BIOEVALUATION OF CABBAGE BASED DESIGNER FOOD TO MODULATE OXIDATIVE STRESS MARKERS

By

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UNIVERSITY OF AGRICULTURE
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2017
DECLARATION

I hereby declare that the work presented in this thesis "Bioevaluation of cabbage based designer food to modulate oxidative stress markers" is my own effort except where other acknowledged and that the thesis is my own composition. No part of the thesis has previously been presented for any other degree. University may take action if information is incorrect at any stage.

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To
The Controller of Examination,
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"We, the Supervisory Committee, certify that the contents and form of thesis submitted by Miss Faiza Ashfaq, Reg. # 2007-ag-1070, have been found satisfactory and recommend that it be processed for evaluation by External Examiner(s) for the award of degree."

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ABBREVIATIONS

Nitrogen Free Extract (NFE)
1, 1-diphenyl-2-picrylhydrazyl (DPPH)
2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); (ABTS)
Ferric Reducing Antioxidant Power (FRAP)
Potassium Ferricyanide Reducing Antioxidant Power (PFRAP)
Aspartate transaminase (AST)
Creatine kinase (CK)
Creatine kinase-MB (CK-MB)
Lactate dehydrogenase (LDH)
Alanine transaminase (ALT)
Alkaline phosphatase (ALP)
γ-glutamyl transferase (γ-GT)
Feed Efficiency Ratio (FER)
Superoxide dismutase (SOD)
Catalase (CAT)
Lipid peroxidation (MDA; Malondialdehyde)
Atherogenic index (AI)
Cardiac risk ratio (CRR)
HTR (HDL-cholesterol to total cholesterol ratio)
Anti-atherogenic index (AAI)
Low density lipoprotein-cholesterol (LDL)
Very low density lipoprotein-cholesterol (VLDL)
Non- High density lipoprotein-cholesterol (non-HDL)
Cholesterol (CHOL)
Triacylglycerol (TAG)
High density lipoprotein-cholesterol (HDL)
hepatosomatic index (HIS)
Nephrosomatic index (NSI)
Cardiosomatic index (CSI)
ABSTRACT
Nowadays, plants bioactive moieties are gaining attention amongst the masses to mitigate lifestyle related dysfunctions owing to their safe nature and functional properties. Considering phytochemistry and cost-effectiveness of cabbage, the current project was designed to probe the antioxidant capacity of locally grown green and red cabbage with special emphasis on modulation of oxidative stress biomarkers in response to hypercholesterolemic diet. Purposely, green and red cabbage samples were analyzed for compositional analyses that depicted high proportion of potassium and vitamin C in both samples. The red cabbage showed higher amount of total polyphenols & flavonoids (224.37±6.96 & 219.15±10.30 mg/100g F.W.) than green cabbage (58.41±3.01 & 34.04±1.06 mg/100g F.W.) along with the existence of anthocyanins (69.86±4.12 mg/100g F.W.) in red cabbage only. Comparative HPLC analysis regarding antioxidant moieties showed significant proportion of kempferol (171.10±5.99 mg/100g F.W.) and vitamin C (139.07±2.23 mg/100g F.W.) in red cabbage however, vitamin C (121.46±3.28 mg/100g F.W.) was found as the major antioxidant in green cabbage. The red cabbage depicted higher free radical quenching and reducing ability in contrast to green cabbage using DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS [2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)], FRAP (ferric reducing antioxidant power) and PFRAP (potassium ferricyanide reducing antioxidant power) reagents. The corresponding assays were ranged from 31.22±1.65 to 87.79±3.69%, 1.51±0.07 to 6.04±0.21 μM Trolox/g F.W., 0.95±0.04 to 1.16±0.04 μM Fe3+/g F.W., 41.16±2.10 to 59.32±2.14% and 48.03±1.68 to 63.45±3.05%. In the product development phase, four types of croquettes based on green and red cabbage leaves & their respective aqueous extracts along with control were stored under frozen conditions and analyzed at three different intervals; 0, 15 & 30 days, employing two types of cooking procedures; baking and frying. The resultant prototypes were assessed for physicochemical analyses (caloric count, color, texture, water activity & antioxidant assays) as well as sensory response. The current study portrayed that designer croquettes remained freezer friendly for one month without employing any preservative. Comparative assessment of cooking methods inferred higher antioxidant potential and taste scores for fried prototypes. Based on antioxidant assays and overall acceptability, red cabbage and its respective aqueous extract were screened for biological evaluation. In animal study (12 weeks), male white New Zealand rabbits were divided into six groups; three groups under normal dietary pattern; normal diet, normal diet+cabbage leaves & normal diet+cabbage extract and next three under hypercholesterolemic dietary regimen; hypercholesterolemic diet, hypercholesterolemic diet+cabbage leaves & high calorie+aqueous cabbage extract. At termination, overnight fasted rabbits were sacrificed to assess serum lipid profile. The percent reduction in serum cholesterol and LDL by red cabbage & its extract was reported up to 10.79 & 15.19 and 12.24 & 18.09 in hypercholesterolemic rabbits, respectively. Furthermore, serum specific and tissues (liver, heart & kidney) oxidative stress biomarkers alongside, their somatic index and histopathology were studied. The red cabbage supplementation suppressed the leakage of liver functioning enzymes in sera up to 15.63% (ALT), 13.88% (ALP), 12.96% (γ-GT) and 10.77% (total bilirubin) besides reduction in hepatic lipid peroxidation up to 29.60% thus improved endogenous antioxidant enzymes. Likewise, red cabbage showed protection against cardiac oxidative stress. Additionally, the red cabbage extract lowered renal MDA (lipid peroxidation) up to 24.07% in oxidative stressed rabbits that ultimately restored renal SOD (11.38%) and CAT (16.72%), accordingly. The hepatosomatic index (HSI) and nephrosomatic index (NSI) expounded a significant impact of treatments on hypercholesterolemic diet fed rabbits except caridiosomatic index (CSI). In hypercholesterolemic rabbits, mild degree of fibrotic and necrotic changes in hepatic parenchyma were rectified by red cabbage supplementation, whereas red cabbage extract based diet showed slower rate of amelioration. Likewise, mild degree of necrotic changes and congestion in cardiac fenestrations were modulated by red cabbage feeding. Moreover, severe necrotic & pyknotic alterations in renal parenchyma along with mild to moderate degree of congestion in hypercholesterolemic rabbits were effectively reversed via extract supplemented group in contrast to cabbage fed group. In the nutshell, dietary inclusions based on red cabbage has proven restorative against hepatic and cardiac compromised conditions, whereas altered renal fenestrations were also effectively restored by red cabbage extract enriched diet.
CHAPTER 1

INTRODUCTION

The poor dietary choice and contemporary lifestyle has become a challenging health dilemma of 21st century. The nutritional transition towards junk foods has become the foremost reason for dramatic prevalence of obesity, coronary heart disease (CHD) and cancer. These diseases have drawn the global interest of scientists towards diet and disease relationship (Popkin et al., 2012; Assad et al., 2014; Al-Dosari, 2014). In addition, medicinal therapies are associated with numerous side effects hence fail to resolve certain complications in the long run (Assad et al., 2014). Furthermore, there is a rising interest in plant extracts and biologically active ingredients for the development of innovative therapies against various maladies (Banjare and Paul, 2014; Khan et al., 2014). In this arena, numerous pharmaceutical companies have started working on crude plant extracts for hypolipidemic and anti-diabetic formulations (Assad et al., 2014).

Furthermore, advancement in food technology has overcome the challenge of food wastage on one hand but increased the access to empty caloric products on the other side. The rising reliance on fast foods, hydrogenated edible oil and sugar rich foods have begun to dominate the globe ultimately shifted the dietary pattern towards metabolic disorders (Sibai et al. 2010; Popkin et al. 2012). In an elaborate manner, consumption of junk food may lead to augmented generation of Reactive Oxygen Species/Metabolites (ROS/ROM) i.e. liable to attack on biological molecules resulting in redox mediated tissue damage (Burton and Jauniaux, 2011). One of these radicals is superoxide anion that scavenges nitric oxide; a potent vasodilator that participates in the homeostasis of vasculature hence leading to atherosclerosis and cardiac stress (Gaafar et al., 2014). Furthermore, hypercaloric diet suppresses superoxide dismutase (SOD) & catalase (CAT); sensitive enzymatic antioxidants involved in the detoxification of free radicals (superoxide anion & hydrogen peroxide) and lipid peroxides to non-toxic metabolites. Moreover, it decreases non-enzymatic glutathione (GSH); reactive non-protein thiol, performing key role in the co-ordination of innate defense mechanisms ultimately up-regulating lipid peroxidation associated oxidative stress (Bhavani et al., 2014; Ji et al., 2015).
High lipid load in hepatocytes and cardiomyocytes is the prime factor for the induction of hepatotoxicity and cardiotoxicity, respectively (Sankhari et al., 2012; Ji et al., 2015). Globally, cardiovascular incidents are considered as the primary cause of mortality (Khan et al., 2014). On the other side, liver is a primary organ i.e. involved in the regulation of numerous biologic activities including energy generation, nutrient availability, disease protection, etc. It detoxifies toxic exogenous & endogenous substances into less toxic components for excretion. Due to its involvement in the detoxification mechanisms, it is at the highest risk to be affected by free radicals (Morsy et al., 2010). The multiple problems associated with liver are on rise, especially non-alcoholic fatty liver diseases have covered up to 3-30% of the globe. Besides, heart ailments have been faced by 27-48% of the Asian Pacific and 40-58% of the western populations. Furthermore, the deaths rate associated with chronic kidney ailments have reached up to 1.5% of the total, world widely (Kim and Kim, 2017; Rajadurai et al., 2017; Webster et al., 2017).

The scientific fraternity is showing keen interest for the preparation of hepato-, cardio- and renal-protective agents from plant extracts with minimal side-effects due to their similar mode of action as that of medicines (Shen et al., 2009; Waqar and Mahmood, 2010). In this context, application of polyphenols in the preparation of designer foods has become the center of focus to improve quality of life by neutralizing the deleterious effects of dietary fat i.e. responsible to exert oxidative stress in hepatic, cardiac and renal tissues (Al-Dosari, 2014; Ji et al., 2015). These healthy dietary interventions provide sufficient amounts of exogenous antioxidants that not only strengthen the endogenous antioxidants but also improve the overall health status and natural defense against numerous ailments including diabetes, obesity, cardiovascular disorders, insulin resistance, etc. Considering the chemical diversity of several antioxidant molecules, it is believed that some behave synergistically while other work in antagonism. Convergent evidences have indicated that individual polyphenol exert protection against oxidative stress however, this effect is usually minor due to poor bioavailability in plasma and tissues thus synergistic effect of several polyphenols is considered for higher efficiency (Bacchetti et al., 2014; Gaafar et al., 2014).

Designer or functional foods refer to normal or processed foods that confer additional health benefits beyond complementary nutrition. These foods are enriched with plethora of health boosting moieties via nutrification process and consumable as part of regular diet. In China,
3000 varieties of designer foods are currently available as therapeutic foods. Recent researches on nutraceuticals have captured the attention of scientific community in designer foods like broccoli containing isothiocyanates, eggs enriched with Ω-3 fatty acids, grains fortified with minerals & vitamins, drinking yogurt containing probiotics, foods enriched with phytosterols or essential amino acids, etc. From health standpoint, designer foods address adjunctive therapeutic benefits under various physiological and pathological conditions. Beyond nutritional enhancement, these foods have the ability to improve health in addition to reduction in disease severity (Kolodziejczyk et al., 2011; Rajasekaran and Kalaivani, 2013).

Large population survey in China has inversely associated the sufficient intakes of vegetables and legumes with diseases owing to the presence of biologically active ingredients including dietary fiber, phytoceutics and minerals that aid in preventing uncontrolled free radical mechanism (Hussein, 2012; Assad et al., 2014; Ji et al., 2015). Amongst various vegetables, brassica vegetables such as broccoli, cabbage, cauliflower and brussels sprouts contribute positively towards human nutrition and health perspectives. Previously, these ingredients have shown their defensive potential against oncogenic events (Oerlemans et al., 2006; Singh et al., 2007; Bacchetti et al., 2014). Recently, the phenolic compounds of cabbage have gained immense attention to limit LDL oxidation i.e. a major determinant of atherosclerotic events and possess higher reducing power as compared to vitamin C (Ji et al., 2015).

Cabbage (Brassica oleracea L.) is an important crop, belongs to family Brassicaceae or Cruciferae containing abundant proportions of fiber, vitamins, minerals and health boosting compounds. Pakistan is an agricultural economy, cultivating various types of fruits and vegetables. In 2008, approx. 71731 tons of cabbage was cultivated in Pakistan (Akbar et al., 2010). The most familiar varieties of cabbage include green, red, chinese and savoy (Khan et al., 2014). Due to the difference in soil conditions, red cabbage is sometimes having purple color hence also named as purple cabbage. It is a fall/winter crop i.e. native to southern Europe where it is being employed in numerous cuisines. It possesses crunchiness along with sweet & peppery taste however, for optimum organoleptic features; it should be cultivated in cold environments (Draghici et al., 2013; Assad et al., 2014). The antioxidant capacity of cabbage heads is strongly influenced by genome, geographical locations and environment (Gaafar et al., 2014; Aires, 2015).
Antioxidants are regarded as first and second line of defense against oxidative stress mediated dysfunctions (Podsędek, 2007; Aires, 2015). Naturally, numerous coloring nutracetics exist such as anthocyanins, carotenoids, quinones, betalains and chlorophylls (Delgado-Vargas and Paredes-López, 2000). In food application, synthetic colors have been questioned resultantly natural coloring compounds are preferred as public safety concerns (Bakowska-Barczak, 2005). According to the previous review, it has been reported that red cabbage possesses maximum amounts of anthocyanins however, these compounds also exist in black carrot, blackcurrant, grape skin and elderberry but in relatively small proportions (Dyrby et al., 2001; Bakowska-Barczak, 2005). Anthocyanins are potent antioxidants; their effective dose in regular diet may reduce various chronic ailments hence regarded as an attractive alternative in comparison to synthetic dyes (Draghici et al., 2013). Besides anthocyanins, cabbage also possesses some other hypolipidemic components including flavonoids, ascorbic acid and isothiocyanates along with other health protective moieties exist like hydroxycinnamic residues, β-carotenes, lutein, zeaxanthin, etc. (Khan et al., 2014; Assad et al., 2014; Al-Dosari, 2014).

Commonly, cruciferous vegetables are consumed as salad (Al-Dosari, 2014). Previous reports have confirmed the positive heath attributes of cabbage as salads, juiced or steamed. The ascorbic acid in cabbage juice and polysaccharides in cabbage leaves has been associated in the management of heart diseases, hypertension and immunity disorders (Priya, 2012). Furthermore, cardioprotective effect of brassica plants is related to ascorbate, α-tocopherol and carotenoids (Singh et al., 2007). In Arabic folkloric practices, cabbage juice is considered effective to protect against hyperlipidemia, obesity, stomach ulcer, liver cirrhosis, obstructive jaundice, hepatitis, renal stress and tachycardia. The cabbage juice possesses agents involve in detoxification mechanisms therefore ameliorative against hepatic stress (Al-Dosari, 2014).

In restaurants or at domestic level, vegetables are normally processed considering the taste regardless of nutritional value and antioxidant potential. In this context, previous scientists have developed broccoli based bars and apple juice enriched with red cabbage extract (Barakat and Rohn, 2014; Radziejewska-Kubzdel and Biegańska-Marecik, 2015). The less eating preference for veganism inspite of health and cost-effective relevance has attracted the attention of food researchers to focus on the concept of designer vegan supplemented meat products to counterbalance the negativities associated with over-consumption of conventional
meat based products. Generally, consumer presumed unprocessed foods relatively healthier than processed. The positivity of processed foods is linked with shelf-life extension or activation of some health boosting compounds (Barakat and Rohn, 2014). Furthermore, for food application, the scientific fraternity seems curious in searching the novel natural antioxidants in order to avoid injudicious incorporation of synthetic equivalents (Kumar et al., 2013; Malav et al., 2015).

Though, previous researchers investigated anthocyanins from the extracts of cruciferous vegetables but the biological impact exerted by cabbage as a whole has not been focused. Furthermore, no systematic study has been carried out so far on Pakistani, locally available green and red cabbage. The interest to explore these aspects is supported by previous studies, that highlighted rich phytochemistry and ameliorative potential of extract of cruciferous vegetables against lifestyle related dysfunctions. Considering the aforementioned facts, the present study was designed to assess the biological proficiency of cabbage on the biomarkers of hepatic, cardiac and renal oxidative stress. The objectives of the current study are herein;

**Objectives:**

- Analyzing composition and quantifying phytochemistry plus antioxidant activity of green and red cabbage
- Evaluating antioxidant potential and sensory appeal of cabbage and its aqueous extract based designer croquettes
- Bioefficacy assessment of cabbage and its aqueous extract against hypercholesterolemic diet induced oxidative stressed rabbits
CHAPTER 2

REVIEW OF LITERATURE

Currently, the oxidant-antioxidant status of the junk food seekers is facing an imbalance in such a way that free radicals are dominating ultimately exhausting the endogenous stores of free radical trapping agents. This condition is further aggravated by the sedentariness resultantly compromising various body organs leading to non-alcoholic fatty liver disease, heart complications and chronic kidney diseases. The prevailing epidemiological shift has increased the consumer curiosity as a response, they are switching to functional or designer foods that not only enhance endogenous antioxidant status but also fight against diseases of modern life. In this regard, cabbage owing to its rich nutritive value and phytochemistry based on anthocyanins, phenolic acids & flavonoids along with hypocaloric nature and economic accessibility is positively linked to heart, liver and kidney health. The literature of the current investigation is focused under following headings:

2.1. Consumer perception for functional/designer foods
   2.1.1. Health-conscious consumers
   2.1.2. Functional/designer food purchasers
   2.1.3. Consumers survey regarding functional/designer foods
   2.1.4. Health related claims and consumer response

2.2. Functional/designer foods
   2.2.1. Historical perspective
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2.5. Impact of processing on antioxidant capacity of vegetables
2.6. Cabbage based designer products
2.7. Epidemiological transition
2.7.1. Translated animal model for metabolic syndromes; Rabbits and rats
2.7.2. Health protective moieties of cabbage
2.7.3. Efficacy of cabbage extracts against oxidative stress biomarkers

2.1. Consumer perception for functional/designer foods

The increasing demand for functional/designer foods is due to the escalating health care cost and desire of older people for optimum life quality in later years. The increase in consumer awareness and focus of health care professionals towards fruits & vegetables for obtaining bioactive ingredients & antioxidant minerals has accelerated the growth of functional ingredients in the marketplace. The scientific discoveries are attracting the interest of food consumers for the attainment of balanced calorific content in addition to health & nutritional value hence introducing the concept of functional/designer foods (Day et al., 2009; Bigliardi and Galati, 2013). The market for these foods is growing and as innovative products are launching continuously, the competition is becoming more intense. In this regard, enrichment of healthy ingredients in non-healthy versions is considered more justified rather enrichment of those products i.e. normally perceived healthy per se. The positive response is achieved when enrichment is done with such functional ingredient i.e. inherent to the product. Furthermore, consumer willingness to try or like the new product depends on carrier or basic ingredient compatibility for that moiety (Ares and Ga´mbaro, 2007).

2.1.1. Health-conscious consumers

There are two groups of consumers who are taking health seriously: (1) Health active; those believing in good health when grow older and concerned about family nutrition. As a response, they consume plenty of fruits & vegetables, take medication only if necessary and exercise twice a week. (2) Health aware; over age 18, they are considering health as the former group but do not exercise regularly. Additionally, these groups underscore their desire for
nutraceuticals or active ingredients from natural plants despite extracted moieties or laboratory sourced synthetic substitutes (Childs, 1997).

2.1.2. Functional/designer food purchasers

In the selection of functional foods, women and middle aged or older people (45 to 55 years) tend to be a little more health oriented as compared to young and men. Multivariate analyses have emphasized that women are more concerned to health due to their heightened responsibility being dominant role in food purchasing and making. Likewise, the reason behind why healthy food is attracting middle aged or older people is due to their appearing disease symptoms or high purchasing potential like in the US. Moreover, claims that emphasizes on improved performance are of the highest interest for men especially breakfast cereals however, claims like constipation prevention & low caloric content are more attracted by the female. On the other hand, youngsters (18 to 34 years) prefer high energy claims. Moreover, consumer’s food choice is changing; they are more likely to accept a food i.e. well known to them than experimenting something new. In this context, ingredients with well-acknowledged health perspectives such as vitamin C, n-3, Fe & Ca are gaining preference over practically unknown moieties such as selenium, xylitol, probiotics, oligosaccharides & phytoestrogens. A few consumers are willing to accept a functional food i.e. inferior in taste this is because general public always evaluate a functional food first as food and its functional attributes could not outweigh its sensory perspectives (Bogue and Ryan, 2000; Bech-Larsen and Scholderer, 2007; Bigliardi and Galati, 2013).

2.1.3. Consumers survey regarding functional/designer foods

In a survey on Irish consumers, 90% of the individuals of age group 35 to 44 responded that they are well aware of the concept of functional foods. Additionally, it was surveyed that while purchasing a functional food, around 47% of the consumers look at health claim, whereas 89% of the individuals look for an expiry date and 72% for price followed by list of ingredients. Price and cooking instructions were more attracted by men or age group 18 to 24 years while 70% female & older were more interested in nutritional information, ingredient list and manufacture name & origin. Furthermore, 63% of the consumers were interested in purchasing functional drinks over pills and those interested in pills were mostly male. However, 69% of the consumers were willing to pay a high cost for functional/designer foods, whereas many
others believe to consume fruits & vegetables over functional/designer foods (Bogue and Ryan, 2000).

2.1.4. Health related claims and consumer response

For legislative purpose, there are two physiologically oriented food claims; (1) enhanced function; health promotion focus e.g. cognitive performance (2) reduced disease risk; specific disease or associated complication prevention focus e.g. cardiac ailments. Amongst the two, enhanced functional claims evoke positive information in memory hence more appealing to consumers. Likewise, for food industry, the enhanced functional health claims have more persuasive impact due to their promotional focus. On the other hand, disease risk reduction claims activate negative perception of the general public because consumer considers the specifically designed foods to confront a particular illness hence finds it more appealing if the relevant illness already exists. In fact, these claims provide an opportunity to avoid disease incidence to which they might fall victim to or from complicating an already existing undesirable health condition (Roberfroid, 2002; Van Kleef et al., 2005; Siroé et al., 2008).

Nowadays, consumers are reducing fat, sodium and cholesterol content in their diets along with adoption of regular exercise for optimal fitness. They are discouraging the concept of extreme dieting or slimming and replacing it with a balanced and healthy lifestyle. They accept positive health claims like Ca build strong bones readily in contrast to negative messages like Ca prevent osteoporosis. Previous surveys have shown that majority of the USA adults are unaware of functional/designer foods, whereas 95% of them believe on diet-health linkages such as oat bran to suppress heart diseases & colorectal cancer, milk to down-regulate osteoporosis and cranberry juice to attenuate urinary tract infections. Additionally, 75% of the females and 72% of the males believe negative association between fat & heart diseases (Bogue and Ryan, 2000).

2.2. Functional/designer foods

2.2.1. Historical perspective

Now, consumers are well aware of the fact that eating right could improve their mental & physical health ultimately promoting life quality & longevity and fight against diseases. The functional/designer foods are recognized as second after fat reduced foods. The concept of
functional foods originated back to Chinese traditional medicine (1000 BC) than the term was initially conceived by Japanese in 1985. Afterwards, the concept was introduced by Paul La Chance who developed Tang beverage with Ca-fortification in US (1960s). However, the first functional food in the US and Europe was fortified breakfast cereals and drinks (Bogue and Ryan, 2000).

2.2.2. Concept and definitions

Inspite of their contribution to positive health outcomes, the concept of functional/designer foods has not yet been emphasized by food industry. As a response, practical application of these foods is somewhat limited in developing countries where nutritional security has become a major challenge for the health related authorities (Rajasekaran and Kalaivani, 2013). Functional/designer food is a food similar to conventional food i.e. consumable on daily basis and ensures health beyond basic provision of nutrients. In Japan (1991), the health ministry has named functional foods as Foods for Specific Health Use (FOSHU). It is not a medicine but normal food i.e. modified to subserve normal physiology or beneficial against modern health worries or technology related diseases. Generally, consumers seem inclined to incorporate healthy foods in their routine but at the same time find it difficult to change their eating pattern. However, they could be convinced by providing detailed information regarding ingredients and their health response (Kwak and Jukes, 2001; Bech-Larsen and Grunert, 2003; Arihara, 2006; Siro’ et al., 2008; Day et al., 2009; Bui, 2015). Designer foods are processed or cooked foods prepared by using scientific intelligence in terms of their specific health benefit, effective dose and impact of processing on antioxidant activity. Furthermore, these foods contain a balance of compositional constituents, nutrients: vitamins & minerals and polyphenols needed for healthy survival (Cencic and Chingwaru, 2010; Pandey et al., 2010). According to the European legislation, functional food is a notion or concept but not any particular food. This concept is based on three points; (1) optimal wellness or health gains and disease prevention for better life, (2) technological process and (3) nutritional function (Bogue and Ryan, 2000; Bigliardi and Galati, 2013).

Besides, functional food is an ambiguous phrase that has undergone revision for several times. There is no legislative or unitary accepted definition for this term that could draw a border line between traditional and functional/designer foods. ADA has recommended an eating plan i.e.
moderation, wholesome and healthier; polyphenols rich diet based on varied nomenclature including nutraceutics, functional or designer foods, hyper-nutritious or super foods, pharmafoods, medical or longevity foods. Though, there is no official term exist in US food regulations except medical foods *i.e.* regulated by FDA (Childs, 1997; Urala and Lahteenmaki, 2003; Day *et al.*, 2009).

In most of the economies, there are no specific rules for functional/designer foods nevertheless, under Food and Drugs Act and Regulations, food is defined as any article represented for use as food, drink, chewing gum or food mixed with ingredients for some purpose (Arihara, 2006; Bigliardi and Galati, 2013; Rajasekaran and Kalaivani, 2013). The designer foods are called as modified conventional foods that ensure health benefits in contrast to un-modified versions (Manjula and Suneetha, 2011). Unique features of functional food concept include: optimum nutrition; a blend of nutrients & non-nutrients, natural routine foods enriched with bioactive molecules or naturally extracted components, and removal of toxicants, aiming to maximize physical & mental health, improve bioavailability & biokinetics and reduce health care cost (Hardy, 2000; Roberfroid, 2000; Roberfroid, 2002; Jones and Jew, 2007; Niva, 2007; Shahidi, 2009; Bigliardi and Galati, 2013).

### 2.2.3. Nutraceutics

Many people are now turning towards nutraceutics owing to their role in controlling various ailments. The term nutraceutic is coined by Stephen Defelice in 1989, derived from two words: nutrition & pharmaceutic. It is a component, extracted from food or part of food that ensures health enhancement and disease protection. Nutraceutical is defined as any pharmacologically active substance or single natural nutrient of food, not a drug that promotes health and prevents disease by modulating metabolic functions of the body. Nutraceuticals encompasses isolated components, dietary supplements and processed designer foods like beverages, soup, etc. Later, Dietary Supplement Health and Education Act (1994) included amino acids, minerals, antioxidant vitamins and plant extracts under the umbrella of nutraceutics. Initially, nutraceuticals are potential nutraceuticals, claiming to have a particular health benefit and transformed to established nutraceuticals, if supported by sufficient clinical data to demonstrate the claimed benefit (Hardy, 2000; Roberfroid, 2000; Aruoma, 2006; Cencic and Chingwaru, 2010; Pandey *et al.*, 2010).
2.2.4. Marketing of nutraceuticals

Nutraceuticals are the fast growing segment of food industry. Nutrition Business Journal (NBJ) has identified US$80 billion market of nutraceuticals in 1995, escalating 5% per annum (Hardy, 2000; Arihara, 2006; Pandey et al., 2010). Globally, the market of functional foods has reached by US$ 33 billion, whereas contribution by the Europe was only 1% of the whole. In 2000, the market value of FOSHU has reached to US$ 2 billion with approvals of 174 FOSHU labels. Most of the functional foods in Germany in 2001 were in the ascending order; soft drinks, confectionary, dairy, bakery items and baby foods (Menrad, 2003; Bigliardi and Galati, 2013). In 2003, the market value of functional foods was around US$ 11.7 billion in Japan whereas, US$ 10.5 billion in USA (Bech-Larsen and Scholderer, 2007). As functional foods are not well defined so global market of these products varied from US$ 25 to 250 billion, whereas US market for such products worth US$ 43 billion with annual growth rate 5-10% and Europe contributed US$ 1.37 billion in 1997 (Bogue and Ryan, 2000). Globally, food regulatory system varies widely, 600 FOSHU products are allowed in Japan (birth place), approved in 2006 while only limited products are acceptable in Canada (Hardy, 2000; Arihara, 2006; Jones and Jew, 2007; Siro´ et al., 2008; Bigliardi and Galati, 2013).

2.2.5. Opportunities and challenges for market success

Scientifically developed functional foods have become feasible thanks in life sciences but unable to meet market acceptance or success. The functional/designer food concept has persuaded people to make healthy food selection and convinced the food industries to attain larger profit margins by reformulating the conventional foods by incorporating clinically proven effective dose of functional ingredients. In this regard, functional beverage is considered as the most popular and easily formulated functional food besides complex food matrix. Most innovative products failed before reaching to marketplace because product development is a difficult & risky process and it can’t be touted to general public without approving its health claims. In order to achieve niche in market place, the road to success need some basic steps such as identify the common diseases in the targeted area, followed by legislative framework to approve a product such as nutrition information on labels, checking functional ingredients for their mode of action, efficacy & safety on biological markers, educating consumers regarding diet-disease linkages and focusing on consumer perception.
regarding taste & trust on health claim (Childs, 1997; German et al., 1999; Urala and Lahteenmaki, 2003; Grunert et al., 2004; Arihara, 2006; Costa and Jongen, 2006; Siro´ et al., 2008; Day et al., 2009; Bigliardi and Galati, 2013).

2.2.6. Classification and examples

The functional foods are classified as essential macronutrients (resistant starch & n-3 fatty acids), essential micronutrients (minerals & antioxidant vitamins) and non-essential nutrients (oligosaccharides & phytochemicals). These are involved in growth & development, maintenance of body weight, defend against free radicals, resist diseases and maintain lipoprotein profile, intestinal physiology & psychological functions (Kwak and Jukes, 2001; Arihara, 2006). Some examples of functional foods include fortified foods containing iodine, vitamin C, E, B₉, Zn, Fe, Ca, n-3 fatty acid, sterols, soluble fiber, oligosaccharides, probiotics (LAB), soy-proteins and peptides to medical foods; administered enterally, intended for specific diseases under the supervision of a medical doctor. The functional meat products prepared by reformulating fatty acid profile, addition of antioxidants, dietary fibers & probiotics, etc. open up a new avenue for the meat industry (Hardy, 2000; Arihara, 2006; Siro´ et al., 2008; Bigliardi and Galati, 2013).

Mainly functional foods have been launched in dairy, confectionary, beverage, bakery and baby food sectors. Normally, the fruit juices are fortified using vitamin C, E, B₉, Zn & Ca, infant foods are enriched with pro-/pre-biotics, food alteration is done by removing deleterious components, incorporation of dietary fiber as fat replacer in meat products (weight loss foods), naturally enhanced foods such as eggs containing n-3 fatty acids and hypoallergenic foods such as lactose or gluten-free products. In Japanese and European market, there is a dominant influence of probiotics especially LAB & bifidobacteria, launching around 370 products all over the globe in 2005. The functional/designer foods enriched with soy could overcome the protein deficiencies faced by vegetarians, lactose intolerant could fulfill nutritional gaps by incorporating Ca-fortified juices in their diet and those who dislike seafood could switch to genetically enriched n-3 eggs. Traditional technology involves formulation/blending procedures to develop functional foods, whereas technologically designed foods are based on (1) microencapsulation to extend the shelf life of functional ingredients and (2) concept of personalized functional food development involving the principles of nutrigenomics;
interaction between foods and human genome (Bogue and Ryan, 2000; Bigliardi and Galati, 2013).

2.2.7. Successfully marketed functional/designer foods

The market for functional food products is divided into 4 groups; enhance performance, self-treatment, lessening disease risk and prevention of existing disease. The new generation functional foods have also been arrived in market with the name of cosmetic foods containing collagen and vitamin C. In Japan, 70% of the functional food market is comprised of drinks while the dominant functional food in US is sport drink carrying vitamins, minerals, amino acids, nucleic acids and electrolytes. In this context, healthy and energy boosting functional beverages are called as ACE vitamin drinks like Red Bull & Lucozade. In Denmark, the active food sector for functional food development is dairy especially pro-biotic to cholesterol lowering yoghurt. Moreover, European functional food market is basically consisting of bakery products; bread, biscuits, pasta enriched with vitamins such as vitamins C & E, minerals like Ca, cholesterol lowering soluble fiber e.g. Kellogg’s products that are now upgraded by adding bran, naming as ‘All Bran Plus’ or n-3 fatty acids (Bogue and Ryan, 2000).

2.3. Vegan and meat based foods

2.3.1. Vegan based foods

Some decades back, man has included processed foods based on high fat & simple sugars and reduced the use of naturally existing fruits & vegetables. This trend has brought negative outcomes in terms of metabolic syndromes (Domínguez-Avila et al., 2011). Some believes that it does not make much difference what we eat until we eat in moderation hence advised to eat less at a time and think about proportion size. Vegetables are considered as natural, wholesome and healthy in terms of dietary fiber, phytoceutics & minerals hence recommended to be consumed @ ½ kg/day (Niva, 2007; Hussein, 2012). The WHO estimates that worldwide consumption of fruits and vegetables is only 20 to 50% of the recommended daily minimum of 400 g per person (Rickman et al., 2007). Most often, isolated nutraceutics derived from fruits and vegetables were assessed against a particular disease but ingesting isolated moieties won’t have similar response as that of eating a whole plant matrix (Domínguez-Avila et al., 2011).
Vegetables are conferred with the status of functional foods and their habitual consumption is capable of delivering health beneficial moieties to restore the physiological needs of the body. *In vitro* studies have strongly suggested that foods enriched with polyphenols (flavonoids especially anthocyanins & catechins) and antioxidant vitamins (ascorbic acid, tocopherol & β-carotene) possess strong free radical scavenging potency hence protect against various ailments (Nicolli *et al.*, 1999; Kaur and Kapoor, 2002). In Pakistan, the polyphenol content of commonly consumed vegetables was investigated and results indicated maximum polyphenols in cabbage followed by cauliflower, spinach, yellow turnip, white turnip, peas and carrot (Sultana and Anwar, 2008).

The minimum of five servings of green & yellow vegetables are recommended (Schieber *et al.*, 2001). The vegetable in relavence to fruits are not that pleasant in taste or texture thus not consumable frequently but their entrance into human alimentation has proved relatively healthy. Among vegetables, garlic, spinach, onion, cruciferous vegetables, green peppers, tomatoes and root vegetables whereas, berries in fruits, walnuts in nuts and spicy aromatic plants have been acknowledged for their nutraceutic worth (Beceanu, 2008). Based on 206 human epidemiological analyses & 22 animal trials, American dietetic association have demonstrated that vegetables including cruciferous, allium and tomatoes have potential to fight against oncogenesis of gastrointestinal tract (Cencic and Chingwaru, 2010). Cruciferous vegetables like cabbage contains isothiocyanates and sulforaphane that are responsible to induce phase II enzymes, involved in the detoxification of reactive DNA metabolites hence prevent cancer initiation or propagation (Morsy *et al.*, 2010).

Different genus of cabbage (green, red, savory & chinese) belongs to the same family and contributes positively towards human nutrition and health perspectives. brassica vegetables are processed or cooked using different methods or may be eaten as raw ingredient in different salads (Podsedek, 2007; Singh *et al.*, 2007). These vegetables contribute glucosinolates in our diet besides the provision of dietary fiber and vitamin C. Americans were reported to consume 3 billion pounds of cabbage in 2001. Around 88% of the consumers relied on fresh cabbage and remaining have adapted taste for sauerkraut (Chun *et al.*, 2004). It is documented that major antioxidants in cruciferous vegetables are hydrophilic in nature while hydrophobic antioxidants are responsible for 20% of total antiradical capacity (Leja *et al.*, 2010). From health point of view, cabbage is considered for its antioxidant, anticancer, antiplatelet,
antihyperthyroidism, antihyperglycemic and antihyperlipidemic effects. It has the ability to attenuate bronchoconstriction and inflammation. In Arab folkloric practices, cabbage juice is recommended against various disorders like hyperlipidemia, obesity, stomach ulcer, liver dysfunctions, nephritis and tachycardia (Al-Dosari, 2014).

Recently, numerous medicinal reasons such as headaches, diarrhea, gout and peptic ulcer are negatively associated with red/purple cabbage being rich in pigmented compounds. According to the findings of previous researchers, suppressive effects of red cabbage were measured against lipid peroxidation and plasma protein carbonylation induced by peroxynitrite (Kolodziejczyk et al., 2011; Park et al., 2014).

Besides health, natural antioxidants in vegetables have been reported to counterbalance microbes especially in meat based products, in this context, use of cabbage powder or extract serves as bio-preservatives in food application (Malav et al., 2015).

2.3.2. Meat based foods

On the other hand, consumers often recognize meat products as unhealthy unlike dairy products due to high fat content or cancer stimulating role of red meat. Alongside, addition of sodium chloride during meat processing is responsible to promote hypertension. These views disregard the fact that functional meat plays an important role in health maintenance. Though, there are some hurdles in designing and marketing functional meat products. Therefore, there is an urgent need to make the consumer well aware of the limited scientific information that meat itself contains valuable healthy minerals like Fe, vitamins; vitamin B\textsubscript{12} & B\textsubscript{9}, bioactives; carnosine, anserine, L-carnitine & conjugated linoleic acid, bioactive peptides; antihypertensive peptides and meat protein with higher biological value & bioavailability. There are number of routes in the development of functional meat such as reduction of cholesterol, fatty acids, calories, sodium & nitrites, addition of vegetables/fish oil & fiber and inclusion of functional ingredients; vegetable or soy proteins, antioxidants & probiotics (Arihara, 2006).
2.4. Phytochemicals & antioxidant vitamins: Antioxidant assays

2.4.1. Extraction of phytochemicals

Healthiness is a key driver in food business today due to escalating demand of functional foods enriched with healthy characteristics. The plants possess a mixture of hydrophilic and lipophilic constituents either in free or bound form thus binary or multiple solvent system is normally employed rather than single solvent for solubilization of varied components however, for a particular component; single solvent may work effectively (Roberfroid, 2002). In the analysis of plant botanicals, extraction is a critical preliminary step necessary to quantify the desired antioxidants. To facilitate this step, there is a need to increase the contact area of the sample with that of solvent involving numerous steps; pre-washing, drying or freeze drying and size reduction. Furthermore, selection of solvent largely depends on nature or type of biologically active molecule. The hydrophilic compounds are extracted through polar solvents particularly methanol, ethanol or ethyl-acetate while the lipophilic compounds need non-polar solvents such as dichloromethane or hexane (Cos et al., 2006; Tsao, 2010). The modern methods including microwave-assisted extraction, pressurized-liquid extraction, supercritical-fluid extraction, etc. are gaining worth in the nutraceutic world due to certain advantages such as extraction proficiency & optimization, less use of organic solvent, less possibility of sample degradation, no supplementary clean-up & concentration stages before characterization and safer for clinical trials in the attainment of particular targets (Huie, 2002; Tsao, 2010).

For the laboratory analyses of plant extracts, solvent extraction is the most frequently employed extraction procedure. The pH of the solvent is a considerable factor for the extraction purpose. Generally, acidic conditions (using weak acid or strong acid of lower concentration) are preferred for higher stability and easy extractability of phenolic compounds via organic solvents. On the other hand, highly acidic conditions may cause hydrolysis of glycosides or acylglycosides altering the native polyphenols picture (Tsao, 2010).

Considering anthocyanins, the glycosylated anthocyanins have better extractability via polar solvents (Ferreiro-González et al., 2014). Earlier, Lapornik et al. (2005) explored that anthocyanins are naturally polar compounds and their mass transfer is related to solvent polarity. They also determined that methanol and ethanol are equally effective and suitable solvents for the extraction of anthocyanins. Earlier researchers found methanol better for
extraction of phenolics and anthocyanins over ethanol while three to four times lower extraction efficiency was achieved through water. The higher concentration of methanol is related to its smaller size, relative to ethanol thereby penetrating deep into plant matrix where ethanol cannot reach. However, ethanol residues are considered safer for food-based applications (Pankaj and Sharma, 1991; Bridgers et al., 2010).

2.4.2. Extraction of cabbage nutraceuticals

Cumulative evidences have suggested that hydrophilic antioxidants are the major active moieties in cruciferous vegetables with total contribution nearly 99% (Podsedek et al., 2006). Accordingly, polar solvents are considered as the preferable choice for mass transfer of hydrophilic compounds (Sasidharan et al., 2011). These compounds include pigmented molecules (anthocyanins), phenolic acids, vitamin C & glucosinolates, whereas hydrophobic substances include carotenoids & vitamin E or some lipophilic flavonoids hence non-polar solvents are employed for their extraction (Podsedek et al., 2006; Sasidharan et al., 2011; Draghici et al., 2013; Gaafar et al., 2014; Park et al., 2014).

2.4.3. Quantification of phytochemicals

For quantification, spectrophotometric and chromatographic techniques are commonly employed to achieve valuable information about phenolic acids and flavonoids (Tsao, 2010). The crude plant extracts contain various bioactive compounds with different polarities that could be detected using different techniques including chromatographic; TLC, column chromatography, Sephadex chromatography & HPLC system and non-chromatographic; polyphenol photometric assay & Fourier-transform infrared spectroscopy (FTIR) for the determination of structural features and bioactivity. Currently, HPLC analysis is gaining popularity due to varying migration rates of active molecules in the column and high resolution. Furthermore, the extent of separation is based on choice of mobile and stationary phase. Generally, isocratic system is employed for mass transfer phytochemicals while gradient elution is desirable if more than one moieties are under focus, varying widely in their retention time under specific conditions. Each molecule possesses a distinctive peak under particular settings. Depending on active compound, the chromatographer chooses conditions to identify a substance of interest via HPLC system (Sasidharan et al., 2011)
In contrary, polyphenols assessment via spectrophotometric method is an easy approach to quantify total active molecules in plants extracts. On the other hand, HPLC is sophisticated equipment i.e. employed to characterize individual moities with high resolution despite the fact that it is hectic in term of sample preparation and system checks (Szóllôsi and Varga, 2002; Sasidharan et al., 2011).

