits are effected significantly due to treatments and time intervals while their interactions were found non-significant.

Zinc fortified apricots were capable of decreasing the serum glucose and increasing the insulin level of experimental rabbits as manifested from their mean values. The minimum value of serum glucose was observed in G_3 group (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 111.79±4.48mg/dL whilst, the values for this trait in group G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$), G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) were 112.42±4.57, 114.80±4.78 and 115.10±4.78mg/dL, respectively whereas, the maximum in G_0 (control) 117.91±4.23mg/dL (Table 32). The percent diminish of serum glucose was 5.19, 4.66, 2.63 and 2.38% in G_3, G_1, G_4 and G_2, respectively as compared to G_0 (Figure 14).

Zinc has potential to ameliorate serum glucose with the passage of time and the recorded values for this trait were 117.60±5.41, 116.14±4.70, 114.57±4.72, 112.45±3.76 and 111.27±3.78mg/dL at 0, 15, 30, 45 and 60 days, correspondingly.

Likewise, means in Table 33 revealed values of serum insulin as 9.38±0.51, 9.34±0.53, 9.27±0.49 and 9.23±0.52μU/mL in G_3, G_1, G_4 and G_2 groups, respectively whilst, minimum in G_0 by 9.01±0.50μU/mL. Similarly, percent increase in insulin was recorded in G_3, G_1, G_4 and G_2 by 4.31, 3.87, 3.08 and 2.54% as compared to control (Figure 15). The uplift in serum insulin at 0, 15, 30, 45 and 60 day was 9.18±0.44, 9.20±0.51, 9.25±0.49, 9.28±0.52 and 9.31±0.61μU/mL, accordingly.

The current results are in harmony with Duzguner and Kaya, (2007), performed rabbit's efficacy trial for assessing blood and serum profile. They highlighted the therapeutic role of zinc against type I & II diabetes. It is a proven fact that zinc has tendency to decrease blood glucose and improves insulin resistance in human and animals. Besides modulates the glucose metabolism, zinc also have potential to manage obesity thus provides protection against cardiovascular complications. They elucidated the modulatory role of Zn

Table 31: Means squares for serum glucose and serum insulin

S.O.V	df	Serum glucose	Serum insulin
Groups (G)	4	88.896**	0.34542**
Duration gap (D)	4	100.929**	0.04450**
D x G	16	5.395^{NS}	0.00440^{NS}
Error	50	1.066	0.00255

($p<0.05$)
** = Highly significant
^{NS} = Non significant

supplementation in diabetic New Zealand rabbits. The animals were distributed in three groups; control, diabetic and diabetic+zinc fed. After the completion of three months study, it was revealed that Zn supplementation 150 mg/L improves the antioxidative status of rabbits by enhancing glutathione, superoxide dismutase and catalase alongside reduction in malondialdehyde content. The higher body weight was observed in control animal 3.1% conversely, body weight was decreased 5.75% in diabetic animals followed by 1.53% in Zn supplemented diabetic rabbits. The results exhibited higher feed and water utilizations in diabetic group as compared to control. However, body weight gain was lesser in diabetics as compared to healthy rabbits. Similarly, the blood glucose level was higher in diabetic group than that of control.

Another possible mechanism behind the control of diabetes by the application of zinc salts is that unrestrained diabetes leads to increase hepatic glucose production. Firstly, liver glycogen reserves are metabolized in the body and then hepatic gluconeogenesis is taking into account for glucose production. Likewise, insulin deficiency also weakens the non-hepatic tissue consumption of glucose. In meticulous, insulin encourages glucose uptake in skeletal muscles and adipose tissues. In the same way, reduced glucose uptake by peripheral tissues leads to decreased rate of glucose metabolism. The amalgamation of increased hepatic glucose formation and reduced peripheral tissues metabolism resulted elevated plasma glucose concentration and weight loss that are frequently seen in diabetic patients. Additionally, glucose reduction up to 6.9% was noticed in Zn administered diabetic group (16.25±2.37 mmol/L) as compared to diabetic group (17.46±2.08 mmol/L). Likewise, biochemical analysis of normal rabbit's showed glucose level *i.e.* 7.92mmol/L in rabbit's serum (Süvegová *et al.,* 2004).

The exploration of Dashti *et al.* (2012) supported the substantial reduction in blood glucose by zinc fed diet. Their results explicated that serum glucose of the experimental rabbits was in the range of 91-95mg/dL. The probable reason for decrease in glucose through zinc is that it elevates the level of hepatic glycogen, improves glycogen synthesis and disturbs glucose-6-phosphatase activity thus reduces glucose production. One of their peers, Ewuola *et al.,* 2012 carried out efficacy trial to assess the glucose profile of normal rabbits and recorded value for this trait as 142mg/dL. Instant results are well supported with the work of Ozkan *et al.,* 2012, noticed 3.83-10.77mmol/L of glucose in the serum of male and 4.94-8.32mmol/L in the

Table 32: Effect of zinc fortified apricots on serum glucose of rabbits (mg/dL)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	118.36±5.39	116.93±5.31	118.42±6.02	116.18±5.56	118.11±5.15	117.60±5.41a
15	118.32±4.71	113.74±4.48	117.91±4.67	113.58±4.42	117.13±4.80	116.14±4.70a
30	117. 92±4.25	111.98±4.95	115.92±4.99	111.62±3.34	115.39±4.69	114.57±4.72b
45	117.49±4.08	110.33±3.36	111.93±3.39	109.98±3.91	112.52±3.14	112.45±3.76bc
60	117.46±3.35	109.12±3.14	111.30±3.51	107.58±3.33	110.87±3.99	111.27±3.78c
Means	117.91±4.23a	112.42±4.57bc	115.10±4.78b	111.79±4.48c	114.80±4.78b	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

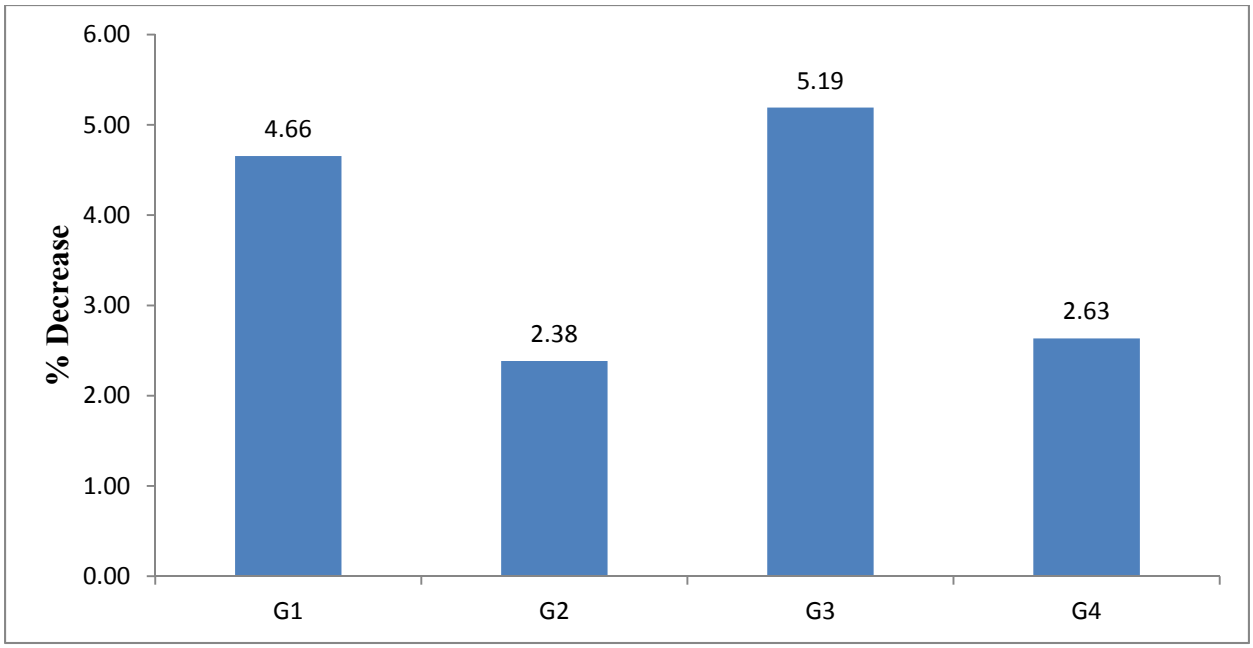

Figure 14: Percent decrease in serum glucose of different rabbits groups as compared to control