2.4.4. Dietary phytoceutics

Antioxidants are considered as the first line of defense against oxidative stress in response to free radicals including vitamin C, E, carotenoids, phenolic acids, polyphenols and flavonoids (Farida and Sari, 2011; Malav et al., 2015). Dietary phytoceutics are secondary metabolites of plants with >8,000 structural variants and divided into four classes; phenolic acids, flavonoids, stilbenes and lignans. The phenolic acids include hydroxybenzoic acids & hydroxycinnamic acid, constituting 1/3rd of total polyphenols. The flavonoid encompasses >4,000 different kinds of components. Amongst which anthocyanins are the most abundant, glycosylated derivative of anthocyanidins exists in colorful plants and anthoxanthins; colorless compounds including flavanols like catechins, flavonols (myricetin, fisetin, quercetin & kaempferol), flavones, isoflavones, flavanones & their glycosides that comprises of 2/3rd of total polyphenols. The health benefits of polyphenols are expected due to their safe, long term administration and defensive role against various degenerative diseases associated with redox mediated tissue damage. The polyphenols have antioxidant activity due to the presence of aromatic rings containing OH moieties or serve as metal chelators hence suppress lipoproteins from oxidation or improve protective HDL levels or down-regulate lipid peroxidation ultimately protect plasma vitamin E. Alongside, these moieties could inhibit the activities of hepatic HMG-CoA reductase, ACAT & stearoyl-CoA desaturase (key enzymes involved in lipid synthesis) or increase fecal cholesterol excretion or up-regulate LDL receptor & genetic expressions of endogenous antioxidant enzymes; SOD, CAT & GPx (glutathione peroxidase). Moreover, they have the potential to activate intracellular defense mechanism by modulating signal transduction pathways and glutathione synthesis (Jeon et al., 2001; Chen et al., 2003; Han et al., 2007; Zhu et al., 2008; Choi et al., 2010; Barakat and Mahmoud, 2011; Wang et al., 2011; Rezq, 2012; Khademi et al., 2013).
2.4.5. Quantification of cabbage phytochemicals

Earlier researchers found total polyphenols in white, green or common cabbage varying between 12.58 to 153.00 mg/100g F.W., whereas 34.41 to 322.00 mg/100g F.W. for red cabbage. Furthermore, the total flavonoids in white and red cabbage were reported from 13.13 to 18.95 and 9.01 to 141.21 mg/100g F.W., respectively. The higher antioxidant activity of red cabbage is attributed to anthocyanin content. Besides, these differences were due to different localities like, USA, England, Poland, Germany & India, variation in extracting solvents; methanol, acetone, etc. and different cultivars & lines of cabbages (Vinson et al., 1998; Chu et al., 2002; Kaur and Kapoor, 2002; Proteggente et al., 2002; Bahorun et al., 2004; Wu et al., 2004; Leja et al., 2005; Leja et al., 2006; Podsędek et al., 2006; Singh et al., 2006; Podsędek, 2007; Singh et al., 2007; Podsędek et al., 2008; Leja et al., 2010; Draghici et al., 2013; Podsędek et al., 2014; Xu et al., 2014).

2.4.6. Anthocyanins

There are about 600 naturally occurring anthocyanins that vary on the basis of number & position of methoxyl & hydroxyl groups on anthocyanidin skeleton and positions & number at which sugar molecules are attached (Cavalcanti et al., 2011; Park et al., 2014). Anthocyanin is the most ubiquitous flavonoids or pigmented compounds, responsible for red, blue and purple hue hence considered as natural choice of coloring drinks, jams & jellies and confectionery products. Their esterification of sinapic-, ferulic- & p-coumaric acids enhances antioxidant activity thus anthocyanins rich phenolic mixture has more nutritional value. Anthocyanins are water soluble glycosylated & non-acetylated compounds that are present abundantly in red cabbage, grapes, berries and apples. However, anthocyanins in red cabbage are basically cyanidin as aglycone; glycosylated with glucose and/or sophorose that are acylated with diverse aromatic and aliphatic acids (Day et al., 2009; Kolodziejczyk et al., 2011). Naturally, anthocyanins are the important and largest group of hydrophilic vacuolar pigments. The name derived from two Greek words; anthos (flower) and kyanos (dark blue). The anthocyanins in red cabbage are negatively related to hepatotoxicity, hyperglycemia, neurotoxicity and hypercholesterolemia (Sankhari et al., 2012; Park et al., 2014). Furthermore, pure or crude extract of anthocyanins demonstrated biomedical benefits against obesity, cardiovascular diseases, visual & brain disorders, ulcer and cancer (Espin et al., 2007).
2.4.7. Anthocyanins in red cabbage

Previous researchers reviewed anthocyanins in different cultivars and lines of red cabbage, ranging from 14 to 495 mg/100g F.W. (Mazza and Miniati, 1993; Hodges et al., 1999; Piccaglia et al., 2002; Podsedek et al., 2006; Wu et al., 2006; Podsedek, 2007; Scalzo et al., 2008; Volden et al., 2009; Yuan et al., 2009; Leja et al., 2010; Xu et al., 2010; Kolodziejczyk et al., 2011; Draghici et al., 2013; Wiczkowski et al., 2014; Zaidel et al., 2014).

2.4.8. Antioxidant vitamins

Ascorbic acid also called as vitamin C is a free radical scavenger, reduce lipid peroxides and function parallel to glutathione hence possesses therapeutic role against CVD, Parkinson’s, Huntington’s and Alzheimer’s disparities. Vitamin E prevents LDL oxidation by fighting against free radical induced oxidative stress especially in hydrophobic environment of cells and its membrane (Niki, 2010; Domínguez-Avila et al., 2011). It serves as an inhibitor of HMG-CoA reductase (Chen et al., 1997). Carotenoids is a large family involving >700 molecules of different structure are hydrophobic pigments, majorly exist in vegetables. They include β-carotene (pro-vitamin A activity & pro-oxidant i.e. itself act as oxidant in higher/toxic dose), lutein, lycopene, zeaxanthin and cryptoxanthin. They are potent free radical scavengers and negatively linked with diabetes, obesity, low sperm motility & hearing loss and function as immune-boosters & anticancer agent (Domínguez-Avila et al., 2011).

2.4.9. Antioxidant vitamins in cabbage

It is documented that almost ½ cup of raw & cooked red cabbage could provide 20 & 25 mg of vitamin C, protecting from lipid peroxidation by-products and oncogenic events (Padayatty et al., 2003; Bacchetti et al., 2014). Moreover, dehydroascorbic acid is considered as the dominant form of vitamin C in cabbage i.e. almost 4 X to that of ascorbic acid (Podsedek, 2007). Red cabbage possesses more vitamin C than oranges, fulfills 61% of vitamin K requirement and 20% of the RDA of vitamin A along with healthy amounts of vitamin Bs, B6 and B1 (Draghici et al., 2013).

The amounts of vitamin C in green & red cabbage were quantified in various lines, ranged from 22.72 to 51.65 & 36.57 to 129.90 mg/100g F.W., respectively (Park et al., 2014). Furthermore, vitamin C in white and red cabbage was reported as 9.65 and 24.38 mg/100g F.W.
(Singh et al., 2006). One of their peers, Podsdek et al. (2006) measured the difference of ascorbic acid in white & red cabbage as 18.00 to 35.64 & 62.00 to 72.52 mg/100g F.W., correspondingly.

The red and green cabbage also contains natural antioxidants including vitamin C 5.7 to 695.6 mg/100g F.W, α-tocopherol 0.03 to 0.69 mg/100g F.W., γ-tocopherol 0.00 to 0.006 mg/100g F.W., α-carotene 0.00 to 0.002 mg/100g F.W., β-carotene 0.01 to 0.13 mg/100g F.W., lutein+zeaxanthin 0.08 to 0.45 and 0.03 to 0.15 mg/100g F.W., apigenin as 0.8 to 6.1 mg/100g F.W., kaempferol 0 to 23.9±0.7 mg/kg F.W. and quercetin 0.9 to 5.1 mg/100g F.W. that not only maintain vegetable quality but also play nutritive role in human diet (Hertog et al., 1992; Kurilich et al., 1999; Ching and Mohamed, 2001; Puupponen-Pimia et al., 2003; Bahorun et al., 2004; Chun et al., 2004; Singh et al., 2006; Podsdek et al., 2006; Podsdek, 2007; Singh et al., 2007; Bushra and Anwar, 2008; Park et al., 2014). Besides, Gaafar et al. (2014) and Al-Dosari (2014) also documented the presence of rutin, ferulic, benzoic, acacetin, myricetin, coumarin, luteolin, genistein, pyrogallol, gallic acid, catechins, p-coumaric acid, chlorogenic acid, vanillic acid, caffeic acid and protocatechuic acid. They also mentioned that flavonols; quercetin and kaempferol are influenced by various externous determinants including variation in type & growth pattern of plant, seasonal variability, degree of maturity and food processing conditions (Chun et al., 2004).

Conclusively, the variation in phenolic content, anthocyanins and vitamin C was attributed to differences in cultivar, geographical conditions, agricultural practices, maturity stage, seasons, storage conditions and analytical methods (Chun et al., 2004; Podsdek, 2007).

2.4.10. Antioxidant assays

Numerous tests have been acknowledged for the assessment of antioxidant potential (Molyneux, 2004). Commonly, radical trapping capacity is measured by ABTS and DPPH (free radical producing) reagents because polyphenols have the ability to react with these free radicals, ensuring stability (Leja et al., 2010). Additionally, FRAP assay is a reducing assay by which reductional potential i.e. ability to donate electron or hydrogen is estimated, also known as total antioxidant capacity (Antolovich et al., 2002). Earlier research on fifty popular fruits & vegetables and beverages showed that antioxidant assessment of pigmented &
hydrophilic phenolics is better represented via ABTS assay than that of DPPH reagent (Floegel et al., 2011).

**2.4.11. Antioxidant assays with special reference to cabbage**

Previously, DPPH scavenging ability of different cultivars of red cabbage were measured as 26.6 to 70.9% however, 2 to 8% in case of white cabbage (Leja et al., 2010). The DPPH scavenging ability of freshly harvested & stored white cabbage were 2.76 to 10.33 & 2.49 to 15.14%, respectively (Leja et al., 2006). Currently, Xu et al. (2014) determined the DPPH quenching ability of fresh-cut red cabbage as 95%. Moreover, the DPPH scavenging ability of conventional & organic white and red cabbage were determined as 16.095 & 22.533 and 66.370 & 73.316%, correspondingly (Sakunasing and Kangsadalampai, 2008).

The TEAC of white cabbage was measured as 1.73 µM Trolox/g F.W. (Bahorun et al., 2004). Furthermore, the TEAC of green and red cabbage were reported as 4.92 and 13.77 µM Trolox/g F.W., correspondingly (Proteggente et al., 2002). Later, Podsdek et al. (2006) measured the ABTS values for white and red cabbage as 1.34 to 1.81 and 9.81 to 12.64 µM TEAC/g F.W., respectively.

Earlier, the FRAP values for green and red cabbage were estimated as 6.94 and 18.70 µM Fe²⁺/g F.W., respectively (Proteggente et al., 2002). Recently, the FRAP value of green and red cabbage were measured as 17.00 and 80.87 µM TE/g F.W., correspondingly (Rokayya et al., 2013). Earlier, the FRAP values of white and red cabbage were found as 1.93-2.25 and 0.20-0.30 mM/100g F.W. (Wold et al., 2006).

These antioxidant assays serve as an effective tool to validate various health benefits hence give an estimation of effective dose of antioxidants and their incorporation in designer foods.

**2.5. Impact of processing on antioxidant capacity of vegetables**

Food is a mixture of nutrients, phytochemicals and fiber like components however, their health promoting ability depends on these ingredients content, activity and bioavailability that alters based on processing history. This aspect is of great consideration, as in Pakistan only small proportions of vegetables are consumed in their raw state, whereas most of them are often processed. Processing and preservation procedures are responsible for the improvement or depletion of health promoting antioxidant vitamins and polyphenols. However, blanching
treatment retains the original antioxidant profile in most of the cases. Other operations including peeling and slicing contributes to enzymatic oxidation leading to modification of inherent antioxidants or decrement of their antioxidant activity. Furthermore, prolonged storage promotes enzymatic and chemical oxidation of phenolic moieties. High antioxidant capacity is attributed to higher ability to donate a hydrogen atom from antioxidant (aromatic hydroxyl group) to unpaired electron system of free radicals (Nicoli et al., 1999). The concept that antioxidant activity of fruits and vegetables are lost during processing is due to the removal of skin (Shahidi, 2009).

There is a general trend of consuming semi-cooked or boiled vegetables in western countries whereas, in Asian countries like Pakistan, the vegetables are normally subjected to various cooking procedure prior to consumption including shallow or deep frying, steaming, roasting and stewing. These treatments affect the efficacy of active constituents in vegetables either positively or sometimes negatively. In Pakistani cuisines, cauliflower, cabbage, spinach, carrot, yellow & white turnip are the most frequently consumed vegans. These vegetables contain rich nutritive but low caloric value along with the presence of myriad of antioxidants. Earlier study revealed that cabbage exhibit higher phenolic compounds as compared to other vegetables thus there is a need to explore its nutraceutic worth and designer food applications (Sultana and Anwar, 2008). The cooking methods such as boiling, steaming and microwaving are generally practiced in western society while stir-frying is considered as a common dietary habit among Chinese. It is assumed that domestic cooking methods resulted in profound alterations in chemical composition and bioavailability of active compounds in cruciferous vegetables (Xu et al., 2014).

In general, processing or cooking methods impact the stability of polyphenols of brassica vegetables. Some of the cooking methods donot cause any significant alteration in antioxidant profile, whereas it may deplet or form novel antioxidant metabolities that may have more or less bioactivity or bioavailability (Turkmen et al., 2005; Volden et al., 2009). Therefore, food manufactures should focus on antioxidant potential of designer foods to ensure health promotion (Barakat and Rohn, 2014). Earlier, Wachtel-Galor et al. (2008) and Roy et al. (2009) found high extractability of broccoli nutraceutics during steaming, resulting in more antioxidant capacity in contrast to raw equivalents. These findings were contradictory to the earlier notion demonstrating reduction of polyphenols in response to thermal procedures.
(Podsedek, 2007). In addition, processing converts glucosinolates to isothiocyanates hence suppresses urinary mutagens derived from meat, tobacco carcinogens and gastric ulcers. Besides, cabbage also comprises of an anti-inflammatory amino acid; glutamine (Draghici et al., 2013).

2.6. Cabbage based designer products

Incorporation of cabbage in meat based product ensures the provision of myriad of natural phytoceuticals including β-carotene, ascorbic acid and α-tocopherol hence overcoming the injudicious incorporation of synthetic antioxidants to prolong meat quality against oxidative degradation. Furthermore, cabbage could overcome the calcium deficiency in meat products. Additionally, the use of fiber from cruciferous vegetables resulted in fat reduced meat products. On the other hand, cabbage could mask the meaty flavor. Besides, cabbage powder or extracts are considered as bio-preservatives (Kumar et al., 2013; Malav et al., 2015). Mostly in dieting programs, cabbage is given a major share of the dietary plan being low in calories (Al-Dosari, 2014). The calories content in brassica vegetables were documented approx. 24-34 kcal/100g as reported by Heimler et al. (2006). Recently, the calorific values of control and 6% cabbage powder based meat patties were reported as 194.65 and 187.18 kcal/100g, respectively (Malav et al., 2015).

The inclusion of cruciferous vegetables enhances DPPH and ABTS radical scavenging activity due to the presence of natural antioxidants such as carotenoids, tocopherols, ascorbic acid and flavonoids (Kumar et al., 2013). On commercial scale, anthocyanins from red cruciferous vegetables have gained immense use in designer foods including jams, juices, preserves and ice-cream to curtail escalating health disparities (Cavalcanti et al., 2011). In this context, Barakat and Rohn (2014) prepared broccoli based bars with higher organoleptic acceptability via frying however; maximum retention of flavonoids were noted in baked and steamed bars. In another study, Radziejewska-Kubzdela and Biegańska-Marecik (2015) incorporated anthocyanin-rich red cabbage extract in apple juice and found increment in antioxidant activity attributed to increase in polyphenols up to 3.1 to 4.9 X.

Later, Banerjee et al. (2012) developed broccoli powder enriched nuggets and found linearly increasing trend in radical scavenging potential and reducing power with increment in broccoli dosage. Earlier, Llorach et al. (2005) prepared soup modified with cauliflower by-products and
found higher antiradical activity attributed to polyphenols. Malav et al. (2015) evaluated the general appearance of cabbage enriched mutton patties and found that decrease in appearance during storage is attributed to breakdown of pigmented compounds, depletion of nitrites and occurrence of browning reactions. Currently, Verma et al. (2016) studied the sensory response of green cabbage @ 15 & 25% incorporated meat balls. They assessed gradual decrement in appearance, flavor and juiciness over prolonged storage.

2.7. Epidemiological transition

The Asian world has shifted from nutritional deficiencies to chronic ailments such as CVD, diabetes and cancer i.e. termed as “epidemiological transition” in response to urbanization. The marked dependence on hypercaloric diets and decrement in energy expenditure has raised the probabilities of non-communicable diseases even below the age of 50 years. Concomitantly, the global influences in terms of television viewing or increased access to junk foods are positively associated with BMI. The higher energy proportion obtained from fat sources has led to escalated rates of hyperlipidemia, hyperglycemia, obesity, insulin resistance, hypertension and strokes (Yusuf et al., 2001; Jakes et al., 2003; Fleischhacker et al., 2011).

Among the modern societies, high reliance on calorie dense foods is responsible for obesogenic environment, generating ROS and weakening of antioxidant defense system hence 3000 kcal need to cut down to 2200 kcal (Kainuma et al., 2006; Ogawa et al., 2010; Waqar et al., 2010; Auberval et al., 2014). According to WHO reports (2007), overweight people all over the globe were estimated around 1.7 billion involving 155 million children (Domínguez-Avila et al., 2011). This situation leads to hyperlipidemia and related disparities. This postulation is found to be consistent with animal models where accumulation of lipids occurs in liver, heart and kidney. Moreover, the available therapies to alleviate hyperlipidemia are responsible to aggravate liver toxicity; more than 900 drugs have been implicated to affect liver. Throughout the world, 5% of all hospitalized and 50% of acute liver failures are the culprits of drug induced liver damage still all these disorders are without appropriate therapies (Pan et al., 2006). Thus, scientists are looking for such hypolipidemic therapies that could increase hepatic LDL receptors expression (Kong et al., 2004). The cross-sectional and longitudinal studies have reported that consumption of junk foods is linked to insulin resistance and obesity that further
exacerbate to non-alcoholic fatty liver disease *i.e.* prevalent world widely ~10-24% (Pan *et al.*, 2006; Qin and Tian, 2010; Van Rooyen *et al.*, 2011).

In normal metabolic processes, Reactive Oxygen/ Nitrogen Species/Metabolites ROSs/RNSs/ROMs are continuously being produced *i.e.* neutralized by detoxification system of antioxidants and antioxidative enzymes. In response to over-consumption of junk food, this system is insufficient to combat free radicals. This results in the generation of ROS/ROM; superoxide anion, hydrogen peroxide and hydroxyl ion via numerous pathomechanisms inducing sequential events of oxidative injury especially in the fragile organs of the body (Burton and Jauniaux, 2011; Sankhari *et al.*, 2012). Basically, the over-accumulation of free radicals and their covalent bonding with bio-molecules induce peroxidative damage of lipophilic (rich in PUFA) membrane structure leading to the synthesis of lipid peroxides that are involved in multiple pathologies. Furthermore, the pro-inflammatory end products of lipid peroxidation; MDA and 4-hydroxynonenal (aldehyde by-products) enhance LDL deposition around the vasculature and responsible for hepato-pathological features. Furthermore, prolonged consumption of hypercaloric diet depletes antioxidant reserves or compromises innate antioxidant defense system of oxygen dependent organisms that fight against pro-oxidants (active oxygen molecules) by converting them to lipid peroxides and finally to non-toxic alcohol. The first line of defense against oxidative damage is SOD; catalyzes superoxide anions; O$_2^-$ (generated by one electron reduction in oxygen molecule by transition metal ion) to hydrogen peroxide (H$_2$O$_2$) that get converted to (OH$^-$) radicals, promptly reacting with cellular components; lipids, proteins & nucleic acids leading to lipid peroxidation & cell death however, presence of CAT (hemeprotein) and GSH; considered as second line of defense and its related enzymes; GPx & GST detoxify (OH$^-$) radicals to non-toxic metabolites like H$_2$O (Jeon *et al.*, 2001; Vijayakumar *et al.*, 2004; Zou *et al.*, 2006; Zhu *et al.*, 2008; Cencic and Chingwaru, 2010; Choi *et al.*, 2010; Dimitrova-Shumkovska *et al.*, 2010; Nader *et al.*, 2010; Mohamed *et al.*, 2011; Suanarunsawat *et al.*, 2011; Rezq, 2012; Wang *et al.*, 2012; Kertész *et al.*, 2013; Adaramoye and Akanni, 2014; Kim *et al.*, 2014; Ragab *et al.*, 2014; Hussein *et al.*, 2015; Ji *et al.*, 2015; Zhong *et al.*, 2015).

Initially, the intracellular enzymatic antioxidants become activated as cells well-being becomes a threat due to the over-generation of ROS being sensitive to redox state. All the enzymes (SOD, CAT, GP$_X$, glutathione peroxidase, GR; glutathione reductase & GST; glutathione-S-
transferase) work in synergy with non-enzymatic antioxidants (endogenous GSH & exogenous polyphenols or antioxidant vitamins; ascorbate, tocopherols, carotenes & retinols in diet) to neutralize redox state of cell by converting free radicals to less toxic/reactive compounds or maintaining the free radicals within the tolerable limits. The supportive team of free radical scavengers; CAT, SOD, GST, GPx & PON1 (paraoxonase 1; an antioxidant enzyme linked with HDL, calcium dependent esterase that detoxifies lipid peroxides and widely distributed in numerous tissues) mutually fights against markers of oxidative stress; products of lipid and protein oxidation include thiobarbituric acid reactive substances & hydroperoxides and carbonyl proteins. The hypertriglyceridemia inactivate antioxidant enzymes by cross linking with malondialdehyde (MDA) or rapid consumption/exhaustion of enzymes in fighting against free radicals further aggravate lipid peroxidation leading to cell injury that in turn causes the release of cytokines like TNF-α; Tumor necrosis factor-α, IL-6; Interleukin-6 and CRP; C-reactive protein (pro-inflammatory marker of cardiac stress). Additionally, cellular proteins are targeted by free radicals resulting in the formation of protein carbonyl (PCO) content (indicator of damaged proteins) in liver, heart and kidney. Under stress, PON-1 get inactivated as a result of impaired synthesis/secretion of HDL due to alteration in lecithin cholesterol acyl transferase (LCAT) activity or S-glutathionylation; redox mechanism forming mixed disulfide linkage between protein thiol & oxidized glutathione (Domínguez-Avila et al., 2011; Noeman et al., 2011; Olorunisola et al., 2012; Xu et al., 2012; Assad et al., 2014; Keyamura et al., 2014).

Hyperlipidemia produces ROS that results in the formation of cardiovascular diseases as well as NAFLDs (Suanarunsawat et al., 2011; Lee et al., 2013; Ragab et al., 2014; Zhong et al., 2015). Previously, high cholesterol diet @ 2% to male white New Zealand rabbits depicted damage to tissues of liver (fatty degeneration, inflammation & necrosis) and heart (vacuolar degeneration, disorganized myofibrils and necrosis). Alongside, previous studies reported that sustained hyperlipidemia is responsible for elevated cholesterol especially LDL (primary risk factor for endothelial dysfunction & atherosclerosis), redox mediated oxidative stress in liver, heart & kidney and lower activities of endogenous radical scavengers. Due to modern lifestyle, people are unable to manage their cholesterol level therefore medication is considered as an option however, these hypolipidemic drugs may contribute liver damage, myopathy and drug-drug interactions. In this context, natural antioxidants are gaining much more attention being
potentially safe, economical and effective radical scavengers to attenuate lipid peroxidation (process involve in the conversion of unsaturated fatty acids to free radicals by subtracting hydrogen) and apoptosis (programmed cell death process involve in the elimination of defective & harmful cells). This revitalized the concept of herbal remedies to prevent hyperlipidemia in individuals with cholesterol at borderline levels and to improve antioxidant status of the body. The antioxidant rich diet particularly targets symptoms of liver, heart and kidney hence termed as hepato-, cardio- and nephro-protective diets (Paul et al., 2010; Qin and Tian, 2010; Al-Naqeep et al., 2011; Mohamed et al., 2011; Suanarunsawat et al., 2011; Yu et al., 2012; Abdel-Rahman, 2014; Hussein et al., 2015).

The experimental evidences have suggested that small changes in attitudes could bring appreciable responses like watching television one hour less per day could result in risk reduction of heart attacks up to 2.5 and 4% in men and women, respectively (Jakes et al., 2003). Moreover, fast food establishments are adversely affecting health quality by offering options that tend to have lower nutritional value; minerals, vitamins and dietary fiber and higher in fat, sodium and sugars. In USA (2007), 37.4% of the food eaten was purchased from fast food establishment (Satia et al., 2004; Morland et al., 2006; Moore et al., 2009; Fleischhacker et al., 2011). The unhealthy meal in poor countries is basically attracted by its low price but there is a need to realize the fact that a cheaper pro-inflammatory diet is not cost saving rather it adds burden in terms of medical costs. Based on obesity survey in America (2010), it was observed that obese men and women besides poor work productivity, pay an extra medical bill up to $1,152 and $3,613, accordingly (Myles et al., 2014).

2.7.1. Translated animal model for metabolic syndromes; Rabbits and rats

Earlier studies on laboratory animals found that cession of cholesterol feeding or introduction of antioxidant rich foods resulted in reverse of oxidative stress, normalizes insulin release and lowers cholesterol accretion in hepatocytes. Therefore, animals are considered as ideal model to induce chronic ailments in response to diet to validate biological efficacy of antioxidant moieties (Auberval et al., 2014).

According to previous review, rat is considered as a resistant animal to cholesterol feeding and moderate hyperlipidemia demand for extra cholic acid with double frequency and time frame. On the other hand, the provision of 1% cholesterol to rabbits could increases plasma
cholesterol, phospholipid & triglyceride levels and liver cholesterol to higher extents. It is found that aortic ACAT (Acyl-CoA:cholesterol acyltransferase; microsomal enzyme catalyzes the formation of esterified cholesterol) activity of rabbits is much higher than rats however, hepatic ACAT activity is similar in both animal models. Furthermore, cholesterol feeding increases plasma lipid peroxidation in rabbits, whereas it may increase, decrease or remained constant in rats. On the other hand, mild hepatic lipid peroxidation is detected in both animals. Alongside, high cholesterol intake decreases rabbit liver GSH concentration and GSH-related enzyme activities; GPx and GST with minor change in hepatic SOD activities while, no changes in GSH and SOD were observed in rats rather decrement in GPx and GST activities. Moreover, antioxidant enzyme activities in the rat liver detected increase in response to high cholesterol diet due to adaptive increase in rats to protect liver but obvious decrease was observed in case of rabbits. Similarly, high levels of aortic and plasma MDA levels for longer time have additive effect in inducement of atherogenesis in rabbits (@ 0.2-1.3% cholesterol in 8 to 14-week period) in contrast to rats (Balkan et al., 2004; Vijayakumar et al., 2004; Arhan et al., 2009 Marinou et al., 2010; Nader et al., 2010; Rezq, 2012; Hussein et al., 2015).

Furthermore, rabbits and rats have different metabolic responses towards cholesterol rich diets due to difference in lipid peroxidation and antioxidant enzyme activities. The basal SOD & CAT activities are 50 & 100% higher in rats than rabbits, respectively thus rabbits are regarded as less resistant animals. In rabbits, cholesterol causes severe hyperlipidemia and increase in oxysterols (7ɑ-, 7β-hydroxycholesterol, α-epoxy, β-epoxycholesterol, cholestanetriol, 7-keto and 27-hydroxycholesterol) in contrast to rats where increment was observed in 7ɑ- & 7β-hydroxycholesterol only. In rabbits, serum thromboxane (TXA2) level increased by cholesterol supplementation however, inverse was observed in rats. The decrease in triglyceride in rabbit liver is attributed to its accumulation in VLDL in larger amounts. The plasma lipid peroxidation was more in rabbits because antioxidant protection by vitamin E is insufficient to capture free radicals. Thus, lower vitamin E:cholesterol ratio in rabbits strongly predicts poor antioxidant protection resulting in higher lipid peroxidation probabilities. Furthermore, cholesterol in hypercholesterolemic rabbits is around 30% polyunsaturated fatty acids thus an easy target for free radicals. According to previous researchers, two types of effects were noted; Firstly, SOD activity in cholesterol fed rabbits remained same however, decrease in GSH-Px that deals with hydrogen peroxide caused increment in CAT activity as an adoption. If
enhancement of CAT is not sufficient than lipid peroxidation would dominate. Secondly, significant decrease in SOD activity of rabbits was in response to higher levels of superoxide anions thus more lipid peroxidation and both GSH-Px and catalase become inactive. On the other hand, fewer changes were observed in hypercholesterolemic rats due to the protective effect of antioxidant defense system against lipid peroxidation and formation of oxysterols (Mahfouz and Kummerow, 2000; Han et al., 2007; Feillet-Coudray et al., 2009; Qin and Tian, 2010; Lee et al., 2013; Hussein et al., 2015).

According to previous researchers, cholesterol fed rabbit models have various advantages such as cholesterol based diet is not a special induction treatment rather it reflects junk food consumption pattern of general public, fatty liver disorder occur in association with β-oxidation in mitochondria without obesity or insulin resistance hence clarifies the mechanism of hyperlipidemia induced NAFLD, slender fibrosis (increase in LPO & number of HSCs) resembles strongly with that observed in NAFLD patients. In NAFLD patients, mixed macrovesicular fats (benign; mild decrease of mitochondrial β-oxidation) and microvesicular fats (severely impaired mitochondrial β-oxidation) were found. On the other hand, microvesicular lipids are more prominent in rabbits representing severe condition of fat deposition. Moreover, cholesterol fed rabbit model could also be used to study hyperlipidemia induced CVD (Kainuma et al., 2006). The rabbits are used as translated animal models for hyperlipidemia induced atherosclerosis because their lipoprotein profile is similar to humans. Some other researchers depicted that feeding cholesterol to rabbits for 12 weeks lead to metabolic syndromes like obesity, hypertension and fibrosis (Karimi, 2012; Yu et al., 2012). Rabbits are the most sensitive specie to dietary cholesterol induction therapy being herbivores and it is the only animal in which hyperlipidemia could be induced within few days (Waqar et al., 2010; Karbiner et al., 2013).

Previously, a study conducted on New Zealand rabbits fed on 1% high cholesterol diet for 4 weeks that depicted increment in total cholesterol (from 74±8.2 to 688.75±20.02 mg/dL), LDL (from 199.5±23.6 to 621.00±20.9 mg/dL), triacylglycerols (from 35.75±2.25 to 168.00±16.04 mg/dL) & TBARS (from 7.39±0.35 to 14.43±0.95 nmol/mL) and decrement in HDL (from 266.50±23.67 to 36.25±3.52 mg/dL) & GSH (from 0.58±0.002 to 0.26±0.01 µmol/mL). Furthermore, kidney functioning test showed significant alteration in response to high cholesterol diet especially in creatinine increased from 0.7±0.16 to 1.73±0.23 mg/dL and BUN
increased up to 59.25±0.48 from 25.25±3.47 mg/dL (Nader et al., 2010). One of their peers, Marinou et al. (2010) elucidated that 1% cholesterol in rabbits resulted in altered biochemical and liver biomarkers. The body weight was increased from 3.17±0.21 to 3.49±0.19 kg, total cholesterol up-regulated from 67.88±5.66 to 1347.00±149.33 mg/dL, HDL decreased from 24.62±2.38 to 46.37±4.20 mg/dL, decrement in triacylglycerol from 97.63±12.93 to 770.13±68.48 mg/dL. However, the increments in MDA levels were from 1.14±0.14 to 3.47±0.47 nmol/L, similarly increase in ALT, AST & γGT was from 7.75±2.12, 11.75±2.87 & 14.25±2.71 to 37.25±5.42, 47.75±6.69 & 215.13±67.34 IU/L, respectively. Later, Al-Naqeep et al. (2011) fed 1% cholesterol to New Zealand male white rabbits that induced hyperlipidemia and related atherosclerosis. Later, Yu et al. (2012) studied the effect of hypercholesterolemic diet induced hyperlipidemia in rabbits in 12-week duration and results depicted increment in body weight from 2.51±0.06 to 2.95±0.07 kg and cardiac stress marker; CRP from 16.76±3.53 to 81.51±27.90 mg/dL. Furthermore, a study was conducted on New Zealand white rabbits fed with 1% cholesterol for 3 months presented early fibrotic lesions in hepatocytes and development of foam cells in arteries due to cholesterol deposition that was further exacerbated in response to systemic inflammation leading to early lesions in liver and aorta of rabbits (Karbiner et al., 2013; Kim et al., 2014).

2.7.2. Health protective moieties of cabbage

Currently, application of polyphenols and designer foods has become the center of focus to improve quality of life by neutralizing deleterious effects of dietary fat responsible to exert oxidative stress in hepatic, cardiac and renal tissues (Al-Dosari, 2014; Ji et al., 2015). In this context, functional ingredients present in cabbage could control negative biological impact of free radicals by enhancing enzymatic as well as non-enzymatic antioxidant defense system hence maintains normal cell structure and functions (Ji et al., 2015).

Numerous studies have depicted that cruciferous vegetables as a whole possesses complex combination of bioactive ingredients, minerals and antioxidant vitamins that could scavenge ROS ultimately improve the levels of GSH, SOD and CAT. Besides other ingredients, glucosinolates-myoisinase system exist inherently in brassica vegetables, myrosinase hydrolyzes glucosinolates to isothiocyanates. The myrosinase is either natively present within the compartments of plant cells or in the mammalian intestine. It is still unclear that these S-
containing compounds are either responsible for lowering cholesterol level or the synergistic effect of several antioxidants in cabbage improve the overall antioxidant status. Recent investigation on broccoli has associated isothiocyanates and sulforaphane with cholesterol-lowering activity (Melega et al., 2013).

Red cabbage contains anthocyanins as main active ingredient predominantly cyanidin-3-diglucoside-5-glucoside derivatives. These are water soluble pigments that contribute in health promotion. However, other natural antioxidants such as ascorbic acid, β-carotene, α-tocopherol and lutein could further improve its disease modulatory role. Previous data directly linked these antioxidants with hypocholesterolemic, hypoglycemic, hepatoprotective, cardioprotective, nephroprotective and neuroprotective activities. Earlier study based on anthocyanins rich diet to oxidative stressed rats showed significant decrement in lipid profile by eliminating cholesterol and triglyceride through feces or by inhibiting intestinal absorption. The anthocyanins were assumed to reduce cholesterol and triglyceride absorption in the intestine. This postulation is further supported by previous reviewers who reported that herbal extracts are primarily responsible for catabolism of cholesterol to bile acids thus eliminating subsequently through feces (Sankhari et al., 2012).

Previously, it was reported that cabbage extract could reverse oxidative damage leading to restoration of GSH. Biological action of phenolics is based on free radical quenching and metal chelating ability (Ji et al., 2015). Cruciferous vegetables are commonly consumed dietary vegetables owing to their consumer preference, easy in accessibility and cost-effectiveness. It is considered for its hypocholesterolemic, hypoglycemic and anticancer activities. The principle antioxidants include ascorbic acid, anthocyanins and isothiocyanates. Animal and human interventional studies suggested its chemo-protective aptitude due to glucosinolates (GLS) and their hydrolytic product; isothiocyanates, responsible in inhibiting phase I enzymes hence prevent the activation of carcinogens whilst, induces phase II enzymes involve in detoxification of xenobiotic (Gaafar et al., 2014). In accordance with previous studies, cabbage extracted moieties are believed to protect plasma protein and lipid profile from hydrogen peroxide induced oxidative stress (Kolodziejczyk et al., 2011).
2.7.3. Efficacy of cabbage extracts against oxidative stress biomarkers

Recent study has presented convincing evidences in the favor of cabbage extract @ 300 to 500 mg/kg B.W. against drug induced hepatotoxicity. Alongside, an inclining trend was observed in endogenous antioxidants; SOD, CAT & GPx by 46.48, 58.89, 27.06 & 26.16%, correspondingly (Bhavani et al., 2014). Earlier, another study depicted dose dependant hepatoprotective role of indole-3-carbinal, present in cruciferous vegetables, on cell viability against oxidative damage and subsequent leakage of oxidative stress biomarkers; ALT; alanine transaminase, AST; aspartate transaminase, ALP; alkaline phosphatase, GST and LDH; lactate dehydrogenase from liver into the blood stream (Guo et al., 2010). One of their peers, Morsy et al. (2010) explored the hepatoprotective properties of red cabbage and broccoli extract @ 10% against hepatic cancer induced by N-Nitrosodiethyamine (NDEA) and carbon tetrachloride (CCl₄). During 30 days of experimentation phase, the common cabbage extract @ 240 mg/kg B.W./day showed an inclining trend in hepatic SOD and CAT up to 28 and 24%, respectively but hepatic lipid peroxidation demonstrated significant decline by 36%. The positive impact of cabbage extract on GSH is attribute to chlorogenic-, gallic-, protocatechuic-, caffeic- and vanillic acid (Ji et al., 2015).

In a rat model trial, co-ingestion of anthocyanins derived from red cabbage plus high fat diet reported decreased serum biochemical parameters including cholesterol 57%, triglyceride 23%, LDL 70%, VLDL 27%, atherogenic index 72%, ALT 32% and ALP 35%, whereas HDL was raised up to 32%. The red cabbage extract was found to suppress hepatic lipid peroxidation by 44% along with significant rise in the activity of enzymatic and non-enzymatic antioxidants; superoxide dismutase 44%, catalase 47% and reduced glutathione as compared positive control animals. However, alterations in histological architecture of oxidative stressed liver tissues were counteracted by co-administration of red cabbage extract. Furthermore, serum creatine kinase (CK), creatine kinase-MB (CK-MB), LDH and AST showed reduction up to 40, 42, 32 and 31%, respectively. The decrement in MDA of heart tissues was up to 40% whereas, momentous increment was observed in the endogenous antioxidants of heart tissues; SOD 46% and CAT 47% as compared to positive control group. Moreover, histopathology of damaged cardiac tissues was significantly reversed with anthocyanin rich diet (Sankhari et al., 2012).
Previous researcher, Al-Dosari (2014) found decrement in cholesterol, triacylglycerol, LDL and VLDL up to 10 & 33, 16 & 28, 12 & 42, 17 & 29% at different doses; 250 & 500 mg/kg/day of lyophilized red cabbage juice, respectively. On the other hand, HDL showed an inclining trend up to 16 & 26% in the corresponding doses. The decrement in cellular leakage of the corresponding liver enzymes ALT, ALP, γ-GT; γ-glutamyl transferase & bilirubin levels were up to 5.3 & 12.3, 11 & 14.9, 14 & 36 and 9 & 23%. The decrement in hepatic MDA was up to 57 to 71%. Briefly, red cabbage has the potential to modulate redox sensitive dyslipidemia and associated hepatic injury in a dose dependent manner. The suppression in cardiac indicators; CK, LDH and AST were reported by 15 & 33, 22 & 33 and 2.3 & 16% at two different doses, accordingly. Apart from this, MDA level in heart tissues decreased to 55 & 66%, whereas creatinine and urea reduced to 25 & 50 and 12 & 18%, correspondingly. According to an earlier scrutiny, ethanolic extract of common cabbage @ 500 mg/kg B.W. along with high fat diet decreased serum cholesterol, triglyceride, LDL and MDA by 23.23, 4.54, 3.81 and 31.7% during 12-week trial although, anti-atherogenic index (AAI) showed an increase up to 49% in comparison with hyperlipidemic rats ultimately protects against life-threatening modalities such as myocardial infarction and atherosclerosis (Waqar and Mahmood, 2010).

In an animal study, red cabbage powder @ 10% and red cabbage extract @ 100 mg/kg B.W. was tested against paracetamol induced hepatotoxicity. The serum cholesterol, LDL and VLDL were found to reduce up to 38 & 43, 53 & 61 and 29 & 29% whereas, ALT, ALP, AST and γ-GT were down-regulated by 20.9 & 24.3, 25 & 32.5, 16.9 & 31.3 and 41 & 44% via red cabbage powder @ 10% & red cabbage extract @ 100 mg/kg B.W., correspondingly. Furthermore, the increment in hepatic SOD and decrement in MDA was up to 35.5 & 54.5 and 47 & 59.5% on feeding red cabbage powder & extract, respectively (El-Mowafy, 2012).

The generally recommended golden standard for lifestyle modifications against obesity and related NAFLD & CVD include; caloric restriction by 50 to 70% (delaying metabolism responsible for redox mediated lipid peroxidation), inclusion of antioxidant & soluble fiber enriched functional foods or dietary supplements along with habit of regular physical exercise (Masoro et al., 2000; Farrell and Larter, 2006; Zou et al., 2006; Savransky et al., 2007; Zivkovic et al., 2007; Wouters et al., 2008; Arhan et al., 2009; Cencic and Chingwaru, 2010;
Ogawa et al., 2010; Qin and Tian, 2010; Barakat and Mahmoud, 2011; Suanarunsawat et al., 2011; Adaramoye and Akanni, 2014).
CHAPTER 3

MATERIALS AND METHODS

The present research was carried out at the National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad (UAF), Pakistan. In the current investigation, locally available green and red cabbage were procured to assess their nutritional and antioxidant status followed by product development and storage response on physicochemical characteristics & sensory attributes. Based on selection criteria, best selected cabbage and its extract were shortlisted to test their effect on liver, heart & kidney biomarkers involving normal and hypercholesterolemic diet induced oxidative stressed rabbits. The materials and protocols followed are elaborated herein.

3.1. Procurement and preparation of raw materials

The green and red cabbage (Brassica oleracea L.), locally grown in Pakistan were procured from local market. The cabbage samples were randomly selected on the basis of quality attributes and washed to remove foreign matters & other impurities followed by refrigeration prior to experimentation. The HPLC grade reagents and standards were acquired from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany). For biological evaluation, male white New Zealand rabbits were housed in the Animal Room of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. For bioassays, diagnostic kits were purchased from Sigma-Aldrich Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

3.2. Compositional analysis

The cabbage samples were analyzed for proximate composition, minerals and antioxidant vitamins. The brief description is given as under:

3.2.1. Moisture content

The percentage moisture in cabbage samples was analyzed according to the standard procedure of AOAC (2006) Method No. 934-01. Accordingly, 5 g fresh cabbage sample was dried in Air Forced Draft Oven (Model: DO-1-30/02, PCSIR, Pakistan) at 105±5 °C till constant weight. Afterwards, moisture content in cabbage was calculated using following expression;
Moisture (%) = \frac{\text{Initial weight of fresh sample (g)} - \text{Final weight of dried sample (g)}}{\text{Initial weight of fresh sample (g)}} \times 100

3.2.2. Crude protein

The protein content of cabbage samples was estimated through Kjeltech Apparatus (Model: D-40599, Behr Labor Technik, GmbH-Germany) as per the guidelines of AOAC (2006) Method No. 984-13. Purposely, dried sample was digested using concentrated H\textsubscript{2}SO\textsubscript{4} plus digestion mixture (K\textsubscript{2}SO\textsubscript{4}:FeSO\textsubscript{4}:CuSO\textsubscript{4} :: 100:5:10; prevent from splashing) till light greenish color, achieved after three to four hours. Afterwards, digested material was diluted with distilled water up to 250 mL in the volumetric flask. For distillation, the digested diluted sample (10 mL) was treated with 10 mL of 40% NaOH using distillation assembly. The liberated ammonia was captured in 4% boric acid solution containing methyl red as an indicator, forming ammonium borate \textit{i.e.} an indicator of nitrogen content the sample. Finally, the distillate was titrated against 0.1 N H\textsubscript{2}SO\textsubscript{4} till light golden color and noted the volume used. The crude protein (%) in the sample was estimated by multiplying percent nitrogen (N\%) with correction factor (5.65) using following formula;

\[
\text{Nitrogen (\%)} = \frac{\text{Vol. of 0.1N H}_2\text{SO}_4 \text{ used } \times 0.0014 \times \text{Dilution volume (250 mL)}}{\text{Weight of sample } \times \text{Vol. of diluted sample taken}} \times 100
\]

\[
\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 5.65
\]

3.2.3. Crude fat

The crude fat in dried samples was determined through Soxhlet System (Model: H-2 1045 Extraction Unit, Hoganas, Sweden) according to the approved procedure of AOAC (2006) Method No. 920-39. For maximum fat extraction, dried cabbage powder (5 g) was given five to six continuous siphon washings by adjusting flow rate; 3-4 drops per second of n-hexane followed by sample drying at 105±5 °C till constant weight. The fat content was expressed as loss in sample weight.

\[
\text{Crude fat (\%)} = \frac{\text{Initial weight of dried sample (g)} - \text{Final weight of dried & defatted sample (g)}}{\text{Initial weight of dried sample (g)}} \times 100
\]

3.2.4. Crude fiber

The crude fiber was measured following standard protocol of AOAC (2006) Method No. 978-10. Accordingly, dried & defatted sample (2 g) was digested with 1.25% 200 mL of boiling
H₂SO₄ for 30 min in Labconco Fibertech apparatus (Labconco Corporation Kansas, USA). After draining the acid, digested sample was filtered followed by washing with boiling distilled water to make it acid free. Later, the resultant sample was treated with 1.25% 200 mL of boiling NaOH solution for 30 min to remove all base solubilized fractions. Again filtration and washing procedure was repeated, remaining residues (containing crude fiber and ash) were dried at 130 °C for 2 hr followed by weighing (W₁) and ignition in Muffle Furnace at 550±15 °C till grayish white ash. After cooling, reweighed the ash achieved (W₂). The percent crude fiber was calculated according to the following mathematical expression:

\[
\text{Crude fiber (\%) = } \frac{\text{Weight of dried sample after digestion } W₁ (g) - \text{Weight of ash } W₂ (g)}{\text{Initial weight of dried & defatted sample (g)}} \times 100
\]

### 3.2.5. Ash content

The inorganic residues in cabbage samples were estimated as outlined in AOAC (2006) Method No. 942-05. Purposely, 5 g finely ground cabbage powder was directly charred on flame in crucible until fumeless followed by incineration in muffle furnace (MF-1/02, PCSIR, Pakistan) at 550±15 °C till grayish white residues were obtained (5-6 hr).

\[
\text{Ash (\%) = } \frac{\text{Weight of residues after incineration (g) }}{\text{Initial weight of sample (g) }} \times 100
\]

### 3.2.6. Nitrogen Free Extract (NFE)

The nitrogen-free extract in cabbage samples was calculated by using following equation;

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

### 3.2.7. Total carbohydrate content

The carbohydrate content in cabbage samples was calculated by using following formula;

\[
\text{Total Carbs (\%) = } 100 - (\% \text{ moisture } + \% \text{ crude protein } + \% \text{ crude fat } + \% \text{ ash})
\]

### 3.2.8. Mineral profile

Minerals like Na, Ca and K were determined using Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge, UK) Method no 956.01. Whilst, Mg, Fe, Zn, Mn, Co and Cu through Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) according to the standard procedures of AOAC (2006) Method no 975.03 (b) and 991.11. Purposely, wet digestion of dried sample (0.5 g) was carried out using di-acid mixture
of nitric acid (HNO₃) and perchloric acid (HClO₄) in the ratio of 7:3 on hot plate till 1 to 2 mL solution left followed by dilution up to 100 mL and quantification using the desired system.

3.2.9. Quantification of vitamin C

The ascorbic acid was extracted and analyzed through High Performance Liquid Chromatography (HPLC) system as described by Podsedek et al. (2006). Purposely, cabbage samples (10 g) were homogenized with 25 mL of extracting solution; HPLC grade water containing 5% metaphosphoric acid for 15 min, at room temperature using shaker. Afterwards, the resultant sample was centrifuged (centrifuge machine; M-3K30, Sigma, Germany) at 4000 rpm for 10 min at 4 °C and supernatant was collected and filtered through 0.45 μm membrane filter followed by storage at -40 °C prior to analysis. After dilution, samples were analyzed using HPLC (Shimadzu LC-20AC, Kyoto, Japan) equipped with Shim-Pack CLC-ODS (C₁₈), 25 cm x 4.6 mm, 5 μm column. For detection of ascorbic acid, an isocratic mobile phase of 70% methanol was used with flow rate 1 mL/min. The elution was detected with UV-Vis detector set at 280 nm. Ascorbic acid was identified by comparing retention time of the sample with ascorbic acid standard and multiplied by dilution factor.

3.2.10. β-carotene assessment

The β-carotene and vitamin E in fresh cabbage samples were extracted and analyzed according to the procedure published by Siriamornpuna et al. (2012). The samples (2 g) were placed in a vessel which was protected from light using Al-foil; then it was mixed with 100 mL of extraction solvent (hexane/ethanol :: 75:25 v/v). The mixture was stirred for 30 min at 320 rpm. Afterwards, 15 mL water was added and upper layer was collected in a round-bottom flask and an aliquot of 10 mL of extract was diluted with methanol/acetonitrile (1:1, v/v) to a final volume of 4 mL. The final solution was filtered via 0.45 μm membrane filters and filtered solution (20 μL) was injected in the HPLC system (Shimadzu LC-20AC, Kyoto, Japan) contained column Shim-Pack CLC-ODS (C₁₈), 25 cm x 4.6 mm, 5 μm chromatographic separation column. The mobile phase was consisted of acetonitrile+water, 10:90 (v/v) at a flow rate of 0.8 mL/min. The absorbance was recorded at 450 nm for β-carotene by comparing with standard peaks.
3.2.11. $\alpha$-Tocopherol determination

The extraction method used for $\alpha$-tocopherol was similar to that of $\beta$-carotene as aforementioned (Singh et al., 2007). The specifications for HPLC (Shimadzu LC-20AC, Kyoto, Japan) analysis include column Shim-Pack CLC-ODS (C\textsubscript{18}), 25 cm x 4.6 mm, 5 μm. The mobile phase was consisted of 100% methanol at a flow rate of 1 mL/min. The absorbance was recorded at 290 nm for tocopherol. The concentration of sample was calculated from peak area of sample & standard and concentration of standard.

3.3. *In vitro* analysis of polyphenols and antioxidant capacity

Green and red cabbage extracts were prepared for the purpose of laboratory analysis including phytochemical & antioxidants assays. Their detailed procedures are elaborated below:

3.3.1. Extraction protocol

The green and red cabbage extracts were prepared using aqueous methanol following the protocol of Naguib *et al.* (2012). Purposely, fresh leaves of each cabbage were treated with binary solvent system; methanol: water (70:30 v/v) and macerated overnight at 4 °C. Afterwards, the extraction was facilitated through orbital shaker (Edmund Buhler Gmg H-Ks 15, Germany) with speed (280 rpm), time (4 hr) and temperature (20 °C). Finally, the extract was filtered and methanol was evaporated at 40±5 °C using rotary evaporator (Eyela, Japan). The resultant aliquots were tested for phytochemistry and antioxidant assays.

3.3.2. Phytochemical screening analyses

3.3.2.1. Total polyphenols (Folin-Ciocalteu method)

The phenolic compounds in cabbage extracts reduces phosphotungstic acids contained in Folin-Ciocalteu reagent (colorless) to phosphotungstic blue under alkaline conditions and change in absorbance gives a measure of total phenolic contents (TPCs) as outlined by Mahboubi *et al.* (2015). In this context, Folin-Ciocalteu reagent was employed to determine the TPCs of extracts. Purposely, 50 μL of extract was mixed with 1.0 mL of Na\textsubscript{2}CO\textsubscript{3} (20%) followed by the addition of 50 μL of Folin-Ciocalteu reagent. The mixture was incubated for 40 min at 25 °C. Absorbance was measured at 765 nm using microplate reader (Model No. ELx-800 BioTek, USA). Different concentrations of gallic acid (standard) were used to attain
calibration curve. Total phenolic contents were calculated in terms of gallic acid equivalent (GAE).

### 3.3.2.2. Total flavonoids (Aluminum chloride colorimetric assay method)

Flavonoids in cabbage extracts were determined, based on the development of flavonoid-aluminium complex (pink colored mixture) as illustrated by Mohammed and Manan (2015). For the purpose, 100 µL extract or different concentrations of quercetin standard were added to glass tube followed by the addition of 300 µL 5% NaNO2. After 5 min, 600 µL of 10% AlCl3 was added and at 6th min, 2 mL of 1 M NaOH was added. The total volume was made up to 5 mL by adding distilled water. Absorbance was recorded immediately at 510 nm and standard curve was made to express the data as Quercetin Equivalents (QE).

### 3.3.2.3. Total anthocyanins determination

The total anthocyanins were determined by pH-differential spectrophotometry method as described by Xu et al. (2010). In this context, 50 µL of extract was mixed separately with 1 mL of sodium acetate and potassium chloride, adjusted to pH 1 and 4.5 by HCl, respectively followed by centrifugation (5000 rpm, 25 min at 4 °C). Afterwards, supernatants were taken in 96-well plate and absorbance of samples was recorded at 510 nm.

\[
A = (A_{510} \text{ pH 1}) - (A_{510} \text{ pH 4.5})
\]

\[
\text{Anthocyanin (mg/L)} = \frac{A \times \text{Molecular weight} \times \text{DF} \times 1000}{\varepsilon \times 1}
\]

\[
y \text{ (mg/100g F.W.)} = \frac{\text{Anthocyanin content} \times V}{m} \times 100
\]

Where, y = content of anthocyanins; A = absorbance; \(\varepsilon\) = extinction co-efficient; DF = dilution factor; V = final volume; m = weight of red cabbage

### 3.3.3. HPLC analyses of phenolic acids and flavonoids

HPLC quantification of phenolic acids and flavonoids were performed as per the guidelines of Sultana and Anwar (2008) and Sultana et al. (2012), respectively. For sample preparation, 5 mL of plant extract was solvated with 6 mL of HPLC grade H2O and 12 mL of methanol. Afterwards, the mixture was shaken for 5 min following addition of 10 mL of 6 M HCL. It was given stay for 2 hr in water bath at 60 °C to free the bound phenolics. Afterwards, the sample was filtered using 0.45µm Millipore filter and filtrate was injected in the HPLC system (Shimadzu LC-20AC, Kyoto, Japan) fitted with column Shim-Pack CLC-ODS (C-18), 25 cm
x 4.6 mm, 5 μm and detected via UV-Vis detector. The mobile phase was gradient solvent system; A (water:acetic acid; 94:6 pH = 2.27) and B (100% acetonitrile) consisted of acetonitrile+water, 10:90 (v/v) at a flow rate of 1 mL/min. The absorbance was recorded at 280 nm for quercetin, gallic acid, p-coumeric acid, vanillic acid, trans-4-hydroxy 3 methoxy-cinnamic acid, 4 hydroxy 3 methoxy benzoic acid and sinapic acid by comparing with standard peaks. Whilst, HPLC specification were different for kaempferol like Shim-Pack CLC-ODS (C-18), 25 cm x 4.6 mm, 5 μm column, acetonitrile:dicholoromethane:methanol-60:20:20 mobile phase at 1 mL/min flow rate and 248 nm as detection absorbance. Quantification of all compounds was done using their respective standards.

For EGCG, sample preparation and HPLC specifications were different. Acetonic cabbage extract 70% along with addition of 1% acetic acid was prepared following the similar extraction procedure as described earlier (3.3.1). The resultant concentrated aliquot was filtered using 0.45 μm Millipore filter and filtrate was injected in the HPLC (Shimadzu LC-20AC, Kyoto, Japan) containing C-18 column Shim-Pack CLC-ODS (25 cm x 4.6 mm, 5.0 μm particle size). The mobile phase was isocratic solvent system; water:methanol:phosphoric acid-70:30:0.1 at flow rate of 0.5 mL/min. The detection was taken at 280 nm and quantification was done using its EGCG standard (Longo et al., 2008).

3.3.4. Antioxidant capacity of extracts

3.3.4.1. DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging assay

The odd electron of DPPH (free radical with deep violet color) gets paired off in the presence of free hydrogen atoms from cabbage polyphenols, forming yellow colored hydrazine (DPPH-H) thus decrease in absorption strength give an estimation of DPPH radical scavenging ability as estimated by Bhakya (2016). DPPH solution was prepared by dissolving 0.004 g of DPPH radical in 100 mL of methanol. The resultant DPPH solution (1 mL) was added in glass tube followed by the addition of 10 μL extract. From the reaction mixture, 200 μL was transferred to 96-well plate and incubated for 20-30 min in dark. The optical density (OD) was measured through ELISA plate reader at 517 nm. The potential of cabbage phenolics to scavenge the DPPH radical was calculated using the following formula:

\[
\text{DPPH radical scavenging potential (\%)} = \left(\frac{\text{OD control} - \text{OD sample}}{\text{OD control}}\right) \times 100
\]
3.3.4.2. ABTS [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay]

The cabbage polyphenols suppress the generation of ABTS·+ (green coloration) by donating electron, inhibiting colored ABTS radical. The decrease in absorbance, indicates more polyphenols as measured by Désiré et al. (2016). Purposely, 5 mL ABTS solution was diluted with 30 mL of methanol till the absorbance reached to 0.700±0.005. The diluted ABTS solution (180 µL) was mixed with 20 µL of cabbage extract and absorbance was noted at 734 nm. This value was then used to calculate antioxidant capacity against standard curve of Trolox, expressing the results in terms of Trolox equivalent (TE).

3.3.4.3. FRAP (Ferric Reducing Antioxidant Power) assay

The cabbage phenolics reduce ferric-tripyridyl triazine complex (colorless) to blue colored ferrous tripyridyltriazine (TPTZ) complex. The increase in absorbance of reaction mixture indicates increase in the reducing power as determined by Koh et al. (2016). The freshly working solutions including 25 mL of acetate buffer (300 mM), 2.5 mL of TPTZ (10 mM) and 2.5 mL of FeCl₃·6H₂O solution (20 mM) were mixed and heated to 37 °C. Afterwards, 200 μL of extract reacted with 2800 μL of FRAP solution for 30 min in the dark and absorbances were taken at 593 nm. The values were expressed in μM Fe (II)/g using linear standard curve based on different concentrations of FeSO₄.

3.3.4.4. PFRAP (Potassium Ferricyanide Reducing Antioxidant Power) assay

The cabbage phenolics reduce ferric (Fe³⁺; potassium ferricyanide) in the presence of FeCl₃ to ferrous (Fe²⁺; potassium ferrocyanide), forming intense Prussian blue complex and increase in absorbance i.e. directly proportional to reducing power of the test sample as elaborated by Singh et al. (2015). Purposely, cabbage extracts (100 µL) was treated with PBS (250 µL; 0.2 M- pH 6.6) and 1% of potassium ferricyanide (250 µL) followed by incubation (50 °C, 20 min). Afterwards, the resultant mixture was treated with 1% of TCA followed by centrifugation (300 rpm, 10 min). After centrifugation, supernatant (250 µL) was mixed with distilled water (250 µL) and 0.1% of ferric chloride (50 µL) and absorbance was taken at 700 nm.

3.3.4.5. Hydrogen peroxide inhibitory activity

The hydrogen peroxide capturing capacity of cabbage extract was analyzed by Bera et al. (2015). For the preparation of H₂O₂ solution, 20 µL of H₂O₂ was treated with 15 mL of PBS.
From the prepared mixture, 0.3 mL of H$_2$O$_2$ solution was mixed with 50 μL of extract. Afterwards, 50 μL of the prepared sample was read at 230 nm. The control sample (H$_2$O$_2$ solution) and blank (PBS) was also read at similar wavelength

$$\text{Scavenging H}_2\text{O}_2 (\%) = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

3.4. Product development

3.4.1. Food product design

Ten treatments of designer croquettes were prepared using two types of cooking procedures (baking; C$_B$ & frying; C$_F$) and four types of formulations; C$_1$ containing green cabbage, C$_2$ enriched with green cabbage aqueous extract, C$_3$ stuffed with red cabbage and C$_4$ enriched with red cabbage aqueous extract along with control (C$_0$). All the prototypes followed the same recipe except the difference of cabbage type & respective aqueous extract (Table 3.1).

3.4.2. Preparation of cabbage aqueous extract

The cabbage and water was subjected to an electric blender to obtain aqueous extract followed by filtration using muslin cloth to remove fiber portion (Al-Dosari, 2014).

3.4.3. Preparation method for croquettes

Designer cabbage based croquettes were prepared using boiled chicken 37 g, boiled potatoes 30 g, cabbage 20 g or cabbage extract 10 mL, carrot 3 g, green capsicum 3 g, cheddar cheese 3 g, corn 2 g and black olives 2 g. The prepared croquette formulations were weighed, mixed with seasonings, shaped and coated properly followed by frozen storage at -40 °C for one month. During storage intervals (0, 15 & 30 days), the prepared treatments were analyzed for physicochemical analyses and hedonic response after cooking. Furthermore, two types of processing procedures were applied; frying and baking to check the response of cooking procedure on antioxidant capacity and sensory attributes. Before baking or frying, the cost analysis of frozen croquettes; control, green cabbage stuffed, red cabbage stuffed, green cabbage extract enriched and red cabbage extract enriched croquettes were 160, 132, 140, 136 and 144 rupees/500 g packing weight.
Table 3.1: Treatment plan for product development module

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baked croquettes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;0B&lt;/sub&gt;</strong></td>
<td>Control (baked croquettes)</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;1B&lt;/sub&gt;</strong></td>
<td>Baked croquettes stuffed with green cabbage</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;2B&lt;/sub&gt;</strong></td>
<td>Baked croquettes enriched with green cabbage aqueous extract</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;3B&lt;/sub&gt;</strong></td>
<td>Baked croquettes stuffed with red cabbage</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;4B&lt;/sub&gt;</strong></td>
<td>Baked croquettes enriched with red cabbage aqueous extract</td>
</tr>
<tr>
<td><strong>Fried croquettes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;0F&lt;/sub&gt;</strong></td>
<td>Control (fried croquettes)</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;1F&lt;/sub&gt;</strong></td>
<td>Fried croquettes stuffed with green cabbage</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;2F&lt;/sub&gt;</strong></td>
<td>Fried croquettes enriched with green cabbage aqueous extract</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;3F&lt;/sub&gt;</strong></td>
<td>Fried croquettes stuffed with red cabbage</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;4F&lt;/sub&gt;</strong></td>
<td>Fried croquettes enriched with red cabbage aqueous extract</td>
</tr>
</tbody>
</table>

3.4.4. Designer food analyses

3.4.4.1. Compositional analysis and calorific value

The developed prototypes were assessed for moisture, protein, ash, crude fat and total carbohydrates (as described in section 3.2.7.) according to the method described by AOAC (2006). Furthermore, energy value of each sample was analyzed by the Atwater factor as described by Neiva et al. (2011).

3.4.4.2. Storage studies

3.4.4.2.1. Color

The croquette samples were assessed for color as per the guidelines of Das et al. (2013). The ground product with inner surface was placed in the plastic cup and light captures the color tonality from the bottom of the cup to determine the values of L (lightness), a* (−a greenness; +a redness) and b* (−b blueness; +b yellowness) using CIE-Lab Color Meter bench-top colorimeter (CIELAB SPACE, Color Tech-PCM, USA). The data obtained was used to calculate chroma and hue angle. After the application of formula for hue angle, the actual range was found; +a & +b= 0-90 (red-yellow), -a & +b= 90-180 (yellow-green), -a & -b= 180-270 (green-blue) and +a & -b= 270-360 (blue-purple).
3.4.4.2. Water activity

The water activity of the resultant samples was analyzed using Hygropalm water activity meter; Rotronic aw-Dio (Fuchs et al., 2013). It is a portable humidity temperature indicator in which ground sample was filled in the plastic cups and water activity reading (0-1), along with temperature was displayed on remote unit after almost three to four minutes.

3.4.4.2.3. Texture

Texture of the samples was measured using compressible probe of Texture Analyzer (TA-XT plus Texture Analyzer by texture technologies corporation and by stable micro systems made in Hamilton, Canada) attached to a software (Feng et al., 2016). A crosshead speed of 2 mm/sec with a load cell of 50 kg was used to compress the prepared treatments. More the distance travelled, more the capacity to endure compression force without breakage. The parameter obtained from the curves was hardness; maximum force required to compress croquettes was noted and means were calculated.

3.4.4.2.4. Phytochemical and antioxidant analyses

During storage, designer croquettes were quantified for total phenolic contents (TPCs) and total flavonoids (TFs) as described by Mahboubi et al. (2015) and Mohammed and Manan (2015). Alongside, total antioxidant capacity was assessed using DPPH, ABTS and FRAP assays (Bhakya, 2015; Désiré et al., 2016; Koh et al., 2016).

3.4.4.2.5. Sensory response

For sensory quality, designer croquettes were evaluated by a panel of judges (n= 15) using 9-point hedonic scale system; 9 = like extremely; 1 = dislike extremely (Fuchs et al., 2015). The hedonic response of the prepared prototypes containing with green & red cabbage and their aqueous extracts were evaluated in the Sensory Evaluation Laboratory of the NIFSAT, University of Agriculture, Faisalabad, involving students and staff members aging 25-45 years. Various sensory attributes including color, taste, odor, tenderness, juiciness and overall acceptability of the prepared products were scored. All the ten treatments were served hot under soft white light. To remove any biasness, the treatments were presented to the judges in transparent plates coded with random numbers. The judges were requested to express their

\[
\text{Chroma} = \sqrt{a^*2 + b^*2} \\
\text{Hue angle} = \tan^{-1} \left( \frac{b^*}{a^*} \right)
\]
opinion by assigning scores to each treatment. For effective response, panelists were provided with mineral water and unsalted crackers to neutralize their mouth receptors. The evaluation was performed after cooking for three consecutive intervals during one month frozen storage.

3.5. Selection of cabbage

Based on highly significant antioxidant capacity and acceptable sensory response, red cabbage & its extract was selected for further evaluation using animal modeling.

3.6. Experimental paradigms

For bioefficacy assessment, 60 male white New Zealand rabbits (aged 8 to 12 months) were housed in the Animal Room of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The bioevaluation was carried out to test the nutraceutical worth of red cabbage against oxidative stress in response to hypercholesterolemic diet. Purposely, experimental animals were acclimatized to environmentally controlled room (temperature & relative humidity of 23±2 °C & 55±5%, respectively) and fed standard laboratory diet and water ad libitum for one week before experimentation. During 12-week trial, rabbits were divided into six groups on the basis of diet fed, each group containing ten animals. Accordingly, Group G0, renamed as “N”; rabbits were administrated with normal diet, whereas hypercholesterolemic diet (i.e. 2% cholesterol for first week followed by 1% for the rest of first month, whereas dietary intervention was given throughout the study plan to harmonize the effect except the first week of the study to alter lipid profile) was given to Group G3: “H”; rabbits to induce hyperlipidemia and associated oxidative stress. In Groups G1, renamed on the basis of selected cabbage type; “NRC”, rabbits relied on the red cabbage leaves, supplemented 20% of their diet while Group G2; “NRCE”, provided with aqueous red cabbage extract equivalent in amount to the cabbage leaves fed to NRC along with normal diet whilst, high calorie diet in conjunction with red cabbage leaves and its aqueous extract was given to Groups G4 and G5; “HRC” and “HRCE”, respectively (Table 3.2). During the experimental phase, physical parameters including feed intake and body weights were measured. At termination, overnight fasted animals were weighed, sacrificed and sera were collected in non-coated tubes (yellow capped vials) to assess serum lipidemic profile and related oxidative stress biomarkers of liver, heart and kidney through Microlab-300, Merck, Germany, whereas EDTA coated tubes (purple and blue capped vials) were employed for
hematological aspects; complete blood count and prothrombin time, correspondingly. Furthermore, hepatic, cardiac and renal tissues were washed with cold saline and then placed in PBS normal saline to analyze indicators of oxidative stress and formalin to fix tissues for histomorphology, respectively.

3.6.1. Feed Efficiency Ratio (FER)

During 12-week experimental period, net feed (as normal fed) intake was recorded daily, whereas body weight gain (wet basis) was measured on weekly basis. Each animal net feed intake and body weight gain during the set duration (12 weeks) was used to calculate FER as described by Al Hamedan (2010).

\[ \text{FER} \% = \frac{\text{Body weight gain (g)}}{\text{Feed intake (g)}} \times 100 \]

<table>
<thead>
<tr>
<th>Table 3.2: Animal study plan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diets</strong></td>
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<tr>
<td>Normal diet</td>
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<tr>
<td></td>
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<tr>
<td>Hypercholesterolemic diet</td>
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</table>

3.6.2. Lipidemic parameters and ratio

The serum lipid profile; high density lipoprotein (HDL) was determined by HDL precipitant method using commercially available Ecolin kits (Merck, Germany), whereas total cholesterol (TC) and triacylglycerol (TAG) were measured using commercial kits; Fluitest Chol (Cholesterin CHOD-PAP) and Fluitest TG (Triglyceride GPO-PAP) kits (Biocon, Vohl-Marienhagen, Germany), respectively. The analyses were performed using semi automated clinical chemistry analyzer; Microlab 300, Merck, Netherland. Furthermore, non-HDL, LDL and VLDL were calculated using Friedewald formula. The lipidemic ratio including Atherogenic Index (AI), Cardiac Risk Ratios (CRRs), HDL to cholesterol ratio (HTR) and Anti-atherogenic Index (AAI) were calculated (Cavallini et al., 2009; Jeong et al., 2010; Hassan et al., 2011; Paun et al., 2015; Vijayasteltar et al., 2016).
3.6.2.1. Friedewald formula;

*Low density lipoprotein-cholesterol (LDL-c)*

\[
\text{LDL concentration} = \text{Total cholesterol} - \text{HDL} - \text{VLDL}
\]

*Very low density lipoprotein-cholesterol (VLDL-c)*

\[
\text{VLDL concentration} = \frac{\text{Triglycerides}}{5}
\]

*Non- High density lipoprotein-cholesterol (non-HDL-c)*

\[
\text{nHDL (LDL+IDL+VLDL)} = \text{Total cholesterol} - \text{HDL}
\]

3.6.2.2. Lipidemic ratio:

*Atherogenic index (AI)*

\[
\text{Atherogenic index} = \frac{\text{nHDL}}{\text{HDL}}
\]

*Cardiac risk ratio (CRR)*

\[
\text{Cardiac risk factor ratios} = \frac{\text{LDL}}{\text{HDL}}
\]

*HTR (HDL-cholesterol to total cholesterol ratio)*

\[
\text{HTR \%} = \frac{\text{HDL}}{\text{Total cholesterol}} \times 100
\]

*Anti-atherogenic index (AAI)*

\[
\text{Antiatherogenic index} = \frac{\text{HDL}}{\text{nHDL}}
\]

3.6.3. Serum specific oxidative stress biomarkers of liver, heart and kidney

These parameters were assessed via semi automated clinical chemistry analyzer; Microlab 300, Merck, Netherland.

3.6.3.1. Biomarkers of liver oxidative stress

The alanine transaminase (ALT) was measured by IFCC method. However, serum alkaline phosphatase (ALP) was measured by Alkaline Phosphates-DGKC method, whereas \(\gamma\)-glutamyl transferase by Gamma GT SL, Merck. However, total bilirubin was analyzed using Jenrassik & Grof method and direct bilirubin by Schellong & Wende method using commercial kit (Ecoline, Merck Germany). The indirect bilirubin was calculated by subtracting direct bilirubin.
from total bilirubin (Park et al., 2012; Nakyinsige et al., 2013; Hussain et al., 2016; Vijayasteltar et al., 2016).

3.6.3.2. Biomarkers of heart oxidative stress

The aspartate transaminase (AST) was measured by IFCC method (EC 2.6.1.1). The creatine kinase (CK) and creatine kinase-MB (CK-MB) were also measured employing commercial kit methods; (CK NAC Innoline, Merck) and (Breuer & Breuer Diagnostic, Germany), respectively. Furthermore, lactate dehydrogenase (LDH) was estimated by optimized test according to German Society of Clinical Chemistry; DGKC kit; Breuer & Breuer Diagnostic, Germany (Narin et al., 2010; Yang et al., 2010; Nakyinsige et al., 2013; Badole et al., 2015).

3.6.3.3. Biomarkers of kidney oxidative stress

The glucose was estimated by enzymatic photometric test method GOD-PAP (Breuer & Breuer Diagnostic, Germany). Moreover, urea by Urease-glutamate dehydrogenase (GLDH) enzymatic UV test and creatinine by Jaffe method were quantified as renal oxidative stress indicators using commercial kits (Adriawan et al., 2015; Hussain et al., 2016; Vijayasteltar et al., 2016).

3.6.4. Endogenous antioxidant activity and lipid peroxidation in sera and tissues

The collected serum from each animal was directly employed to SOD & CAT activities and MDA assay. On the other hand, liver, heart & kidney from all animals were harvested, washed with ice cold saline water, weighed, homogenized and centrifuged (high speed cooling centrifuge at 5000 rpm for 20 min) by placing in 10% w/v chilled potassium phosphate buffer; PBS. The clear supernatant was transferred to Eppendorf tubes and stored at -40°C to assess enzymatic oxidative stress indicators; superoxide dismutase (SOD) & catalase (CAT) activities and non-enzymatic biomarker; lipid peroxidation by adopting the method of Kakarla et al. (2005) and Umarani et al. (2015).

3.6.4.1. Superoxide dismutase (SOD)

The O$_2^{•-}$; superoxide anion, generated by xanthine-xanthine oxidase, reacts with NBT (yellow coloration), forming blue colored formazan i.e. measured at 560 nm. The percent inhibition of NBT reduction is a measure of SOD. For the determination, 50 μL of tissue extract was mixed with 1 mL of 250 μL of PBS (pH 5), 100 μL of methionine, 100 μL of trition X, 50 μL of
nitroblue tetrazolium (NBT) and 400 μL of distilled water. Afterwards, the reaction mixture was kept under UV light for 15 min. Then the resultant mixture (50 μL) was added in 96-well plate along with 50 μL of riboflavin and absorbance was measured against blank. One unit of SOD represents the amount of enzyme required to inhibit the rate of NBT oxidation by 50% at 25 °C. The enzyme activity was calculated in Unit/mL for serum and Unit/g for tissue.

3.6.4.2. Catalase (CAT)

The principle is based on decomposition rate of H$_2$O$_2$ by catalase at 240 nm. Purposely, 100 μL of tissue extract was treated with 100 μL of 5.9 mM of H$_2$O$_2$ i.e. freshly prepared in phosphate buffer saline. The disappearance of H$_2$O$_2$ was monitored at 240 nm for 1 min at 25 °C. Catalase activity was calculated using extinction co-efficient of 0.0436 mM$^{-1}$ cm$^{-1}$. The enzyme activity was calculated in Unit/mL for serum and Unit/g for tissue.

3.6.4.3. Lipid peroxidation (MDA)

Malondialdehyde (MDA) is a dialdehyde of malonic acid, determined by thiobarbituric acid reactive substances (TBARS) assay. MDA, present in the sample reacts with thiobarbituric acid (TBA) in the kit at high temperature under acidic conditions, forming MDA-TBA adduct (pink coloration) whose concentration was estimated by absorbance using spectrophotometer. Purposely, 300 μL of MDA solution (20% TCA solution containing 0.5% thiobarbituric acid) was vortex along with 50 μL of supernatant of tissue extract and heated on water bath at 50 °C for 50 min. Afterwards, the reaction mixture was cooled in iced water bath for 10 min followed by stay for 20 min at room temperature. After stabilizing, each sample mixture was centrifuged at 10 °C, 3000 rpm for 15 min. The resultant supernatant i.e. an organic layer was placed in a 96-well plate then the absorbance was recorded at 532 and 600 nm. The similar procedure was employed for blank & standard and final values were measured using the standard curve.

3.6.5. Somatic index

It gives an idea of fat deposition within a particular organ. The organs such as liver, heart and kidneys were harvested and properly rinsed with physiological saline solution and weighed on an electronic balance to calculate somatic index (Chan et al., 2015).

\[
\text{Somatic index} \% = \frac{\text{Weight of organ}}{\text{Final body weight}} \times 100
\]
3.6.6. Histopathology of liver, heart and kidney tissues

The microscopic evaluation of liver, heart & kidney tissues were carried out as described by Amal and El-Sanaa (2012). After the completion of the experimental trial, liver, heart and kidney of rabbits were excised and cuts were made using sharp blade followed by fixation in 10% w/v neutral buffered formalin (10 X to that of tissue bulk). After 30 days of fixation, the samples were trimmed and blocks were made. Afterwards, the blocks were processed using higher concentration series of alcohol, xylene and paraffin wax for dehydration, clearance and penetration, employing for particular time period. After processing, the samples were embedded on melted wax on perforated plastic holder. After cooling, the paraffin blocks were separated from the plastic holder. Now, the block carrying hard tissue was decalcified by placing in water for 1 hr followed by rapid washing, kept under frozen condition till trimmed via microtome. After sectioning the tissue at a thickness of 5 μm, it was placed on a water bath at 40 °C and finally picked up over the marked glass slide. After incubation, the sections on glass slide were stained using hematoxylin & eosin dye and mounted on neutral deparaffined xylene medium. The stained slides were viewed under light microscope for digital photomicrographs (MCX 100, Micros Austria).

3.6.7. Hematological aspects

The erythrocytes (hemoglobin & hematocrits), absolute values (total red blood count, MCV, MCH & MCHC), total white blood count and DLC; differential leukocyte count (neutrophiles, lymphocytes, monocytes, eosinophils & basophils), thromobocytes (platelets count) were determined using Medonic M Series; Boule Diagnostics Int AB Stockholm, Sweden (Al-Shehri, 2013; Karbiner et al., 2013). Besides, prothrombin time as clotting time and erythrocyte sedimentation rate (ESR) were determined using Soluplastin kit (Wiener Lab, Rosario, Argentina) and Westergren’s method using automated ESR system, respectively (Gerard et al., 2015; Hattori and Ishihara, 2015).

3.7. Statistical analysis

The resultant data from each parameter were subjected to statistical modeling through completely randomized design (CRD) using Statistix 8.1. Furthermore, level of significance was also estimated (p<0.05 & p<0.01) by using analysis of variance (ANOVA) technique followed by Tukey’s HSD multiple comparison tests for means separation. The two factor
factorial under CRD was applied in product development, having treatment and storage as two factors, whereas simple CRD was applied in compositional analyses as well as animal study, considering means at termination of the trial (Mason et al., 2003).
CHAPTER 4

RESULTS AND DISCUSSION

Currently, consumers are shifting their preference from refined, hypercaloric diets to natural, safe, wholesome & hypocaloric diets with special emphasis on fruits and vegetables. The fruits are relished owing to their sweet taste but they are costly. On the other hand, vegetables are easily accessible by the low income individuals being cost-effective besides relatively healthier than fruits. Thus, the present focus is to enhance the awareness regarding vegetable based designer foods over conventional hypercaloric foods. In this regard, bulk of review articles are available discussing the health benefits of cabbage though, there is a need of detailed scientific researches. Besides, vegetables are normally consumed as a whole hence its bioefficacy assessment is necessitated to validate the associated health implications. This is because plant matrix matters a lot, as various ingredients may undergo degradation during extraction besides the un-extracted bound moieties remained within the plant matrix. Considering these points, the present study was basically divided into three parts; 1st part based on compositional and antioxidant characterization of green and red cabbages, grown in Pakistan to assess the expected variabilities based on geographical region. Whilst, in 2nd part, two types of cooking procedures; baking & frying were employed to process cabbage enriched croquettes and in 3rd part, the selected cabbage and its aqueous extract in equivalent dosage were assessed for their free radical trapping ability within the physiological system using rabbits as animal models.

The sections and subsections of the present study are described herein;

Section 4.1: Compositional and antioxidant indices
Section 4.2: Designer prototype analyses
Section 4.3: Bioassessment trial

Subsection: 4.3.1. Lipidemic profile & serum oxidative stress biomarkers
Subsection: 4.3.2. Liver oxidative stress biomarkers
Subsection: 4.3.3. Heart oxidative stress biomarkers
Subsection: 4.3.4. Kidney oxidative stress biomarkers
Subsection: 4.3.5 Hematological aspects & inflammatory indicator
SECTION 4.1:
COMPOSITIONAL AND ANTIOXIDANT INDICES
4.1.1. Proximate composition

The compositional analysis is an imperative tool to assess the nutritive value of food commodities. According to Table 4.1., the moisture content (g/100g F.W.) was found slightly higher in green cabbage 91.29±4.29 than red cabbage 89.84±4.04 as evidenced by statistics. Resultantly, other components including crude protein, crude fat, crude fiber, ash and Nitrogen Free Extract (NFE) were found significantly higher in red cabbage as compared to green counterpart, ranging from 0.92±0.03 to 1.21±0.05, 0.02±0.01 to 0.04±0.00, 2.95±0.11 to 3.49±0.17, 0.69±0.01 to 0.87±0.03 and 4.13±0.14 to 4.55±0.17 g/100g F.W. correspondingly. On dry weight basis, the current results explicated crude protein, crude fat, crude fiber, ash and NFE in green & red cabbage samples as 11.84 & 10.92, 0.23 & 0.35, 37.77 & 31.56, 8.83 & 7.87 and 52.85 & 41.17 mg/100g, respectively.

The current results are in close harmony with the work of previous researchers, who measured crude fiber, carbohydrate, fat, protein, ash and NFE in white, green or common cabbage, varying between 21.64-33.31, 62-71.26, 0.52-4.4, 14.3-30.09, 5.82-10.5 and 35.12% D.W., whereas in red cabbage, the said traits were reported as 14.61-44.87, 64.54, 0.85, 13.99-26.67, 9.77 and 17.84% on dry weight basis. The total dietary fiber in dried cabbage was found varying from 27.3 to 29.9% however, the moisture content in fresh red cabbage was varying from 91.0 to 91.7% (Wu et al., 2006; Kahlon et al., 2007; Kahlon et al., 2008; PeñAs et al., 2010; Hussein, 2012; Amnah, 2013; Mohammed and Luka, 2013). Previously, the compositional profile of outer leaves of cabbage (Brassica oleracea L. var. capitata) portrayed protein 18.43±0.60%, lipid 1.02±0.11%, ash 9.02±0.90%, carbohydrate 30.46±0.99% and total dietary fiber 40.89±2.25%; soluble dietary fiber 7.35±0.81% and insoluble dietary fiber; 33.54±1.44% on dry mass basis (Tanongkankit et al., 2012). Earlier scrutiny documented that cabbage (Brassica oleracea L. var. capitata) possesses approx. 4.6 g carbohydrate, 1.8 g protein, 0.1 g fat, 0.6 g minerals and 36.6 mg vitamin C, 98 IU vitamin A & 76 µg vitamin K per 100 g F.W. (Tiwari et al., 2003; Park et al., 2014). The ranges of almost all attributes in the aforementioned studies are covering the data obtained in the current investigation.

In another attempt, the compositions of fresh and blanched cabbage (Brassica oleracea L. var. capitata) samples were compared by Nilnakara et al. (2009). The study outcomes
Table 4.1. Compositional profiling of cabbage

<table>
<thead>
<tr>
<th>Compositional analyses</th>
<th>Green cabbage</th>
<th>Red cabbage</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximate composition (g/100g F.W.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture</td>
<td>91.29±4.29</td>
<td>89.84±4.04</td>
<td>0.6919$^{NS}$</td>
</tr>
<tr>
<td>Crude protein</td>
<td>0.92±0.03</td>
<td>1.21±0.05</td>
<td>0.0012$^{**}$</td>
</tr>
<tr>
<td>Crude fat</td>
<td>0.02±0.01</td>
<td>0.04±0.00</td>
<td>0.0103$^*$</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.95±0.11</td>
<td>3.49±0.17</td>
<td>0.0099$^{**}$</td>
</tr>
<tr>
<td>Ash</td>
<td>0.69±0.01</td>
<td>0.87±0.03</td>
<td>0.0011$^{**}$</td>
</tr>
<tr>
<td>Nitrogen Free Extract (NFE)</td>
<td>4.13±0.14</td>
<td>4.55±0.17</td>
<td>0.0313$^*$</td>
</tr>
</tbody>
</table>

$P$ value < 0.05 = Significant ($^*$); $P$ value < 0.01= Highly significant ($^{**}$); $P$ value > 0.05 = Non-significant ($^{NS}$)
presented protein, crude fat, crude fiber, ash and carbohydrates in fresh & blanched cabbage as 19.48±0.19 & 21.15±0.02, 0.97±0.06 & 1.85±0.05, 19.92±1.08 & 47.47±3.34, 7.82±0.38 & 5.26±0.18 and 51.36±1.89 & 24.61±1.11% on dry weight basis, accordingly. These findings are also in corroboration with the results of the instant study. Recently, Malav et al. (2015) determined the characteristics of cabbage powder; moisture 10.94±0.60%, protein 4.56±0.29%, fat 2.80±0.01% and ash 7.71±0.07%. Likewise, the protein, fat and ash contents of cabbage (tronchuda) powder were reported as 4.19, 3.01 and 7.98%, respectively (Batista et al., 2011). Mostly in dieting programs, cabbage is given a major share in the diet plan being low in calories (Al-Dosari, 2014). The calories content in brassica vegetables as reported by Heimler et al. (2006) were approx. 24-34 kcal/100g as the protein, fat and fiber were 1.44-2.82, 0.12-0.37 and 2.5 g per 100g F.W., respectively.