female rabbits. One of the certain mechanisms proving zinc as hypoglycemic agent is its ability to modulate the activity of glucose transporters (GLUTs); major carriers that maintain the glucose homeostasis and require IRβ and AMPKR proteins for their translocations. During zinc deficiency, there is reduction in GLUT4 and associated proteins thereby glucose incorporation in the cells is disturbed resulted diabetes and insulin resistance. Zinc enhances translocation proteins IRβ, AMPKR and GLUT4 expressions thus maintains glucose homeostasis.

Earlier, Thompson and Godin (1995) reported 50% reduction in blood glucose level by supplementing $ZnCl_2$. The fructose or sucrose rich diets trigger abnormal glucose production that affect different hormones in plasma like adiponectin and intestine GLUT1 linked with insulin resistance. The high fat and sugar diets initiate the cascade of coronary complications, obesity and diabetes by disturbing glucose homeostasis and insulin sensitivity (Kimberly and Lambert, 2010; Jong, 2010).

In the nut shell, zinc plays a pivotal role to attenuate serum glucose and improves insulin sensitivity and resistance. However, the proposed route of action is the inhibition of α-amylase & α-glucosidase activities in the intestine that balanced the glucose and insulin levels thus enhance insulin binding to the adipocytes and promote intracellular glucose transporter in the myocytes. It might also be that insulin release is improved by better zinc status, because zinc is required for insulin packaging and release in the pancreatic beta cells. The higher insulin response can lead to greater glucose uptake and lower serum glucose.

Table 33: Effect of zinc fortified apricots on serum insulin of rabbits (µU/mL)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	8.92±0.43	9.30±0.40	9.21±0.32	9.25±0.44	9.21±0.43	9.18±0.44b
15	8.98±0.52	9.31±0.50	9.20±0.41	9.29±0.43	9.24±0.53	9.20±0.51b
30	9.01±0.42	9.34±0.53	9.24±0.54	9.39±0.33	9.28±0.44	9.25±0.49ab
45	9.03±0.51	9.37±0.56	9.22±0.47	9.48±0.53	9.30±0.55	9.28±0.52a
60	9.05±0.62	9.40±0.65	9.25±0.64	9.52±0.54	9.34±0.56	9.31±0.61a
Means	9.01±0.50c	9.34±0.53ab	9.23±0.52b	9.38±0.51a	9.27±0.49ab	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

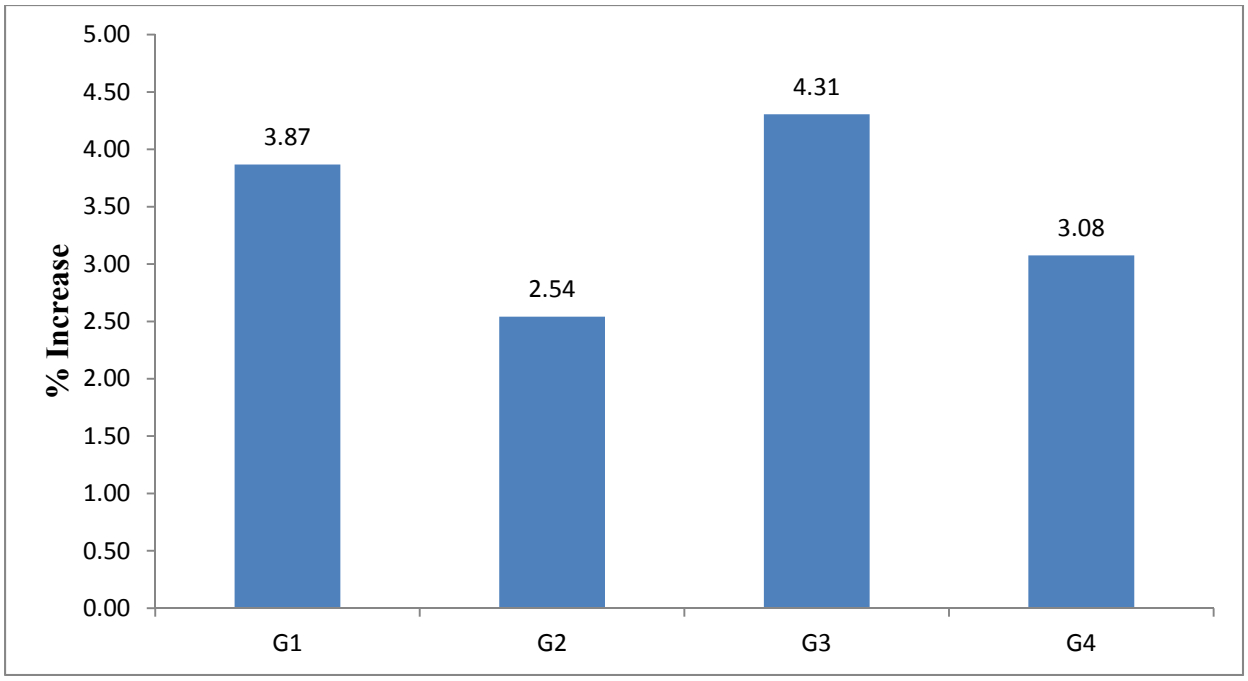

Figure 15: Percent increase in serum insulin of different rabbits groups as compared to control

4.5.6. Liver function tests

The mean squares in Table 34 indicated that aspartate transaminase (AST), alanine transaminase (ALT) and total bilirubin of experimental rabbits were effected non-significantly due to treatments and time intervals whereas significant differences were observed for alkaline phosphatase (ALP).

The results indicated values for AST in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$), G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$), G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) groups 26.79±1.57, 26.87±1.79, 26.95±1.76 and 27.02±1.84IU/L, respectively nevertheless, in G_0 (control) 27.08±1.88IU/L. However, the AST level was 27.16±1.87, 27.11±1.99, 26.96±1.68, 26.76±2.04 and 26.72±1.52IU/L at 0, 15, 30, 45 and 60 days of trial, respectively (Table 35).

Similarly, the results enlightened values for ALT in G_3, G_1, G_4 and G_2 groups as 25.69±1.33, 25.73±1.43, 25.81±1.76 and 25.86±1.39IU/L, respectively whilst, 26.08±1.48IU/L in G_0 (Table 36). Results delineated that the ALT level varied from 26.19±2.01 to 25.52±1.56IU/L from start to end of efficacy study.

Conversely, significant differences for ALP as 15.07±0.43, 15.14±0.49, 15.24±0.59 and 15.32±47IU/L were observed in G_3, G_1, G_4 and G_2, respectively whilst, 15.58±0.76IU/L in G_0 (Table 37). During rabbits modeling, the decline in ALP was observed by 15.46±0.53, 15.44±0.79, 15.28±0.37, 15.13±0.28 and 15.06±0.55IU/L at 0, 15, 30, 45 and 60 days, respectively.

The results of total bilirubin elucidated minimum value in G_3 0.42±0.03 whilst, the results for this attribute in G_1, G_4 and G_2 groups were 0.43±0.03, 0.44±0.04 and 0.44±0.02mg/dL, respectively nevertheless, maximum in G_0 by 0.45±0.04mg/dL (Table 38). The decrease in total bilirubin at initiation to end of trial was 0.44±0.04 to 0.42±0.01mg/dL, correspondingly.

The current explorations are in agreement with the findings of Ozkan *et al.* (2012), carried out hematological analysis and blood efficacy through rabbits modeling. Their results exposed values 6-20, 6-9 and 12-26U/L for AST, ALT and ALP in male rabbits and 7-19, 5-8 and 13-26U/L in female, respectively.

Table 34: Means squares for liver function tests

S.O.V	df	AST	ALT	ALP	Total bilirubin
Groups (G)	4	0.19318^{NS}	0.34925^{NS}	0.59668*	0.00168^{NS}
Duration gap (D)	4	0.60116^{NS}	1.40673^{NS}	0.49045*	0.00318^{NS}
D x G	16	0.03772^{NS}	0.08401^{NS}	0.04500^{NS}	0.00003^{NS}
Error	50	0.02616	0.00094	0.00080	0.00008

($p<0.05$)
* = Significant
^{NS} = Non significant

Table 35: Effect of zinc fortified apricots on AST of rabbits (IU/L)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	27.18±2.02	27.24±2.03	27.24±2.07	27.05±1.54	27.08±1.76	27.16±1.87
15	27.14±2.03	27.19±1.72	27.18±1.68	27.03±1.50	27.03±2.12	27.11±1.99
30	27.08±1.94	26.88±1.82	27.11±1.95	26.82±1.67	26.93±1.38	26.96±1.68
45	27.03±2.06	26.58±2.02	26.80±1.09	26.54±1.07	26.86±2.03	26.76±2.04
60	26.98±1.02	26.48±1.96	26.74±1.25	26.54±1.52	26.84±1.49	26.72±1.52
Means	27.08±1.88	26.87±1.79	27.02±1.84	26.79±1.57	26.95±1.76	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Earlier, biochemical profile of rabbits was assessed by determining AST and ALT levels that were 0.27 and 1.01μkat/L, accordingly (Süvegová *et al.,* 2004). One of the researchers groups, Ewuola *et al.,* 2012 recorded AST, ALT and ALP levels or rabbits serum as 0.14, 0.18 and 2.41μmol/L, respectively. The instant results are also in harmony with the work of Archetti *et al.* (2008), elucidated that AST and ALT level of experimental rabbits are 51±24 and 31±9IU/L, correspondingly.

In another study, efficacy of Zn was measured against renal disease in weaned male Wistar rats. The results presented reduced glomerular filtration, activity of nitric oxide synthase and NADPH diaphorase in renal system hence protects against oxidative stress (Tomat *et al.,* 2007).

Earlier, Keeling *et al.* (1980) analyzed Zn status in 27 patients with liver disease, showing an inverse correlation between serum zinc and liver disease. Accordingly, Zn levels were found as 0.9±0.03 (control), 0.64±0.06 (alcoholic cirrhosis), 0.7±0.03 (primary biliary cirrhosis) and 0.78±0.07 μg/L (active chronic hepatitis).

One of their peers, Gil *et al.* (2010) recorded that total bilirubin of experimental rabbits was varied from 0.01 to 0.74mg/dL. Liver is a site for accumulation and synthesis of various endogenous and exogenous substances. A sound liver allowed low level of ALT and AST in the serum however, during diseased state the elevated concentrations of these enzymes are an indication of liver malfunctioning. For healthy liver, minerals and active ingredients are gaining popularity due to their high antioxidant, anti-inflammatory, anti-oncoganic and immune boosting instincts (Bárta *et al.,* 2006; Shin *et al.,* 2006; Noori *et al.,* 2009; Ramesh *et al.,* 2009).

Table 36: Effect of zinc fortified apricots on ALT of rabbits (IU/L)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	26.21±2.03	26.09±2.05	26.27±1.96	26.14±1.85	26.25±2.02	26.19±2.01
15	26.08±1.06	26.04±1.23	26.25±1.45	26.04±1.28	26.21±1.24	26.13±1.52
30	25.94±2.11	25.65±1.28	25.64±1.09	25.76±1.40	25.65±1.27	25.73±1.34
45	26.15±1.28	25.49±1.44	24.61±1.61	25.31±1.43	25.50±1.87	25.61±1.78
60	26.01±1.21	25.41±1.28	25.55±1.37	25.18±1.33	25.44±1.84	25.52±1.56
Means	26.08±1.48	25.73±1.43	25.86±1.39	25.69±1.33	25.81±1.76	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 37: Effect of zinc fortified apricots on ALP of rabbits (IU/L)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	15.67±0.51	15.39±0.53	15.47±0.43	15.37±0.42	15.40±0.55	15.46±0.53a
15	15.64±1.02	15.38±0.52	15.45±0.63	15.36±0.54	15.36±0.83	15.44±0.79a
30	15.55±0.82	15.21±0.67	15.38±0.53	14.94±0.12	15.30±24	15.28±0.37ab
45	15.51±0.12	14.81±0.17	15.31±0.26	14.80±0.37	15.20±0.41	15.13±0.28ab
60	15.56±1.01	14.89±0.22	14.99±0.31	14.89±0.36	14.96±0.52	15.06±0.55b
Means	15.58±0.76a	15.14±0.49ab	15.32±0.47ab	15.07±0.43b	15.24±0.59ab	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 38: Effect of zinc fortified apricots on total bilirubin of rabbits (mg/dL)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	0.46±0.04	0.44±0.03	0.45±0.02	0.43±0.03	0.45±0.04	0.44±0.04
15	0.44±0.02	0.42±0.01	0.43±0.03	0.41±0.04	0.43±0.02	0.43±0.03
30	0.45±0.03	0.43±0.04	0.44±0.01	0.42±0.02	0.44±0.03	0.44±0.02
45	0.46±0.02	0.45±0.01	0.46±0.04	0.44±0.01	0.46±0.02	0.45±0.03
60	0.43±0.04	0.41±0.03	0.42±0.01	0.40±0.02	0.42±0.04	0.42±0.01
Means	0.45±0.04	0.43±0.03	0.44±0.02	0.42±0.03	0.44±0.04	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

4.5.7. Renal functioning tests

The mean squares in Table 39 depicted that serum urea and creatinine of experimental rabbits are effected non-significantly in various groups because of treatments and time intervals.

The results exhibited lowest value of serum urea by 25.30±1.44mg/dL in group G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) whereas, the value for this feature was 25.38±1.62, 25.40±1.41 and 25.44±1.59mg/dL in G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$), G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$), respectively nevertheless, highest as 25.75±1.51mg/dL in G_0 (control). The serum urea decreased non-momentously with the passage of time and values for this trait were 26.03±2.03, 25.79±1.69, 25.36±1.42, 25.10±1.71 and 24.99±1.75mg/dL at 0, 15, 30, 45 and 60 days, correspondingly (Table 40).

Likewise, mean values in Table 41 showed minimum creatinine level in G_3 followed by G_1, G_4 and G_2 groups by 1.04±0.05, 1.05±0.06, 1.06±0.04 and 1.07±0.08mg/L, respectively though, maximum 1.08±0.07mg/L in G_0. The non-significant decrease in creatinine was detected 1.08±0.07 to 1.04±0.3mg/L at initiation to the end of efficacy trial.