4.1.2. Minerals

Statistical analysis indicated that minerals including potassium, sodium, iron, cobalt & manganese differed momentously in both cabbages, especially on higher side in red cabbage 96.44±3.38, 12.33±0.55, 1.11±0.04, 0.20±0.01 & 0.18±0.01 mg/100g F.W. than green cabbage 53.42±1.87, 9.87±0.39, 0.75±0.03, 0.14±0.00 & 0.12±0.01 mg/100g F.W. Furthermore, calcium, magnesium, zinc and copper explicated non-significant variations however, calcium was found more in red cabbage 20.56±1.11 than green equivalent 19.88±1.29 mg/100g F.W. whereas, magnesium, zinc and copper were detected higher in green cabbage 22.6±0.81, 0.31±0.01 & 0.05±0.00 mg/100g F.W. as compared to red cabbage 20.56±0.99, 0.29±0.01 and 0.04±0.00 mg/100g F.W., accordingly (Table 4.2).

The green cabbage consumed in southern Brazil, raw & cooked counterparts were analyzed for minerals (mg/100g F.W.) and findings disclosed higher amounts of potassium 266±87 & 275±103 followed by calcium 44±6 & 46±5, magnesium 14±2 & 15±3 and sodium 3±1 & 3±1. The K & Ca were on the higher side, whereas Mg and Na were quantified lesser than the outcomes of the current exploration. Furthermore, trace minerals like iron 0.14±0.03 & 0.16±0.04, manganese 0.2±0.1 & 0.2±0.1, copper 0.05±0.05 & 0.04±0.04 and zinc 0.2±0.1 & 0.2±0.1 mg/100g F.W. were nearly within the ranges as noted in the present study. After cooking, the percent increase in potassium was 7% while, percent decrease in rest of the minerals was observed as sodium 21, calcium 2, magnesium 2, iron 1, manganese 11, copper
3 and zinc 5. Furthermore, it is worth mentioning that calcium absorption from cabbage is higher due to the presence of citric and malic acids (Kawashima and Soares, 2003). Recently, Malav et al. (2015) reported calcium content in cabbage up to 45 mg/100g F.W. that was higher than the current findings. Similarly, another researcher reported calcium content in green cabbage in the range of 45 to 106 mg/100g F.W. Furthermore, he noted higher calcium content in outer leaves of cabbage; 476 to 998 mg/100g F.W. as compared to inner yellowish leaves, varying from 26 to 53 mg/100g F.W. (Cowell, 1932).

The outcomes of the current research are comparable to another previous study that reported Ca 29, Fe 0.8 and Na 14.1 mg/100g F.W. in common cabbage (Tiwari et al., 2003; Park et al., 2014). In another study, the present data is in agreement with the work of Lucarini et al. (2000), they determined iron and zinc in green cabbage as 0.71±0.23 and 0.53±0.12 mg/100g, respectively. In a research work, Zarembski and Hodgkinson (1962) measured the Ca, Mg and P contents in cabbage (Brassica oleracea L. var. capitata), ranging from 19.5 to 87.7, 11.6 to 2.6 and 13.9 to 97.6 mg/100g F.W., correspondingly. Moreover, Warman and Havard (1997) analyzed Ca from 341 to 603 and 312 to 614 mg/100g in conventional and organic cabbage samples, whereas Mg was varying from 125 to 144 & 125 to 154 and Cu was found as 19 mg/100g in the respective samples. Previously, Mohammed and Luka (2013) compared the green & red cabbage for different nutrients including calcium and phosphorous. They noted values for the said minerals as 1.76±0.04 & 0.96±0.09 and 0.86±0.09 & 1.89±0.19 mg/100g D.W., respectively that were far lower than results obtained in the current study.

Furthermore, red cabbage was found with higher proportions of fiber, vitamin C, A, B₆ & K and Mn (Amnah, 2013). One of their peers, Draghici et al. (2013) found higher amounts of minerals especially Ca and Mg in white and red cabbage. The Ca and Mg in white cabbage were found as 40 and 12 mg/100g F.W., respectively. Besides, cabbage juice is considered as a rich source of potassium that could fulfill 17% of the daily requirement hence maintains body’s fluid balance especially for those who consume higher amounts of sodium in their diet or facing nutrient malabsorption. Apart from this, calcium fulfills 19% of 1000 mg of daily suggested intake, contributing structure to bones and teeth. It is also considered as an excellent source of vitamin C in addition to considerable amounts of glutamine and indole-3-carbinol that are involved in anti-inflammation and liver detoxification, accordingly. However, significant reductions are expected in vitamin C while cooking thus raw counterparts should

60
need to be consumed to ensure the integrity of delicate nutrients. It also contains vitamin A, fulfilling 19% of the daily needs thus maintains teeth, skeletal tissue, skin and mucous membranes. Furthermore, the presence of selenium in cabbage is attributed to enhance immune responses. Considering Fe, the shredded green and red cabbage could supply 2 and 3% of the daily value, maintaining red blood cells function i.e. carrying oxygen to all the cells ultimately protecting from anemia (Priya, 2012).

4.1.3. Antioxidant vitamins

The HPLC quantification of vitamin C, vitamin E and β-carotene were significantly higher in red cabbage than green counterpart ranged between 121.46±3.28 & 139.07±2.23, 0.32±0.01 & 0.44±0.00 and 0.41±0.01 & 1.05±0.02 mg/100g F.W., respectively as indicated by statistical values (Table 4.3).

The cabbage also contains natural antioxidants including ascorbic acid, α-tocopherol, β-carotene and lutein that not only maintain vegetable quality but also impart nutrients in human diet (Singh et al., 2006; Park et al., 2014). Vitamin C has potential to protect cells from free radical damage and fights against cancer and eye diseases. The concentration of vitamin C in cruciferous vegetables might be varied based on climatic conditions, extraction procedure or analytical method (Chun et al., 2004; Park et al., 2014). Current US-RDA of ascorbic acid for adults is 100 to 200 mg/day (Naidu, 2003). The ascorbic acid contributes stability to anthocyanins hence inherently benefiting anthocyanins existence in red cabbage (Cavalcanti et al., 2011).

According to previous investigations, the vitamin C in white or green and red cabbage was found to vary between 9.65 & 51.65 and 24.38 & 129.90 mg/100g F.W., respectively. The ascorbic acid content in different cultivars and localities of white or green cabbage and red cabbage was ranging between 13.5 & 51.48 and 38.4 & 73.5 mg/100g F.W., correspondingly. On dry weight basis, the vitamin C in cabbage inner and outer leaves was reported as 325.78-532.85 and 639.55 mg/100g, accordingly (Lucarini et al., 2000; Proteggente et al., 2002; Naidu, 2003; Puupponerr-Pimia et al., 2003; Bahorun et al., 2004; Chun et al., 2004; Podsedek et al., 2006; Singh et al., 2006; Leja et al., 2007; Podsedek, 2007; Podsedek et al., 2008; Volden et al., 2008; Nihnakara et al., 2009; PeñAs et al., 2010; Tanongkankit et al., 2010; Draghici et al., 2013). According to an earlier study, eighteen different cultivars of cabbage
were assessed for vitamin C, ranged from 5.7 to 23.5 mg/100g F.W. (Singh et al., 2007). Additionally, Vanderslice and Higgs (1991) analyzed ascorbic acid, dehydroascorbic acid and vitamin C in fresh cabbage as 54±3.0 to 77.3±1.2, 4.3±0.6 to 6.7±1.2 and 54±5 to 83±1 mg/100g, respectively. Later, Leja et al. (2006) found increment in ascorbic acids during storage from 40.96 to 55.88 mg/100g. In another attempt, Xu et al. (2014) determined the vitamin C content in fresh, stir-fried, boiled, microwave and steamed red cabbage and elucidated the contents as 79, 28, 50, 71 and 80 mg/100g F.W., respectively. Earlier, Kurilich et al. (1999), Davey et al. (2000), Chu et al. (2002), Priya (2012) quantified relatively lower proportion of vitamin C in cabbage, varying between 32 and 47 mg/100g F.W. as compared to the present analyses results.

One of the researchers groups, Podsedek et al. (2008) assessed the leeching of vitamin C from red cabbage ranged from 16.3 to 36.2% on domestic cooking, varying on the basis of cultivars. One of their peers, Volden and his coworkers (2008) determined 24% loss of ascorbic acid during boiling of red cabbage in water (1:1 ratio) for 10 min. Earlier, Rickman et al. (2007) and Leong and Oye (2012) measured the losses during blanching and freezing of vegetables up to 60 and 20%, respectively. During blanching, provision of high temperature causes release of ascorbic acid oxidase, localized in cytosol and vacuoles, to interact with ascorbic acid followed by thermal oxidation/degradation. On the other hand, formation of large ice crystals during freezing ruptures the cells. Moreover, Puupponerr-Pimia et al. (2003) studied the reduction of vitamin C content in cabbage from 43 to 30 mg/100g F.W. while processing, whereas the content reduced to 26 & 21 mg/100g F.W. during 6 & 12-month storage. Recently, Xu et al. (2014) found that thermal processing increased oxidation of ascorbic acid to dehydroascorbic acid and the effect was more pronounced in case of stir frying and boiling due to leeching in surrounding water, whereas lowest in fresh-cut, microwave and steamed cabbage.

Conclusively, it was found that red cabbage possesses higher proportion of phenolics as compared to other cruciferous vegetables however, in case of ascorbic acid, red cabbage ranked second after brussels sprout. On the other hand, white cabbage demonstrated lower amounts of both phenolics as well as ascorbic acid contents (Podsedek et al., 2006). One of their peers, Wold et al. (2006) compared the red and white cabbage in 2001 and 2002 and found minor variations.
According to an earlier scrutiny, Podsedek et al. (2006) determined α-tocopherol in white & red cabbage, differing from 0.008±0.001 to 0.022±0.005 and 0.061±0.003 to 0.111±0.008 mg/100g F.W., accordingly. One of their peers, Singh and his co-workers (2006) assessed variation in cabbage cultivars for DL-α-tocopherol, the red cabbage possesses higher amounts 0.261 mg/100g in comparison to white counterparts; 0.107 mg/100g. One of the researchers groups, Tanongkankit et al. (2010) analyzed α-tocopherol in cabbage outer leaves as 5.45±0.09 mg/100g D.W. Previously, Warman and Havard (1997) determined vitamin E in conventional and organic cabbage samples to be varying from 0.32 to 0.44 and 0.31 to 0.43 µg/g F.W., respectively. Furthermore, the total tocopherols and tocotrienols in cruciferous vegetables were detected maximum in broccoli 0.82 trailed by brussels sprouts 0.40, cauliflower 0.35, chinese cabbage 0.24, red cabbage 0.05 and white cabbage 0.04 mg/100g (Podsedek, 2007). The previously published findings regarding α-tocopherol in cabbage cultivars were found in the range of 0.03 to 0.69 mg/100g F.W. (Kurilich et al., 1999; Ching and Mohamed, 2001; Singh et al., 2006; Singh et al., 2007).

Earlier, Singh and his co-workers (2006) determined β-carotene and lutein on higher side in white cabbage; 0.050 and 0.137 mg/100g as compared to red cabbage 0.044 and 0.046 mg/100g. Furthermore, white and red cabbage indicated β-carotene ranged from 0.01 to 0.13 and 0.02 to 0.05 and lutein+zeaxanthin 0.08 to 0.45 and 0.03 to 0.15 mg/100g of edible portion (Podsedek et al., 2006; Podsedek, 2007). According to an earlier scrutiny, eighteen different cultivars of cabbage were found to possess β-carotene in the range of 0.01 to 0.12 and lutein 0.02 to 0.26 mg/100g F.W. (Singh et al., 2007). Later, β-carotene in outer cabbage leaves was assessed by Tanongkankit et al. (2010), as 9.44±0.13 mg/100g D.W. In contrary, Puupponen-Pimia et al. (2003) did not report the presence of β-carotene in cabbage.

Amongst, 22 vegetables, carotenoids in red and white cabbage were ranked on 20th (0.43 mg/100g edible portion) and 21st position (0.26 mg/100g edible portion), respectively (Müller, 1997). However, Podsedek et al. (2006) assessed total carotenoids in white & red cabbage varying in the range of 0.009±0.001 to 0.051±0.003 & 0.013±0.001 to 0.016±0.002 mg/100g F.W., respectively. Earlier, Takagi (1985) analyzed total carotenoids in cabbage (Brassica oleracea L. var. capitata) as 1731.3 µg/100g F.W. with different percentages of each fraction; carotene 17.9%, lutein 36.3%, antheraxanthin 15.3%, violaxanthin 14.2%, neoxanthin 13.5% and crocetin 0%. In another study, Heinonen et al. (1989) measured carotenoid fractions in
Table 4.2. Mineral contents of cabbage

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Green cabbage</th>
<th>Red cabbage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (K)</td>
<td>53.42±1.87</td>
<td>96.44±3.38</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>19.88±1.29</td>
<td>20.56±1.11</td>
<td>0.5268NS</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>22.60±0.81</td>
<td>20.61±0.99</td>
<td>0.0545NS</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>9.87±0.39</td>
<td>12.33±0.55</td>
<td>0.0033**</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>0.75±0.03</td>
<td>1.11±0.04</td>
<td>0.0002**</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.31±0.01</td>
<td>0.29±0.01</td>
<td>0.0913NS</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.05±0.00</td>
<td>0.04±0.00</td>
<td>0.5185NS</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.14±0.00</td>
<td>0.20±0.01</td>
<td>0.0005**</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.12±0.01</td>
<td>0.18±0.01</td>
<td>0.0011**</td>
</tr>
</tbody>
</table>

Table 4.3. Antioxidant vitamins of cabbage

<table>
<thead>
<tr>
<th>Antioxidant vitamins</th>
<th>Green cabbage</th>
<th>Red cabbage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>121.46±3.28</td>
<td>139.07±2.23</td>
<td>0.0015**</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.32±0.01</td>
<td>0.44±0.00</td>
<td>0.0002**</td>
</tr>
<tr>
<td>β-carotene</td>
<td>0.41±0.01</td>
<td>1.05±0.02</td>
<td>0.0000**</td>
</tr>
</tbody>
</table>

P value < 0.05 = Significant (*); P value < 0.01 = Highly significant (**); P value > 0.05 = Non-significant (NS)
white & red cabbage; α-carotene (traces), β-carotene (0.066 & 0.015 mg/100g F.W.), γ-carotene (not detected; nd), cryptoxanthin (nd), lutein (0.15 & 0.026 mg/100g F.W.) and lycopene (nd). Furthermore, Kurilich et al. (1999) reported α- and β-carotene as 0.06 to 0.27 and 0.00 to 0.006 mg/100g F.W., correspondingly.

### 4.1.4. Phytochemistry of cabbage

The statistical analysis depicted that phenolic acids and flavonoids in both cabbages were varying significantly. The total polyphenols in green and red cabbage samples were 58.41±3.01 & 224.37±6.96 mg/100g F.W., respectively. Furthermore, the HPLC analysis of phenolic acids in green cabbage showed higher proportion of chlorogenic acid 17.47±0.63 followed by vanillic acid 10.98±0.15, gallic acid 6.78±0.10 and sinapic acid 4.50±0.11 mg/100g F.W. On the other hand, eight varied phenolic acid moieties were detected in red cabbage, demonstrating abundant quantity of vanillic acid 13.20±0.33 trailed by syringic 8.78±0.31, gallic acid 4.36±0.20, chlorogenic acid 3.32±0.19, cinnamic acid 2.53±0.04, ferulic acid 2.37±0.01, sinapic acid 2.02±0.03 and p-coumeric acid 1.25±0.05 mg/100g F.W.

Moreover, total flavonoids in green & red cabbage were quantified as 34.04±1.06 & 219.15±10.30 mg/100g F.W., accordingly. Further, total anthocyanins were merely detected in red cabbage as 69.86±4.12 mg/100g F.W. as expected from red coloration. Besides, flavonoid fractions analyzed via HPLC system quantified quercetin, epigallocatechin gallate, catechins and kaempferol in green & red cabbage as 4.31±0.06 & 2.60±0.04, 4.63±0.11 & 28.80±0.71, 1.11±0.04 & 4.45±0.20 and 24.30±0.97 & 171.10±5.99 mg/100g F.W., accordingly (Table 4.3.).

On the basis of low caloric value and high phenolic content & antiradical activity, the vegetables consumed in Pakistan are ranked in ascending order; cabbage>cauliflower>spinach>yellow turnip>white turnip>peas>carrot. Besides, the dietary antioxidants such as carotenes, ascorbate and α-tocopherol in vegetables vary largely on the basis of genotypes (Singh et al., 2007; Sultana and Anwar, 2008). However, the quantity of polyphenols in vegetables is influenced by various factors including variety, geographical conditions, agricultural practices, maturity stage, seasons, storage conditions and analytical methods (Chun et al., 2004; Podsdek, 2007). Briefly, red cabbage potrayed 3 to 4 times higher phenolic contents as compared to green equivalent, whereas total flavonoid of red cabbage was
25 times higher than that of green cabbage and the major contribution is by anthocyanins (Chun et al., 2004; Hassimotto et al., 2005). Thus, large variations were measured in flavonoid content of leafy vegetables, depending on the basis of variety, vegetable portion, seasonal and agronomic conditions because flavonoid formation is light-dependent (DuPont et al., 2000; Hussein, 2012).

Phytochemical screening assay is a simple, quick and inexpensive procedure to assess various types of phytonutrients in plants extracts (Sasidharan et al., 2011). Earlier findings illustrated that total polyphenols in white, green or common cabbage were found to vary between 12.58 to 153.00 mg/100g F.W. On the other hand, red cabbage was within the range of 34.41 to 322.00 mg/100g F.W. Furthermore, the total flavonoids & flavonols in white and red cabbage were reported to vary from 13.13 to 18.95 & 4.4 to 8.8 and 9.01 to 141.21 & 23.3 to 57.7 mg/100g F.W., respectively. The higher antioxidant activity of red cabbage is attributed to anthocyanin content. Besides, differences also exist in cultivars & lines of cabbages, nature of extraction solvent; methanol, acetone, etc. and localities; USA, England, Poland, Germany & India like white cabbage grown in Mauritius depicted 40% less phenolic compounds as compared to Poland cultivars (Vinson et al., 1998; Chu et al., 2002; Kaur and Kapoor, 2002; Proteggente et al., 2002; Bahorun et al., 2004; Wu et al., 2004; Leja et al., 2005; Leja et al., 2006; Podsędek et al., 2006; Singh et al., 2006; Podsędek, 2007; Singh et al., 2007; Podsędek et al., 2008; Leja et al., 2010; Draghici et al., 2013; Podsdek et al., 2014; Xu et al., 2014). On dry weight basis, the TPC in green, white and red cabbage was 1917.91, 1144 and 2913 mg/100g, whereas the flavonoids were quantified as 437 and 1144 mg/100g in white and red cabbage, respectively (Chaisamlitpol et al., 2014; Gaafar et al., 2014).

Previously, one of the researchers groups, Chu et al. (2002) found that free phenolics in cabbage were higher than bound phenolics; free (67.1%), bound-E (ethyl acetate Et-Ac soluble fraction; 26.8%) and bound-W (water soluble fraction; 6.1%). Amongst bound phenolics, they assessed relatively higher amounts of bound-E phenolics in cabbage in contrast to other vegetables that could be extracted via basic hydrolysis and solvent soluble extraction procedures.

Previously, a study conducted on green, red, napa and savoy cabbage depicted higher total phenolics and total flavonoids in red cabbage 393.1±10.8 mg GAE/100g and 108.1±9.3 mg
CE/100g followed by green cabbage 97.8±0.8 mg GAE/100g and 3.9±0.4 mg CE/100g, correspondingly (Chun et al., 2004). Later, total polyphenols in fresh red cabbage were measured as 210±9 mg GAE/100g F.W. (Volden et al., 2008). Moreover, total polyphenols and flavonoids in methanolic extract of cabbage without & with the addition of 1.2 M HCl were recorded as 3.16 & 10.17 mg GAE/g and 1.67 & 3.22 mg CE/g, respectively (Gorinstein et al., 2009). Similarly, total polyphenols in methanolic extract of white and red cabbage were reported between 53.51 & 114.63 and 174.259 & 195.74 mg GAE per liter (Sakunasing and Kangsadalampai, 2008). In another study, the total phenolic content in red cabbage was estimated up to 160 mg/g F.W. that faced reduction while stir-frying, boiling, microwaving and steaming as 115, 120, 155 and 153 mg/g F.W., respectively (Xu et al., 2014). This was supported earlier by Podsędek et al. (2008), they reported that boiling (1:1 ratio, 20 min) causes more loss of cabbage polyphenols over steaming for 20 min.

One of the exceptional cases, Deng et al. (2013) measured the total polyphenols in common cabbage as 624 mg GAE/100g F.W. however, Nilnakara et al. (2009) determined total polyphenol as 1600 mg GAE/100g F.W. in red cabbage; these values were far higher as analyzed in the current investigation. In addition, Bushra and Anwar (2008) determined total flavonoids in common cabbage as 2.39 mg/100g that was too low as noted in the present study or as described by earlier scientists.

Previous researchers determined the antioxidant activity of different vegetables and noticed striking differences between red and green cabbage, apparently due to the presence of pigmented moieties; anthocyanins in red cabbage i.e. responsible for higher antioxidant ability (Singh et al., 2007; Walkowiak-Tomczak and Czapski, 2007). One of the scientists groups, Park et al. (2014) found that red cabbage (Brassica oleracea var. capitata f. rubra) contains abundant amounts of anthocyanins (13 residues detected) especially cyanidin-3-(sinapoyl) diglucoside-5-glucoside.

Previous scientists analyzed different amount & types of anthocyanins in red cabbage varieties, ranged from 14 to 495 mg/100g F.W. (Mazza and Miniati, 1993; Hodges et al., 1999; Piccaglia et al., 2002; Podsedek et al., 2006; Wu et al., 2006; Podsedek, 2007; Scalzo et al., 2008; Volden et al., 2009; Yuan et al., 2009; Leja et al., 2010; Xu et al., 2010; Kolodziejczyk et al., 2011; Draghici et al., 2013; Wiczkowski et al., 2014; Zaidel et al., 2014).
According to the reports of Wu et al. (2006), Walkowiak-Tomczak and Czapski (2007), Posmyk et al. (2009), Kolodziejczyk et al. (2011) and Park et al. (2014), the major anthocyanin fraction in red cabbage was revealed as cyanidin 3, 5-diglucoside that exist as non-acylated, mono-acylated or di-acylated with p-coumaric, caffeic, ferulic and sinapic acids. Furthermore, Wu & Prior (2005) found 23 different cyanidin derivatives in red cabbage, either conjugated with sugars or acylated with phenolic acids. According to Wu et al. (2006), around 30 different anthocyanins have been identified in red cabbage, conjugated with tri-glycoside; 15% non-acylated and 85% acylated. In this context, McDougall et al. (2007) demonstrated acetylated cyanidin glycosides and pelargonidin-3-glucoside as the major moieties and detected 18 anthocyanins in extracts of fresh and processed red cabbage. Conversely, Lin et al. (2008) demonstrated the qualitative picture of red cabbage anthocyanins through HPLC technique and found malvidin glycosides as the predominant moiety. Later, Podsedek et al. (2014) analyzed Cy3-(feruloyl) diglucoside-5-glucoside as the major anthocyanin fraction in red cabbage 13.10±0.3 mg/100g, whereas in extract the major moiety was Cy3-(sinapoyl) (feruloyl) diglucoside-5-glucoside i.e. 11.77±0.51 mg/100mL of extract. Additionally, Valenti et al. (2013) studied the anthocyanin constituents in red cabbage and found cyanidin as 11.8 mg/100g, whereas other fractions including delphindin, malvidin, pelargonidin, peonidin & petunidin were not identified. Afterwards, Wiczkowski et al. (2013) detected 20 different types of cyanidin derivatives with major moiety; cyanidin-3-diglucoside-5-glucoside. Afterwards, Wiczkowski et al. (2015) assessed 20 different fractions of anthocyanin, contributing non-acylated, mono-acylated and di-acylated fractions up to 20.6, 45.1 and 34.3%, respectively. Amongst various vegetative portions, anthocyanins were found maximum in head tissues (Yuan et al., 2009).

Furthermore, Kolodziejczyk et al. (2011) studied the phenolic compounds in crude methanolic extracts of red cabbage and demonstrated 64% anthocyanins, 21% hydroxycinnamic acids and 14% hydroxybenzoic acids. After semi-purification, 84% anthocyanins and 16% hydroxycinnamic acids were detected. Previous researchers quantified phenolic acids like quercetin, kaempferol glycosides and hydroxycinnamic acids in white cabbage (Heimler et al., 2006; Leja et al., 2010). Earlier researchers found that cabbage is a mixture of >20 phenolic compounds, out of which seven has been identified as 3-O-sophoroside-7-O-glucosides of kaempferol and quercetin that remains either unmodified or acetylated with hydroxycinnamic.
acids such as sinapic, ferulic or caffeic acid (Podsedek et al., 2006; Podsedek, 2007). Later, Podsedek et al. (2008) analyzed two different varieties of red cabbage; Koda & Kissendrup for hydroxybenzoic acid (15.48 & 25.59 mg/100g) and hydroxycinnamic acid (19.91 & 28.87 mg/100g).

Furthermore, Podsedek et al. (2006) determined more amounts of hydroxycinnamic acid in white and red cabbage ranged from 3.01±0.11 to 12.59±0.56 and 33.96±4.22 to 45.13±3.21 mg/100g F.W. than hydroxybenzoic acids 5.91±0.21 to 10.09±0.18 and 15.52±1.10 to 19.92±1.31 mg/100g F.W., respectively. Hence, they found that red cabbage is a rich source of dietary antioxidant; phenolic acids and anthocyanins, whereas kale, broccoli and brussels detected carotenoids (major moieties; lutein & zeaxanthin) and antioxidant vitamins in higher proportions. Furthermore, they documented that hydroxycinnamic acids were higher in cruciferous vegetables except a variety naming white cabbage Vestri, where hydroxybenzoic acids were measured 3.4 folds higher than hydroxycinnamic acids. However, the flavonols were found in white cabbage, ranging from 0.03±0.01 to 0.45±0.06 mg/100g F.W., whereas no flavonols were detected in red cabbage. Likewise, Bahorun et al. (2004) revealed higher levels of flavonols in white cabbage as 5.9 mg/100g F.W. They confirmed the presence of quercetin as 5.1 mg/100g F.W. and apigenin as 0.8 mg/100g F.W. while failed to detect myricetin, kaempferol and luteolin in white cabbage. On the other hand, Chu et al. (2000) reported higher amounts of flavonols in red cabbage 0.15 than white counterpart 0.11 mg/100g.

In another study, total phenolics and flavonoids in white cabbage extracts were noted as 18.4 mg GAE/g D.W. and 8.75 mg QE/g D.W., respectively. Furthermore, the hydroxybenzoic acids in the white cabbage extract were reported as 4.86 mg/g D.W., whereas hydroxycinnamic acids as 0.57 mg/g D.W. (Jaiswal et al., 2012).

In another study conducted on green, red, napa and savoy cabbage, the outcomes depicted that red & green cabbage possess good flavonoid profile (80.7 & 1.9 mg/kg F.W.); cyanidin 73.6±6.0 & 0.0±0.0, quercetin 1.1±0.2 & 0.9±0.2, apigenin 6.1±0.8 & 0.0±0.0 and kaempferol 0.0±0.0 &1.0±0.2 mg/kg F.W. Moreover, they did not found myricetin and luteolin in green and red cabbage. In the nutshell, thy found cyanidin as the major moiety in red cabbage while quercetin, kaempferol & apigenin contribute a major share in total polyphenols in other cabbage types. They also mentioned that flavonols; quercetin and kaempferol are influenced by extrinsic factors such as variation in cabbage type, season, degree of ripeness and food
<table>
<thead>
<tr>
<th>Phytochemistry (mg/100g F.W.)</th>
<th>Green cabbage</th>
<th>Red cabbage</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Total phenolic content</td>
<td>58.41±3.01</td>
<td>224.37±6.96</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>-</td>
<td>2.37±0.01</td>
<td>-</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>4.50±0.11</td>
<td>2.02±0.03</td>
<td>0.0000**</td>
</tr>
<tr>
<td>p-coumeric acid</td>
<td>-</td>
<td>1.25±0.05</td>
<td>-</td>
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<tr>
<td>Chlorogenic acid</td>
<td>17.47±0.63</td>
<td>3.32±0.19</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>-</td>
<td>2.53±0.04</td>
<td>-</td>
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<tr>
<td>Caffeic acid</td>
<td>9.42±0.38</td>
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<td>-</td>
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<tr>
<td>Gallic acid</td>
<td>6.78±0.10</td>
<td>4.36±0.20</td>
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<td>Vanillic acid</td>
<td>10.98±0.15</td>
<td>13.20±0.33</td>
<td>0.0004**</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>-</td>
<td>8.78±0.31</td>
<td>-</td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>34.04±1.06</td>
<td>219.15±10.30</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Quercetin</td>
<td>4.31±0.06</td>
<td>2.60±0.04</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>24.30±0.97</td>
<td>171.10±5.99</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>4.63±0.11</td>
<td>28.80±0.71</td>
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<tr>
<td>(+)-Catechins</td>
<td>1.11±0.04</td>
<td>4.45±0.20</td>
<td>0.0000**</td>
</tr>
<tr>
<td>Total anthocyanins</td>
<td>-</td>
<td>69.86±4.12</td>
<td>-</td>
</tr>
</tbody>
</table>

*P value < 0.05 = Significant (*); P value < 0.01 = Highly significant (**); P value > 0.05 = Non-significant (NS)*
processing & preparation conditions (Chun et al., 2004). Conclusively, previous researchers documented a range of kaempferol in cabbage 0 to 11.9 mg/kg F.W., whereas quercetin presented variations from 0.4 to 10.5 mg/kg F.W. (Hertog et al., 1992; Aherne and O’Brien, 2002; Chun et al., 2004). Afterwards, Bushra and Anwar (2008) determined the flavonols in common cabbage and detected kaempferol as 23.9±0.7 mg/kg, whereas myricetin and quercetin were not detected.

Conversely to the current study, Puupponen-Pimia et al. (2003) did not report the presence of kaempferol in cabbage. Moreover, Bilyk and Sapers (1985) measured quercetin in red cabbage as 2 mg/kg F.W. however, kaempferol was not found. Later, Miean and Mohamed (2001) reported myricetin as the major contributor in total flavonoid content of cabbage with value reported as 147.5±0.05 mg/100g F.W, whereas quercetin, luteolin, kaempferol and apigenin were not detected.

In a recent research work, Gaafar et al. (2014) measured the phenolic compounds in methanolic red cabbage; rutin (58.36 mg/100g), ferulic (7.82 mg/100g), benzoic (14.40 mg/100g), acacetin (8.06 mg/100g), myricetin (7.61 mg/100g), coumarin (5.36 mg/100g), luteolin (119.65 mg/100g), quercetin (36.33 mg/100g), genistein (2.57 mg/100g) and kaempferol (1.88 mg/100g). They compared methanolic extraction with aqueous extraction and found a different picture of phenolic acids. The aqueous extract of red cabbage presented pyrogallol (5.12 mg/100g), gallic acid (3.67 mg/100g), catechins (22.34 mg/100g) and p-coumaric acid (10.72 mg/100g) that were absent in methanolic extract. One of their peers, Bacchetti et al. (2014) found phenolic acids; caffeic-, coumeric- & ferulic acids as 0.42, 0.63 & 0.09 mg, flavonols; kaempferol, quercetin & isorhamnetin as 11.19, 3.66 & 0.36 mg and flavone; luteolin 0.63 mg, accordingly in red or black cabbage samples.

According to Hounsome et al. (2009), the antiradical activity of cabbage is related to the presence of phenolic acids as well as vitamin C. In another investigation carried out by Leja et al. (2007), it came into the limelight that vitamin C did not affect lipid peroxidation and hydroxyl radical activity because the antioxidant potential of red cabbage was far higher than white cabbage; the major moieties were anthocyanins and phenylpropanoids though, vitamin C was comparable in both types of cabbages. Furthermore, it is reported by Leja et al. (2010) that antiradical activity of phenolic acids is related with number of OH-groups bonded to aromatic ring that provides an ideal structure to scavenge free radicals over antioxidant vitamins. Recently, Al-Dosari (2014) related
cabbage polyphenols predominantly hydroxycinnamic acids; chlorogenic acid, vanillic acid, caffeic acid, gallic acid and protocatechuic acid with anti-atherogenesis due to their potential to limit LDL oxidation.

4.1.5. Antioxidant potential of cabbage extracts

In the current exploration, the green and red cabbage extracts demonstrated significant variations for free radical scavenging assays; DPPH-, ABTS- & H$_2$O$_2$ assays and reducing power assays; FRAP- and PFRAP assays as evident from statistics. The DPPH scavenging potential of red cabbage extracts was almost three times higher i.e. 87.79±3.69% than that of green cabbage extracts 31.22±1.65%. Moreover, the values for ABTS assay were 1.51±0.07 & 6.04±0.21 µM Trolox/g F.W., whereas H$_2$O$_2$ scavenging capacities were recorded as 48.03±1.68 & 63.45±3.05% in green & red cabbage samples, respectively. Furthermore, the FRAP (µM Fe$^{+2}$/g F.W.) and PFRAP (%) assays showed obvious differences, from 0.95±0.04 and 41.16±2.10 (green cabbage) to 1.16±0.04 and 59.32±2.14 (red cabbage), correspondingly (Table 4.4).

The radical scavenging capacity is based on chemical structure of antioxidants and normally measured by using different reagents like DPPH & ABTS (Leja et al., 2010). On the other hand, reducing power is related to electron donating ability of antioxidants to free radicals, controlling lipid peroxidation reactions in food in body system (Podsedek et al., 2006; Gaafar et al., 2014). Common cabbage is normally associated with lower antioxidant potential however, some reports have mentioned that antioxidant activity of red cabbage is comparable to that of broccoli (Podsedek, 2007). Later, Floegel et al. (2011) investigated antioxidant activity of 50 popular fruits, vegetables & beverages and mentioned that foods rich in pigmented & hydrophilic antioxidants possess higher antioxidant capacity. Earlier, Kurilich et al. (1999) ranked brassica vegetables on the basis of total antioxidant capacity (TEAC) and order was reported as red cabbage>brussels>savoy cabbage>white cabbage, whereas superoxide anion scavenging potential was in the following order; brussels>red cabbage>savoy cabbage>white cabbage. This concluded that red cabbage and brussels have comparable antioxidant activity that was 5 to 4.5 and 2.4 to 2.2 folds higher than white and savoy cabbage, respectively. The total antioxidant capacity (expressed as TEAC value) of cabbage is related to hydrophilic antioxidants (phenolic compounds+vitamin C). They noticed 1.6 folds higher scavenging potential of red cabbage against peroxyl radicals as
### Table 4.5. Antioxidant assays of cabbage extracts

<table>
<thead>
<tr>
<th>Antioxidant assays</th>
<th>Green cabbage extract</th>
<th>Red cabbage extract</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH scavenging potential (%)</td>
<td>31.22±1.65</td>
<td>87.79±3.69</td>
<td>0.0000**</td>
</tr>
<tr>
<td>ABTS test (µM Trolox/g F.W.)</td>
<td>1.51±0.07</td>
<td>6.04±0.21</td>
<td>0.0000**</td>
</tr>
<tr>
<td>FRAP assay (µM Fe²⁺/g F.W.)</td>
<td>0.95±0.04</td>
<td>1.16±0.04</td>
<td>0.0030**</td>
</tr>
<tr>
<td>PFRAP assay (%)</td>
<td>41.16±2.10</td>
<td>59.32±2.14</td>
<td>0.0005**</td>
</tr>
<tr>
<td>H₂O₂ scavenging assay (%)</td>
<td>48.03±1.68</td>
<td>63.45±3.05</td>
<td>0.0016**</td>
</tr>
</tbody>
</table>

*P value < 0.05 = Significant (*); P value < 0.01 = Highly significant (**); P value > 0.05 = Non-significant (NS)*
compared to common cabbage owing to the presence of myriad of antioxidants especially flavonoids, ascorbic acid, isothiocyanates and anthocyanins, may contribute synergistic effects. Furthermore, polyphenols and flavonoids have direct impact on antioxidant capacity of cabbage while slight effect was observed by vitamin C & E (Chun et al., 2004; Al-Dosari, 2014; Gaafar et al., 2014).

The earlier scientists found comparable results of antioxidant assays to that of present study findings. In this context, Leja et al. (2010) found more than 50% higher DPPH radical scavenging potential of red cabbage than white cabbage. They elucidated DPPH scavenging ability of different varieties of red cabbage as 26.6 to 70.9% whilst, 2 to 8% in case of white cabbage. Furthermore, Leja et al. (2006) demonstrated DPPH scavenging ability of freshly harvested & stored white cabbage as 2.76 to 10.33 & 2.49 to 15.14%, respectively. Currently, Xu et al. (2014) determined the DPPH scavenging activity of fresh cut, stir-fried, boiled, microwaved and steamed red cabbage as 95, 92, 91, 90 and 96%, correspondingly. The DPPH scavenging activities of different concentrations (0, 10, 20 & 30 µg/mL) of common cabbage extract were estimated as 0, 80, 85 & 80%, respectively (Ji et al., 2015). One of their peers, Al-Dosari (2014) determined the DPPH capturing abilities of different concentrations; 10, 50, 100, 500 & 1000 µg/mL of red cabbage extract and measured the values as 12.9, 30.7, 67.1, 80.3 & 86.0%, correspondingly. Previously, Waqar and Mahmood (2010) estimated the percent inhibition of DPPH of 80% ethanolic extract and aqueous extracts of cabbage as 92.4 and 92.1%, respectively. Moreover, Sakunasing and Kangsadalampai (2008) estimated the DPPH scavenging ability of conventional & organic white and red cabbage as 16.095 & 22.533 and 66.370 & 73.316%, correspondingly. Previously, Shyamala et al. (2005) quantified the DPPH scavenging potential of ethanolic extract 50 & 100 ppm of green cabbage as 48 & 49%, accordingly. Recently, Malav et al. (2015) found that cabbage possesses DPPH scavenging potential up to 41.87±2.81% however, the reducing power absorbance was reported as 0.29±0.04.

The TEAC of white cabbage was measured as 1.73 µM Trolox/g F.W. (Bahorun et al., 2004). Furthermore, Proteggente et al. (2002) estimated TEAC of green and red cabbage as 4.92 and 13.77 µM Trolox/g F.W., correspondingly. They measured the antioxidant activity (TEAC) of various fruits and vegetables in the order of strawberry>raspberry>red plum>red cabbage>grape fruit>orange>green cabbage. Later, Leja et al. (2010) measured the ABTS radical scavenging
activity 30% higher in red cabbage than white; the values were 45 to 99.9% (red cabbage) and 38 to 43% (white cabbage). Previously, Podesek et al. (2006) measured the ABTS values for white and red cabbage as 1.34 to 1.81 and 9.81 to 12.64 µM TEAC/g F.W., respectively. Recently, Rokayya et al. (2013) analyzed ABTS values of red and green cabbage as 1.11 and 4.00 µM TE/g F.W., respectively. One of the researchers groups, Gorinstein et al. (2009) analyzed the ABTS value of 50% methanolic and 50% methanolic+1.2 M HCl extracts of white cabbage as 10.50 and 21.80 µM TE/g F.W., respectively. Furthermore, Deng et al. (2013) analyzed the TEAC of cabbage as 8.24 µM Trolox/g F.W. Additionally on dry weight basis, Chaisamlitpol et al. (2014) measured the ABTS value of common cabbage as 382.89 mM Trolox/g D.W. Currently, Wiczkowski et al. (2014) reported the TEAC of red cabbage varied from 87.99 to 169.46 µM Trolox/g D.W. Furthermore, Wiczkowski et al. (2013) measured the ABTS value for red cabbage as 86.51 µM Trolox/g D.W.

Earlier, Proteggente et al. (2002) estimated the FRAP values for green and red cabbage as 6.94 and 18.7 µM Fe$^{+2}$/g F.W., respectively. Moreover, the fresh red cabbage FRAP value was measured as 29.4 µM Fe$^{+2}$/g F.W. (Volden et al., 2008). One of the scientists groups, Deng et al. (2013) quantified FRAP for cabbage as 5.74 µM Fe$^{+2}$/g F.W. Earlier, Wold et al. (2006) determined the FRAP values of white and red cabbage as 19.3-22.5 and 2.0-3.0 µM/g F.W., accordingly. Additionally, Nilsson et al. (2005) tested ABTS and FRAP assays for water soluble & insoluble fractions of red cabbage and determined the corresponding values as 54.2 & 0.83 and 30.6 & 0.87 µM/g F.W. Besides, Yuan et al. (2009) found that red cabbage possesses ten folds higher antioxidant activity (measured by FRAP) as compared to green cabbage.

The reducing power absorbance for cabbage extracts concentrations; 0, 10, 20 & 30 µg/mL were 0, 0.22, 0.43 & 0.65, correspondingly (Ji et al., 2015). According to Shyamala et al. (2005), the reducing power of cabbage was found more than spinach but lower than coriander.

Previous researchers revealed the hydroxyl radical scavenging potential of cabbage solvent extracts at various concentrations; 0, 20, 40, 60, 80 & 100 µg/mL as 0, 10, 40, 62, 77 & 80%, respectively (Ji et al., 2015). Furthermore, Shyamala et al. (2005) measured the % hydroxyl radical scavenging abilities of ethanolic extracts of cabbage at different concentrations; 25, 50 & 100 ppm as 48, 50 & 53 ppm, accordingly.
Earlier, the compositional & phytochemical screening assays for cruciferous vegetables were carried out by previous researchers however, the geographical differences resulted in greater variations. Thus, Pakistani cabbage was probed in order to recommend their incorporation in interventional therapies.
SECTION 4.2:
DESIGNER FOOD ANALYSES
4.2.1. Proximate composition and caloric count

In the product development module, two types of cooking procedures; baking and frying were compared to assess the effectiveness of cooking procedures on antioxidant indices and hedonic response.

Mean squares (Table 4.6 and 4.7) indicated that the proximate components of fried and baked cabbage based croquettes differed significantly.