The present results are in accordance with the work of Süvegová *et al.* (2004), they demonstrated that rabbit's sera contain 5.23mmol/L urea and 62.66µmol/L of creatinine. Afterward, Ewuola *et al.,* 2012 assessed rabbit's serum urea and creatinine levels *i.e.* 92.9µmol/L and 5.90mmol/L, respectively. Additionally, they concluded that antioxidative action of zinc is a considerate factor for the management of renal functionality.

Earlier, in a bioevaluation trial, urea and creatinine levels in rats were measured and their findings showed decreased urea and creatinine from 15.91 to 14.58mg/100mL and 3.82 to 3.38mg/100mL by Zn supplementation, respectively (El Hendy *et al.,* 2001). The instant results are in line with the work of Archetti *et al.* (2008), expounded that serum urea of experimental rabbits ranged from 3.6 to 6.6mmol/L and creatinine from 37 to 65µmol/L.

Table 39: Means squares for serum urea and creatinine

S.O.V	df	Serum urea	Serum creatinine
Groups (G)	4	0.44012^{NS}	0.00293^{NS}
Duration gap (D)	4	2.95246^{NS}	0.00421^{NS}
D x G	16	0.15443^{NS}	0.00014^{NS}
Error	50	0.03212	0.00013

($p<0.05$)
* = Significant
^{NS} = Non significant

Table 40: Effect of zinc fortified apricots on serum urea of rabbits (mg/dL)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	25.89±2.02	26.17±2.05	26.16±1.04	25.97±1.03	25.95±1.95	26.03±2.03
15	25.86±1.97	25.73±1.82	25.83±1.53	25.69±1.57	25.81±1.26	25.79±1.69
30	25.74±1.88	25.09±1.53	25.61±1.01	25.01±1.18	25.35±1.33	25.36±1.42
45	25.69±1.28	24.87±1.92	24.94±1.67	24.93±1.29	25.08±1.43	25.10±1.71
60	25.55±1.03	25.03±1.29	224.67±1.84	24.91±1.63	24.80±1.25	24.99±1.75
Means	25.75±1.51	25.38±1.62	25.44±1.59	25.30±1.44	25.40±1.41	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

One of their peers, Ozkan *et al.* (2012) observed that male rabbit's sera contain 0.06-0.14mmol/L of creatinine and female 0.06-0.14mmol/L. The creatinine is a break down product of creatinine phosphate and an indicator of kidney functioning. During healthy state, creatinine is filtered by the kidney but during diseased condition its level increased drastically. The elevated blood creatinine is an indicator for impaired glomerulus filtration that is a first sign of CKD. One of the possible mechanisms for the proper functioning of kidney is that zinc is supportive for managing the abnormalities of glomerulus filtration by reducing toxic impact of reactive oxygen species and improving the overall antioxidant status (Di *et al.,* 2012; Theeshan *et al.,* 2012).

Table 41: Effect of zinc fortified apricots on creatinine of rabbits (mg/L)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	1.09±0.09	1.08±0.06	1.09±0.04	1.06±0.02	1.08±0.08	1.08±0.07
15	1.08±0.02	1.05±0.01	1.07±0.05	1.04±0.06	1.05±0.03	1.06±0.04
30	1.09±0.01	1.07±0.10	1.08±0.09	1.05±0.07	1.07±0.02	1.07±0.06
45	1.07±0.10	1.03±0.08	1.04±0.03	1.01±0.07	1.06±0.08	1.04±0.05
60	1.05±0.04	1.04±0.05	1.05±0.01	1.02±0.02	1.04±0.07	1.04±0.3
Means	1.08±0.07	1.05±0.06	1.07±0.08	1.04±0.05	1.06±0.04	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

4.5.8. Hematological aspects

Mean squares in Table 42 showed that T-lymphocytes, b-lymphocytes, neutrophils and hemoglobin of experimental rabbits effected significantly due to treatments and time intervals however, non-significant behavior for leukocytes, monocytes and eosinophils was observed.

The Table 43 elucidated highest value for T-lymphocytes in group G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 22.34±1.29% followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 22.21±1.35%, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 22.02±1.14% and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 21.50±1.14%, respectively whilst, lowest in G_0 (control) 21.22±1.13%. The T-lymphocytes momentously influenced due to time intervals and recorded values for this parameter were 21.32±1.19, 21.55±1.08, 21.97±1.15, 22.17±1.26 and 22.28±1.34% at 0, 15, 30, 45 and 60 days, respectively.

The observed values for leukocytes were 7240.60±76.86, 7234.87±75.85, 7229.13±75.96 and 7226.20±76.80cu mm in G_3, G_1, G_4 and G_2 groups, respectively whereas, 7204.47±76.79cu mm in G_0 (Table 44). The leukocytes contents were not significantly influenced due to time interval and the recorded values from beginning to end of trial were 7202.67±74.04 to 7248.87±75.17cu mm. Contrarily, the highest value of b-lymphocytes was noticed in G_3 trailed by G_1, G_4 and G_2 as 47.29±3.15, 46.87±3.12, 46.62±3.47 and 46.58±3.13%, respectively although, lowest in G_0 45.74±3.46%. The b-lymphocytes were varied from 45.46±3.38 at beginning to 47.41±2.34% at the end of trial (Table 45).

The results in Table 46 reflected that values for monocytes in group G_3, G_1, G_4, G_2 and G_0 were 1.79±0.14, 1.78±0.011, 1.77±0.10, 1.76±0.13 and 1.74±0.09%, respectively. The augment in monocytes at 0, 15, 30, 45 and 60 days was 1.74±0.13, 1.75±0.14, 1.77±0.05, 1.78±0.15 and 1.79±0.03%, accordingly. Similarly, values for eosinophils in G_3, G_1, G_4 and G_2 groups were 1.07±0.08, 1.06±0.06, 1.05±0.06 and 1.04±0.04%, respectively whilst, 1.03±0.07% in G_0 (Table 47). During rabbit's experimental modeling, the eosinophils were varied from 1.03±0.07 to 1.07±0.03% at initiation to the end of trial.

Table 42: Means squares for hematological aspects of rabbit's blood

S.O.V	df	T-lymphocytes	Leukocytes	b-lymphocytes	Monocytes	Eosinophils	Neutrophils	Hemoglobin
Groups (G)	4	3.45379*	2849.15^{NS}	4.83116*	0.00605^{NS}	0.00291^{NS}	4.77692*	1.27513*
Duration gap (D)	4	2.52948*	5594.58^{NS}	8.53510*	0.00629^{NS}	0.00317^{NS}	3.87307*	0.23167*
D x G	16	0.19589^{NS}	113.89^{NS}	0.56605^{NS}	0.00052^{NS}	0.00018^{NS}	0.07292^{NS}	0.01774^{NS}
Error	50	0.03405	23.09	0.00383	0.00012	0.00009	0.00438	0.00055