The means regarding proximate composition of baked croquettes (Figure 4.1 & 4.2) depicted that the maximum moisture content was found in C1B= Baked croquettes stuffed with green cabbage followed by C3B= Baked croquettes stuffed with red cabbage, C2B= Baked croquettes containing green, cabbage extract, C4B= Baked croquettes containing red cabbage extract by 61.85±2.47, 60.95±2.07, 58.20±2.74 and 56.26±2.81%, respectively. Nevertheless, the moisture content was minimum in C0B= Baked croquettes (Control) i.e. 54.09±2.54%. Likewise, the ash content was highest in red cabbage based treatments; C3B and C4B by 3.99±0.24 and 3.46±0.42%, respectively followed by C1B 3.43±0.24 and C2B 2.97±0.59%. However, the minimum value was reported in C0B as 2.52±0.50%. The crude fat in red cabbage extract enriched baked croquettes C4B was maximum (3.96±0.29%) followed by control; C0B (3.28±0.30%) and green cabbage extract enriched baked croquette; C2B (3.21±0.26%) whereas, cabbage stuffed treatments; C3B and C1B lagged behind by 3.15±0.30 and 3.07±0.25%, respectively. The crude protein was lower in cabbage stuffed croquettes i.e. C1B 13.68±1.23% and C3B 14.41±1.34% as compared to other treatments; C2B 17.19±1.39, C0B 17.06±1.19 and C4B 16.77±0.92%. The carbohydrate was highest in C0B 23.05±0.81 followed by C4B 19.55±0.65, C2B 18.43±1.69, C1B 17.97±0.88 and C3B 17.50±0.72, correspondingly. The calorie count was lowest in cabbage stuffed croquettes; C1B 155.65±10.82 and C3B 157.67±11.05 kcal/100g trailed by cabbage extract enriched croquettes; C1B 155.65±10.82 and C4B 182.99±8.92 kcal/100g whereas, the highest calories were found in C0B as 191.64±10.8 kcal/100g.

The means regarding compositional profile of fried croquettes (Figure 4.1 & 4.2) showed that the moisture content was higher in baked croquettes as compared to fried croquettes except control prototype though the trend with respect to treatments was similar. The means regarding moisture content of fried croquettes; C0F= Fried croquettes (Control), C1F= Fried croquettes stuffed with
green cabbage, C_2F= Fried croquettes containing green cabbage extract, C_3F= Fried croquettes stuffed with red cabbage and C_4F= Fried croquettes containing red cabbage extract were 55.49±2.08, 60.35±1.99, 58.31±2.39, 60.01±2.17 and 56.63±2.21%. In comparison, the ash content was high than baked croquettes and the corresponding values were 4.27±0.09, 4.06±0.27, 3.91±0.33, 3.52±0.35 and 3.31±0.33%. As expected, crude fat was far higher than baked samples and values in increasing trend are in the following pattern: C_2F 17.78±0.43>C_4F 16.51±0.51>C_0F 16.23±0.48>C_3F 11.82±0.39>C_1F 11.20±0.27%. The protein content in fried croquettes was ranging between 12.91±0.63% (C_1F) to 14.86±0.74% (C_0F). Furthermore, the means for crude fiber were on lower side in fried samples than baked and lie in the range of 5.94±0.27 to 11.48±0.47%. Due to higher fat content, the calories were varying from 199.80±8.19 kcal/100g (green cabbage stuffed fried croquettes) to 247.21±8.16 kcal/100g (control fried croquettes).

In accordance to the current study, Verma et al. (2016) utilized green cabbage @ 15 & 25% to develop chicken meatballs. With the increment in the levels of green cabbage, significant decrease in fat, crude protein, ash and caloric content was observed in the designed meatballs, whereas non-significant increase in carbohydrate content was viewed. They also noticed increase in the moisture content and moisture protein ratio in the respective product as compared to control. The increment in moisture content as well as carbohydrate content and decrement in fat, protein & ash was related to the addition of green cabbage in contrast to chicken meat. In fried green cabbage (0, 15 and 25%) incorporated meatballs, the percent moisture content reported as (53.81±0.26, 57.35±0.28 & 60.08±0.39), protein (18.39±0.22, 16.89±0.23 & 15.07±0.32), fat (16.55±0.20, 14.35±0.19 & 13.09±0.18) ash (2.48±0.03, 2.31±0.01 & 2.11±0.03) and carbohydrates (8.76±0.37, 9.15±0.49 & 9.34±0.48), respectively. Furthermore, energy (kcal) in different levels of green cabbage 0, 15 and 25% stuffed meat balls were 257.64±1.29, 233.31±1.67 and 215.52±1.66, accordingly. Apart from this, decrement in lipid oxidation and microbial growth was found in response to antioxidants present in green cabbage. Earlier, Kumar et al. (2013) investigated the nutritional perspectives of chicken nuggets enriched with green banana flour at three different levels; 3, 4 & 5% and their findings were inharmony with the current investigation. The treated samples faced decrease in protein and fat along with increment in ash and fiber. The moisture, protein, fat, crude fiber & ash and energy values of the control samples were 64.50±0.40, 20.90±0.57, 11.90±0.60, 0.50±0.03 &
Table 4.6. Means squares for compositional analyses of baked croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude fat</th>
<th>Crude protein</th>
<th>Carbohydrates</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.92439*</td>
<td>0.38529*</td>
<td>8.16351*</td>
<td>14.9181**</td>
<td>742.417*</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>6.4482</td>
<td>0.17794</td>
<td>0.07884</td>
<td>1.50062</td>
<td>1.0455</td>
<td>131.307</td>
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<tr>
<td>Total</td>
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<td></td>
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</tr>
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</table>

**= Highly significant
*= Significant

Table 4.7. Means squares for compositional analyses of fried croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Moisture</th>
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<th>Crude protein</th>
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<th>Calories</th>
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</thead>
<tbody>
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<td>13.2832*</td>
<td>0.46359*</td>
<td>26.7356**</td>
<td>1.72449*</td>
<td>13.4392**</td>
<td>1617.47**</td>
</tr>
<tr>
<td>Error</td>
<td>10</td>
<td>2.8701</td>
<td>0.08426</td>
<td>0.1801</td>
<td>0.43530</td>
<td>0.1397</td>
<td>79.95</td>
</tr>
<tr>
<td>Total</td>
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</tr>
</tbody>
</table>

**= Highly significant
*= Significant
Figure 4.1. Compositional profiling of croquette prototypes

C₀B= Baked croquettes (Control); C₁B= Baked croquettes stuffed with green cabbage; C₂B= Baked croquettes containing green cabbage extract; C₃B= Baked croquettes stuffed with red cabbage; C₄B= Baked croquettes containing red cabbage extract; C₀F= Fried croquettes (Control); C₁F= Fried croquettes stuffed with green cabbage; C₂F= Fried croquettes containing green cabbage extract; C₃F= Fried croquettes stuffed with red cabbage; C₄F= Fried croquettes containing red cabbage extract
0.30±0.02% and 193.9±3.20 kcal/100g, respectively. The mentioned traits at 5% green banana were 66.30±0.37, 18.70±0.21, 8.80±0.18, 3.20±0.03, 2.70±0.07 and 167.50±3.10, correspondingly.

Recently, Malav et al. (2015) assessed the nutritional worth of functional meat patties, prepared using 6, 9 & 12% cabbage powder. They measured moisture, protein, fat & ash in control treatment as 60.34±0.34, 17.64±0.34, 15.01±0.45 & 2.57±0.15%, respectively, whereas the corresponding parameters in the treated samples, carrying 6, 9 & 12% cabbage powder, were recorded as 62.03±0.42, 62.89±0.61 & 63.46±0.35% (moisture), 16.26±0.16, 15.32±0.23 & 14.33±0.42% (protein), 14.32±0.50, 13.78±0.30 & 13.05±0.14% (fat) and 2.72±0.05, 2.75±0.10 & 2.77±0.07% (ash), accordingly. The 6% cabbage powder based meat patties was selected for relatively optimal characteristics and calorific values of control and 6% cabbage samples were reported as 194.65 and 187.18 kcal/100g, respectively. Moreover, Kumar et al. (2013) determined the nutritional value of broccoli powder incorporated meat nuggets. The moisture, protein, fat and ash of the prepared prototypes were 67.43±0.32 & 62.08±0.48, 19.85±0.37 & 17.86±0.72, 8.52±0.75 & 7.38±0.22 and 3.28±0.02 & 3.95±0.08% in control & broccoli powder @ 8% enriched meat nuggets, respectively.

4.2.2. Color tonality

The mean squares for color tonality of baked croquettes (Table 4.8 and 4.9) depicted momentous effect of treatments, storage and their interaction on a*, b*, chroma and hue angle values except L value that was significantly different with respect to treatment and storage only. Whilst, fried croquettes showed significant impact on L, a*, b* & chroma except hue angle that was varying significantly with respect to treatments however, storage imparted non-momentous influence.

Means pertaining to L* value (Figure 4.2) for baked and fried croquettes delineated highest score for C₀B 61.65±1.74 followed by C₃B= Baked croquettes containing green cabbage extract 58.57±1.52, C₄B= Baked croquettes containing red cabbage extract 58.38±2.21, C₁F = Fried croquettes stuffed with green cabbage 57.19±2.47, C₁B= Baked croquettes stuffed with green cabbage 56.87±2.45, C₀F = Fried croquettes (Control) 55.03±2.38, C₄F Fried croquettes containing red cabbage extract 53.14±2.41, C₃F = Fried croquettes stuffed with red cabbage 52.18±2.28, C₂F= Fried croquettes containing green cabbage extract 50.76±2.06 and C₃B= 
Baked croquettes stuffed with red cabbage 50.12±2.19. However, momentous reduction was noted from 58.93±2.17 to 55.49±2.34 and 56.29±2.47 to 51.37±2.42 in baked and fried croquettes during 0 to 30-day storage. Briefly, baked croquette prototypes were darker than their fried counterparts.

Means regarding a* value for cabbage based croquettes depicted that positive values for the said parameter was maximally recorded in fried red cabbage based treatments; C4F and C3F as 4.37±0.15 and 4.33±0.17 followed by baked red cabbage based prototypes; C3B 4.05±0.12 and C4B 3.18±0.11 however, the lower positive a* values were noted in control treatments; C0F 1.74±0.05 and C0B 1.17±0.05. On the other hand, negative a* values showing greenish hue were observed highest in baked croquettes over fried counterparts with maximum value recorded as -3.02±0.13 in C1B trailed by -2.82±0.08, -2.67±0.10 and -1.94±0.07 in C1F, C2B and C2F, respectively. During 30 days frozen storage followed by cooking resulted in significant decline in the mentioned trait from 3.22±0.11 to 2.46±0.10 in baked prototypes and from 3.73±0.12 to 2.27±0.08 in fried products, correspondingly.

From means, it was deduced that all the treatments were towards yellowish tone as their b* values were having positive signs. Though, the maximum b* value was noted in control treatments; C0F 15.74±0.53 and C0B 13.98±0.51. Afterwards, the maximum value amongst green cabbage based treatments were reported in C1F 13.70±0.41 followed by C2B 12.58±0.39, C2F 12.36±0.31 and C1B 10.42±0.49, whereas the lowest values were observed in red cabbage based treatments including C3F 12.60±0.37, C4F 8.55±0.33, C4B 7.20±0.14 and C3B 6.50±0.24 in the decreasing order. Furthermore, storage intervals explicated significant decrement from 10.45±0.40 to 9.76±0.42 (baked croquettes) and 14.14±0.52 to 9.84±0.31 (fried croquettes) from day 1st to 30th, respectively.

The means relating to chroma value of designer croquettes (Figure 4.2.) presented higher value for control treatments; C0F 15.85±0.42 and C0B 14.03±0.33 trailed by C1F 13.99±0.47, C3F 13.33±0.55, C2B 12.86±0.24, C2F 12.51±0.40, C1B 10.86±0.55, C4F 9.60±0.35, C4B 7.90±0.12 and C3B 7.72±0.04. During storage, the decrement was from 11.15±0.30 to 10.13±0.25 in baked croquettes and 14.74±0.49 to 10.20±0.37 in fried treatments.

For hue angle, the means were varying from 83.42±2.79 to 84.99±3.18 in fried and baked control treatments. Whereas, the green cabbage stuffed treatments were in the range of
101.42±3.28 (C₁F) to 106.43±5.00 (C₁B) and green cabbage extract enriched prototypes were varying from 99.62±4.35 (C₂F) to 103.14±3.20 (C₂B). Furthermore, the hue angle for red cabbage stuffed treatments was in the range of 56.99±2.63 (C₃B) to 71.03±2.68 (C₃F) and 62.90±2.69 (C₄F) to 66.91±1.65 (C₄B), respectively. Besides, the storage depicted significant decline from day 1 to 30 for baked croquettes with values varying from 79.89±3.21 to 86.60±3.96 whereas, storage imparted non-momentous effect on fried treatments i.e. from 81.97±3.70 to 99.51±3.48 over the storage.

Previously, L, a*, b*, chroma and hue angle for red cabbage were reported as 42.7±1.0, +8.3±2.0, +10.8±2.0, 13.6±1.0 and 52.5±1.0, correspondingly (Zaidel et al., 2014). Whilst, L, a* & b* values for green cabbage were determined as 48.55±1.98, -9.77±0.90 & 18.55±0.70, respectively (Nilnakara et al., 2009). In another study, L, a* & b* values for green cabbage were noted as 40.58±0.51, -7.49±0.13 & 16.52±0.23, accordingly (Tanongkankit et al., 2012). L, a* & b* values for freeze dried cabbage demonstrated as 60.01±0.32, -1.53±0.19 & 27.71±1.33, correspondingly (Gong et al., 2007).

Recently, Malav et al. (2015) documented that L*, a* & b* values are the indicators to assess oxidative stability. They found decrease in redness (a* value) & yellowness (b* value) in cabbage based meat products during storage and related this decrement with lower consumer acceptability as evident in the current research. Moreover, Kumar et al. (2013) found decrement in lightness (L* value), redness (a* value) and yellowness (b* value) as green color of broccoli turned darker on cooking in contrast to control samples. Later, Xu et al. (2014) found significant impact of conventional cooking on anthocyanins, reduction in a* and L* values while minor changes were reported in b* value. Earlier, Kumar et al. (2013) determined the effect of green banana flour @ 5% on nuggets color tonality. The formulated samples were lighter and comparatively less dark as compared to control samples however, the yellowness was reported maximum in treated samples as observed for green cabbage stuffed croquettes in the present scrutiny. The L*, a* & b* values for control treatments were 53.6±0.25, 10.3±0.13 & 17.9±0.18, whereas these values reached to 54.2±0.09, 9.4±0.10 & 18.9±0.17 after adding green banana @ 5% in the preparation of nuggets. Later, Banerjee et al. (2012) determined the redness, yellowness, hue and chroma in control samples as 2.83, 3.23, 50.34 and 4.31 that changed to 2.73, 3.05, 51.31 and 4.10 on addition of broccoli powder @ 2%, respectively.
Table 4.8. Means squares for color tonality of baked croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>L</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>164.729**</td>
<td>4.94483**</td>
<td>95.9107**</td>
<td>73.6259**</td>
<td>4249.54**</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>44.627**</td>
<td>2.15054**</td>
<td>1.8450**</td>
<td>3.9009**</td>
<td>174.87**</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>6.678NS</td>
<td>0.51266**</td>
<td>15.1450**</td>
<td>13.8389**</td>
<td>46.80**</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>4.594</td>
<td>0.01321</td>
<td>0.1641</td>
<td>0.1128</td>
<td>12.82</td>
</tr>
</tbody>
</table>

**= Highly significant

Table 4.9. Means squares for color tonality of fried croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>L</th>
<th>a*</th>
<th>b*</th>
<th>Chroma</th>
<th>Hue angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>56.6776**</td>
<td>14.3264**</td>
<td>61.9847**</td>
<td>47.2334**</td>
<td>2611.22**</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>92.4695**</td>
<td>8.0429**</td>
<td>85.3689**</td>
<td>92.5401**</td>
<td>33.80NS</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>14.8708*</td>
<td>0.8381**</td>
<td>35.6496**</td>
<td>36.0897**</td>
<td>6.81NS</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>5.6404</td>
<td>0.0155</td>
<td>0.2165</td>
<td>0.2312</td>
<td>11.72</td>
</tr>
</tbody>
</table>

**= Highly significant
NS= Non significant
Figure 4.2. Effect of treatments on color tonality of baked and fried croquettes

* C0B = Baked croquettes (Control); C1B = Baked croquettes stuffed with green cabbage; C2B = Baked croquettes containing green cabbage extract; C3B = Baked croquettes stuffed with red cabbage; C4B = Baked croquettes containing red cabbage extract; C0F = Fried croquettes (Control); C1F = Fried croquettes stuffed with green cabbage; C2F = Fried croquettes containing green cabbage extract; C3F = Fried croquettes stuffed with red cabbage; C4F = Fried croquettes containing red cabbage extract
Figure 4.3. Effect of storage on color tonality of baked and fried croquettes

CB= Baked croquettes; CF= Fried croquettes
Later, Kitcharoenthawornchai and Harnsilawat (2015) noticed that increasing textured vegetable protein from 10 to 70% resulted in significant increase in lightness (from 64.40±0.95 to 66.59±0.93) and redness (from 3.38±0.38 to 4.95±0.39) while reduction in yellowness (from 20.46±0.58 to 18.96±0.57). The chroma faced a decrement (from 21.04±1.10 to 19.53±0.67) with the addition of vegetable protein, higher would be the intensity of product color. However, hue angle was ranged from 75.52±0.85 to 80.9±1.21 that was within the range of yellowish to orange tone (45°-90°).

4.2.3. Hardness and water activity

The mean squares relating to hardness indicated significant variations as a function of treatments, storage and their interaction in baked as well as fried prototypes. Moreover, mean squares for water activity elucidated significant effect of treatments and storage except interaction effect that was behaving non-substantially in both cooking methods (Table 4.10 & 4.11).

Means regarding the effect of treatments (Figure 4.4 & 4.5) showed that hardness (N) for designer croquettes was highest in baked treatments over fried counterparts. Though, the maximum value was reported in control treatments; C₀B 23.11±0.84 and C₄F 14.70±0.29 followed by cabbage extract enriched and cabbage stuffed samples in the decreasing order; C₃B 12.38±0.42, C₃B 11.19±0.28, C₄B 10.73±0.21, C₂F 9.90±0.36, C₄F 9.27±0.35, C₃F 9.12±0.37, C₁F 8.43±0.25 and C₁B 8.42±0.22. It is evident from (Figure 4.4 & 4.5) that water activity (aₒ) decreased significantly as a function of different cabbage based treatments. The baked treatments were on higher side; C₀B 0.80±0.03 > C₁B 0.80±0.01 > C₂B 0.76±0.02 ~ C₄B 0.76±0.02 > C₃B 0.75±0.03 followed by fried prototypes; C₀F 0.77±0.07 > C₃F 0.75±0.02 ~ C₄F 0.75±0.07 > C₁F 0.74±0.08 > C₂F 0.73±0.03. However, the hardness was increasing significantly for baked & fried croquettes during storage with values mentioned as 10.80±0.34 & 9.10±0.30, 14.51±0.40 & 10.63±0.38 and 14.18±0.45 & 11.13±0.29 N at 1ˢᵗ, 15ʰ and 30ʰ day, correspondingly. Alongside, the water activity (aₒ) was decreasing significantly from 0.89±0.04 to 0.70±0.02 in baked treatments and 0.82±0.02 to 0.67±0.02 in fried samples during storage period. The decrease in water activity might be due to the diffusion of moisture from centre to the coating material resulting in toughening or staling of coat.
Table 4.10. Means squares for hardness and water activity of baked croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Hardness</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>296.951**</td>
<td>0.00452**</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>63.234**</td>
<td>0.15162**</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>245.635**</td>
<td>0.00145NS</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.269</td>
<td>0.00085</td>
</tr>
</tbody>
</table>

**= Highly significant  
NS= Non significant

Table 4.11. Means squares for hardness and water activity of fried croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Hardness</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>57.2876**</td>
<td>0.00233*</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>16.7646**</td>
<td>0.08550**</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>75.7845**</td>
<td>0.00155NS</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>0.1260</td>
<td>0.00079</td>
</tr>
</tbody>
</table>

*= Significant  
**= Highly significant  
NS= Non significant
Figure 4.4. Effect of treatments on hardness and water activity of baked and fried croquettes

\[ \text{C}_0^B = \text{Baked croquettes (Control)}; \text{C}_1^B = \text{Baked croquettes stuffed with green cabbage}; \text{C}_2^B = \text{Baked croquettes containing green cabbage extract}; \text{C}_3^B = \text{Baked croquettes stuffed with red cabbage}; \text{C}_4^B = \text{Baked croquettes containing red cabbage extract}; \text{C}_0^F = \text{Fried croquettes (Control)}; \text{C}_1^F = \text{Fried croquettes stuffed with green cabbage}; \text{C}_2^F = \text{Fried croquettes containing green cabbage extract}; \text{C}_3^F = \text{Fried croquettes stuffed with red cabbage}; \text{C}_4^F = \text{Fried croquettes containing red cabbage extract} \]

Figure 4.5. Effect of storage on hardness and water activity of baked and fried croquettes

Figure 4.5. Effect of storage on hardness and water activity of baked and fried croquettes
The green banana and soybean hulls flours were added into chicken nuggets and the hardness of samples was reported in the range of 18.0±1.1 to 20.8±1.0 N (Kumar et al., 2013). The hardness in cooked meat balls were reported to vary from 14.3 to 23.7 N. They found change in hardness due to the difference of one or more ingredient or storage condition (Ulu, 2006). Later, Devatkal et al. (2014) determined the firmness of chicken nuggets varying from 12.8 to 19.8 N.

Due to the addition of broccoli powder at three different levels; 4, 6 & 8% in meat nuggets, there observed a decrease in a\textsubscript{w} from 0.85±0.01 (control) to 0.83±0.01, 0.79±0.002 & 0.71±0.01, respectively (Kumar et al., 2013). Previously, Karpinska-Tymoszczyk (2008) measured the water activity of control and rosemary enriched meatballs as 0.985 & 0.982 and 0.984 & 0.978 at 0 & 15\textsuperscript{th} day, respectively. In contrast, Malav et al. (2015) incorporated 6% cabbage in mutton patties and determined the water activity as 0.97 against control \textit{i.e.} 0.96.

One of the researchers groups, Thomas et al. (2007) found that water activity of buffalo meat nuggets was affected by different processing temperatures; 16 °C 0.95, 19 °C 0.95, 27 °C 0.94 and 34 °C 0.94.

4.2.4. Product phytochemistry and antioxidant assays

The basic desire was to assess the antioxidant potential of developed designer croquettes using two different cooking procedures; baking and frying. Different cabbage based treatments were analyzed for TPC, TF, DPPH-, ABTS- & FRAP assays. The mean squares showed momentous effect of treatments on these traits in both cooking methods. However, storage demonstrated significant effect with respect to TPC and ABTS in baked croquettes while ABTS assay for fried croquettes due to frozen storage of uncooked croquettes (Table 4.12 & 4.13).

The data pertaining to TPC (Table 4.14, 4.15, 4.16 & 4.17) demonstrated maximum values for fried croquettes in contrast to baked croquettes with maximum value as 125.82±6.09 C\textsubscript{3}F followed by 123.45±4.92 C\textsubscript{4}F, 121.61±5.85 C\textsubscript{3}B, 118.09±6.33 C\textsubscript{4}B, 92.54±4.68 C\textsubscript{1}B, 88.04±4.37 C\textsubscript{1}F, 87.14±2.73 C\textsubscript{2}B and 82.41±1.83 C\textsubscript{2}F mg GAE/100g F.W. Furthermore, control treatments lagged behind in both cooking methods with TPC (mg GAE/100g F.W) values as 71.17±3.06 C\textsubscript{0}F and 70.59±3.25 C\textsubscript{0}B. During storage, TPC of baked treatments showed momentous increment from 99.03±4.33 to 99.78±5.11 followed by decrement to
Table 4.12. Means squares for phytochemistry and antioxidant assays of baked croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Total phenolic content</th>
<th>Total flavonoids</th>
<th>DPPH scavenging potential</th>
<th>FRAP assay</th>
<th>ABTS assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>4184.32**</td>
<td>1727.56**</td>
<td>306.651**</td>
<td>0.34968**</td>
<td>1.78993**</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>91.95*</td>
<td>22.83NS</td>
<td>3.528NS</td>
<td>0.00842NS</td>
<td>0.05714*</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>20.76NS</td>
<td>4.61NS</td>
<td>0.324NS</td>
<td>0.00117NS</td>
<td>0.00649NS</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>23.53</td>
<td>9.94</td>
<td>1.138</td>
<td>0.00257</td>
<td>0.01245</td>
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</tbody>
</table>

* = Significant  
**= Highly significant  
NS= Non significant

Table 4.13. Means squares for phytochemistry and antioxidant assays of fried croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Total phenolic content</th>
<th>Total flavonoids</th>
<th>DPPH scavenging potential</th>
<th>FRAP assay</th>
<th>ABTS assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>5589.02**</td>
<td>1453.27**</td>
<td>449.220**</td>
<td>0.37753**</td>
<td>3.56253**</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>52.82NS</td>
<td>27.60NS</td>
<td>3.393NS</td>
<td>0.00672NS</td>
<td>0.07323*</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>6.68NS</td>
<td>5.79NS</td>
<td>0.121NS</td>
<td>0.00210NS</td>
<td>0.00440NS</td>
</tr>
<tr>
<td>Error</td>
<td>30</td>
<td>21.66</td>
<td>8.39</td>
<td>1.542</td>
<td>0.00257</td>
<td>0.01628</td>
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</tbody>
</table>

**= Highly significant  
NS= Non significant
Table 4.14. Effect of treatments on phytochemistry and antioxidant assays of baked croquettes

<table>
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<tr>
<th>Parameters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CoB</td>
</tr>
<tr>
<td>TPC (mg GAE/100g)</td>
<td>70.59±3.25c</td>
</tr>
<tr>
<td>TF (mg QE/100g)</td>
<td>64.88±2.77c</td>
</tr>
<tr>
<td>DPPH assay (%)</td>
<td>13.72±0.49a</td>
</tr>
<tr>
<td>ABTS assay (µM Trolox/g F.W.)</td>
<td>1.74±0.09d</td>
</tr>
<tr>
<td>FRAP assay (µM Fe²⁺/g F.W.)</td>
<td>0.71±0.04d</td>
</tr>
</tbody>
</table>

Two-way ANOVA followed by Tukey’s HSD multiple comparison tests; C0B= Baked croquettes (Control); C1B= Baked croquettes stuffed with green cabbage; C2B= Baked croquettes containing green cabbage extract; C3B= Baked croquettes stuffed with red cabbage; C4B= Baked croquettes containing red cabbage extract.

Table 4.15. Effect of storage on phytochemistry and antioxidant assays of baked croquettes

<table>
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<tr>
<th>Parameters</th>
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</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>TPC (mg GAE/100g)</td>
<td>99.03±4.33ab</td>
</tr>
<tr>
<td>TF (mg QE/100g)</td>
<td>78.48±3.16</td>
</tr>
<tr>
<td>DPPH assay (%)</td>
<td>22.33±0.92</td>
</tr>
<tr>
<td>ABTS assay (µM Trolox/g F.W.)</td>
<td>2.38±0.12a</td>
</tr>
<tr>
<td>FRAP assay (µM Fe²⁺/g F.W.)</td>
<td>1.02±0.05</td>
</tr>
</tbody>
</table>

Two-way ANOVA followed by Tukey’s HSD multiple comparison tests; TPCs = Total phenolic contents (mg GAE/100g F.W.); TFCs = Total flavonoids (mg QE/100g F.W.); DPPH assay (%); ABTS assay (µM Trolox/g F.W.); FRAP assay (µM Fe²⁺/g F.W.)
Table 4.16. Effect of treatments on phytochemistry and antioxidant assays of fried croquettes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>CoF</th>
<th>C1F</th>
<th>C2F</th>
<th>C3F</th>
<th>C4F</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC (mg GAE/100g)</td>
<td></td>
<td>71.17±3.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>88.04±4.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.41±1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>125.82±6.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.45±4.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TF (mg QE/100g)</td>
<td></td>
<td>51.84±1.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.17±2.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.77±2.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.17±3.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.11±2.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DPPH assay (%)</td>
<td></td>
<td>14.49±0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.83±1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.45±0.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.00±1.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.51±1.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ABTS assay (µM Trolox/g F.W.)</td>
<td></td>
<td>1.61±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.14±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.03±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.05±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>FRAP assay (µM Fe&lt;sup&gt;2+&lt;/sup&gt;/g F.W.)</td>
<td></td>
<td>0.86±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.02±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.98±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.37±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.23±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Two-way ANOVA followed by Tukey’s HSD multiple comparison tests
CoF= Fried croquettes (Control)
C1F= Fried croquettes stuffed with green cabbage
C2F= Fried croquettes containing green cabbage extract
C3F= Fried croquettes stuffed with red cabbage
C4F= Fried croquettes containing red cabbage extract

Table 4.17. Effect of storage on phytochemistry and antioxidant assays of fried croquettes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Storage intervals (days)</th>
<th>1</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC (mg GAE/100g)</td>
<td></td>
<td>99.86±3.08</td>
<td>98.51±4.31</td>
<td>96.16±4.77</td>
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<tr>
<td>TF (mg QE/100g)</td>
<td></td>
<td>65.15±2.39</td>
<td>65.24±2.99</td>
<td>62.85±2.37</td>
</tr>
<tr>
<td>DPPH assay (%)</td>
<td></td>
<td>24.92±1.19</td>
<td>24.48±1.23</td>
<td>23.97±0.97</td>
</tr>
<tr>
<td>ABTS assay (µM Trolox/g F.W.)</td>
<td></td>
<td>2.40±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.29±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FRAP assay (µM Fe&lt;sup&gt;2+&lt;/sup&gt;/g F.W.)</td>
<td></td>
<td>1.10±0.05</td>
<td>1.11±0.05</td>
<td>1.07±0.05</td>
</tr>
</tbody>
</table>

Two-way ANOVA followed by Tukey’s HSD multiple comparison tests; TPC = Total phenolic contents (mg GAE/100g F.W.); TF = Total flavonoids (mg QE/100g F.W.); DPPH assay (%); ABTS assay (µM Trolox/g F.W.); FRAP assay (µM Fe<sup>2+</sup>/g F.W.)
95.17±5.03 mg GAE/100g F.W. at 1st, 15th and 30th day. The increment at 15th day was attributed to cabbage stuffed designer croquettes; their TPC was increasing while decrease was viewed in remaining treatments. Conversely, the different trend was viewed in fried treatments with values statistically at par, varying from 99.86±3.08 to 98.51±4.31 and 96.16±4.77 mg GAE/100g at 1st, 15th, 30th day, respectively. Thus, storage and cooking methods are the deciding factors relating phenolic constituents and antioxidant potency.

The readings related to total flavonoid content (Table 4.14, 4.15, 4.16 & 4.17) revealed maximum value for C3B 92.30±3.60 followed by C4B 92.83±4.13, C3F 78.17±3.77 and C4F 78.11±2.78 mg QE/100g. Furthermore, the green cabbage based treatments were ranked on 2nd position after red cabbage treated samples and their values came out as 69.63±1.58 (C1B), 67.87±2.83 (C2B), 57.17±2.43 (C1F) and 56.77±2.03 (C2F) mg QE/100g. Whilst, the lowest values were in control treatments; C0B and C0F i.e. reported as 64.88±2.77 and 51.84±1.93 mg QE/100g, accordingly. Storage of baked & fried croquettes resulted in slight changes in total flavonoids from 78.48±3.16 & 65.15±2.39 at 1st day to 77.91±3.15 & 65.24±2.99 at 15th day and 76.12±3.04 & 62.85±2.37 mg QE/100g at termination of storage study, respectively.

Means for DPPH assay (Table 4.14, 4.15, 4.16 & 4.17) explicated varied trend due to different cabbage based formulations; maximum value for the radical scavenging potential was observed in red cabbage based preparations; C3F 32.00±1.75, C4F 30.51±1.55, C3B 28.16±1.25 and C4B 26.56±1.27% trailed by green cabbage based samples; C1F 22.83±1.08, C2F 22.45±0.74, C1B 22.35±1.07 and C2B 19.04±0.90%. However, the control treatments explicated minimum DPPH scavenging ability (%) in C0F and C0B as 14.49±0.55 and 13.72±0.49, respectively. During storage, non-significant decrease was reported, varying from 22.33±0.92 (day 1) to 21.41±0.98 (day 30) and 24.92±1.19 (day 1) to 23.97±0.97 (day 30) in baked and fried croquette samples, accordingly.

With respect to treatments, ABTS scavenging ability was maximum in C3F 3.05±0.15 followed by C4F 2.97±0.14, C3B 2.93±0.14, C4B 2.44±0.12, C1B 2.47±0.13, C1F 2.14±0.11, C2B 2.09±0.10 and C2F 2.03±0.11 µM Trolox/g F.W. However, minimum free radical capturing capacity was measured in C0B 1.74±0.09 and C0F 1.61±0.08 µM Trolox/g F.W. Regarding ABTS assay, progressive decrease was noted among all treatments except cabbage stuffed croquettes where an increase was observed at 15th day followed by a decrease at 30th day.
Storage resulted in marked decrease in ABTS assay value from 2.38±0.12 to 2.36±0.09 & 2.26±0.08 and 2.40±0.11 to 2.39±0.11 & 2.29±0.35 µM Trolox/g F.W. from Day 1 to 15th & 30th in baked and fried croquettes, respectively (Table 4.14, 4.15, 4.16 & 4.17).

Mean values indicated highest FRAP value (µ M Fe^{2+}/g F.W.) in red cabbage based treatments; C_{3}F (1.37±0.05), C_{4}B (1.25±0.07), C_{4}F (1.23±0.06) and C_{3}B (1.11±0.05) followed by green cabbage based samples; C_{1}F (1.02±0.05), C_{1}B (0.99±0.04) ~ C_{2}B (0.99±0.03), C_{2}F (0.98±0.04) whilst, minimum values were that of control tratments; C_{0}F (0.86±0.05) and C_{0}B (0.71±0.04). However, non-momentous decline was observed in reducing power with respect to storage duration in baked croquettes, the recorded results depicted slight increase at 15th day 1.03±0.05 followed by decrement up to 0.98±0.04 at 30th day from 1st day 1.02±0.05. In fried croquettes, decrement in reducing power was from 1.10±0.05 Day 1 to 1.11±0.05 Day 15 and 1.07±0.05 Day 30 (Table 4.14, 4.15, 4.16 & 4.17).

Antioxidants are considered as the first line of defense against free radicals whereas, dietary fiber serves as a bulking agent thus normalizes the gastrointestinal motility. In this regard, incorporation of cabbage in meat based product could balance the nutritive value, ensuring the provision of dietary fiber along with a myriad of antioxidant vitamins including β-carotene, ascorbic acid and α-tocopherol. Furthermore, calcium content of cabbage could improve the nutritional value of calcium deficient meat products. Additionally, the use of fiber from cruciferous vegetables or dehydrated powder in meat based products could offer an opportunity for the development of fat reduced meat products (@ 20-40 and 2-13%, respectively). On the other hand, cabbage powder has low acceptability *i.e.* up to 6% due to poor textural characteristics and mask meaty flavor. Besides, cabbage powder or extracts are considered as bio-preservatives, carrying better antioxidant activity over synthetic counterparts (Malav *et al*., 2015). Injudicious incorporation of synthetic antioxidants in meat products may leads to numerous disorders hence scientific fraternity need to explore novel natural antioxidants. In this context, incorporation of cruciferous vegetables in meat not only prolongs its quality by improving oxidative stability but also imparts various health attributes (Kumar *et al*., 2013).

In harmony with the current study, DPPH scavenging potential of control and 6% cabbage fortified patties were reported as 5.76 and 22.51%, respectively (Malav *et al*., 2015). The inclusion of broccoli powder in nuggets enhances DPPH radical scavenging activity owing to
more hydroxyl groups of phytoceutics. The DPPH radical scavenging activity of broccoli powder 3 mg was comparable to that of BHT 100 ppm. Furthermore, 8% broccoli powder revealed highest percent inhibition of ABTS radicals due to the presence of natural antioxidants such as carotenoids, tocopherols, ascorbic acid and flavonoids (Kumar et al., 2013). Previously, it is documented that boiling (thermal treatment) of green cabbage caused 31% increase in total carotenoids owing to release of lutein from matrix though, β-carotene faced thermal destruction (Podsedek, 2007).

Similarly, Banerjee et al. (2012) quantified total polyphenol content in control, broccoli powder; 1, 1.5 & 2% and BHT treated nuggets as 0.06±0.01, 0.09±0.01, 0.12±0.01, 0.16±0.01 and 0.16±0.01 mg GAE/g, respectively. Alongside, they found linearly increasing trend in radical scavenging potential and reducing power with the increase in broccoli dosage. Earlier, Llorach et al. (2005) prepared soup modified with cauliflower by-products and analyzed total phenolics & flavonoids as 33.8 & 32 mg/g dry extract and 0.18 & 0.16 mg/mL soup, accordingly. Furthermore, the ABTS & FRAP values of control and modified soup were measured as 0.17 & 0.035 and 0.59 & 0.80 mg TEAC/mL, respectively. Recently, Das et al. (2015) analyzed total phenolics of control and bael pulp (0.25 & 0.50%) treated nuggets as 855.83±15.29, 1171.18±9.96 & 1437.06±10.48 μg/g, correspondingly. The ABTS and FRAP values for free & bound shrimp nuggets (control treatment) were 1.54 & 36.05 and 0.98 & 1.21 μM Trolox/g, respectively. The corresponding antioxidant indices for β-glycan treated samples were 1.72 & 51.17 and 1.36 & 2.58 μM Trolox/g (Haghshenas et al., 2015). Later, Kumar et al. (2016) measured the total phenolics and DPPH radical-scavenging activity of control & peanut based functional nuggets as 367.0±26.52 & 635.0±35.15 mg TA eq./g and 13.33±1.06 & 26.40±1.86%, respectively.

The earlier study conducted on Broccoli Based Bars (BBBs) compared frying and baking procedures and its findings are in justification with the present investigation. They found higher TPC in fried bars 38.2 mg/g D.W. as compared to baked bars 33.9 mg/g D.W. Furthermore, flavonoids were up to 912.7 and flavonols 349.1 mg/100g D.W. in baked as compared to fried bars with corresponding values reported as 730.4 and 292.6 mg/100g D.W. They also determined quercetin, kaempferol and protocatechuic acid in fried & baked bars as 126.4 & 146.8, 78.4 & 91.9 and 117.7 & 132.5 mg/100g D.W., correspondingly. The lower availability of these moieties in fried bars is associated to their leeching or thermal degradation.
Briefly, 25 to 33% of flavonoids were lost during frying as compared to baking 16%. On the other hand, fried BBBs indicated maximum scavenging potential against ABTS radicals 146.6 μM/g D.W. in contrast to baked versions 130.0±0.2 μM/g D.W. Conclusively, antioxidant activity of the BBBs was able to scavenge approx. 51-72% of free radicals, more in uncooked followed by fried and baked prototypes. The increase in antioxidant activity of fried BBBs was in response to high extractability or release of bound bioactive molecules due to the disruption of plant cells (Barakat and Rohn, 2014).

One of the scientists groups, Sultana and Anwar (2008) reported appreciable effect of varying cooking treatments (frying, boiling and microwaving) on antioxidant capacity of some vegetables including cabbage. Frying reported significant increase in reducing power; this effect was almost similar as viewed in the existing study. The increase in reducing power was maximally reported in cauliflower followed by cabbage, spinach, yellow turnip, white turnip, carrot and peas. The increase in reducing power was related to the fact that pre-cooking or cooking bruised the tissue and exposed the bound phytoceutics. However, maximum deleterious impact on antioxidant activity was noted during microwaving, attributed to the breakdown of heat-labile phenolic substances up to 71%. Likewise, Lin and Chang (2005) documented increase in reducing power of vegetables after cooking. Earlier, Ismail et al. (2004) observed non-significant effects of cooking on antioxidant capacity of cabbage. Likewise, Bernhardt and Schlich (2006) enumerated higher amounts of β-carotene and α-tocopherol in broccoli after steaming due to protein denaturation resulting in easy extractability from plant matrix. These researches noted contradictory to the previous theory that processed vegetables possess low nutritive value in contrast to their equivalent unprocessed parts.

Earlier, Volden et al. (2008) studied the effect of thermal processing on antioxidant capacity of red cabbage. They found a drop in ferric reducing ability power (42, 17 & 0%) and oxygen radical absorbance capacity (51, 19 & 0%) via blanching, boiling & steaming due to loss in total phenols (43, 16 & 0%), total monomeric anthocyanins (59, 41 & 29%) and L-ascorbic acid (48, 24 & 11%), accordingly. Later, Xu and his coworkers (2014) reported considerable reduction in anthocyanins, phenolics, ascorbic acids and DPPH radical-scavenging capacity of red cabbage due to domestic cooking methods; stir frying & boiling. The losses in anthocyanins were reported up to 62, 55.5, 46.1 and 17.5% through stir-frying, boiling, microwave heating and steaming, respectively. They also elaborated various mechanisms, involved in the
degradation of polyphenols such as thermal degradation, activation of polyphenol oxidase and leeching in cooking medium. Previously, Turkmen et al. (2005) depicted higher amounts of anthocyanins in red cabbage in contrast to other vegetables. Furthermore, they found higher DPPH scavenging capacity of red cabbage 95.64% than broccoli 78.17%. Furthermore, Gliszczynska-Swiglo et al. (2006) reported accelerated thermal oxidation of ascorbic acid to dehydroascorbic acid, resulting in 29% losses via boiling, whereas non-significant affects were viewed through steaming process. Besides, another study found that red cabbage polyphenols reduced significantly during 4-month storage while no decline in antioxidant capacity was observed in white cabbage (Leja et al., 2010).

In a recent study, Radziejewska-Kubzdela and Biegańska-Marecik (2015) investigated the effect of red cabbage incorporation in apple juice in frozen, puree or freeze-dried format. The results reflected significant increase in phenolic content from 3.1 to 4.9 folds. The total phenolic contents in unpasteurized juice were ranging from 143 to 221 mg/L than pasteurized counterpart 126 to 186 mg/L. Furthermore, ascorbic faced significant decrement during thermal processing ranged from 3.5 to 7.9 mg/L as compared to un-pasteurized treatments (5.1 to 25.1 mg/L). Additionally, they reported nine anthocyanin fractions with cyanidin 3-(sinapoyl) diglucoside-5-glucoside and cyanidin 3-(sinapoyl) (sinapoyl) diglucoside-5-glucoside as the 1st and 2nd dominant moieties, varying from 6.8 to 19.4 and 8.5 to 25.7 mg/L, respectively. With the addition of red cabbage in apple juice, they found increment in chlorogenic acid and decrement in p-coumaryl-quinic acid as compared to apple juice. Moreover, cryptochlorogenic, p-coumaric, sinapic and ferulic acids were not present in apple juice initially however, the red cabbage enriched treatments detected these moieties. The antioxidant capacity measured by ABTS assay was varying from 454 to 469 μM Trolox/L in apple juices, whereas it raised to 50% higher on addition of purée, frozen and freeze dried red cabbage i.e. 675 to 962 μM Trolox/L. This study showed positive association between phenolic compounds and antioxidant activity.

4.2.5. Sensory response

The mean squares corresponding to sensory response of baked croquettes (Table 4.18) showed significant effect of treatments on color, taste, odor, juiciness and overall acceptability except tenderness. During storage, odor and tenderness of the resultant prototypes affected
substantially whilst, all other attributes were behaving non-significantly. Regarding hedonic response of fried croquettes, the effect of treatment was significant with respect to color, taste, odor, juiciness and overall acceptability except tenderness. The 30 days storage impacted non-momentously on these characteristics. Furthermore, the interaction impact was non-momentous in baked as well as fried croquettes.

The maximum score for color (Figure 4.6) was assigned to C₂B 7.66±0.44 followed by C₀B 7.56±0.54, C₀F 7.36±0.68, C₂F 7.20±0.42, C₄B 7.05±0.59, C₄F 7.04±0.45, C₁F 6.66±0.39, C₃F 6.63±0.38 ~ C₁B 6.63±0.38 and C₃B 6.28±0.36. However, storage did not impart any significant impact on color of baked and fried designer croquettes. In baked croquettes, the decrement in color score was from 7.13±0.45 to 7.03±0.52 and 6.96±0.41 over the storage. Likewise, the slight decrement was observed in fried croquettes from 7.08±0.52 to 6.89±0.42 during 30 days frozen storage.

Treatments have substantial effect on taste (Figure 4.6) in baked and fried croquettes with maximum scores allotted to C₀F, trailed by C₁F, C₄F, C₃F ~ C₂F, C₁B, C₀B, C₄B, C₂B and C₃B with scores attained as 7.25±0.47, 7.13±0.53, 6.99±0.59, 6.94±0.39, 6.94±0.40, 6.92±0.39, 6.90±0.67, 6.87±0.57, 6.65±0.50 and 6.60±0.37, accordingly. Briefly, maximum scores for taste were achieved by fried treatments taste wise as compared to baked counterparts.

During storage, a slight decrement was noted in the said attribute score, varying from 6.85±0.46 to 6.79±0.47 in baked prototypes, whereas in fried samples, the variations were minor from 7.07±0.43 (1st day) to 7.03±0.45 (30th day).

The scores for odor indicated significant variations amongst treatments (Figure 4.7). The highest score assigned to C₁F 7.41±0.65 followed by C₂F 7.31±0.61, C₀B 7.25±0.9, C₄B 7.14±0.46, C₃F 7.03±0.41, C₁F 6.99±0.93, C₂B 6.79±0.60, C₃B 6.74±0.40 and C₁B 6.23±0.46. The recorded mean scores in baked croquettes depicted decline in odor from 6.98±0.57 to 6.67±0.58, whereas from 7.32±0.74 to 7.07±0.57 in fried croquettes during 30 days frozen storage.

Figure 4.7 indicating the sensory scores of tenderness in baked and fried croquettes showed that the scores were at par ranging from 6.62±0.40 (C₁B) to 7.04±0.84 (C₄F) though the effect of storage was significantly different, ranging from 6.78±0.46 to 6.58±0.31 in baked croquettes.
Table 4.18. Means squares for sensory response of baked croquettes

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Color</th>
<th>Taste</th>
<th>Odor</th>
<th>Tenderness</th>
<th>Juiciness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>15.9483 **</td>
<td>0.66434 *</td>
<td>7.62261 **</td>
<td>0.35898 NS</td>
<td>0.92604 *</td>
<td>2.11264 **</td>
</tr>
<tr>
<td>Storage (B)</td>
<td>2</td>
<td>0.4409 NS</td>
<td>0.06404 NS</td>
<td>1.65891 *</td>
<td>0.68631 *</td>
<td>0.23513 NS</td>
<td>0.39095 NS</td>
</tr>
<tr>
<td>A x B</td>
<td>8</td>
<td>0.0174 NS</td>
<td>0.44506 NS</td>
<td>0.12515 NS</td>
<td>0.28900 NS</td>
<td>0.53068 NS</td>
<td>0.11329 NS</td>
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* = Significant  
** = Highly significant  
NS = Non significant

Table 4.19. Means squares for sensory response of fried croquettes

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<tr>
<th>SOV</th>
<th>df</th>
<th>Color</th>
<th>Taste</th>
<th>Odor</th>
<th>Tenderness</th>
<th>Juiciness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (A)</td>
<td>4</td>
<td>4.75194 **</td>
<td>0.63877 *</td>
<td>2.11774 **</td>
<td>0.11481 NS</td>
<td>1.49629 **</td>
<td>0.68072 *</td>
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<tr>
<td>Storage (B)</td>
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<td>0.67656 NS</td>
<td>0.03102 NS</td>
<td>1.25488 NS</td>
<td>1.10450 NS</td>
<td>0.00849 NS</td>
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<tr>
<td>A x B</td>
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<td>0.35328 NS</td>
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<td>Error</td>
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<td>0.24344</td>
<td>0.50044</td>
<td>0.55197</td>
<td>0.29629</td>
<td>0.27161</td>
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</table>

* = Significant  
** = Highly significant  
NS = Non significant
Figure 4.6. Effect of treatments and storage on color and taste of baked & fried croquettes

C₀B= Baked croquettes (Control); C₁B= Baked croquettes stuffed with green cabbage; C₂B= Baked croquettes containing green cabbage extract; C₃B= Baked croquettes stuffed with red cabbage; C₄B= Baked croquettes containing red cabbage extract; C₀F= Fried croquettes (Control); C₁F= Fried croquettes stuffed with green cabbage; C₂F= Fried croquettes containing green cabbage extract; C₃F= Fried croquettes stuffed with red cabbage; C₄F= Fried croquettes containing red cabbage extract
Figure 4.7. Effect of treatments and storage on odor and tenderness of baked & fried croquettes

C₀B = Baked croquettes (Control); C₁B = Baked croquettes stuffed with green cabbage; C₂B = Baked croquettes containing green cabbage extract; C₃B = Baked croquettes stuffed with red cabbage; C₄B = Baked croquettes containing red cabbage extract; C₀F = Fried croquettes (Control); C₁F = Fried croquettes stuffed with green cabbage; C₂F = Fried croquettes containing green cabbage extract; C₃F = Fried croquettes stuffed with red cabbage; C₄F = Fried croquettes containing red cabbage extract
Figure 4.8. Effect of treatments and storage on juiciness and overall acceptability of baked & fried croquettes

C₀B= Baked croquettes (Control); C₁B= Baked croquettes stuffed with green cabbage; C₂B= Baked croquettes containing green cabbage extract; C₃B= Baked croquettes stuffed with red cabbage; C₄B= Baked croquettes containing red cabbage extract; C₀F= Fried croquettes (Control); C₁F= Fried croquettes stuffed with green cabbage; C₂F= Fried croquettes containing green cabbage extract; C₃F= Fried croquettes stuffed with red cabbage; C₄F= Fried croquettes containing red cabbage extract
whilst, minor variations were noted in fried samples, differing from $7.08\pm0.50$ to $6.84\pm0.38$ over the storage.

It is noted from Figure 4.8 regarding panelist preferences for juiciness in baked and fried croquettes that assigned scores were significantly different within the treatments and fried treatments were having an upper hand comparative to baked prototypes. The maximum judges score was allotted to $C_3 F$ 7.11\pm0.50, however the minimum scoring was attained by $C_3 B$ 6.49\pm0.35. With progression over storage, a slight depression was assigned, ranging from $6.73\pm0.54$ (Day 1) to $6.72\pm0.45$ (Day 30) in baked samples whilst, $6.88\pm0.49$ to $6.86\pm0.48$ in fried croquettes.

Hedonic rating for overall acceptability of baked and fried croquettes depicted that maximum scores were assigned to fried treatments at initiation. Amongst treatments, the panelist rating was varying from $7.17\pm0.67$ ($C_0 F$) to $6.55\pm0.42$ ($C_3 B$). With the progression in storage, there was a significant decline in hedonic response for the mentioned trait, lowering from $6.89\pm0.54$ to $6.73\pm0.57$ in baked and $7.09\pm0.66$ to $6.94\pm0.46$ in fried prototypes (Figure 4.8).

In accordance to the current findings, Verma et al. (2016) studied the sensory response of green cabbage incorporated meatballs. They observed a declining trend in all the organoleptic attributes during storage. They evaluated appearance/color scores for control and 15 & 25% green cabbage stuffed meatballs as $6.30\pm0.12$ and $7.44\pm0.12$ & $6.83\pm0.15$ at 0 day that changed to $6.00\pm0.11$ and $7.06\pm0.15$ & $6.39\pm0.12$ at 9th day under refrigerated storage, accordingly. Likewise, the odor, juiciness, texture, tenderness, flavor and overall acceptability in control, 15 & 25% green cabbage based prototypes were scored as $6.50\pm0.12$, $7.33\pm0.11$ & $7.06\pm0.15$, $6.50\pm0.17$, $7.33\pm0.14$ & $6.89\pm0.13$, $7.33\pm0.14$, $6.67\pm0.11$ & $6.39\pm0.12$ and $6.39\pm0.12$, $7.33\pm0.13$ & $6.57\pm0.13$ at 0 day, accordingly. The respective scores changed over refrigerated storage duration of 9 days to $6.06\pm0.15$, $6.89\pm0.14$ & $6.56\pm0.12$, $6.17\pm0.15$, $6.44\pm0.12$ & $7.11\pm0.14$, $6.22\pm0.13$, $6.89\pm0.16$ & $6.44\pm0.12$, $6.89\pm0.16$, $6.28\pm0.14$ & $6.00\pm0.14$, $5.94\pm0.13$, $6.89\pm0.14$ & $6.17\pm0.15$. During storage, the gradual decrement in color was due to degradation of pigmented compounds, lipid oxidation or non-enzymatic browning reactions between lipids & amino acids. The decline in flavor was in response to oxidative rancidity, formation of fatty acids or microbial growth. Moreover, diminution in juiciness of meat based product is related to LDPE packaging i.e. responsible for high permeability to moisture.
Previously, Malav et al. (2015) evaluated a shift in general appearance of cabbage powder enriched mutton patties from 7.15±0.05 (control) to 7.05±0.04, 7.00±0.05 & 6.81±0.07 by incorporating 6, 9 & 12% levels of cabbage powder, respectively. Furthermore, flavor, texture, binding, juiciness and overall acceptability scores were 7.17±0.04, 7.18±0.06, 7.23±0.04, 7.15±0.04 and 7.21±0.04 in control, whereas the scores for cabbage powder containing mutton patties rated lower as 7.06±0.06, 6.85±0.08 & 6.46±0.09 (flavor), 7.01±0.06, 6.88±0.05 & 6.71±0.05 (texture), 7.09±0.05, 6.90±0.06 & 6.68±0.06 (binding), 7.04±0.06, 6.90±0.05 & 6.67±0.05 (juiciness) and 7.08±0.06, 6.89±0.05 & 6.61±0.08 (overall acceptability) at three different doses; 6, 9 & 12%, correspondingly. During storage, the decrease in appearance was attributed to breakdown of pigmented compounds, depletion of nitrites and browning reaction. Similarly, Banerjee et al. (2012) assessed negative consumer perception by adding broccoli powder @ 2% in nuggets, demonstrated momentous decrement in appearance, flavor, texture and overall acceptability from 7.23, 7.06, 7.15 and 7.18 (control) to 7.12, 6.94, 7.02 and 7.05 (treated samples) except juiciness that improved from 7.07 to 7.17, respectively. The sensory responses were almost similar to that viewed in the present research plan.

In a research work, Kumar et al. (2013) developed meat nuggets by incorporating broccoli powder at different levels; 4, 6 & 8%. The treated nuggets concluded significant improvement in oxidative stability and total fiber. They also noticed loss in sensory characteristics; texture & juiciness as the level of broccoli powder increases. The color, flavor, tenderness, juiciness and overall acceptability scores for control samples were 7.14±0.08, 7.17±0.08, 7.06±0.08, 7.03±0.09 and 7.11±0.09, correspondingly. The mentioned characteristics scores lowered to 6.00±0.08, 5.67±0.08, 5.26±0.08, 5.00±0.09 and 5.11±0.09 by the addition of 8% broccoli powder. Recently, Fuchs et al. (2015) found increment in sensory acceptability of control croquette samples over storage (240 days), from 80.1 to 85.3%, whereas the inclusion of flaxseed flour reduced the acceptability from 80 to 71% (70% considered as the minimum acceptable limit for commercialization).

Considering cooking methods, Barakat and Rohn (2014) found higher overall acceptability of Broccoli Based Bars (BBBs) via frying method. The highest score for color was achieved by steamed BBBs followed by fried and baked versions. On the other hand, taste score was best for fried bars due to vaporization of sulfurous compounds while non-significant effect on odor was observed in microwaved, fried or baked bars. The improvement of texture during frying
was associated with reduced water content, denaturation of proteins and browning reactions. The overall acceptability was scored highest for frying followed by baking as noticed in the present study. Later, Radziejewska-Kubzdela and Biegańska-Marecik (2015) introduced red cabbage (purée, frozen and freeze dried format) to apple juice that lowered the score for taste and aroma however, the samples were still within acceptable limits. The purée and frozen red cabbage enriched juice samples were better in sensory response followed by apple juice treatments and freeze dried red cabbage containing juice. Furthermore, Stojceska et al. (2008) incorporated cauliflower trimmings @ 5-20% in extrudate formulations for the enhancement of functional and textural characteristics of snacks. The outcomes presented significant increase in dietary fiber and protein contents of raw cauliflower snacks as compared raw control samples. The maximum acceptability was attained @ 10% cauliflower. At the same level, extrusion cooking significantly raised phenolic compounds and total antioxidant capacity along with decrease in fiber content and protein in vitro digestibility.

In the current study, comparative analyses of fried and baked croquettes inferred higher antioxidant potential and taste scores for fried prototype. The lower existence of flavonoids in fried croquettes might be due to their leeching in frying oil however, the antioxidant activity in fried croquettes was comparable to that of baked counterparts owing the presence of synthetic antioxidants in frying oil. Furthermore, high temperature during baking could be responsible for more disruption and release of sulfurous compounds hence lower acceptability. During storage, slight variations in tested parameters were noted, being freezer friendly.
SECTION 4.3:
BIOASSESSMENT TRIAL
subsection 4.3.1: Serum lipidemic and oxidative stress biomarkers

4.3.1.1. Feed Efficiency Ratio (FER)

In normal diet fed rabbits, the F values relating to FER expounded significant impact of red cabbage & its extract supplemented groups with respect to normal control. Likewise, the F value for FER in hypercholesterolemic diet administrated animals depicted momentous influence of cabbage based treatments against positive control group. The Table 4.20 elucidated maximum FER in N; normal diet group (0.78±0.01%) followed by NRCE; normal diet+red cabbage extract (0.67±0.03%) and NRC; normal diet+red cabbage (0.52±0.02%) fed groups. The mean feed consumption during 12-week trial by N, NRC and NRCE group animals were 16.09, 19.46 and 17.26 kg that resulted in average body weight gain up to 125, 102 and 116 g, respectively. In hypercholesterolemic diet administered groups, the values for FER were comparatively higher than normal animal groups. Amongst hypercholesterolemic groups, the maximum FER was observed in H; hypercholesterolemic diet control group i.e. 1.20±0.05% that decreased to 1.11±0.04% in HRC; hypercholesterolemic diet+red cabbage and 1.13±0.05% in HRCE; hypercholesterolemic diet+red cabbage extract administered groups. The average feed intake and body weight gain for 12 weeks by H, HRC & HRCE groups were 15.11, 14.04 & 14.63 kg and 181, 158 & 163 g, correspondingly.

The FER of normal and hyperlipidemic rabbits were ranged from 0.08 to 0.12 (Lee et al., 2013). The over-accumulation of hepatocellular lipids (HCLs), exceeding 5-10% of liver weight may lead to hepatic steatosis. This condition causes overflow of free fatty acids and triggers inflammatory pathways such as protein kinase C, nuclear factor κB and c-Jun N-terminal kinase 1, resulting in cirrhosis. Numerous animal studies have presented that hypercaloric diet resulted in more liver & total weight due to fat deposition. In contrary, hypocaloric diets have proved effective in overcoming body lipids leading to weight loss (Roden, 2006; Wang et al., 2012). Moreover, Shanmuganayagam et al. (2007) found that hypercaloric diet raised the body weight of rabbits from 2.75 to 3.46 kg with decrement in feed consumption from 141.70 to 140.80 g/day at 0 and 96th day, respectively.

Furthermore, Geremias et al. (2006) determined the impact of hydrosoluble chitosan and Brassica oleracea (1:4) @ 4% of diet on Wister rats for 35 days. The tested diet raised water & feed intakes up to 10 & 7% as compared to control group, respectively. According to an
Table 4.20. Effect of diets on Feed Efficiency Ratio (FER) of rabbits

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>FER (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diet</td>
<td>N</td>
<td>0.78±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NRC</td>
<td>0.52±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>0.67±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>317.68**</td>
</tr>
<tr>
<td>Hypercholesterolic diet</td>
<td>H</td>
<td>1.20±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HRC</td>
<td>1.11±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>1.13±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>9.86**</td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; ** = Highly significant; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract
earlier study, anthocyanins in red cabbage promote weight loss by suppressing the formation of adipose tissues (Priya, 2012). In contrast to the present findings, Annah (2013) studied the impact of red cabbage powder & extract on body weight. The results depicted an increase in feed efficiency ratio, feed intake and weight gain by 42 & 73.68, 7.2 & 8.29 and 53.5 & 89.5%, respectively in contrast to diseased group. Likewise, another rat trial reported contrary results by assessing the impact of red cabbage powder and extract against paracetamol induced hepatotoxicity. The improvement in weight gain, feed intake and feed efficiency ratio was observed up to 31 & 35, 7.5 & 14.8 and 26 & 16.5% by administrating red cabbage powder @ 10% & red cabbage extract @ 100 mg/kg B.W., correspondingly (El-Mowafy, 2012).

The presence of anthocyanins and phenolic acids in the current study is normally associated with free radical trapping ability. In this connection, Shanmuganayagam et al. (2007) assessed the effect of concord grape juice @ 225 mL/day, rich in anthocyanins, proanthocyanidins & hydroxycinnamic acids on hypercholesterolemic rabbits. The body weight (kg) was reported as 3.46±0.03 and 3.19±0.06 in control and juice treated group however, the fluid (mL/day) & feed (g/day) intakes were 140.8±0.8 and 249.0±1.5 & 141.0±0.6 and 245.3±1.7 in the corresponding groups. The presence of polyphenols in juice is attributed to alter GI absorption of cholesterol or down-regulate SGLT-1 thereby reducing glucose uptake in the GI tract. Apart from this, insulin sensitivity and fat oxidation could be the possible factors but need confirmation. Furthermore, the potassium level; 1500-1700 mg/L in juice may normalizes the blood pressure.

Earlier, Finné Nielsen et al. (2005) revealed the restorative potential of anthocyanins against hyperlipidemia in rabbits for 16 wk. The gain in body weight via juice containing anthocyanins @ 48±6.5 mg/kg/day, purified anthocyanins @ 47±3.1 mg/kg/day and normal control were 1.66±0.17, 1.51±0.18 and 1.43±0.20 kg with relative feed intakes as 45.2±3.8, 47±3.1 and 48.6±3.6 g/kg/day, respectively. This study also found that food matrix is responsible for higher absorption of anthocyanins than purified format. One of their peers, Chen et al. (2005) measured the effect of anthocyanin rich aqueous mulberry extract (containing 2.5% anthocyanins) against atherosclerotic lesions in high caloric (cholesterol 1.3% & lard oil 3%) diet fed rabbits for 10 weeks. The weight (kg) & daily feed (g) intake of rabbits were determined in normal, aqueous mulberry extract @ 1%, high cholesterol and high
cholesterol+aqueous mulberry extract as 2.25±0.32 & 149±23, 2.12±0.24 & 154±28, 2.29±0.34 & 155±18 and 2.23±0.28 & 156±13, respectively.

4.3.1.2. Serum lipidemic profile

The F values pertaining to cholesterol (Table 4.21) indicated significant effect of treatments in different groups of normal and hypercholesterolemic diet fed rabbits during the experimentation period. Means for normal rabbits depicted maximum cholesterol level (mg/dL) as 73.02±3.69 in (N) that significantly reduced to 70.09±3.16 (NRCE) and 69.12±3.56 (NRC). In hypercholesterolemic diet fed rabbits, the maximum decrement was viewed in HRC as 124.09±5.56 followed by 130.52±6.94 in HRCE as compared to control (146.31±7.29). The Figure 4.9 demonstrated that RC; Red cabbage was more effective in lowering cholesterol as compared to RCE; Red cabbage extract in both studies. In normal rabbits, the dietary interventions based on RC and RCE resulted in 5.34 and 4.01% decrease in cholesterol level, accordingly. In hypercholesterolemic rabbits, provision of RC and RCE depicted obvious reductions up to 15.19 and 10.79%, respectively.

The statistical study (Table 4.21) showed that tested diets imparted non-significantly on triacylglycerol in normal rabbits nonetheless, the impact of treatments was momentous in hypercholesterolemic diet treated animals. The mean value for triacylglycerol (mg/dL) in normal rabbits was 89.10±4.61 that decreased to 85.06±3.81 and 86.14±4.45 in NRC and NRCE groups, correspondingly. The triacylglycerol in hypercholesterolemic dietary pattern was maximally reduced to 105.08±5.22 (HRC) followed by 109.37±4.90 (HRCE) as compared to positive control group (116.01±5.20). The graphical depiction (Figure 4.9) presented that RC was more potent for reducing triacylglycerol as compared to RCE in normal as well as hypercholesterolemic animals. In hypercholesterolemic rabbits, the RC and RCE treatments decreased triacylglycerol to 9.42 and 5.72%, respectively whereas, the decrement in normal study was minor though the trend was similar.

It is obvious from the F value (Table 4.21) that treatments imparted significant impact on LDL-c in both dietary modules; normal as well as hypercholesterolemic. The maximum reduction in LDL-c (mg/dL) was observed in red cabbage fed rabbits followed by red cabbage extract administered group from 20.09±1.13 (N) & 92.99±4.36 (H) to 18.47±1.41 (NRC) & 72.01±3.87 (HRC) and 18.92±1.40 (NRCE) & 77.16±4.21 (HRCE). It is evident from Figure
Table 4.21. Effect of diets on lipidemic profile of rabbits

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>Parameters (mg/dL)</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CHOL</td>
<td>TAG</td>
<td>LDL-c</td>
<td>VLDL-c</td>
<td>n-HDL-c</td>
<td>HDL-c</td>
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<tr>
<td>Normal diet</td>
<td>N</td>
<td>73.02±3.69</td>
<td>89.10±4.61</td>
<td>20.09±1.13a</td>
<td>17.82±0.86</td>
<td>39.51±2.73a</td>
<td>33.51±1.54a</td>
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<tr>
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<td>NRC</td>
<td>69.12±3.56</td>
<td>85.06±3.81</td>
<td>18.47±1.41b</td>
<td>17.01±0.75</td>
<td>35.48±2.32b</td>
<td>33.64±1.52b</td>
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<tr>
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<td>NRCE</td>
<td>70.09±3.16ab</td>
<td>86.14±4.45</td>
<td>18.92±1.40ab</td>
<td>17.23±0.97</td>
<td>36.02±2.51b</td>
<td>34.07±1.92b</td>
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</tr>
<tr>
<td></td>
<td>F value</td>
<td>3.42**</td>
<td>2.36NS</td>
<td>4.01*</td>
<td>2.33NS</td>
<td>7.51**</td>
<td>0.32NS</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolic diet</td>
<td>H</td>
<td>146.31±7.29</td>
<td>116.01±5.20a</td>
<td>92.99±4.36a</td>
<td>23.20±1.52a</td>
<td>116.19±6.24a</td>
<td>30.12±1.32</td>
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</tr>
<tr>
<td></td>
<td>HRC</td>
<td>124.09±5.56b</td>
<td>105.08±5.22b</td>
<td>72.01±3.87c</td>
<td>21.02±1.68b</td>
<td>93.03±5.95b</td>
<td>31.06±1.73</td>
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</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>130.52±6.94b</td>
<td>109.37±4.90b</td>
<td>77.16±4.21b</td>
<td>21.87±1.46ab</td>
<td>99.03±6.33b</td>
<td>31.49±1.26</td>
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<tr>
<td></td>
<td>F value</td>
<td>29.66**</td>
<td>11.63**</td>
<td>69.35**</td>
<td>5.01*</td>
<td>37.86**</td>
<td>2.32NS</td>
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</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey's HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); CHOL = Cholesterol; TAG = Triacylglycerol; LDL-c = Low density lipoprotein cholesterol; VLDL-c = Very low density lipoprotein cholesterol; n-HDL-c = Non-High density lipoprotein cholesterol; HDL-c = High density lipoprotein cholesterol; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract
Figure 4.9. Percent reduction in serum lipid profile

RC = Red cabbage; RCE = Red cabbage extract
CHOL = Cholesterol; TAG = Triacylglycerol; LDL-c = Low density lipoprotein cholesterol; VLDL-c = Very low density lipoprotein
Figure 4.10. Percent increase in serum HDL-c levels
RC = Red cabbage; RCE = Red cabbage extract
HDL-c = High density lipoprotein cholesterol
4.9 that maximum reduction in LDL-c was in response to red cabbage fed diet trailed by red cabbage extract. The RC treatments reduced the LDL-c up to 8.07 & 18.09% in normal as well as hypercholesterolemic diet dependent rabbits, respectively whereas, RCE down-regulated the LDL-c by 6.46% in normal rabbits and 12.24% in hypercholesterolemic diet induced oxidative stressed rabbits.

The F values for VLDL-c in normal rabbits differed non-significantly with respect to treatments, whereas for hypercholesterolemic rabbits, the VLDL-c in different groups varied significantly as a function of treatments (Table 4.21). In normal animals, the VLDL-c (mg/dL) values were ranging from 17.01±0.75 to 17.82±0.86 however, in hypercholesterolemic rabbits, the said parameter decreased to 21.02±1.68 (HRC) and 21.87±1.46 (HRCE) from 23.20±1.52 (H).

The F value relating to n-HDL was following the similar trend in both normal as well as hypercholesterolemic animals i.e. highly significant as presented in Table 4.21. The means of n-HDL-c (mg/dL) in normal rabbit groups; N, HRC & NRCE were 39.51±2.73, 35.48±2.32 and 36.02±2.51, whereas the means for the said trait were 116.19±6.24, 93.03±5.95 and 99.03±6.33 in H, HRC and HRCE groups, respectively.

The F values pertaining to HDL-c expounded non-substantial difference in normal and hypercholesterolemic diet fed rabbits. Means related to the said trait in normal & hypercholesterolemic rabbits were 33.51±1.54 & 30.12±1.32 that increased to 33.64±1.52 (NRC) & 34.07±1.92 (NRCE) and 31.06±1.73 (HRC) & 31.49±1.26 (HRCE) mg/dL hence results depicted that the treatments are not effective in increasing HDL-c. It is evident from the Figure 4.10 that RC and RCE raised HDL-c non-significantly in both dietary patterns.

4.3.1.3. Serum lipidemic ratio

The F values in Table 4.22 delineated that treatments exhibited significant impact on lipidemic ratios including; AI, CRR, HTR (%) and AAI in both normal and hypercholesterolemic animals. In case of AI and CRR, the maximum values were reported by control groups N & H i.e. 1.18±0.05 and 0.60±0.02 & 3.86±0.18 and 2.92±0.17, respectively. The treated diets fed normal animals; NRC & NRCE decreased these values to 1.05±0.06 & 1.06±0.05 and
Table 4.22. Effect of diets on lipidemic ratio

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Diets</th>
<th>NA</th>
<th>NRC</th>
<th>NRCE</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal diet</td>
<td>N</td>
<td>1.18±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.05±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NRC</td>
<td>0.60±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NRCE</td>
<td>45.89±2.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.67±1.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.61±2.31&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.85±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hypercholesterolemic diet</td>
<td>H</td>
<td>3.86±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00±0.160&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.14±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRC</td>
<td>2.92±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.45±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HRCE</td>
<td>20.59±0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.03±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.13±1.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>0.26±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F value: 19.02<sup>**</sup> 13.91<sup>**</sup> 4.94<sup>*</sup> 17.07<sup>**</sup>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); AI = Atherogenic index ((CHOL-HDL-c)/HDL-c); CRR = Cardiac risk ratio (LDL-c/HDL-c); HTR = HDL-cholesterol to total cholesterol ratio (HDL-c/CHOL*100); AAI = Anti-atherogenic index (HDL-c/(CHOL-HDL-c)); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract
0.55±0.03 & 0.55±0.02 in AI and CRR, correspondingly. However, in hypercholesterolemic animals; HRC & HRCE, the marked decrement was observed with values reported as 3.00±0.160 & 3.14±0.14 and 2.32±0.10 & 2.45±0.09, accordingly. In contrast, the minimum values were reported in normal control groups in HTR (%) & AAI as 45.89±2.63 & 0.85±0.04 that increased up to 48.67±1.74 & 0.95±0.05 in NRC and 48.61±2.31 & 0.95±0.04 in NRCE, respectively. Means pertaining to HTR and AAI, showed minimum values in positive control animals feeding hypercholesterolemic diet that were 20.59±0.87% and 0.26±0.02. On feeding red cabbage & administrating its respective extract, the mentioned traits presented an inclining trend with values reported as 25.03±1.34 & 24.13±1.07% and 0.33±0.01 and 0.32±0.01. Briefly, red cabbage fed animals found relatively more effective in assuaging atherogenic markers as compared to extract supplemented groups.

**4.3.1.4. Serum oxidative stress biomarkers**

The statistical analysis (F value) in Table 4.23 displayed significant variations in CAT and MDA as a function of treatments during the experimental trial in both normal and hypercholesterolemic diet fed animals. On the other hand, SOD depicted non-significant variation in normal rabbits, whereas the impact of the respective trait was momentous in hypercholesterolemic diet fed rabbits. Means relating to SOD (U/mL) and CAT (U/mL) in normal rabbits were 187.39±9.64, 193.18±9.99 & 192.54±10.35 and 98.02±4.39, 104.69±5.53 & 100.16±5.13 in N, NRC & NRCE groups, respectively. The similar increasing trend with respect to treated diets was observed in SOD & CAT enzymatic activities of hypercholesterolemic animals with maximum values of the discussed variables reported as 90.06±3.02 & 74.35±4.26 (HRC) followed by 86.79±4.48 & 70.83±3.11 (HRCE) and 78.53±3.78 & 61.24±2.77 (H), respectively. On the other hand, means regarding MDA (nM/mL) revealed maximum lipid peroxidation in control groups; in N and H groups as 6.29±0.24 and 11.02±0.52 that later decreased to 5.94±0.24 (NRC) & 6.01±0.23 (NRCE) and 7.95±0.36 (HRC) & 8.66±0.49 (HRCE) on provision of red cabbage & red cabbage extract, correspondingly. Briefly speaking the intensified values of SOD and CAT via red cabbage based treatments was related to decrement in MDA (lipid peroxidation) in the respective groups. According to the demographic representation (Figure 4.11), it is evident that red cabbage based treatments presented higher amelioration against lipid peroxidation hence responsible for increase in SOD & CAT levels. In this context, red cabbage supplementation
Table 4.23. Effect of diets on oxidative stress biomarkers in sera of rabbits

<table>
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<tr>
<td></td>
<td>HRCE</td>
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<tr>
<td></td>
<td>F value</td>
<td>24.36**</td>
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</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract.
Figure 4.11. Percent modulation in serum oxidative stress biomarkers

RC = Red cabbage; RCE = Red cabbage extract
SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation)
showed enhancement of SOD & CAT up to 3.00 & 6.37 and 12.80 & 17.63% along with decrement up to 5.56 and 27.86% in MDA levels in normal and hypercholesterolemic diet fed groups, accordingly. Furthermore, the red cabbage extracts administered groups showed relatively poor control on lipid peroxidation i.e. 4.45 and 21.42% in normal and hypercholesterolemic animals resultantly escalating SOD & CAT by 2.67 & 2.14% in normal animals and up to 9.52 & 13.54% in hypercholesterolemic animals, correspondingly.

Cholesterol is a lipophilic compound that requires different carriers; chylomicron (CM), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) for its circulation in the blood (Yang et al., 2012). In liver, there are two physiologic pathways involved in the homeostasis of plasma cholesterol; liver cholesterol synthesis and intestinal cholesterol absorption. The dramatic rise in plasma cholesterol is attributed by HMG-CoA reductase activity and other enzymes involved in lipid synthesis; acyl CoA cholesterol acyltransferase (ACAT) & stearoyl-CoA desaturase (Suanarunsawat et al., 2011; Wang et al., 2011; Hussein et al., 2015). For cholesterol, the best philosophy is "the lower, the better" because it is considered as the leading cause of death for next decades. With increasing age, the production of LDL increases due to increase of VLDLs (precursors of LDLs) in parallel with decrease in LDL receptors or clearance. LDL called as bad cholesterol because it carries the cholesterol from liver to the body due to the presence of Apo-B. In contrary, Apo-A1 is a main protein of HDL and considered as 2-3 times more effective than HDL in lowering CVDs. Recent evidences supported that HDL and ApoA-1 render LDL resistant to lipoxygenase oxidation by responding against transition metal ions, reversing cholesterol transport; removing excess cholesterol from extra-hepatic tissues back to liver for the formation of bile acids, preventing 12-lipoxygenase provoked generation of lipid hydroperoxides and suppressing the expression of adhesion molecules & monocyte recruitment. Conclusively, increase of HDL up to 1 mg/dL is linked with 6% decrement in CVD risk. The serum paraoxonase (PON) associated with HDL is also recognized as an antioxidant enzyme due to its ability to hydrolyze specific lipid peroxides. However, PON get inactivated by ox-LDL or end-products of lipid peroxidation, whereas remained preserved in the presence of endogenous or exogenous antioxidants (Grundy, 1986; Basu et al., 2007; Nader et al., 2010; Van Rooyen et al., 2011; Asgary et al., 2012; Michos et al., 2012; Wang et al., 2012; Gadi et al., 2013; Lee et al., 2013; McEneny et al., 2013; Adaramoye and Akanni, 2014; Zhong et al., 2015).
According to previous review, it has been studied that cholesterol feeding induces hypercholesterolemia and redox mediated plasma & hepatic lipid peroxidation in rabbits. The end-product of lipid peroxidation is MDA; a reactive 3 carbon di-aldehyde, formed when free radicals attack on polyunsaturated fatty acyl groups on membrane lipids, increasing membrane rigidity, osmotic fragility & cellular deformation ultimately compromising membrane integrity leading to necrosis. In this context, increasing the concentration of antioxidants could decrease lipid peroxidation related oxidative damage. Naturally, the free radicals are countered by inherent antioxidant defenses; vitamin E i.e. a major lipophilic scavenger in plasma & tissues, superoxide dismutase (SOD; catalyze superoxide anions into hydrogen peroxide) and catalase (CAT; heme containing oxidoreductase, transforms hydrogen peroxide into water & oxygen molecule) or glutathione peroxidase (GSH-Px); detoxifies hydrogen peroxide & lipid hydroperoxides to nontoxic alcohol (Balkan et al., 2004; Vijayakumar et al., 2004; Arhan et al., 2009; Marinou et al., 2010; Nader et al., 2010; Setorki et al., 2010; Domínguez-Avila et al., 2011; Olusola, 2011; Asgary et al., 2012; Rezq, 2012; Hussein et al., 2015). The rabbits are the most sensitive specie to induce hyperlipidemia within few days (Karbinier et al., 2013). Furthermore, rabbits possess lower vitamin E: cholesterol ratio along with abundance of polyunsaturated fatty acid bodies (easy target for free radicals) leading to significant lipid peroxidation (Mahfouz and Kummerow, 2000; Han et al., 2007; Feillet-Coudray et al., 2009; Qin and Tian, 2010; Lee et al., 2013; Hussein et al., 2015).

Previous researchers worked on different types and cultivars of cabbage using their extracts at varying levels however, scarce information is available regarding cabbage bioevaluation as a whole food matrix, format in which it is normally consumed; the matrix effect on bioavailability as well as on bioactivity of active molecules in the body. The present findings pertaining to lipid modulatory role of cabbage are supported by the earlier outcomes of Sankhari et al. (2012). The authors worked on rats for 56 days, the animals were fed on red cabbage extract @ 100 mg/kg B.W. along with atherogenic diet based on 3% cholesterol, 0.5% cholic acid, 0.2% 6-propyl-2-thiouracil, 5% sucrose, 10% lard and 81.3% laboratory chow. The co-ingestion of extract plus high fat diet reported decrease in serum biochemical parameters including cholesterol 57%, triglyceride 23%, LDL 70%, VLDL 27% and atherogenic index 72%, whereas HDL was raised to 32% as compared to positive control group. In another animal study, red cabbage powder and -extract were tested against
paracetamol induced hepatotoxicity. The serum cholesterol, LDL and VLDL were found to reduce up to 38 & 43, 53 & 61 and 29 & 29% by feeding red cabbage powder @ 10% & red cabbage extract @ 100 mg/kg B.W., respectively (El-Mowafy, 2012). Likewise, a study regarding red cabbage powder & extract depicted pronounced decline in cholesterol (32 & 35%), triacylglycerol (32 & 36%), LDL (44 & 46%), VLDL (75 & 75.6%) and MDA (47 & 55%) along with increment in HDL (32 & 30%) and SOD (47 & 51%) as compared to streptozotocin injected rats (Amnah, 2013). The percent reductions in aforementioned studies were higher as compared to the current experimental trial results, might be due to the concentration of extract, dose or cultivar differences.

Similarly, Al-Dosari (2014) conducted a research based on lyophilized red cabbage juice fed to albino rats in combination with 1% cholesterol+0.2% cholic acid for 6 weeks. The current study is also validated by the findings of previous researchers that reported significant decrement in cholesterol, triacylglycerol, LDL and VLDL up to 10 & 33, 16 & 28, 12 & 42, 17 & 29% at two different doses of freeze-dried red cabbage juice; 250 & 500 mg/kg/day, respectively. On the other hand, HDL showed increase up to 16 & 26% at the corresponding doses. Earlier investigation found that white & red cabbage crude extracts to diabetic rats resulted in reduction of serum cholesterol, triacylglycerol, LDL and MDA by 44.4 & 48.09, 62.5 & 70.09, 62.5 & 82.7 and 41 & 45%, respectively. However, significant increments in serum HDL & GSH levels were also noticed as compared to positive control group (Gaafar et al., 2014). The differences in percent reduction in contrast to the current outcomes could be attributed to variations among cultivars or geographical conditions.

The animal studies carried out on black cabbage samples also explicated their ability to lower high lipidemic profile. In this context, the bioavailability of carotenoids in red and black cabbage was assessed on 38 healthy volunteers against LDL peroxidation. Daily intake of red or black cabbage for two weeks @ 300 g, containing 1 mg lutein and 0.6 mg β-carotene resulted in increment in plasma β-carotene and lutein concentration up to 80 and 204%, respectively as compared to baseline values. Hence, favorable effects of cabbage supplementation were observed on serum lipid profile with significant decrease in total cholesterol, LDL and oxidized LDL levels. The positive effects on lipid metabolism and oxidative status were related to increase in plasma carotenoids & ORAC values (Bacchetti et al., 2014). Likewise, another study conducted on rats using black cabbage extract @ 150 mg/kg plus 23% fat based diet.
The reductions in cholesterol and triacylglycerol were up to 7.4 and 24%, respectively as compared to positive control group. Thus, cruciferous vegetables, even at low doses, could counteract the lipidemic alterations associated with fat rich diet (Melega et al., 2013).

Besides red and black cabbage, numerous studies were conducted on common cabbage or cabbage; without identifying its type, still these studies also depicted hypolipidemic potential. According to an earlier scrutiny, rats were administered with ethanolic extract of cabbage @ 500 mg/kg B.W. along with high fat diet (20% animal fat) that resulted in reduced serum cholesterol, triacylglycerol, LDL and MDA by 23.23, 4.54, 3.81 and 31.7%, respectively during 12-week trial although, anti-atherogenic index (AAI) showed an increase up to 49% in comparison with hyperlipidemic rats ultimately protects against life-threatening modalities such as myocardial infarction and atherosclerosis (Waqar and Mahmood, 2010). The mechanistic study in terms of cholesterol regulating ability of cabbage was conducted by Assad et al. (2014). They tested on methanolic extract of cabbage @ 500 mg/kg using diabetic rabbits. The study resulted in lowering of total cholesterol 39.24%, triacylglycerol 28.03% and LDL 63.24%, whereas HDL showed momentous increment up to 44% as compared to positive control group (30-day trial). Furthermore, the irrefutable scientific proofs have confirmed that consuming vegetables as a major portion of diet along with minimum intake of saturated fat could lower the risk of cardiovascular and oncogenic events. In this regard, a study (28 days) conducted on hypercholesterolemic rats using cabbage powder @ 7.5%, containing total polyphenols 81.58±3.65 mg GAE/100g. The outcomes of the study reported significant suppression of total lipids, cholesterol, triacylglycerol, LDL and VLDL up to 50, 46, 55, 59 and 55%, correspondingly. The lipid lowering potential of cabbage is attributed to phenolics and organic acids. Furthermore, serum MDA decreased by 20.8% while increased GSH levels up to 26.9% (Hussein, 2012). Afterwards, Al-Jawadi (2013) found the hypolipidemic effects of aqueous cabbage extract in alloxan induced diabetic rats, resulted in reduction of total cholesterol and total lipids by 28.4 and 26.9%, respectively. One of the recent studies conducted on cabbage has explored chlorogenic acid in cabbage extract with powerful reducing capacity against DPPH-, hydroxyl- & superoxide anion radicals. During 30 days of experimentation, the cabbage extract @ 240 mg/kg B.W./day+2% cholesterol based diet showed significant hypocholesterolemic potential by lowering cholesterol, triglyceride and
LDL up to 36, 44 and 46%, respectively along with increment in HDL level up to 17% against hypercholesterolemic control group (Ji et al., 2015).