($p<0.05$)
* = Significant
^{NS} = Non significant

Table 43: Effect of zinc fortified apricots on T-lymphocytes of rabbits (%)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	21.07±1.04	21.43±1.33	21.16±1.06	21.54±1.47	21.40±1.38	21.32±1.19c
15	21.11±1.06	21.73±1.15	21.49±1.07	21.70±1.26	21.71±1.11	21.55±1.08b
30	21.19±1.03	22.37±1.30	21.52±1.37	22.52±1.09	22.25±1.13	21.97±1.15b
45	21.30±1.24	22.75±1.41	21.57±1.13	22.88±1.08	22.34±1.06	22.17±1.26a
60	21.44±1.09	22.78±1.31	21.74±1.24	23.06±1.46	22.41±1.12	22.28±1.34a
Means	21.22±1.13b	22.21±1.35ab	21.50±1.14ab	22.34±1.29a	22.02±1.14ab	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Likewise, the neutrophils count was 38.37±2.09, 38.27±2.11, 38.09±2.23 and 38.03±2.14% in G_3, G_1, G_4 and G_2, accordingly whereas, 36.96±2.15% in G_0 (Table 48). The neutrophils increased slightly at 0 to 60 days by 37.44±2.55 to 38.65±2.16%, correspondingly. Similarly, the maximum value of hemoglobin was noticed in G_3 group (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 13.07±0.53g/dL followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 12.94±0.47g/dL, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 12.78±0.45g/dL and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 12.61±0.51g/dL, respectively whilst, minimum in G_0 (control) 12.32±0.36g/dL. Current findings explicated that hemoglobin level was gradually enhanced *i.e.* 12.60±0.41, 12.66±0.50, 12.72±0.39, 12.83±0.43 and 12.90±0.71g/dL at 0, 15, 30, 45 and 60 days, respectively (Table 49).

The instant results are in agreement with the findings of Archetti *et al.* (2008), worked on the hematological aspects of rabbits and recorded values for T-lymphocytes, leukocytes, b-lymphocytes, monocytes, eosinophils, neutrophils and hemoglobin were in the range of 20-79%, 2.6-12.7%, 20-79%, 0.5-28%, 0.0-0.5% and 10-66% and 6.7-12.7g/dL, respectively. The results are also supported with the work of Ewuola *et al.* (2012), documented that rabbits blood have 60.6% of T-lymphocytes, 2.4% monocytes, 2.2% eosinophils, 34.8% neutrophils and 6.32mmol/L hemoglobin.

Previously, various bioefficacy studies have proven that hypozincemia increases the incidence of bacterial attack due to over-expression of NF-*k*B and targeted genes like IL-1β, ICAM-1 and TNF-α in Zn deficit mice. It has been noticed that Zn deficiency increases the inflammatory response resulting in reduced activity of vital organs including lung and liver. During a bioevaluation trial, Zn supplementation was found effective to mediate innate immunity by downregulating NF-*k*B & TNF-α signaling pathway. It has also been observed that Zn provision reversed the dysregulation of immune expression thus reduces the rate of morbidity (Bao *et al.,* 2010).

In a biological study, comparison was made between healthy and common variable immunodeficient (CVID) patients to assess their serum Zn concentration. The lower zinc concentration was noticed in immunodeficient patients as compared to control group. It was further highlighted that lower zinc status worsens the disease condition thereby triggering the

Table 44: Effect of zinc fortified apricots on leukocytes of rabbits (cu mm)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	7191.33±76.81	7199.33±75.03	7205.67±76.03	7215.00±74.04	7202.00±75.69	7202.67±74.04
15	7198.00±75.03	7220.00±76.66	7212.00±75.57	7223.00±77.94	7212.67±73.61	7213.13±76.64
30	7205.33±76.43	7239.00±76.56	7224.67±76.03	7242.67±77.21	7231.00±74.51	7228.53±76.38
45	7211.00±76.11	7255.00±75.13	7239.00±77.51	7258.33±75.29	7247.00±76.00	7242.07±75.94
60	7216.67±75.13	7261.00±75.03	7249.67±77.00	7264.00±73.79	7253.00±74.73	7248.87±75.17
Means	7204.47±76.79	7234.87±75.85	7226.20±76.80	7240.60±76.86	7229.13±75.96	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 45: Effect of zinc fortified apricots on b-lymphocytes of rabbits (%)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	45.54±3.06	45.23±3.03	45.48±3.06	45.76±3.54	45.28±3.12	45.46±3.38c
15	45.62±4.04	46.41±3.13	46.59±3.16	47.02±4.05	46.33±4.03	46.39±3.95b
30	45.66±3.26	46.65±3.46	46.79±3.29	47.38±3.28	47.11±3.41	46.72±3.44ab
45	45.92±3.02	47.73±2.94	46.87±3.13	47.87±3.61	47.16±2.98	47.11±2.87a
60	45.94±3.17	48.32±3.14	47.15±2.49	48.42±2.51	47.23±2.18	47.41±2.34a
Means	45.74±3.46c	46.87±3.12ab	46.58±3.13b	47.29±3.15a	46.62±3.47ab	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

inflammatory response. Subsequently, Zn deficiency induces apoptosis in β cells and impairs antibodies & lymphocytes action in the body (Santos-Valente *et al.,* 2012).

Similarly, zinc in the blood of experimental rabbits was measured as 11.2, 1.2 & 0.17 mg/L for serum, red blood cells and whole blood (Jenner *et al.,* 2007; Rashtchizadeh *et al.,* 2008). The anti-atherogenic effect of Zn supplementation was assessed on New Zealand white rabbits that were divided in to three groups on the basis of diet *i.e.* control, high cholesterol diet (HCD) and Zn+HCD. The data showed reduction by 20.9, 56.9, 18.89 and 2.2% in total cholesterol, triglyceride, LDL and WBC of Zn treated group, respectively.

Later, an increasing trend in RBC and platelets by 33.88 and 11.4% was observed in Zn+HCD treated groups, correspondingly (Ren *et al.,* 2006). The data showed an increasing trend in hemoglobin by 25.5% in Zn+HCD treated groups.

Earlier, Evans and Halliwell (2001) recorded lower concentration of Zn in serum, erythrocytes and glutathione in zinc deprived rats. The instant results are in agreement with the work of Ozkan *et al.* (2012), found hemoglobin in the range of 147-208g/L in male and 108-175g/L in female rabbits. Similarly, Süvegová *et al.* (2004) noticed that hemoglobin of experimental rabbits was varied from 7.90 to 14.10g/dL.

One of the scientists groups, El Hendy *et al*. (2001) recorded the effects of different zinc levels *i.e.* (38mg/kg diet, control) and the concentration that developed hypozincemia (19mg/kg diet, 1/2 of control & 3.8mg/kg diet, 1/10 of control) in male and female rats. The hematological parameters were significantly effected by zinc insufficiency included hemoglobin, packed cell volume and total erythrocyte count. Similarly, serum concentrations of globulin, glucose, total protein and high density lipoprotein decreased in a dose-dependent manner. Whilst, total leukocyte count, serum albumin, cholesterol, triglycerides, total lipids and low density lipoprotein were increased. Their results delineated that zinc deficiency has depressing effect on body growth, organ weights and hematological traits.

Table 46: Effect of zinc fortified apricots on monocytes of rabbits (%)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	1.72±0.17	1.75±0.11	1.75±0.12	1.75±0.10	1.74±0.09	1.74±0.13
15	1.74±0.15	1.77±0.14	1.76±0.11	1.76±0.16	1.75±0.13	1.75±0.14
30	1.73±0.06	1.79±0.08	1.78±0.04	1.79±0.02	1.78±0.03	1.77±0.05
45	1.74±0.08	1.81±0.12	1.76±0.17	1.82±0.16	1.79±0.13	1.78±0.15
60	1.75±0.06	1.81±0.01	1.77±0.04	1.83±0.02	1.79±0.03	1.79±0.03
Means	1.74±0.09	1.78±0.011	1.76±0.13	1.79±0.14	1.77±0.10	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 47: Effect of zinc fortified apricots on eosinophils of rabbits (%)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	1.02±0.10	1.04±0.09	1.04±0.02	1.04±0.01	1.03±0.06	1.03±0.07
15	1.04±0.01	1.06±0.03	1.05±0.04	1.08±0.08	1.05±0.06	1.06±0.05
30	1.03±0.03	1.05±0.04	1.03±0.01	1.05±0.09	1.04±0.02	1.04±0.04
45	1.04±0.08	1.07±0.07	1.06±0.04	1.09±0.02	1.06±0.05	1.06±0.06
60	1.05±0.01	1.08±0.03	1.05±0.02	1.10±0.09	1.07±0.10	1.07±0.03
Means	1.03±0.07	1.06±0.06	1.04±0.04	1.07±0.08	1.05±0.06	

G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 48: Effect of zinc fortified apricots on neutrophils of rabbits (%)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	36.66±2.02	37.78±2.15	37.54±2.34	37.68±2.67	37.53±2.58	37.44±2.55b
15	36.69±2.76	37.81±2.16	37.62±2.84	37.79±2.17	37.64±1.82	37.51±2.23b
30	36.83±2.24	38.08±2.19	38.01±2.41	38.46±2.91	38.08±2.48	37.89±2.37ab
45	36.87±1.37	38.67±1.29	38.28±1.20	38.83±1.46	38.47±1.73	38.22±1.64ab
60	37.77±1.10	38.99±2.04	39.70±1.05	39.08±2.20	38.73±3.06	38.65±2.16a
Means	36.96±2.15b	38.27±2.11a	38.03±2.14ab	38.37±2.09a	38.09±2.23ab	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

Table 49: Effect of zinc fortified apricots on hemoglobin of rabbits (g/dL)

Days	Groups					Means
	G_0	G_1	G_2	G_3	G_4	
0	12.24±0.32	12.73±0.53	12.55±0.44	12.81±0.33	12.68±0.34	12.60±0.41b
15	12.26±0.49	12.83±0.64	12.57±0.72	12.90±0.41	12.71±0.44	12.66±0.50b
30	12.31±0.38	12.91±0.31	12.61±0.23	13.01±0.19	12.77±0.74	12.72±0.39ab
45	12.35±0.26	13.07±0.16	12.64±0.64	13.26±0.71	12.83±0.28	12.83±0.43a
60	12.45±0.69	13.17±0.72	12.67±0.86	13.34±0.77	12.89±0.64	12.90±0.71a
Means	12.32±0.36b	12.94±0.47a	12.61±0.51ab	13.07±0.53a	12.78±0.45ab	

Means sharing the same letter in a row are not significantly different
G_0: Control (without fortificant)
G_1: Apricot containing 2% alginate coating having 50 ppm $ZnSO_4$
G_2: Apricot containing 2% alginate coating having 50 ppm $ZnCl_2$
G_3: Apricot containing 2% chitosan coating having 50 ppm $ZnSO_4$
G_4: Apricot containing 2% chitosan coating having 50 ppm $ZnCl_2$

CHAPTER 5

SUMMARY

Globally, the nutritionists are focusing their attention towards food security & safety, malnutrition and diet-health linkages. Every nutrient has immense importance for the body and plays a specific role for normal functioning. Various segments of the developing world consume food that is deficient in micronutrients ultimately leads to numerous health problems. For the achievement of one of the Millennium Development Goals a strong political assurance, nutritional guidelines and awareness programs are required to reduce morbidity and mortality in mother and child due to micronutrients deficiencies. The zinc deficiency is appeared as one of the alarming issues amongst the poor nations due to various socio-economic factors like unavailability of ample food, unhealthy dietary practices and lack of nutrition knowledge.

Zinc deficiency symptoms include growth retardation, anemia, hypogonadism, hepatosplenomegaly, geophagia etc. The affected patients have dysfunctions of central nervous, immune, reproductive, epidermal, and skeletal systems. Presently, Pakistan is facing the problem of micronutrients imbalance in a wide segment of population. To overcome zinc deficiency in Pakistani peoples, different fortification strategies are being in practice as 54% pregnant women, 37.2% babies and 62% of peoples are zinc deficient.

Various nutritional strategies are emerging to combat these micronutrients discrepancies. Amongst, edible coatings have potential to protect fresh agriculture produce from harvesting to users consumption with improved keeping quality. Edible coatings are used as a carrier for fortification and enrichment of vitamins and minerals. These novel coatings are perceived as an efficient medium to add nutritional supplements to meet the daily dietary requirements of individuals. The advantages of edible films over conventional packaging materials are improved structural properties, ability to incorporate pigments, flavoring & food additives and maintenance of quality during shipping & storage thus extend the shelf life. These edible coatings are consumer friendly, cost effective and versatile in their application. Nevertheless, alginate and gellan biopolymers are considered much appropriate due to their ability to form strong gels with unique colloidal properties.

The instant research is an effort to overcome the alarming situation of micronutrients deficiency among the population as well as to decrease the postharvest losses with special reference to apricots. Purposely, different zinc salts (zinc sulphate and zinc chloride) were applied on apricots with the help of chitosan and alginate coatings. Afterwards, zinc fortified apricots were placed in controlled climate chamber for checking their storage behavior and accordingly performed different sort of tests. During the whole storage period, the environmental conditions were maintained to attain precise results.

For the purpose, zinc fortified alginate and chitosan based coatings (1 and 2% each) were prepared using various levels of zinc sulfate ($ZnSO_4$) and zinc chloride ($ZnCl_2$) as fortificants. The alginate film forming solution was prepared by dissolving alginate powder (2g) in 100mL of distilled water and heated at 70°C with continuous stirring until the clear solution was formed. The citric acid was added in the coating formulation to avoid enzymatic browning. Glycerol was added as plasticizer (1.5g/100mL) in alginate solution. Film forming solution was emulsified with sunflower oil (0.025g/100mL) followed by the addition of N-acetyl L-cysteine (1g/100mL) and calcium chloride (2g/100mL water) for cross linking of carbohydrate polymers. Chitosan based formulation was prepared by dissolving chitosan (crab shell chitosan) in distilled water (100mL) with the addition of glacial acetic acid (1g) to dissolve it. After the development of coatings, various lots of zinc fortified edible coated apricots were made. The analyses was performed by assessing the physico-chemical and sensory characteristics.

Apricots were subjected to different quality traits assessment and revealed crude fat, crude protein, crude fiber, ash and NFE as 2.17±0.16, 6.91±0.29, 11.19±0.74, 9.15±0.35 and 70.57±2.35%, respectively whereas, minerals like sodium, calcium, potassium, iron, cobalt, zinc and manganese were 14.28±0.63, 112.58±3.19, 1322.06±35.21, 7.35±0.58, 0.17±0.01, 1.61±0.03 and 1.72±0.05mg/100g in respective manner.

Mean squares regarding weight loss, pH, titratable acidity, total soluble solids and moisture loss of zinc fortified apricots were significantly affected due to treatments and storage.

During storage, there was a significant decrease in weight varied from 57.56±3.42 at initiation to 54.63±3.54, 52.12±3.45, 49.45±3.52 and 47.54±3.39g at 15^{th}, 30^{th}, 45^{th} and 60^{th}

day, respectively. Amongst treatments, the maximum reduction in weight was reported in T_0 (control) 49.03±3.51g however, the minimum loss was in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 55.05±2.53g followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 53.36±3.16g, T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 53.35±3.41g and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 53.35±3.18g, correspondingly.