Regular consumption of vegetables helps in controlling hyperlipidemia, hypertension and diabetes owing to the presence of (1) health boosting phytochemicals, (2) antioxidant vitamins like vitamin C (56% RDA by red cabbage); defends against inflammatory pathways & chronic cell injury and (3) minerals such as Mn; cofactor for antioxidant enzyme; MnSOD (Amnah, 2013). In this context, the authors associate anthocyanins as well as other flavonoids, ascorbic acid and isothiocyanates (glucosinolates) in red cabbage with hypolipidemic potential, contribute to down-regulate HMG-CoA reductase; a rate limiting enzyme in the de novo biosynthesis of cholesterol (Priya, 2012; Al-Dosari, 2014). Furthermore, cabbage extract comprised of hydroxycinnamic acids, flavonoids, β-carotenes, lutein, zeaxanthin, alkaloids, glycosides, saponins, titerpenoids, tannins and polysaccharides. These moieties are also associated with other hypolipidemic mechanisms such as down-regulation of cholesterol 7alpha-hydroxylase activity; rate-limiting enzyme of bile acid biosynthesis in the liver resultantly enhances fecal excretion of bile acids. Besides, these moieties are also responsible to lower hepatic gluconeogenesis. Normally, a positive relationship exists between lipogenesis and gluconeogenesis. Thus, any medicine or extract interfering gluconeogenesis is also expected to affect lipogenesis in the similar way. This is the proposed mechanistic approach that how cabbage controls alterations in lipid profile still further confirmation is required to reach a conclusive approach (Priya, 2012; Assad et al., 2014). Previously, Komatsu et al. (1998) also revealed a compound “S-Methyl-L-Cysteine Sulfoxide” in cabbage extract, involved in cholesterol catabolism and fecal excretion. Another researcher Gaafar et al. (2014) mentioned that phenolic acids, vitamin C and α-tocopherol in red cabbage however, quercetin derivatives along with high levels of total GLS in white cabbage were found protective against oxidative injury. Likewise, red and black cabbage inclusion in the diet not only enhances fiber but also contain a myriad of bioactive compounds like cyanidin, quercetin, kaempferol, luteolin & vitamin C, responsible to reduce cholesterol absorption, LDL modification and ox-LDL/LDL-c levels to significant levels (Bacchetti et al., 2014).

Several scientific evidences endorsed the cholesterol lowering ability of anthocyanins. Considering its existence in red cabbage, some studies are described in this regard. Qin et al. (2009) found percent reductions in cholesterol, triacylglycerol and LDL levels in dyslipidemic
individuals up to 2.65, 4.06 and 12.57 by administration of 80 mg of anthocyanins, whereas HDL rose to 10.35% as compared to baseline value. Earlier, Kabiri et al. (2010), Setorki et al. (2010), Asgary et al. (2012), Setorki et al. (2013) and Ali et al. (2016) determined the effect of anthocyanins on cardiac parameters of New Zealand white rabbits in response to high cholesterol diet @ 1%. The extract showed significant reductions in Apo-B, cholesterol, triacylglycerol, LDL, ox-LDL, MDA, AST, ALT, CRP and AI however, increments were observed in HDL & Apo-A thus effective in reducing hyperlipidemia. The hypolipidemic mechanisms related to anthocyanins include suppression in HMG-CoA reductase or lower cholesterol absorption or increase in fecal excretion of cholesterol and bile acids, reduce LDL modification or oxidation by trapping free oxygen radicals such as superoxide anion & peroxynitrates thus protects against protein & lipid oxidation, up-regulation of lipoprotein lipase, reduction in acyl cholesterol acyl transferase; involved in cholesterol esterification & absorption and secretion of hepatic VLDL, Apo-B & VLDL packaging and suppress triacylglycerol accumulation hence prevent fatty liver, insulin resistance & obesity (Finné Nielsen et al., 2005; Kabiri et al., 2010; Setorki et al., 2010; Asgary et al., 2012; Asgary et al. 2013; Setorki et al., 2013; Jawi et al. 2015). Furthermore, protocatechuic acid, a breakdown molecule of anthocyanin, possesses high antioxidant activity as compared to anthocyanins and fights against oncogenesis and atherogenesis (Chan et al., 2013).

As per the current study plan, the whole fruit was tested previously on rabbits and compared against its pure extract. In this study, Sozanski et al. (2014) tested the impact of cornelian cherry @ 100 mg/kg/day containing anthocyanins along with other active ingredients on triacylglycerol and cardiac risk markers in cholesterol @ 1% fed rabbits. The marked decrement was observed in MDA that resultantly preserved hepatic glutathione level in cornelian cherry fed rabbits. In contrast to whole fruit, previous researchers demonstrated that pure anthocyanins extracted from cornelian cherry significantly lowered lipid profile and formation of foam cells in experimental animals. Moreover, they reported a positive correlation between anthocyanin intake and increase in serum antioxidant status. Thus, comparison between whole fruit and pure extract need to consider for final decision. According to the present study, the whole cabbage fed to rabbits demonstrated an upper hand in modulating serum lipidemic & oxidative stress biomarkers as compared to cabbage extract based dietary regimen. Similarly, another investigation by Finné Nielsen et al. (2005) compared purified
anthocyanin and black currant juice containing anthocyanins on hyperlipidemic rabbits. They found increment in LDL and cholesterol via purified antioxidants not by black currant juice. However, the supplementation of whole fruit or juice did not present any adverse effects. The study also depicted improvement in antioxidant enzymes; control, purified anthocyanins & juice supplemented groups were tested for SOD and CAT, the values presented as 1338±108, 1401±162 & 1490±148 and 9.21±1.54, 9.97±1.04, 9.17±1.97 U/g hemoglobin for the respective groups. The study also found similarities in excretion or absorption of anthocyanins in rabbits and humans. Conclusively, anthocyanins showed higher absorption through juice than purified anthocyanins.

Furthermore, the synergistic role of various antioxidant moieties, inherently existing in fruit matrix, was assessed using rabbits as model animals. In this investigation, Yanni et al. (2015) studied the impact of corinthian currant (10%) on the biological markers of New Zealand white rabbits supplemented with cholesterol @ 0.5% for 8 weeks. As whole fruit was fed to the rabbits thus impact of various moieties; anthocyanins, catechins, epicatechin, gallic acid, vanillic acid, caffeic acid as well as quercetin cannot be ignored due to their implication on metabolic responses. The gallic acid, catechins and epicatechin has the potential to inhibit pancreatic cholesterol esterase or bind to bile acids resultantly delaying cholesterol absorption. Apart from this, the metabolites of flavonoids in in vivo system may have more or less antioxidant activity. Besides, the effect of dietary fiber in the plant matrix was analyzed by, Lecumberri et al. (2007). They studied the effect of dietary fiber from cocoa @ 165 g/kg to reduce lipid profile and MDA in hypercholesterolemic rats during 3 wk. The hypolipidemic effects were observed, reducing LDL and restoring triacylglycerol to normal levels along with reduction in lipid peroxidation, whereas no impact was noticed on serum antioxidant enzymes.

The flavonoids (antioxidant activity depends on OH groups in the benzene ring) in the presence of ascorbic acid protect against lipid peroxidation (Setorki et al., 2009; Kabiri et al., 2010). Besides, the co-existence of anthocyanins and vitamin E in addition to vitamin C has the ability to raise plasma antioxidant capacity of rabbits significantly, strengthens endogenous vitamin E and scavenges superoxide anion resultantly preventing lipid peroxidation (Rafieian-Kopaei et al., 2011). Furthermore, Setorki et al. (2009) and Al-Bayati et al. (2012) found synergistic effect of moieties; cyanidin-3-glucoside, quercetin, kaempferol, catechins, epicatechin, organic acids, antioxidant vitamins, minerals and amino acids against atherosclerosis in high
cholesterol @ 1% fed rabbits during 8 wk. They also reported decrement in Apo-B by delaying fat re-absorption or synthesis in enterocytes. Additionally, Duchnowicz et al. (2012) found hypolipidemic potential of cyanidin 3-glucoside, quercetin and hydroxycinnamic acids against cholesterol induced oxidative stress in erythrocytes in vitro. Furthermore, these components were found effective in controlling lipid peroxidation in in vivo system. The results depicted higher antioxidant activity of cyanidin followed by quercetin and hydroxycinnamic acids. Later, Chan et al. (2013) worked on hyperlipidemic New Zealand white rabbits and reported that quercetin has inhibitory role on enzymes involved in oxidative damage such as xanthine oxidase, cyclooxygenase and lipoxygenase whereas, galocatechin has also confirmed its potency against xanthine oxidase.
Subsection 4.3.2: Liver oxidative stress biomarkers

4.3.2.1. Serum specific oxidative stress biomarkers of heart

The results (F values) illustrated non-significant impact of cabbage based treatments on liver specific oxidative stress indicators including ALT, γ-GT and TB, DB & IDB in sera of normal rabbits except ALP. Whilst, all the said traits depicted significant influence of treated diets in hypercholesterolemic animals (Table 4.24).

In normal animal groups; N, NRC & NRCE, the values for ALT were 51.13±2.75, 48.54±2.34 & 49.82±2.48 U/L, ALP 90.11±4.76, 85.24±3.85 & 86.95±3.90 U/L, γ-GT 6.26±0.35, 5.98±0.28 & 6.01±0.28 U/L, TB 0.52±0.03, 0.50±0.02 & 0.51±0.02 mg/dL, DB 0.23±0.01, 0.22±0.01 & 0.23±0.01 mg/dL & IDB 0.30±0.02, 0.28±0.01 & 0.29±0.01 mg/dL, correspondingly. In hypercholesterolemic animals, the effect of treatments was significant and red cabbage showed an upper hand over red cabbage extract whilst, maximum values were reported in hypercholesterolemic control animals i.e. 89.06±4.73 U/L (ALT), 165.08±7.40 U/L (ALP), 9.18±0.50 U/L (γ-GT), 0.65±0.03 mg/dL (TB), 0.25±0.01 mg/dL (DB) and 0.40±0.02 mg/dL (IDB). The decrement was higher via red cabbage followed by red cabbage extract; (75.14±3.75 & 77.75±3.93 U/L) ALT, (142.17±6.37 & 149.31±7.72 U/L) ALP, (7.99±0.38 & 8.37±0.43 U/L) γ-GT, (0.58±0.04 & 0.61±0.03 mg/dL) TB, (0.23±0.02 & 0.24±0.01 mg/dL) DB and (0.35±0.02 & 0.37±0.02 mg/dL) IDB.

The pictorial demonstration (Figure 4.12) showed higher effect of red cabbage in contrast to red cabbage extract in attenuating risk markers of liver stress. In normal rabbits, the percent reduction was minor in all parameters except ALP that ranges from 3.51% (red cabbage extract) to 5.40% (red cabbage) whereas, the percent decrease was far higher in case of hypercholesterolemic animals fed on red cabbage & supplemented with red cabbage extract i.e. 15.63 & 12.70 (ALT), 13.88 & 9.55 (ALP), 12.96 & 8.82 (γ-GT), 10.77 & 6.15 (TB), correspondingly.

4.3.2.2. Oxidative stress indicators of liver tissues

It is revealed from statistical analysis (F values) that endogenous enzymatic markers; SOD & CAT in hepatocytes behaved non-significantly in normal animals however, the effect was momentous in hypercholesterolemic rabbits. Furthermore, F values regarding MDA showed
Table 4.24. Effect of diets on liver specific oxidative stress biomarkers in sera of rabbits

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<td>NRC</td>
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<td>F value</td>
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Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); ALT = Alanine transaminase; ALP = Alkaline phosphatase; γ-GT = Gamma-glutamyltransferase; TB = Total bilirubin; DB = direct total bilirubin; IDB = indirect total bilirubin; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract
Figure 4.12. Percent reduction in serum biomarkers of liver oxidative stress

RC = Red cabbage; RCE = Red cabbage extract
ALT = Alanine transaminase; ALP = Alkaline phosphatase; γ-GT = Gamma-glutamyl transferase; TB = Total bilirubin
### Table 4.25. Effect of diets on oxidative stress biomarkers in liver tissues of rabbits

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<th></th>
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<tr>
<td></td>
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<td>SOD (U/g wet weight)</td>
<td>CAT (U/g wet weight)</td>
<td>MDA (nM/g wet weight)</td>
<td></td>
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<tr>
<td>Normal diet</td>
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<td>2.95NS</td>
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<td>HRC</td>
<td>215.07±9.69a</td>
<td>939.24±42.09a</td>
<td>28.69±1.26c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>209.84±10.46a</td>
<td>898.67±46.42a</td>
<td>31.12±1.46b</td>
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</tr>
<tr>
<td></td>
<td>F value</td>
<td>50.08**</td>
<td>93.17**</td>
<td>168.18**</td>
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</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract.
Figure 4.13. Percent modulation in liver oxidative stress biomarkers

RC = Red cabbage; RCE = Red cabbage extract
SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation)
significant change in both normal and hypercholesterolemic diet fed animals (Table). Means regarding SOD (U/g wet weight), CAT (U/g wet weight) and MDA (nM/g wet weight) in N, NRC & NRCE groups were 223.16±11.54, 232.04±11.04 & 226.21±12.16, 969.07±49.62, 1017.81±53.72 & 1005.03±33.73 and 20.50±0.94, 18.98±1.09 & 19.63±1.11, correspondingly. In hypercholesterolemic diet fed animals, the means for SOD were increasing by feeding red cabbage 215.07±9.69 and red cabbage extract 209.84±10.46 from that of hypercholesterolemic control 176.43±7.74. Likewise, for CAT, the higher increment was viewed in red cabbage based treatments i.e. 939.24±42.09 relatively to that of red cabbage extract supplementation 898.67±46.42 whilst, the control values were at minimum 710.12±29.77. Inverse relation was observed with MDA where maximum lipid peroxidation was observed in H group i.e. 40.75±1.88 followed by 31.12±1.46 in HRCE and 28.69±1.26 in HRC.

The Figure 4.13 explicated non-significant increase in SOD and CAT by administering red cabbage and red cabbage extract in normal rabbits whilst in hypercholesterolemic animals, red cabbage increased SOD and CAT by 17.97 and 24.39% followed by red cabbage extract 15.92 and 20.98%, respectively. On the other hand, the percent reductions were 4.24 and 7.41 via red cabbage extract and red cabbage in normal rabbits, whereas 23.63 and 29.60% in hypercholesterolemic animals, correspondingly.

A sound liver allows very low level of liver functioning enzymes in the serum. During liver disease or in the presence of hepatotoxic compounds, lipid peroxidation occurs, structural integrity of hepatocytes become compromised, rupturing cellular membrane ultimately causing leakage of enzymes; ALT, ALP, γ-GT, AST & LDH along with existing endogenous antioxidants into blood or exhaustion or reduction in their expression (Noori et al., 2009; Morsy et al., 2010; Olusola, 2011; Bhavani et al., 2014). The outcomes of numerous studies indicated that hypercholesterolemic diet serve as a gateway for the production of reactive oxygen species (ROS) that interact with polyunsaturated fatty acids in the cell membranes leading to structural and functional damage (McEneny et al., 2013). The high fat diet increases plasma & liver lipid peroxidation that diffuses from origin site to distant cells, amplifying oxidative stress. The hepatic events are based on two hit models (i) first hit; benign accumulation of hepatic lipids leading to fatty liver (ii) second hit; dominance of pro-oxidant resulting in lipid peroxidation; products of lipid peroxidation in liver further impair respiratory
chain by damaging mitochondrial genome leading to mitochondrial dysfunctions simultaneously suppresses mRNA expression of SOD and GSH & its related metabolic enzyme like GST or exhaustion and leakage into blood stream. Furthermore, continuous inflammation in liver cell induces activation of stellate cells via LPS due to the increase in expressions of TLR4/CD14/MD2, TLR2 & cytokine signaling and collagen formation leading to fibrosis (Kainuma et al., 2006; Milagro et al., 2006; Zou et al., 2006; Savransky et al., 2007; Zivkovic et al., 2007; Wouters et al., 2008; Arhan et al., 2009; Ogawa et al., 2010; Qin and Tian, 2010; Barakat and Mahmoud, 2011; Noeman et al., 2011; Van Rooyen et al., 2011; Wang et al., 2012; Kim et al., 2014; Ragab et al., 2014; Abulnaja and El Rabey, 2015; Zhong et al., 2015). The sources of oxidative stress include cytochrome P-450 2E1, even in the absence of alcohol consumption, generates ROS/ROM from ketones, aldehydes & dietary N-nitrosamines (Arhan et al., 2009). Additionally, hypercholesterolemic diet establishes obesity related leptin resistance leading to lipotoxicity, decrease in fatty acid oxidation and increase in free fatty acids uptake from adipose to non-adipose tissues especially liver (Zivkovic et al., 2007; Torre-Villalvazo et al., 2008; Feillet-Coudray et al., 2009).

The early investigations determined the hepatoprotective effects of red cabbage extracts. Previous researchers determined the hepatoprotective properties of red cabbage and broccoli extract @ 10% against hepatic cancer induced by N-Nitrosodiethyamine (NDEA) and carbon tetrachloride (CCl4). The red cabbage extract significantly down-regulated serum specific markers of liver oxidative stress; ALT (36.7%), ALP (25.9%), AST (29.7%), total bilirubin (35.2%), direct bilirubin (35%) and MDA (35.58%) while momentous increase was observed in serum SOD (19.18%) and CAT (18.39%) as compared to positive control rats during 12-week trial (Morsy et al., 2010). In another study, the potential of red cabbage powder & extract were evaluated against liver toxicity. The markers of hepatic stress including ALT, ALP, AST and γ-GT were suppressed by 20.9 & 24.3, 25 & 32.5, 16.9 & 31.3 and 41 & 44% via red cabbage powder @ 10% & red cabbage extract @ 100 mg/kg B.W., correspondingly. Furthermore, percent increment in SOD and decrement in MDA in liver tissues were 35.5 & 54.5 and 47 & 59.5% on feeding the respective diets (El-Mowafy, 2012).

Earlier, various studies based on cabbage extracts were tested against hepatic toxicity without mentioning the type of cabbage. In this connection, 30-day trial based on cabbage extract @ 240 mg/kg B.W./day was administered to high fat fed rats. The high fat diet resulted in
significant increment in ALT and ALP by 49 and 60% due to hepatic lesions however, the said traits were reduced up to 40.5 and 40% via cabbage extract. Likewise, tested extract raised the SOD and CAT in hepatocytes up to 28 and 24%, respectively but hepatic lipid peroxidation demonstrated significant decline by 36% (Ji et al., 2015). Previous study has presented convincing evidences in the favor of cabbage extract @ 300 to 500 mg/kg B.W. against drug induced hepatotoxicity. In this context, cabbage extract @ 500 mg/kg B.W. depicted reduction in ALT, γ-GT, AST and bilirubin levels up to 67.68, 33.96, 76.3 and 74.7%, respectively. On the other hand, levels of SOD and CAT increased by 58.89 and 27.06%, correspondingly (Bhavani et al., 2014). The current study results are in corroboration with the research work of earlier scientists, Ahmed and his co-workers (2012). The hepatoprotective properties of cabbage extract @ 300 and 500 mg/kg for 30 days restored the oxidative stress biomarkers towards normalization. The cabbage extract reduced the serum indicators of liver oxidative stress; ALT 58 & 66.9%, AST 57 & 76%, ALP 44.6 & 55.9%, direct bilirubin 68 & 77.6% and total bilirubin 68 & 77.6%, whereas increased SOD in liver tissues up to 22 & 36% and CAT to 24.8 & 37.5% at two different levels; 300 and 500 mg/kg, respectively.

In an experimental trial of 21 days, the increments in hepatic SOD and CAT were reported up to 31 and 0.8% via administration of black cabbage @ 150 mg/kg plus high fat fed diet as compared to fat fed group (Melega et al., 2013). In an experimental trial, the effect of dried cabbage @ 7.5% plus high cholesterol diet 1% was assessed in in vivo system and found marked suppression in ALT up to 57% as compared to cholesterol fed rats (Hussein, 2012). Later, Ahmed and Rao (2013) tested the efficacy of Brassica oleracea @ 5000 mg/kg B.W. against simvastatin induced liver toxicity. The percent decrement in ALT, AST, ALP and total bilirubin after administrating cabbage were 69, 78, 56 and 77%, whereas increment in SOD and CAT were 36 and 35.9% as compared to positive control group, accordingly. In another study, antioxidant activity of cabbage extract @ 5 mg/kg B.W. was tested against aflatoxin diet fed diseased rats and results showed lower ALT, ALP and total bilirubin up to 74.9, 61 and 57%, respectively as compared to diseased group, serving as positive control. Furthermore, the decrement in lipid peroxidation was up to 74.5%, whereas SOD increased by 46% in liver homogenates (Abdel-Wahhab and Aly, 2003).

Various mechanisms explicated compounds present in cabbage such as polyphenols, phenolic acids (chlorogenic acid, caffeic acid, vanillic acid, gallic acid & protocatechuic acid),
flavonoids (2 phenolic benzene rings linked to a heterocyclic pyre), sulfurous compounds (glucosinolates, isothiocyanates & indole-3-carbinal), glycosides, amino acids, saponins, tannins, alkaloids & terpenoids act as electron donors hence down-regulate free radical chain reactions. Thus, cabbage has membrane stabilizing effects; restores hepatocytes membrane permeability resultanty suppresses collagen degradation or lipid peroxidation (by suppressing the conversion of hydroperoxides into free radicals) ultimately controlling liver damage and leakage of intracellular enzymes alongside, preventing depletion of hepatic endogenous antioxidants; SOD & CAT. Most of the studies indicated dose dependent response of polyphenols of cruciferous vegetables in protecting cell viability (Guo et al., 2010; Ahmed et al., 2012; Bhavani et al., 2014; Ji et al., 2015). Furthermore, several evidences reported that cabbage phenolics are involved in the activity of γ-glutamyl cysteine synthetase i.e. a rate limiting enzyme of glutathione synthetic pathway (Ji et al., 2015). Furthermore, it is still unclear that either glucosinolates or isothiocyanates or the synergy of various moieties in crude extract is responsible for the observed effects (Melega et al., 2013).

Numerous researchers found that disturbed indicators in response to high cholesterol diet were restored due to anthocyanins. In this context, anthocyanin+cholesterol administration to rabbits presented reduction in ALT, AST and ALP up to 27, 23.7 and 11% as compared to hypercholesterolemic control group (Chen et al., 2005; Setorki et al., 2010). In harmony with present investigation, Chan et al. (2013) tested the synergistic effect of various antioxidants (protocatechuic acid, gallicatechin, gallicatechin gallate, caffeic acid, rutin, quercetin & naringenin) in crude extract along with high caloric diet on rabbits. The results demonstrated decrement up to 69% in ALT as compared to high caloric diet fed rabbits. In accordance with the plan of current study, rabbits were supplemented with anthocyanins rich corinthian currant (10%) in addition to cholesterol @ 0.5%. The dietary intervention improved oxidation resistance by attenuating MDA and reduced ALT up to 29.7% at 4th wk as compared to positive control group (Yanni et al., 2015). Previously, Olusola et al. (2012) administered pre-treated anthocyanins and ascorbic acid, each @ 100 mg/kg B.W. against DNPH @ 28 mg/kg B.W. induced oxidative stress in rabbits. The results demonstrated lower levels of serum ALT, AST and MDA. However, anthocyanins have proved more protective against liver stress than ascorbic acid. This is because antioxidant activity of anthocyanins is attributed to free OH-groups that scavenges free radicals, preventing cell necrosis and protects antioxidant enzymes.
resultantly avoiding the leakage from cytoplasm to blood. In addition, Alia et al. (2003) studied the effect of antioxidant dietary fiber (AODF); a combination of antioxidants plus dietary fiber against oxidative stress in liver during 3 wk. The results depicted non-significant protection in balancing oxidant-antioxidant status as compared to baseline values.

The mechanism of bilirubin and anthocyanin in lowering hepatic MDA was explained by Olusola (2011), who found that bilirubin exist in conjugated form in hepatocytes and absorbs free radicals resulting in MDA reduction. On the other hand, anthocyanin protects against MDA because of numerous mechanisms such as function as an antioxidant in polar cell environment, allows intra-membrane movement i.e. potential to interdigitate with molecules of membrane phospholipids and induces expression of antioxidant enzymes. Thus, collective effect of bilirubin, exogenous antioxidants and liver detoxification system protects against liver damage. Moreover, before toxicity induction, the pretreatment of anthocyanins could block MDA production.

4.3.2.3. Somatic index and histopathology of liver tissues

4.3.2.3.1. Hepatosomatic index

The F value regarding hepatosomatic index (HSI) expounded significant impact of treatments on hypercholesterolemic diet fed rabbits, whereas F value for the said trait illustrated non-momentous influence of treatments in normal dietary pattern. In normal diet fed rabbits, the means concerning to HSI among different groups ranged from 2.41±0.13 to 2.50±0.11% whereas, in hypercholesterolemic diet fed rabbits, the variations were significant from 2.86±0.15 to 3.13±0.14% (Table 4.26).

4.3.2.3.2. Histopathology of liver tissues

Normal diet fed rabbits (N)

The hepatic parenchyma (functional tissue of an organ) was normal. The spaces between hepatocytes (building blocks of liver) i.e. sinusoidal spaces were clear. Hepatic nuclei, nucleolus and chromatin material were also normal. Fine pinkish cytoplasm was present that indicates normal parenchyma.
Normal diet + red cabbage fed rabbits (NRC)

Hepatic parenchyma was normal in appearance. The hepatocytes were normal, arranged in hepatic cords; mass of cells, originating from central vein and irregularly arranged in columns. Each cord contains two rows of hepatocytes arranged back to back. Many such cords join together to form hepatic parenchyma. Furthermore, hepatocytes contain one or two distinct nucleus that contains normal chromatin material and nucleolus. Sinusoidal spaces were also normal. The presence of black dots in sinusoids could be endothelial cells or kupffer cells. The three types of cells could be distinguished as described; (1) Hepatocytes; Nuclei red and round (2) Endothelial cells; Nuclei red and flat (3) Kupffer cells; Nuclei red and cytoplasm blue, located in the lining of sinusoids. The kupffer cells are critical components involved in phagocytic activity; resident macrophages in liver that are involved in innate immune response. Their localization in hepatic sinusoids allows them to efficiently phagocytize pathogens entering into systematic circulation. They normally prevent inflammation induced by immune-reactive substances by attacking & engulfing these particles. However, activation of kupffer cells increases pro-inflammatory cytokines.

Normal diet + red cabbage extract administered rabbits (NRCE)

Hepatic parenchyma was normal in appearance along with normal arrangement of hepatocytes in hepatic cords. Moreover, hepatocytes were having normal nucleus, nucleolus and chromatin material. The portal area was visible, indicating central vein with portal vein in the center and hepatic artery also located nearby carrying bile duct.

Hypercholesterolemic diet fed rabbits (H)

Mild degrees of fibrotic and necrotic changes were obvious. Necrosis is the cell injury, cell degeneration and premature death of cells. Ballooning hepatocytes were present as the name indicates, it is the cell swelling; the cell becomes lighter in color and enlarged in size. Ballooning hepatocytes could be visualized especially in case of fatty liver. At some places, diffused sinusoidal spaces were found due to fat deposition that increases cell size, covering area of sinusoidal spaces.

Fibroblastic changes initiates inflammation or inflammatory zones. Normal synthesis of extracellular matrix and collagen play an important role in wound healing. Liver fibrosis occurs
in response of excessive accumulation of extracellular matrix protein including collagen in hepatic sinusoids, resulting in blood flow resistance. Inflammatory hepatocytes infiltrate the hepatic parenchyma; some of the hepatocytes undergo apoptosis. Kupffer cells get activated, releasing fibrogenic mediators. Hepatic Stellate Cell (HSC) starts secreting extracellular protein in larger amounts. Sinusoidal cells lose their fenestrations and tonic contractions of HSC.

**Hypercholesterolemic diet + red cabbage fed rabbits (HRC)**

The hepatic parenchyma was normal in appearance. However, redness was found at some places, depicting congestion.

**Hypercholesterolemic diet + red cabbage extract administered rabbits (HRCE)**

Fibrotic changes were present. Fibroblastic changes and inflammatory zones were existent in the hepatic parenchyma. However, at few places, hepatocytes were normal in appearance i.e. an indication of mild ameliorative effect of red cabbage extract (Figure 4.14).

Earlier, Jurgonski *et al.* (2014) worked on NZW rabbits fed with hypercholesterolemic diet for 4 weeks and found body weight gain as 41 and 58.9 g. In addition, liver, kidney & heart to body weight ratio values for normal rabbits were 24.80, 6.36 & 2.58 that changed to 34.30, 6.11 & 2.28 g/kg B.W. on consumption of hypercholesterolemic diet. One of their peers, Ram *et al.* (2014) determined the liver, heart and kidney to body weight ratio (g/kg B.W.) as 26.34, 2.12 and 6.74 that reached to 40.01, 2.85 and 7.23 on administrating hypercholesterolemic diet. Furthermore, Purohit and Vyas (2006) analyzed liver & heart to body weight ratio of normal and hyperlipidemic rabbits as 40.62 & 2.24 and 44.49 & 2.31 g/kg, respectively. Earlier, Jeon *et al.* (2002) measured the weight gain as 20.06 and 25.71 g/week however, 17.93 and 23.39 g/kg B.W. liver/body weight ratio in normal and hyperlipidemic rabbits, correspondingly.

In a study conducted on Sprague Dawley rats, fed with Tuscan black cabbage sprout @ 150 mg/kg in combination with high fat diet supported the current exploration. The experiment results reported decrement in weight gain and liver weight by 20 and 15%, respectively along with decrease in feed intake by 13% as compared to high fat fed positive control group (Melega *et al.*, 2013). Previous investigators observed that high cholesterol diet is responsible to
Table 4.26. Effect of diets on hepatosomatic index (HSI) of rabbits

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<th>Diets</th>
<th>Experimental groups</th>
<th>HSI (%)</th>
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<td>NRC</td>
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<tr>
<td></td>
<td>NRCE</td>
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<td>F value</td>
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<td>Hypercholesterolic</td>
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<tr>
<td>diet</td>
<td>HRC</td>
<td>2.86±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>2.98±0.14&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>8.73**</td>
</tr>
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</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); HSI (%) = (liver wt. (g)/ body weight (g)×100; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract.
Figure 4.14. Histomorphological examination of liver tissues of rabbit

N: Normal diet fed rabbits indicating normal hepatic parenchyma; NRC: Red cabbage supplemented rabbits indicating normal hepatic parenchyma without any histological alterations; NRCE: Red cabbage extract administered rabbits showing normal hepatocytes in appearance; H: Hypercholesterolemic diet fed rabbits depicting mild degree of fibrotic and necrotic changes; HRC: Hypercholesterolemic diet + red cabbage fed rabbits portraying normal hepatic parenchyma due to restorative effects of red cabbage; HRCE: Hypercholesterolemic diet + red cabbage extract administered rabbits representing fibrotic and inflammatory zones however, most of the hepatocytes are normal in appearance i.e. an indication of mild ameliorative effect of red cabbage extract
increase organ as well as body weight due to lipid deposition e.g. in hepatic cytoplasm leading to hepatic ballooning ultimately causing chronic ailments such as hyperlipidemia, hypertension and fatty liver disease. The increment in weight of steatotic liver or 2X higher hepatosomatic index as compared to normal animals further leads to hepatic edema and related inflammation. Earlier researchers described the gross morphology of fatty liver as large sized pale, soft, mottled and fatty. Furthermore, they displayed the histology of liver with features like more microvesicles as compared to macrovesicles; vacuolated hepatocytes pushing the nucleus towards periphery, fatty cysts with foamy degeneration and dilated sinusoids. In contrary, antioxidant based dietary therapies have the potential to attenuate such pathological changes by lowering somatic index, less fatty or yellowish color and well-preserved architecture including normal cytoplasm, nucleus, nucleolus & central veins however, mild degree of fatty alterations to moderate degree of inflammation, lesser vacuolar degeneration of hepatocytes and mild steatosis still persisted. Restoring hepatic structure to normal in hyperlipidemic animals could be possible to some extent via antioxidant or dietary fiber based interventional therapies i.e. related to various mechanisms; fecal excretion of cholesterol or reduction in intestinal cholesterol absorption ultimately overcoming fatty liver. Additionally, antioxidants have the ability to protect cell from redox mediated damage and lipid peroxidation (Chan et al., 2015).

The hepatoprotective effects of cabbage based dietary inclusion are consistent with the outcomes of Sankhari et al. (2012). An animal trial (56 days) was conducted in which red cabbage extract @ 100 mg/kg B.W. to high cholesterol diet fed rats reported significant reduction in serum biochemical parameters; ALT 32% and ALP 35%. The red cabbage extract was found to suppress lipid peroxidation by 44% along with significant rise in the activity of enzymatic (superoxide dismutase 44% & catalase 47%) and non-enzymatic antioxidants (reduced glutathione) as compared to control atherogenic rats. The histological architecture of oxidative stressed liver tissues depicted lipid accumulation & fatty streaks, ballooning of hepatocytes and inflammatory alterations in response to high fat diet. However, these alterations were counteracted by co-administration of red cabbage extract (Sankhari et al., 2012).
Later, Al-Dosari (2014) employed cholesterol rich diet to exert hepatic oxidative stress during 6-week period using albino rats and tested the impact of lyophilized red cabbage juice. The findings of their work depicted significant increment in serum liver oxidative stress markers in response to high cholesterol diet including ALT (73%), ALP (38%), \( \gamma \)-GT (53%) and bilirubin (64%). In contrast, the decrement in cellular leakage of the aforementioned liver enzymes was up to 5.3 & 12.3, 11 & 14.9, 14 & 36 and 9 & 23% at two different doses of freeze dried red cabbage extracts; 250 & 500 mg/kg/day, respectively. Furthermore, histological alterations such as inflammation, fibrosis and necrosis were observed due to the generation of reactive oxygen species however, high dose of red cabbage extract protected liver from oxidative damage in a more effective manner. This effect was also confirmed by decrement in MDA level in liver tissues that rose to 94% via cholesterol based diet but reduced to 57 to 71% via different doses of red cabbage extracts; 250 & 500 mg/kg/day. Briefly, red cabbage has the potential to modulate redox sensitive dyslipidemia and associated hepatic injury in a dose dependent manner due to its significant impact on detoxifying enzymes. However, less reduction was viewed in the respective parameters of the current study plan as reported by Sankhari et al. (2012).

The outcomes of the current investigation are in harmony with Gaafar et al. (2014). They conducted a study on Wister rats, the aqueous extract of white and red cabbage to diabetic rats indicated decrease in ALT, ALP and bilirubin levels by 27.3 & 30.9, 44.7 & 50 and 35 & 31%, respectively as compared to diabetic control animals. The histopathological manifestations depicted that liver of diabetic animals showed degeneration of hepatocytes, cell proliferation and congestion of central vein. In contrast, anthocyanin isolates and mixtures provide protection against numerous physiological failures including lipid peroxidation, declined capillary permeability & fragility ultimately improve membrane strengthening. Thus, the effect of cabbage on hepatic fenestrations in the present research is in line with previous outcomes. In addition, selenium from cabbage depicted its high bioavailability thus up-regulates GPx activity, being its co-enzyme. Moreover, histopathological studies showed that cabbage extract protects against fatty streak lesions in hepatocytes steatosis or fibrosis (Ahmed et al., 2012).

Previous researchers, Khoo and his followers (2015) elicited the therapeutic potential of anthocyanins against histomorphological abnormalities via administration of cholesterol rich diet to rabbits. The cholesterol expanded the cytoplasm of hepatic cells due to fatty liver disease
*i.e.* named as micro-vesicular steatosis. Indistinctly, a slight necrosis was also seen with diffused parenchyma. The coupling of inflammation with fiber extension and collagen accumulation resulted in liver fibrosis. In group supplemented with statin, though no heavy fat infiltration but marked hepatic fibrosis was seen because hepatotoxicity is itself a limitation of statin. On the other hand, anthocyanin supplemented models depicted more improvements, characterized by mild steatosis or less possibility of ‘ghost cells’ (absence of nucleus and eosinophilic granular cytoplasm) that are responsible of fatty necrosis.
Subsection 4.3.3: Heart oxidative stress biomarkers

4.3.3.1. Serum specific oxidative stress biomarkers of heart

The F values indicated non-significant effect of treatments on CK, CK-MB and LDH in normal rabbits however, momentous behavior of treatments was observed for these traits in hypercholesterolemic animals. Furthermore, F value pertaining to AST as an indicator of heart depicted significant effect of treatments in normal as well as hypercholesterolemic animals (Table 4.27). In normal rabbits, CK level (U/L) was 465.31±20.85 (N), 458.29±20.54 (NRC) & 462.72±23.90 (NRCE), whereas CK-MB, LDH and AST were 51.28±2.68, 48.71±2.87 & 49.46±2.17, 306.15±13.55, 295.47±18.90 & 299.04±16.90 and 42.03±1.90, 39.76±2.24 & 40.71±1.71 U/L in N, NRC & NRCE groups, respectively. Nevertheless, in hypercholesterolemic animals, the values were substantially higher in hypercholesterolemic control animals with values reported as 980.15±61.73, 106.04±6.19, 547.09±16.32 & 67.12±2.96 U/L for CK, CK-MB, LDH & AST, respectively. In hypercholesterolemic animals, the values for the said markers decreased markedly by red cabbage as 914.07±52.72, 95.63±6.02, 491.64±21.96 and 58.04±2.44 U/L followed by red cabbage extract supplemented group; 917.32±53.58, 97.19±5.61, 509.83±32.61 and 62.18±3.56 U/L, correspondingly. It is depicted from the Figure 4.15 that red cabbage is more effective in ameliorating CK, CK-MB, LDH and AST in hypercholesterolemic rabbits i.e. 6.74, 9.82, 10.14 and 13.53% followed by red cabbage extract 6.41, 8.35, 6.81 and 7.36% as compared to hypercholesterolemic control group whereas, the percent reduction in normal rabbits was non-significant among all other traits except AST, ranging from 3.14 to 5.40%.

4.3.3.2. Oxidative stress indicators of heart tissues

The F value regarding cardiac SOD and CAT elucidated non-momentous effect of treatments in normal rabbits, whereas significant variations were noticed in hypercholesterolemic animals. Furthermore, F value relevant to MDA in myocytes explicated significant effect in normal as well as hypercholesterolemic experimental animals (Table 4.28). In normal dietary pattern, the means for SOD (U/g wet weight), CAT (U/g wet weight) and MDA (nM/g wet weight) were 195.76±9.42, 667.03±30.17 and 52.72±2.51 (N) that modulated by the provision of red cabbage & red cabbage extract in addition to normal diets and the corresponding values were reported as 201.82±9.01 & 197.15±7.05 (SOD), 701.16±39.53 & 685.52±35.41 (CAT) and
Table 4.27. Effect of diets on heart specific oxidative stress biomarkers in sera of rabbits

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<th>Diets</th>
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<th>Parameters (U/L)</th>
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<td>CK-MB</td>
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<td>AST</td>
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<td>NRC</td>
<td>458.29±20.54</td>
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<td>NRCE</td>
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<td>Hypercholesterolic diet</td>
<td>H</td>
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<td>HRCE</td>
<td>917.32±53.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.19±5.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>509.83±32.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.18±3.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>4.40&lt;sup&gt;*&lt;/sup&gt;</td>
<td>8.92&lt;sup&gt;**&lt;/sup&gt;</td>
<td>13.23&lt;sup&gt;**&lt;/sup&gt;</td>
<td>22.58&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); CK = Creatine kinase; CK-MB = Creatine kinase-MB; LDH = Lactate dehydrogenase; AST = Aspartate aminotransferase; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract.
Figure 4.15. Percent reduction in serum biomarkers of heart oxidative stress

RC = Red cabbage; RCE = Red cabbage extract
CK = Creatine kinase; CK-MB = Creatine kinase-MB; LDH = Lactate dehydrogenase; AST = Aspartate aminotransferase
<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>Parameters</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD (U/g wet weight)</td>
</tr>
<tr>
<td>Normal diet</td>
<td>N</td>
<td>195.76±9.42</td>
</tr>
<tr>
<td></td>
<td>NRC</td>
<td>201.82±9.01</td>
</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>197.15±7.05</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>1.38&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypercholesterolemic</td>
<td>H</td>
<td>107.49±4.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>diet</td>
<td>HRC</td>
<td>120.06±6.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>113.58±4.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>12.69&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract.
Figure 4.16. Percent modulation in heart oxidative stress biomarkers

RC = Red cabbage; RCE = Red cabbage extract  
SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation)
49.67±2.56 & 50.88±2.24 (MDA). Likewise, hypercholesterolemic animal models were administered with red cabbage that showed maximum increment in SOD 120.06±6.76 and CAT 314.17±13.17 due to significant decrement in MDA 74.51±3.44 followed by red cabbage extract; 113.58±4.99 (SOD), 301.86±12.66 (CAT) and 79.45±3.66 (MDA), whereas minimum SOD & CAT were found in hypercholesterolemic control animals; 107.49±4.82 & 280.79±16.52 in response to maximum lipid peroxidation in this group with value reported as 89.17±3.76. The Figure 4.16 depicted maximum percent decline in lipid peroxidation (MDA) in myocardial cells i.e. reported up to 16.44 and 10.90 in hypercholesterolemic diet fed animals by administrating red cabbage and red cabbage extract, respectively. In hypercholesterolemic interventional module, the treated groups; RC & RCE with lower levels of MDA revealed higher activity of SOD and CAT; 10.47 & 5.36 and 10.62 & 6.98%, correspondingly, whereas the percent reductions were minor in normal animals except for MDA ranging from 3.49 to 5.79%.

Heart is continuously facing ROS under normal as well as severe conditions. Under normal circumstances, heart muscles are involved in converting one substrate to another for the production of energy (ATP). During energy metabolic processes, ROS are generated by mitochondrial respiratory chain, reduces mitochondrial membrane fluidity; ease in release resultantly induces oxidative stress. Furthermore, during obesity, decrease in oxygen consumption blocks mitochondrial oxidative phosphorylation ultimately increases lipid deposition in adipocytes (Ebaid et al., 2010). According to Kertész et al. (2013), consumption of high fat diet has become a leading cause of cardiomyopathy and mortality amongst middle and high income countries. In severe oxidative environment, the excessive generation of ROS could inactivate cytochrome c oxidase that inhibits mitochondrial electron transport complexes leading to the generation of ROS, tissue injury and leakage of electrons. Besides, some researchers elaborated the mechanism in such a way that the presence of lipid substrates or free radicals in myocardial cells modifies lipid & protein structure leading to oxidative stress in heart. Due to established oxidative stress, myocardial workload increases consuming more oxygen, accelerating mitochondrial electron flux in need of energy (ATP) ultimately increasing electron leakage (superoxide anions) from electron transport chain (Noeman et al., 2011; Xu et al., 2012; Zhong et al., 2015). In this regard, CK & its isoenzyme; CK-MB, AST and LDH are considered as the indicators of cardiac oxidative stress because they get leaked into blood
stream on rupturing of cardiac cell membrane (Adaramoye and Akanni, 2014; Chan et al., 2015).