The results demonstrated that pH was significantly increased during storage ranged from 4.18±0.13 at beginning to 4.58±0.37 at 60th day. Likewise, the maximum rise of pH was observed in T_0 4.76±0.39 though the minimum in T_{12} 4.18±0.31 however, the value for this trait in T_{16}, T_4 and T_8 were 4.22±0.34, 4.25±0.32 and 4.27±0.24, respectively. Conversely, alginate and chitosan coatings have significant decreasing effect on titratable acidity that varied from 0.27±0.02 at start to 0.19±0.01 (% malic acid) at the end of storage. Likewise, the maximum titratable acidity loss was noticed in T_0 (0.18±0.02) whilst the minimum as 0.27±0.02, 0.26±0.02, 0.25±0.02 and 0.24±0.02 (% malic acid) in T_{12}, T_{16}, T_4 and T_8, accordingly.

It is obvious from the means that there was a significant increase in total soluble solids ranged from 12.24±0.41 at initiation to 12.55±0.26 (15thday), 12.72±0.46 (30th day), 12.83±0.59 (45th day) and 13.02±0.66^{o}Brix (60th day). Similarly amongst treatments, the lowest value of total soluble solids was observed in T_0 (11.95±0.56^{o}Brix) whilst, the maximum in T_{12} (13.39±0.64^{o}Brix) followed by T_{16} (12.98±0.46^{o}Brix), T_4 (13.94±0.32^{o}Brix) and T_8 (12.89±0.26^{o}Brix).

For the period of storage, there was a momentous rise in moisture loss that differed from 5.05±0.47 at 15th day to 9.40±0.83, 14.05±0.78 and 17.39±1.57g at 30th, 45th and 60th day, respectively. Whereas, the maximum moisture loss amongst the treatments was reported in T_0 (control) 21.48±1.88 whilst the minimum in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$), T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) as 8.27±0.74, 8.67±0.62, 8.87±0.57 and 8.34±0.39g, correspondingly.

The mean squares of ascorbic acid, citric acid, total sugars, color, texture and mold count of zinc fortified edible coated apricots were effected momentously due to treatments and storage.

It is apparent from the results that there was a gradual decline in ascorbic acid contents from 56.10±4.17mg/100g at beginning to 45.58±3.72mg/100g at the end of study. In treatments, the highest loss in ascorbic acid was observed in T_0 as 40.35±2.73mg/100g whereas the lowest in T_{12}, T_{16}, T_4 and T_8 as 53.71±3.51, 53.40±3.62, 53.31±3.37 and 53.29±3.79mg/100g, respectively. The results delineated that there was a progressive reduction in citric acid contents of fortified apricot treated with various levels of alginate and chitosan coatings that varied from 470.63±12.65mg/100g at initiation to 411.08±14.38mg/100g at final day of storage period. Likewise amongst treatments, the maximum turn down in citric acid was observed in T_0 422.28±11.60mg/100g whilst the minimum 468.83±13.59, 465.23±11.12, 461.51±12.00 and 457.81±12.62mg/100g in T_{12}, T_{16}, T_4 and T_8, correspondingly.

There was a gradual rise in total sugars of fortified edible coated apricots; 65.48±3.87 at beginning to 66.98±4.55 (15^{th} day), 68.62±3.89 (30^{th} day), 69.78±3.53 (45^{th} day) and 70.62±4.53mg/100g (60^{th} day). The treatments showed the lowest sugars in T_0 (59.61±3.23mg/100g) while the highest in T_{12} (71.49±4.54mg/100g) chased by T_{16} (71.06±3.71mg/100g), T_4 (70.62±4.02mg/100g) and T_8 (70.33±4.88mg/100g).

There was a substantial decline in color scores ranged from 119.86±6.69 at initiation to 117.56±5.92, 109.15±4.96, 105.46±5.05 and 101.34±4.37ctn at 15^{th}, 30^{th}, 45^{th} and 60^{th} day, respectively. Coating treatments have direct impact on color and recorded maximum reduction in T_0 (control) 98.61±5.45ctn whereas the minimum in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 118.42±5.28ctn followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 116.26±5.44ctn, T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 115.12±5.26ctn and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 115.07±5.33ctn, correspondingly. Similarly, there was a momentous decline in texture of coated apricots, ranged from 121.46±6.68g at start to 103.61±3.82g at 60^{th} day. Likewise, the maximum decline in texture was observed in T_0 (102.36±4.55g)

whilst the minimum in T_{12}, T_{16}, T_4 and T_8 as 120.11±5.29g, 117.51±5.23g, 115.87±4.88g and 114.59±5.40g, respectively.

Edible coatings put forth considerable effect for controlling the mold growth. During storage, there was a mild uplift in mold growth ranged from 0.29±0.01 at start to 27.12±0.71cfu at final day of storage. Similarly within treatments, maximum mold growth was observed in T_0 as 30.13±1.37 whilst, the minimum as 8.00±0.21, 8.53±0.67, 8.87±0.53 and 9.27±0.49cfu in T_{12}, T_{16}, T_4 and T_8, correspondingly.

The results depicted that there was a non-consistent decrease in zinc contents of edible coated zinc fortified apricots during storage which ranged from 3.53±0.21 at beginning to 3.51±0.18 (15^{th} day), 3.52±0.16 (30^{th} day), 3.49±0.18 (45^{th} day) and 3.50±0.19mg/100g (60^{th} day). Whereas amongst the treatments, the minimum zinc contents was noticed in T_0 (control) 0.26±0.02mg/100g however, the maximum in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 4.83±0.24mg/100g trailed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 4.82±0.21mg/100g, T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 4.78±0.23mg/100g and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 4.77±0.12mg/100g, respectively.

Mean squares regarding sensory parameters showed significant variations in all sensory traits. It has been observed that the maximum scores were assigned at the initiation of the trial. With the ingress in storage, there was decline in the scores. Amongst treatments, lowest sensory scores was reported in T_0 (control) while the highest in T_{12} (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) followed by T_{16} (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$), T_4 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) and T_8 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$), correspondingly. The current research enlightened that chitosan coating along with $ZnSO_4$ was efficient for preserving the apricots, ultimately enhancing the shelf life by maintaining the keeping quality of the fruit.

On the basis of physico-chemical characteristics, sensory behavior, HPLC characterization (organic acids & sugars) and zinc content; four best treatments G_1 (apricots coated with 2% alginate with 50 ppm $ZnSO_4$), G_2 (apricots coated with 2% alginate with 50 ppm $ZnCl_2$), G_3

(apricots coated with 2% chitosan with 50 ppm $ZnSO_4$) and G_4 (apricots coated with 2% chitosan with 50 ppm $ZnCl_2$ along with the control) were selected for bio-evaluation trial. For the purpose, rabbits were housed in the Animal Room of National Institute of Food Science and Technology. The experimental animals were acclimatized on basal diet for the period of seven days under controlled climate conditions. The temperature (23±2 ºC) and relative humidity (55±5%) was maintained throughout the experimental period with 12 hr light-dark period. At the initiation of trial, some rabbits were sacrificed to establish a baseline trend. During 56 days study span, the rabbits were randomly divided into five groups, ten in each and provided with selected uncoated (control) and zinc fortified apricots (150 g/day/rabbit) along with normal diet. The blood was collected from the overnight fasted rabbits at 0, 15^{th}, 30^{th}, 45^{th} and 60^{th} day of modeling trial. For serum collection, blood samples were subjected to centrifugation. The sera samples were examined for total zinc contents, glucose & insulin levels and serum biochemistry (liver & renal function tests) using respective protocols. The collected organs including liver, kidneys and heart were used for the determination of zinc contents and weighed to calculate organ to body weight ratio. Earlier, collected blood samples were analyzed for hematological parameters with special reference to red and white blood cells indices. The entire biological trial was repeated to draw a conclusive inference.

The results explicated that treatments and time intervals affected the feed & drink intake and body weight gain significantly in all groups. Similarly, mean squares of organs weight of experimental rabbits administered by zinc fortified edible coated apricots demonstrated significant variation due to groups and time intervals.

Zinc fortification was effectual in increasing the sera zinc status of experimental rabbits and results showed highest sera zinc in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 89.71±2.26µg/dL followed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 87.43±2.14µg/dL, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 83.51±2.41µg/dL and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 81.49±2.46µg/dL, respectively however, the lowest 72.56±2.85µg/dL in G_0 (control). The percent increase in serum zinc was 24.63, 20.50, 15.09 and 12.31% in G_3, G_1, G_4 and G_2, respectively as comparison to G_0.

The provision of zinc fortified edible coated apricots was effective in enhancing the organs zinc status of rabbits by deposition in the organs. Mean values of liver zinc indicated maximum level in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 23.97±1.41µg/g trailed by G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$) 23.71±1.15µg/g, G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) 23.53±1.28µg/g and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) 23.36±1.45µg/g while minimum in G_0 (control) as 22.42±1.36µg/g. Results showed that liver zinc was increased by 6.91, 5.72, 4.95 and 4.19% in G_3, G_1, G_4 and G_2, respectively compared to control. Whilst in heart, zinc deposition as 17.59±0.55, 17.46±0.54, 17.22±0.42 and 17.18±0.46µg/g was observed in G_3, G_1, G_4 and G_2 whereas, minimum in G_0 as 17.11±0.50µg/g. In case of heart, zinc was increased by 2.84, 2.08, 0.66 and 0.43% in G_3, G_1, G_4 and G_2 as contrast to G_0. Likewise in case of kidneys, means indicated maximum zinc as 25.18±1.23, 24.97±1.56, 24.65±1.11 and 24.40±1.30µg/g in G_3, G_1, G_4 and G_2 though, lowest as 23.53±1.47µg/g in G_0. Kidney zinc was also influenced by the fortification practices and noticed increased was 7.03 (G_3) followed by 6.12 (G_1), 4.76 (G_4) and 3.68% (G_2) as association to control.

Zinc fortified apricots was capable of decreasing the serum glucose and increasing the insulin level of experimental rabbits as manifested from the mean values. Notable decrease of serum glucose was observed in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) 111.79±4.48mg/dL whereas the values for this feature in G_1 (apricot containing 2% alginate coating with 50 ppm $ZnSO_4$), G_4 (apricot containing 2% chitosan coating with 50 ppm $ZnCl_2$) and G_2 (apricot containing 2% alginate coating with 50 ppm $ZnCl_2$) were 112.42±4.57, 114.80±4.78 and 115.10±4.78mg/dL, respectively whereas, highest as 117.91±4.23mg/dL in G_0 (control). The percent diminish of serum glucose was 5.19, 4.66, 2.63 and 2.38% in G_3, G_1, G_4 and G_2, accordingly as comparison to G_0. On the other hand, maximum value of serum insulin was reported in G_3, G_1, G_4 and G_2 as 9.38±0.51, 9.34±0.53, 9.27±0.49 and 9.23±0.52µU/mL, correspondingly however, minimum in G_0 as 9.01±0.50µU/mL. Likewise, percent elevation in insulin was noticed in G_3, G_1, G_4 and G_2 as 4.31, 3.87, 3.08 and 2.54% as contrast to control.

Zinc was affected non-significantly on kidney and renal functioning of experimental rabbits fed on fortified edible coated apricots however, their values remained within normal range. The maximum values of AST, ALT, ALP and total bilirubin levels were recorded as 26.79±1.57IU/L, 25.69±1.33IU/L, 15.07±0.43IU/L and 0.42±0.03mg/dL in G_3 (apricot containing 2% chitosan with 50 ppm $ZnSO_4$) while minimum in G_0 (control) as 27.08±1.88IU/L, 26.08±1.48IU/L, 15.58±0.76IU/L and 0.45±0.04mg/dL, respectively. Similarly, the administered zinc fortified edible coated apricots slightly suppressed the serum urea and creatinine level of rabbits. The results exhibited serum urea and creatinine as 25.30±1.44mg/dL and 1.04±0.05mg/L in G_3 whereas 25.75±1.51mg/dL and 1.08±0.07mg/L in G_0, correspondingly.

The hematological profile depicted that red and white blood cell indices were within range due to treatments and time intervals. The results showed maximum value of T-lymphocytes, leukocytes, b-lymphocytes, monocytes, eosinophils, neutrophils and hemoglobin in G_3 (apricot containing 2% chitosan coating with 50 ppm $ZnSO_4$) as 22.34±1.29%, 7240.60±76.86cu mm, 47.29±3.15%, 1.79±0.14%, 1.07±0.08%, 38.37±2.09% and 13.07±0.53g/dL while minimum in G_0 (control) as 21.22±1.13%, 7204.47±76.79cu mm, 45.74±3.46%, 1.74±0.09%, 1.03±0.07%, 36.96±2.15% and 12.32±0.36g/dL, respectively.

It has been observed that zinc has potential to ameliorate numerous infirmities due to its diverse role in physiological functioning's. The present study showed that edible coating formulations are the economical way to incorporate micronutrients like zinc and other sort of additives to the food commodities. Chitosan based coatings were more effective to preserve the keeping quality of food in comparison to alginate coatings due to their better functional behavior. Similarly, zinc fortified edible coated apricots were found effective to increase serum and organs zinc contents of rabbits. Furthermore, zinc was also helpful to enhance the overall insulin status of tested animals. The $ZnSO_4$ performed better with reference to bioavailability. Decisively, zinc fortified edible coated apricots are effectual to improve serum and organs zinc level thereby have potential to use as a tool in diet based therapy.

RECOMMENDATIONS

- Edible coating should be encouraged as one of the emerging food preservation and fortification techniques owing to growing consumer interest towards healthy foods
- Nutritionist should focus on appropriate strategies to ameliorate micronutrient deficiencies with special reference to zinc
- Concept of designer food has to be promoted to combat health related discrepancies
- Fortification should be launched at mass level through edible film based fruit to alleviate hidden hunger in the vulnerable segments
- $ZnSO_4$ (50 ppm) along with chitosan (2%) should be considered for further fortification purposes to alleviate hypozincemia
- Nutritional support programs ought to be launched to manage metabolic syndromes and create awareness among the masses
- Industry and academia should collaborate to tailor research projects related to public health nutrition
- Contemporary nutritional guidelines should be adopted to improve the health status of target population
- Community based trials and cohort studies should be conducted for further diligence regarding the concern

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APPENDIX

Performa for sensory evaluation of zinc fortified edible coated apricots

Name of the judge…… Date…….....

Parameter	Treatments																
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}
Color																	
Flavor																	
Taste																	
Firmness																	
Overall acceptability																	

Signature……..

INSTRUCTIONS

Eat zinc fortified edible coated apricots and score for color, flavor, taste, firmness and overall acceptability using the following 9-point Hedonic Scale:

Extremely poor	1
Very poor	2
Poor	3
Below fair above poor	4
Fair	5
Below good above fair	6
Good	7
Very good	8
Excellent	9

Note:

1. Eat zinc fortified edible coated apricots and score for color, flavor etc.

2. Before proceeding to the next sample, rinse mouth with water.

3. Make inter comparison of the sample and record the score.

4. Don't disturb the order of samples.