Previously, red cabbage extract based studies were conducted on rats, whereas the aspects of red cabbage as a whole containing both antioxidant mixtures and dietary fiber were not assessed. The present study is in harmony with a rat trial conducted by Sankhari et al. (2012), they found the impact of red cabbage extract @ 100 mg/kg B.W. on atherogenic diet fed rats. The outcomes explicated momentous reduction in biomarkers of cardiac oxidative stress. In this context, serum CK, CK-MB, LDH and AST showed reduction up to 40, 42, 32 and 31%, respectively. Furthermore, the decrement in MDA of heart tissues was up to 40%, whereas momentous increment was observed in the endogenous antioxidants of heart tissues; SOD 46% and CAT 47% as compared to positive control group. In this context, another study indicated that red cabbage extract has the ability to reduce lipid profile hence modulates cardiac damage.

The different doses of red cabbage extracts; 250 & 500 mg/kg/day fed to albino rats in conjunction with cholesterol rich diet for 6 weeks restored the elevated CK, LDH and AST to 15 & 33, 22 & 33 and 2.3 & 16%, accordingly in contrast to cholesterol fed control group. Apart from this, raised cardiac lipid peroxidation (MDA) in response to hypercholesterolemic diet declined to 55 & 66% on administrating 250 & 500 mg of lyophilized red cabbage extract/kg/day, respectively (Al-Dosari, 2014). Earlier, Amnah (2013) found decrement in serum AST up to 38% on administration of red cabbage powder @ 10% in streptozotocin injected rats as compared to diseased group.

Besides red cabbage extract based studies, some animal trials were documented in which cabbage extract was fed to rats without mentioning its type. Most probably, these studied were based on common cabbage according to the general presumption. In these studies, AST level was studied after the provision of cabbage extracts at different doses and duration. Recently, a 30-day trial was conducted on rats fed on high fat plus cabbage extract @ 240 mg/kg B.W./day based diet. The study found that hydroxycinnamic acids in cabbage extract could mitigate increased serum leakage of AST to 45% as compared to positive control animals (Ji et al., 2015). In another trial, white and red cabbage extracts (each 1 g/kg B.W.) were administered to streptozotocin injected animals. The study depicted significant decrement in AST by 25.7 & 39.9%, accordingly as compared to diseased animal (Gaafar et al., 2014). Furthermore, the effect of dried cabbage @ 7.5% plus high cholesterol diet 1% showed reduction in AST up to
57% as compared to positive control group (Hussein, 2012). In another study, the cabbage extract @ 5 mg/kg B.W. were administered to aflatoxin diet fed rats and results depicted reduction in increased serum levels of AST, LDH and CK to 68.8, 69 and 54.7%, correspondingly in comparison to positive control group (Abdel-Wahhab and Aly, 2003).

Thus, cabbage consumption protects the health by lowering the incidences of cardiovascular diseases i.e. credited by polyphenols, phenolic acids, flavonoids and vitamins C & E; contribute to high free radical trapping ability. Besides, anthocyanins in red cabbage possess potential to disintegrate various disease contributing agents like peroxynitrite hence considered as a promising approach against cardiovascular complications without causing any adverse effects (Singh et al., 2007; Kolodziejczyk et al., 2011; Draghici et al., 2013; Park et al., 2014; Xu et al., 2014).

The restorative potential of anthocyanins in red cabbage is also in corroboration with previous research work done by Qin et al. (2009). They reported decrease in AST up to 7.88% during 12-week supplementation of anthocyanins (80 mg/person/day) to dyslipidemic individuals as compared to baseline values. Furthermore, obvious decrement in AST (17.56%) was observed in rabbits owing to the presence of anthocyanins i.e. positively associated to cardiac health (Madihi et al., 2013). Recently, Khoo et al. (2013) studied the efficacy of anthocyanin predominantly cyanidin-3-glucoside on cardiac markers of New Zealand white rabbits for 8 wks. The anthocyanins depicted decrement in lipids peroxidation (MDA) along with elevation in cellular antioxidant enzymes; SOD & GPx in the tested animal model. Earlier researchers explained the mechanism that anthocyanins are negatively related to serum lipid profile via two possibilities; either cholesterol excreted out of the body or its biosynthesis down-regulated. Furthermore, anthocyanins are scavengers of reactive oxygen species hence prevent lipid peroxidation and LDL oxidation or formation of complex fatty lesion Chen et al. (2005). Besides preventing LDL modification, cyanidin (one of the anthocyanins) also form cyanidin-DNA co-pigmentation, resultanty overcoming DNA damage.

Previous studies indicated that the whole fruit or juice administered to rabbits also showed ameliorative potential. Though, the whole fruit or juice was rich in anthocyanins besides, they were carrying other moieties along with dietary fiber in whole plant matrix. In this regard, Yanni et al. (2015) found that corinthian currant @ 10% (containing anthocyanins, catechins
& phenolic acids) plus cholesterol @ 0.5%, lowered the serum AST concentration from 94.8±13.8 to 35.4±12.8 U/L at 4th week as compared to hypercholesterolemic rabbits (control), accordingly.

Previously, Aboulgasem and Azab (2014) determined the effect of pomegranate juice (anthocyanins, ellagic acid and phenolic acids) against nicotine induced oxidative stress on cardiac biomarkers of male New Zealand rabbits for 6 wks. The serum activities of LDH (U/L) and creatine kinase (U/L) in normal control, juice administered, nicotine injected & nicotine injected+juice administered groups were 141.00±3.70, 137.17±3.44, 234.33±7.59 & 161.83±4.74 and 150.83±5.15, 146.83±2.41, 280.33±4.64 & 183.83±3.89, correspondingly.

4.3.3.3. Somatic index and histopathology of heart tissues

4.3.3.3.1. Cardiosomatic index

The F value regarding cardiosomatic index (CSI) explicated non-significant impact of treatments of both types of dietary patterns; normal & hypercholesterolemic. The values of CSI were varying between 0.27±0.01 to 0.28±0.01% and 0.29±0.01 to 0.31±0.01% in normal and hypercholesterolemic diet based groups, respectively (Table 4.29).

4.3.3.3.2. Histopathology of heart tissues

Normal diet fed rabbits (N)

Mild degree of congestion; hindrance or blockage of passage was present, indicated by red coloration. Furthermore, pyknotic; fragmented nuclei and necrotic changes; degenerating cells, swollen with pale cytoplasm were present. The spectrum of changes resulted in cell death in response to enzymatic digestion of cells or denaturation of intracellular protein.

Normal diet + red cabbage fed rabbits (NRC)

The cardiac parenchyma and nucleic material were normal in appearance.

Normal diet + red cabbage extract administered rabbits (NRCE)

Overall cardiac parenchyma was normal in appearance. However, at fewer places, pyknotic nuclei were present.
Table 4.29. Effect of diets on cardio-somatic index (CSI) of rabbits

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>CSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Diet</td>
<td>N</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td></td>
<td>NRC</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>2.91 NS</td>
</tr>
<tr>
<td>Hypercholesterolic diet</td>
<td>H</td>
<td>0.30±0.02</td>
</tr>
<tr>
<td></td>
<td>HRC</td>
<td>0.29±0.01</td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>1.96 NS</td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); CSI (%) = (heart wt. (g)/ body weight (g)×100; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract
Figure 4.17. Histomorphological examination of heart tissues of rabbits

N: Normal diet fed rabbits showing mild degree of congestion along with pyknotic and necrotic changes in myocardium; NRC: Red cabbage supplemented rabbits depicting normal cardiac parenchyma with no alterations; NRCE: Red cabbage extract administered rabbits presenting overall normal parenchyma alongside pyknotic nuclei at few places; H: Hypercholesterolemic diet fed rabbits indicating mild degree of necrotic changes and mild degree of congestion; HRC: Hypercholesterolemic diet + red cabbage fed rabbits presenting most of the cardiac parenchyma as normal in appearance; HRCE: Hypercholesterolemic diet + red cabbage extract administered rabbits demonstrating mild to moderate degree of congestion and hemorrhage along with pyknotic nuclei in myocardial cells.
**Hypercholesterolemic diet fed rabbits (H)**

The cardiac parenchyma was indicating mild degree of necrotic changes. Alongside, mild degree of congestion was also present.

**Hypercholesterolemic diet + red cabbage fed rabbits (HRC)**

Most of the cardiac parenchyma was normal in appearance.

**Hypercholesterolemic diet + red cabbage extract administered rabbits (HRCE)**

Mild to moderate degree of congestion and hemorrhage; blood loss externally/internally was present. Pyknosis of myocardial cells were indicated by pyknotic nuclei (Figure 4.17).

Previous researchers found that myocardial cells are strong and naturally built for pumping action. Thus, hypercholesterolemic diet presented minor abnormality or cellular degeneration resultantly microscopic appearance of heart was normal (Chan *et al.*, 2015). In accord to the previous study, histopathological evaluation of damaged cardiac tissues indicated extensive damage to myocyte membrane, necrosis and infiltration of inflammatory cells in response to atherogenic diet but the reversed effects were noticed via anthocyanin rich diet (Sankhari *et al.*, 2012). Recently, Khoo *et al.* (2015) tested the role of anthocyanins rich extract against oxidative stressed (@ 0.5% cholesterol) New Zealand white rabbits using histological evaluation. The extract suppressed inflammation or atherosclerosis plaque formation in the right ventricle of heart in a dose dependent manner. The arteries and right ventricle wall of heart in control animals were normal however, thick layers of plaques were seen in hypercholesterolemic rabbits. Moreover, they mentioned that anthocyanins scavenge free radicals especially ROS/RNS, suppress COX-2, INOS & ICAM-1 and modulate NF-κB ultimately prevent oxidative stress and inflammation.
4.3.4: Kidney oxidative stress biomarkers

4.3.4.1. Serum specific oxidative stress biomarkers of kidney

The F value in Table 4.30 showed non-substantial variation with respect to treatments on glucose, creatinine and urea levels in normal rabbits whilst, the significant response was observed in creatinine and urea except glucose in hypercholesterolemic diet fed rabbits. In normal animals, the maximum reduction was viewed via red cabbage in glucose (mg/dL); 69.13±3.27 trailed by extract supplemented animals 69.85±3.13 whereas, in urea and creatinine levels (mg/dL), the maximum decrement was found by red cabbage extract supplemented groups 39.97±1.98 and 0.79±0.03 followed by cabbage fed groups 40.03±2.33 and 0.80±0.04, correspondingly. However, the normal control animal values for glucose, creatinine and urea were 72.20±4.38, 0.81±0.04 and 41.06±2.12, respectively. The similar trend was viewed in hypercholesterolemic animals with highest values recorded in hypercholesterolemic control animals for glucose 83.42±3.74, creatinine 1.03±0.05 and urea 53.97±3.72. The red cabbage & extract reduced the respective values to 79.11±4.31 & 80.34±3.64, 0.90±0.05 & 0.88±0.04 and 50.04±3.45 & 49.11±3.40 in hypercholesterolemic diet fed animals, accordingly. The Figure 4.18 indicated negligible percent decline in glucose, creatinine and urea in treated groups of normal animals, whereas in hypercholesterolemic diet section, the percent decrement was obvious in creatinine and urea ranging from 12.62 (RC) to 14.45 (RCE) and 7.28 (RC) to 9.01 (RCE). However, the treatments responded with slight fall in glucose level among hypercholesterolemic animals as well.

4.3.4.2. Oxidative stress indicators of kidney tissues

From F values (Table 4.31), it is deduced that treatments imparted non-momentous impact on CAT in normal animals, whereas significant effect was noted in SOD & MDA. Furthermore, F values in hypercholesterolemic diet induced animal models explicated significant effect of treatments on all the three said biomarkers of renal oxidative stress. Means pertaining to SOD (U/g wet weight) in normal animals expounded 238.06±7.99, 246.15±11.03 and 250.83±11.70 in N, NRC and NRCE groups, respectively. Similarly, the activities of CAT enzyme (U/g wet weight) in the corresponding groups were 678.53±33.90, 697.08±36.09 and 706.61±32.15. The higher activity of SOD and CAT by administrating red cabbage extract over red cabbage was due to better control on lipid peroxidation reactions. Means pertaining to MDA (nM/g wet weight) recorded significant
Table 4.30. Effect of diets on kidney specific oxidative stress biomarkers in sera of rabbits

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>Parameters (mg/dL)</th>
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<td>Glucose</td>
<td>Creatinine</td>
<td>Urea</td>
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<tr>
<td>Normal diet</td>
<td>N</td>
<td>72.20±4.38</td>
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<td>41.06±2.12</td>
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<tr>
<td></td>
<td>NRC</td>
<td>69.13±3.27</td>
<td>0.80±0.04</td>
<td>40.03±2.33</td>
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</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>69.85±3.13</td>
<td>0.79±0.03</td>
<td>39.97±1.98</td>
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</tr>
<tr>
<td></td>
<td>F value</td>
<td>1.95&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.70&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>0.81&lt;sup&gt;NS&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemic</td>
<td>H</td>
<td>83.42±3.74</td>
<td>1.03±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.97±3.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRC</td>
<td>79.11±4.31</td>
<td>0.90±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.04±3.45&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>HRCE</td>
<td>80.34±3.64</td>
<td>0.88±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.11±3.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>3.23&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>33.67&lt;sup&gt;**&lt;/sup&gt;</td>
<td>5.36&lt;sup&gt;*&lt;/sup&gt;</td>
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</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract
Figure 4.18. Percent reduction in serum biomarkers of kidney oxidative stress

RC = Red cabbage; RCE = Red cabbage extract
<table>
<thead>
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<th>Parameters</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD (U/g wet weight)</td>
<td>CAT (U/g wet weight)</td>
<td>MDA (nM/g wet weight)</td>
<td></td>
</tr>
<tr>
<td>Normal diet</td>
<td>N</td>
<td>238.06±7.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>678.53±33.90</td>
<td>20.62±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>NRC</td>
<td>246.15±11.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>697.08±36.09</td>
<td>19.85±0.81&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
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<td>NRCE</td>
<td>250.83±11.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>706.61±32.15</td>
<td>19.67±0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>3.91&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.76&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>4.31&lt;sup&gt;”&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemic</td>
<td>H</td>
<td>193.42±12.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>375.80±16.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51.39±2.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRC</td>
<td>207.59±9.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>420.37±24.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.11±1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>218.27±9.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>451.25±26.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.02±1.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>13.90&lt;sup&gt;**&lt;/sup&gt;</td>
<td>26.97&lt;sup&gt;**&lt;/sup&gt;</td>
<td>95.27&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract.
### Figure 4.19. Percent modulation in kidney oxidative stress biomarkers

RC = Red cabbage; RCE = Red cabbage extract  
SOD = Superoxide dismutase; CAT = Catalase; MDA = Malondialdehyde (Indicator of lipid peroxidation)
decline in red cabbage extract group 19.67±0.76 as compared to red cabbage fed group 19.85±0.81 however, the mean value was higher in normal control animals i.e. 20.62±0.74. Likewise, maximum lipid peroxidation (MDA) was noted in hypercholesterolemic control animals with mean value recorded as 51.39±2.55 that reduced to 43.11±1.82 (HRC) and 39.02±1.64 (HRCE). This reduction in lipid peroxidation preserves the SOD & CAT enzymes from being consumed in red cabbage extract group; 218.27±9.78 & 451.25±26.55 trailed by red cabbage fed group; 207.59±9.30 & 420.37±24.74, respectively. From Figure 4.19, maximum decrement in MDA 24.07% and increment in SOD 11.38% was observed in red cabbage extract supplemented hypercholesterolemic animal group however, red cabbage was also effective (16.11% decrease in MDA & 6.83% increase in SOD) but relatively poor in attenuating oxidative stress in renal tissues. However, in normal animals, the percent reduction in MDA was ranging from 3.73 to 4.61% resultantly improving SOD activity by 3.29 to 5.09%. Similarly, the CAT increased by 10.60 to 16.72% in hypercholesterolemic red cabbage and its extract supplemented groups, respectively whereas, treatment effect on CAT was negligible in normal animals.

Kidneys perform numerous life sustaining functions including body homeostasis, regulation of electrolyte balance & blood pressure and removal of toxins through urination (Garcia et al., 2012). During the recent era, there is a rapid increase in the Chronic Kidney Disease (CKD) especially among the developing states with special reference to poor dietary habits. In CKD, nephrons lost their structural and functional integrity leading to reduced glomerulus filtration and increase in blood urea & creatinine concentrations (Agarwal et al., 2012). This peril is more prevalent in patients suffering from high blood pressure, diabetes and cardiovascular complications (Chauhan and Vaid, 2009). In renal stress, there are several mechanistic pathways that are involved however, lipid peroxidation mediated oxidative damage is the most acknowledged one. Consumption of fat loaded foods alters renal lipid metabolism due to imbalance between lipogenesis & lipolysis and renal endogenous antioxidants & lipid peroxidation. Furthermore, adipose tissues around kidneys infiltrate into medullary sinuses resultanty increase intrarenal pressures causing renal damage. The damaged tissues further accelerate the production of ROS leading to lipid peroxidation. The concurrence of lipid peroxidation with that of altered LDL & VLDL concentrations are involved to onset renal lesions (Noeman et al., 2011). Normally, creatinine is synthesized in the liver and passes from
circulatory system into skeletal muscles however, its retention in the systemic circulation of hyperlipidemic subjects is an evidence of kidney dysfunctions. Likewise, urea is the end product of protein catabolism, whereas increment in urea concentration in serum is an indicator of hyperlipidemia induced kidney malfunction leading to renal injury (Barakat and Mahmoud, 2011).

The current study results are in line with the findings of Al-Dosari (2014), they conducted 6-week trial on cholesterol fed renal oxidative stressed rats and tested the impact of administered lyophilized red cabbage juice. The cholesterol fed diet raised the serum creatinine and urea up to 78 and 63% that reduced to 25 & 50 and 12 & 18% at 250 & 500 mg of freeze-dried red cabbage extract/kg/day, correspondingly. The present results are also in corroboration with the findings of Gaafar et al. (2014). They conducted a study on streptozotocin induced diabetic Wister rats, the white and red cabbage aqueous extracts 1g/kg B.W. showed decrement in glucose, urea and creatinine up to 75 & 77, 62 & 63 and 34 & 37%, respectively. In agreement with previous investigation, hypoglycemic aspects of cabbage extract @ 500 mg/kg were studied using alloxan induced diabetic rabbits during 30 days. The findings showed a decrement of fasting blood glucose by 43.28% in contrast to positive control group (Assad et al., 2014). The current results are also in strong agreement with Onwuka et al. (2010), they found that dried cabbage pellets @ 0.5 kg/day supplementation to Cd-induced oxidative stressed rats for 28 days suppressed the lipid peroxidation in kidney tissues up to 8.3% along with decrement in ALP up to 17%. Earlier experimentation results revealed that red cabbage powder and extract explicated significant modulatory role against markers of oxidative stress induced by streptozotocin injection. The reductions in glucose, creatinine and urea were up to 38 & 44, 42 & 49, 31 & 34% via red cabbage powder and extract based diet to diabetic rats as compared to positive control group however, insulin faced increments up to 24 & 32%, correspondingly (Amnah, 2013).

Earlier, Kataya and Hamza (2008) determined the impact of Brassica oleracea extract @ 1 g/kg B.W. to attenuate diabetic nephropathy in Wister rats during a period of 8 weeks. The combination of red cabbage extract plus streptozotocin induced diabetes resulted in reduction of kidney weight, fasting blood glucose, serum creatinine and serum urea by 27, 44, 10.5 and 29.2%, correspondingly. Furthermore, renal markers of oxidative stress including MDA was decreased to 41.1%, whereas increment in SOD & CAT was 21.6 & 36.5%, respectively on
administrating red cabbage extract. In an animal study, the aflatoxin contaminated diet was fed to rats along with cabbage extract @ 5 mg/kg B.W., the serum urea and creatinine were restored to normal by reducing the said traits to 20.6 and 61.5%, correspondingly in contrast to positive control animals. Moreover, the lipid peroxidation in kidney tissues decreased to 64%, whereas SOD raised by 40% (Abdel-Wahhab and Aly, 2003). The current study outcomes also corroborate to the work done by Al-Jawadi (2013), who assessed the percent reduction in glucose up to 29.7 by feeding aqueous cabbage extract to alloxan induced diabetic rats.

The hypoglycemic mechanisms of cabbage are related to its active molecules including isothiocyanates, ascorbic acids, hydroxycinnamic acid, flavonoids, β-carotenes, lutein, zeaxanthin, alkaloids, glycosides, saponins, titerpenoids, tannins and polysaccharides (Assad et al., 2014). Furthermore, anthocyanins in red cabbage have the potential to capture free radicals, prevent the LDL oxidation and modulate the markers of diabetic nephropathy in rats (Gaafar et al., 2014). Apart from this, ascorbic acid is a natural antioxidant in cruciferous vegetables that protects against renal toxicities (Onwuka et al., 2010).

Previously, anthocyanins rich extracts were supplemented to the animals that confirmed their hypolipidemic and related renoprotective effects. In a trial on dyslipidemic patients (12 weeks), anthocyanins @ 80 mg showed declining trend on glucose, creatinine and urea levels up to 0.88, 0.267 and 4.8%, respectively as compared to baseline value (Qin et al., 2009). Furthermore, Chen et al. (2005) found creatinine and Blood Urea Nitrogen (BUN) in high cholesterol and high cholesterol+anthocyanin rich extract fed groups as 1.48±0.15 & 1.92±0.18 and 19.1±3.32 & 20.4±4.32 mg/dL, correspondingly. This showed that disturbed indicators in response to high cholesterol diet could be restored due to anthocyanins (Sozanski et al., 2014). Afterwards, a study was conducted by Yanni et al. (2015) on whole corinthian currant @ 10% fed to hypercholesterolemic (cholesterol @ 0.5%) New Zealand white rabbits in order to analyze the synergistic effects of various moieties. The serum glucose concentration was differing from 133.5±5.2 to 145.2±4.7 & 127.0±4.8 to 147.9±4.3 mg/dL from 0 to 4th weeks in cholesterol & cholesterol+corinthian currant, respectively.

The current findings are also in harmony with the outcomes of Sharifiyan et al. (2016), they demonstrated that anthocyanin rich extract with total polyphenol contents (1g/kg of diet) showed significant increment in antioxidant status but non-significant impact was reported on
lipid variables alongside, fail to protect kidney from the damages of hypercholesterolemic diet or from the development of lipidemic plaques in renal arteries. Previously, Setorki et al. (2013) documented that glucose reduction is characterized by the presence of flavonoids that inhibit absorption of glucose in the intestine or block G₆-phosphatase; facilitate catalysis of phosphate from phosphorylated glucose, releasing glucose in the blood. Furthermore, anthocyanins are preventive against hyperglycemia because of their potential to reduce glucose but this reduction is obvious in rats however, triacylglycerol decreased to a slight extent. In case of rabbits, non-significant suppression was observed in glucose along with slight decrement in triacylglycerol (Madihi et al., 2013). Additionally, the anti-diabetic effects of anthocyanins have also been established due to their preventive effects against insulin resistance in diabetic and obese animals (Sozanski et al., 2014).

4.3.4.3. Somatic index and histopathology of kidney tissues

4.3.4.3.1. Nephrosomatic index

The F value 4.32 regarding nephrosomatic index (NSI) explicated non-significant impact of treatments in normal animals, whereas F value related to NSI in hypercholesterolemic diet fed rabbits varied significantly. The values of NSI for normal rabbits were varying between 7.26±0.38 to 7.40±0.42% however, 7.26±0.24 to 8.24±0.39% in hypercholesterolemic diet based group (Table 4.32).

4.3.4.3.2. Histopathology of kidney tissues

Normal diet fed rabbits (N)

The renal parenchyma in normal group N was normal in appearance. Tubular epithelial cells nuclei were normal; larger than granulocytes, round/oval nucleus. Alongside, urinary space was normal. However, at few places mild degree of congestion was viewed.

Normal diet + red cabbage fed rabbits (NRC)

Renal parenchyma in group NRC was indicating normal appearance of tubular epithelium. The tubular cells were having normal nucleus along with nucleolus and chromatin material. Furthermore, urinary spaces or Bowman space; present between the parietal and visceral layer of Bowman capsule surrounding the glomerular was also normal in the glomeruli.
Table 4.32. Effect of diets on nephrosomatic index (NSI) of rabbits

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>NSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>N</td>
<td>7.39±0.42</td>
</tr>
<tr>
<td></td>
<td>NRC</td>
<td>7.40±0.42</td>
</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>7.26±0.38</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>0.37NS</td>
</tr>
<tr>
<td>Hypercholesterolemic diet</td>
<td>H</td>
<td>8.24±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HRC</td>
<td>7.63±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HRCE</td>
<td>7.26±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>F value</td>
<td>22.26**</td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NSI (%) = (kidney wt. (g)/ body weight (g)×100; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract
Figure 4.20. Histomorphological examination of kidney tissues of rabbit

N: Normal diet fed rabbits showing mild degree of congestion; NRC: Red cabbage supplemented rabbits indicating normal renal parenchyma without any histological alterations; NRCE: Red cabbage extract administered rabbits representing no significant alterations and most of it is normal in appearance; H: Hypercholesterolemic diet fed rabbits identifying severe necrotic changes in renal parenchyma along with pyknotic nuclei and mild to moderate degree of congestion; HRC: Hypercholesterolemic diet + red cabbage fed rabbits reflecting ameliorative potential of red cabbage in reversing severe congestion to mild to moderate degree of necrotic changes; HRCE: Hypercholesterolemic diet + red cabbage extract administered rabbits depicting normal renal parenchyma along with mild to moderate degree of pyknotic changes in nuclei of tubular epithelial cells
**Normal diet + red cabbage extract administered rabbits (NRCE)**

Renal parenchyma was normal in appearance. Tubular epithelium was normal with normal nucleus, nucleolus and chromatin material. In glomeruli, urinary space was normal. However, mild degree of congestion was present at fewer places.

**Hypercholesterolemic diet fed rabbits (H)**

Severe necrotic changes were present in renal parenchyma. The changes were indicated by pyknotic nuclei. Mild to moderate degree of congestion was also present.

**Hypercholesterolemic diet + red cabbage fed rabbits (HRC)**

Mild to moderate degree of necrotic changes were present in the tubular epithelial cells indicated by condensed and pyknotic nuclei in the tubular epithelial cells. Mild to moderate degree of congestion was also present.

**Hypercholesterolemic diet + red cabbage extract administered rabbits (HRCE)**

Renal parenchyma was almost normal in appearance. At few places, mild to moderate degree of pyknotic changes in nuclei of tubular epithelial cells was an indication of poor amelioration.

Earlier study depicted that nefrosomatic index of normal and hypercholesterolemic animals were non-significant, showing normal color & consistency with no momentous abnormality. However, increase in kidney weight was associated with edema that showed recovery in response to low sodium or high potassium diets (Chan et al., 2015). Similar outcomes were obtained by Khoo et al. (2015), they tested anthocyanins ability to restore renal architecture. The renal morphology of hyperlipidemic animals showed severe steatosis, accumulation of fat within the cell wall of collecting ducts of renal medulla. Conversely, anthocyanins depicted mild nephritis alongside, protective against steatosis in collecting ducts of renal medulla.
4.3.5: Hematological aspects and inflammatory indicator

4.3.5.1. Erythrocytes indices

F values in Table 4.33 demonstrated non-significant impact of treatments on Hb, RBC, MCV and MCH in normal animals, whereas significant effect was viewed in Hct and MCHC. On the other hand, F values of hematological traits in hypercholesterolemic dietary section depicted momentous effect except for RBC and MCH. Means pertaining to Hb were varying from 12.58±0.43 to 12.92±0.33 and 11.21±0.52 to 12.03±0.57 g/dL in normal and hypercholesterolemic diet fed animals, respectively. The Hct (%) in normal animals were in the range of 32.16±1.14 to 33.53±1.13, whereas in hypercholesterolemic animals, minimum was reported in extract based diet 36.43±1.22 and higher in hypercholesterolemic control animal group; 38.37±1.72. The RBC (M/μL) was within the limit of 6.62±0.24 to 6.72±0.31 (normal animals) and 6.24±0.37 to 6.56±0.38 (hypercholesterolemic animals). The calculated values for MCV (fL), MCH (Pg) and MCHC (%) were varying between 48.36±1.75 to 49.90±1.95, 18.76±0.67 to 19.52±0.83 and 37.61±1.37 to 39.23±1.37 in normal rabbits, respectively. However, the corresponding traits were differing from 55.53±2.48 to 61.49±2.59, 17.96±1.15 to 18.34±0.95 and 29.22±1.46 to 33.02±1.45 via different diets in hypercholesterolemic animals.

4.3.5.2. Leukocyte indices

The statistical analysis revealed significant effect of dietary interventions on WBC in normal as well as hypercholesterolemic diet fed animals. In normal animals, the effect of treatments was non-momentous on differential leukocyte count (DLC) including NEU, LYM, MONO, EOS and BASO. In hypercholesterolemic animals, the treatments explicated significant impact on LYM, nevertheless dietary response was non-momentous on all other traits of DLC. For normal rabbits, the values for WBC (k/μL), NEU (%), LYM (%), MONO (%), EOS (%) & BASO (%) were in the range of 8.21±0.32 to 8.63±0.37, 30.92±1.77 to 32.16±1.44, 67.31±3.02 to 68.29±3.05, 1.77±0.09 to 1.82±0.08, 2.95±0.16 to 3.03±0.15 and 0.00±0.00, respectively. In hypercholesterolemic diet fed animals, the WBC was reduced from 15.51±0.70 (control) to 13.73±0.77 and 13.65±0.61 in cabbage extract and cabbage supplemented groups, respectively whilst, increment in LYM was viewed from 60.07±1.56 to 62.51±2.69 and 63.02±2.65 among the aforementioned groups. Moreover, NEU, MONO, EOS & BASO were 36.42±2.14 to
<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>Parameters</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
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<td></td>
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<td>Erythrocytes</td>
<td>Absolute values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hb (g/dL)</td>
<td>Hct (%)</td>
<td>RBC (M/μL)</td>
<td>MCV (fL)</td>
<td>MCH (Pg)</td>
</tr>
<tr>
<td>Normal diet</td>
<td>N</td>
<td>12.92±0.33</td>
<td>32.93±1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.62±0.24</td>
<td>49.74±1.78</td>
<td>19.52±0.83</td>
</tr>
<tr>
<td></td>
<td>NRC</td>
<td>12.58±0.43</td>
<td>32.16±1.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.65±0.35</td>
<td>48.36±1.75</td>
<td>18.92±0.84</td>
</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>12.61±0.46</td>
<td>33.53±1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.72±0.31</td>
<td>49.90±1.95</td>
<td>18.76±0.67</td>
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<tr>
<td></td>
<td>F value</td>
<td>2.08&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>3.44&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2.57&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypercholesterolemic diet</td>
<td>H</td>
<td>11.21±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.37±1.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.24±0.37</td>
<td>61.49±2.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.96±1.15</td>
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<tr>
<td></td>
<td>HRC</td>
<td>11.76±0.59&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>36.82±1.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.49±0.30</td>
<td>56.73±2.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.12±0.76</td>
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<tr>
<td></td>
<td>HRCE</td>
<td>12.03±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.43±1.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.56±0.38</td>
<td>55.53±2.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.34±0.95</td>
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<tr>
<td></td>
<td>F value</td>
<td>5.58&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.40&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.30&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>15.63&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); Hb = Hemoglobin; Hct = Hematocrit; RBC = Total red blood cell count; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE= Hypercholesterolemic diet + red cabbage extract.
<table>
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<tr>
<th>Diets</th>
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<th>Parameters</th>
<th>White blood cells (WBC)</th>
<th>Differential Leukocyte Count (DLC)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>WBC (k/μL)</td>
<td>NEU (%)</td>
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<td>Normal diet</td>
<td>N</td>
<td>8.63±0.37^a</td>
<td>32.16±1.44</td>
<td>67.31±3.02</td>
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<td></td>
<td>NRC</td>
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<td>30.92±1.77</td>
<td>68.29±3.05</td>
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<td></td>
<td>NRCE</td>
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<td>68.04±3.38</td>
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<tr>
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<td>F value</td>
<td>5.08^*</td>
<td>1.95^NS</td>
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<td>63.02±2.65^a</td>
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<td>HRCE</td>
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<td>62.51±2.69^ab</td>
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<td>F value</td>
<td>22.74**</td>
<td>3.27^NS</td>
<td>4.46^*</td>
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Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); ^NS = Non significant (p≥0.05); WBC = Total White blood cells count; NEU = Neutrophils; LYM = Lymphocytes; MONO = Monocytes; EOS = Eosinophils; BASO = Basophils; N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolic diet; HRC = Hypercholesterolic diet + red cabbage; HRCE= Hypercholesterolic diet + red cabbage extract
Table 4.35. Effect of diets on thrombocytes, prothrombin time and inflammatory indicator

<table>
<thead>
<tr>
<th>Diets</th>
<th>Experimental groups</th>
<th>Parameters</th>
<th>Thrombocytes</th>
<th>Prothrombin time (PT)</th>
<th>Inflammatory indicator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Platelets count (k/μL)</td>
<td></td>
<td>PT (sec)</td>
<td>ESR (mm/hr.)</td>
</tr>
<tr>
<td>Normal diet</td>
<td>N</td>
<td>143.75±6.09a</td>
<td>14.59±0.82</td>
<td>5.96±0.34</td>
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</tr>
<tr>
<td></td>
<td>NRC</td>
<td>135.95±5.63b</td>
<td>14.63±0.85</td>
<td>6.03±0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NRCE</td>
<td>137.31±5.95ab</td>
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<td>5.74±0.30</td>
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<tr>
<td></td>
<td>F value</td>
<td>5.00*</td>
<td>0.01NS</td>
<td>2.32NS</td>
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<td>Hypercholesterolemic diet</td>
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<td>296.13±17.42a</td>
<td>14.41±0.92</td>
<td>8.09±0.42a</td>
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<tr>
<td></td>
<td>HRC</td>
<td>268.05±15.15b</td>
<td>14.48±0.82</td>
<td>7.73±0.37ab</td>
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<td></td>
<td>HRCE</td>
<td>271.19±14.18b</td>
<td>14.43±0.64</td>
<td>7.54±0.38b</td>
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<td>F value</td>
<td>9.67**</td>
<td>0.02NS</td>
<td>5.06*</td>
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</table>

Data values represent mean±SD (n = 10); One-way ANOVA followed by Tukey’s HSD multiple comparison tests; * = Significant (p<0.05); ** = Highly significant (p<0.01); NS = Non significant (p≥0.05); N = Normal diet; NRC = Normal diet + red cabbage; NRCE = Normal diet + red cabbage extract; H = Hypercholesterolemic diet; HRC = Hypercholesterolemic diet + red cabbage; HRCE = Hypercholesterolemic diet + red cabbage extract.
38.73±1.82, 1.92±0.12 to 2.01±0.08, 1.92±0.09 to 2.01±0.10 and 0.00±0.00, correspondingly.

4.3.5.3. Thrombocytes, prothrombin time and inflammatory indicator

It is evident from the F value that thrombocytes (platelets) affected substantially as a function of treatments in normal as well as hypercholesterolemic animals. On the other hand, prothrombin time was non-significant thorough out the trial in both types of dietary patterns. Furthermore, F values with reference to inflammatory biomarker (ESR) illuminated substantial effect of diets in hypercholesterolemic animals whilst, non-momentous impact of treatments was related to ESR values in normal animals (Table 4.35). Means related to thrombocytes (k/μL) in N, NRC & NRCE groups were 143.75±6.09, 135.95±5.63 and 137.31±5.95, whereas the mean values of this attribute in hypercholesterolemic animals were higher in control 296.13±17.42 that reduced to 268.05±15.15 and 271.19±14.18 in HRC and HRCE groups, correspondingly. Likewise, in normal and hypercholesterolemic diet fed animals, the values for prothrombin time (sec) were varying from 14.59±0.82 to 14.63±0.85 and 14.41±0.92 to 14.48±0.82, accordingly. Means pertaining to ESR (mm/hr.) were ranging from 5.74±0.30 to 6.03±0.30 in normal animals and 7.54±0.38 to 8.09±0.42 in hypercholesterolemic animals.

Earlier, the abnormalities in RBC and WBC indices were reported by numerous scientists during hyperlipidemia, hyperglycemia and oncogenic events (Kumar, 2000; Hoffman et al., 2004; Madjid et al., 2004). Rabbits are the most sensitive specie to dietary cholesterol induction therapy and it is the only animal in which hyperlipidemia and related redox state induces anemia within fewer days. The cholesterol overload modifies the lipid composition of RBCs membrane, reducing erythrocyte membrane fluidity or rigidifying, altering ionic motion or restricting random membrane permeability of lipids & proteins. Furthermore, it increases lipid peroxidation and decreases enzymatic & non-enzymatic antioxidant protection system of RBCs leading to significant decrement in RBC, PCV, hemoglobin, hematocrit & MCHC levels. Additionally, high lipid load induces inflammation resulting in enhanced activation of WBC & neutrophils, accelerating platelet count & platelet mediated thrombus formation (Olusola, 2011; Karbiner et al., 2013). Moreover, fatty diet increases prothrombin catabolism that in turn stimulates the synthesis of clotting factors; II, VII & X in liver or oxidation of fibrinogen stimulates platelet aggregation and inflammatory cytokines like IL-1 & IL-6; inducing hepatic fibrinogen to 4X and formation of fibrin coagulum leading to arterial injury,
thrombus formation, resulting in atherosclerosis, inflammation, metastasis and acute myocardial infarction (Khan et al., 2014; Khan et al., 2015; Riaz and Khan, 2016).

Earlier, a study evaluated the anticoagulant effect of cabbage extract using three different levels; 200, 300 & 500 mg/kg for 30 days in white rabbits. The extracts displayed an inclining trend in prothrombin time (from 11.86 to 12.99, 13.00 & 16.14 s), activated partial thrombin time (from 8.06 to 15.23, 14.12 & 32.13 s), thrombin time (from 9.33 to 9.99, 12.75 & 13.41 s) and fibrinogen (from 23.41 to 31.45, 33.35 & 52.2 s), respectively. Hence, the research has examined the positive impact of cabbage on coagulation parameters hence a defensive approach in the integrity of vasculature. The adequate intake of plant based diets that contain indole-3-carbinol and anthocyanins are identified as potent anticoagulant and antithrombotic agents. Furthermore, flavonoids like kaempferol and quercetin in cabbage leaves are atheroprotective moieties being involved in the inhibition of cyclooxygenase and lipoxygenase pathways. The elaborative mechanism describes that cruciferous vegetables has similar effects as that of heparin and forms complex with antithrombin III, resultantly removes various activated coagulation factors. These plants have also been found to reduce plasma and hepatic fibrinogen level, contributing to thromboembolism, inflammation and platelet aggregation. Alongside, reduces the activity of thrombin associated coagulation factors; IX, X, XI and XII ultimately delays thrombin time and down-regulates fibrinogen level. Conclusively, it is postulated that decrement in coagulation factors via cabbage extract may be attributed to its hypolipidemic aspects (Khan et al., 2015).

Recent study depicted antiplatelet effects of ethanolic extract of red cabbage by increasing bleeding time to 165±18.4% through a dose of 38.76 mg/kg B.W. as compared to positive control 129±8.2% (Putri et al., 2014). Furthermore, dried cabbage pellets @ 0.5 kg/day supplemented to Cd-induced oxidative stressed rats for 28 days, resulted in increment in Hb, PCV & WBC up to 23, 29 & 59%, respectively. Briefly, it is observed that cabbage supplementation could restore significant distortion in hematological indices in response to oxidative stress (Onwuka et al., 2010). In the nutshell, vegetarian diets are involved in the hemostatic balance of coagulation and fibrinolysis by increasing prothrombin time or suppressing fibrinogen (Setorki et al., 2013).
Recently, Ali et al. (2016) found significant increment in RBC and Hb alongside, decrement in Plt count on administering anthocyanins to rabbits. Furthermore, non-momentous effect was noted for WBC, lymphocytes, monocytes and granulocytes. In a trial on humans (12 weeks), the effect of anthocyanins @ 80 mg was assessed against dyslipidemia. The anthocyanin intake reported increment in RBC, WBC and Hb up to 2.73, 3.10 and 5.80%, respectively (Qin et al., 2009). Afterwards, Olusola et al. (2012) compared the effect of pre-treated anthocyanins and ascorbic acid, each 100 mg/kg B.W. against DNPH @ 28 mg/kg B.W. induced oxidative stress in rabbits. The results demonstrated delaying and protective effects against oxidative stress induced hematoxicity. Their pretreatment increased PCV, Hb & RBC and decreased WBC to a significant extent due to antioxidant activity of anthocyanins, characterized by free OH-groups. The mechanism of anthocyanins in increasing PCV, hemoglobin & RBC and decreasing WBC count is explained by Olusola (2011) and Ali et al. (2016). The reduction in lipid peroxidation resulted in increased PCV. Alongside, anthocyanins induce erythropoietin synthesis and its release from renal cells, whereas anthocyanins and vitamin C increase iron absorption hence enhance Hb; constituent of RBCs involves in the supply of oxygen and removal of carbon dioxide. Conclusively, increase in RBCs production is due to the stimulating effect on erythropoietin, a regulator of RBC. Additionally, the presence of vitamins and polyphenols is characterized by increase in blood cells owing to stimulatory effect on bone marrow and lymphoid organs. The antioxidants and high protein are responsible to increase Hb in response to activation of hemopoietic tissues.

The fruit as a whole possesses numerous antioxidants, minerals and vitamins hence their synergy need to be assessed. In this context, Riaz and Khan (2016) studied the impact of pomegranate juice for 60 days on hematological aspects of rabbits. The pomegranate is a rich source of polyphenols; anthocyanins, ellagic acid and tannins, antioxidant vitamins predominantly ascorbic acid and minerals; K, Mg, Ca, Fe, Zn, P & Cu. These ingredients showed improvement in hematological aspects such as RBC and Hb along with slight decrease in Hct; responsible for inhibiting platelets aggregation and no change in platelet count. Furthermore, the fruit showed non-significant impact on prothrombin time ultimately coagulation (extrinsic factors; I, II, V, VII & X), lacking of these factors is attributed to prolonged prothrombin time.
Earlier, Asgary et al. (2010), Rafieian-Kopaei et al. (2011), Riaz and Khan (2013) and Khan et al. (2014) reported that anthocyanins cause significant decrement in fibrinogen level and preventive against platelet aggregation by reducing coagulation factors or stimulating fibrinolysis ultimately increasing prothrombin time. Furthermore, anthocyanins could prevent fibrinogen oxidation by direct excretion or scavenging of free radicals. According to previous evidences, endothelium matrix contains pre-coagulation compounds like collagen that releases platelets granules containing VWF (Von Willebrand Factor) and fibrinogen; serve as a bridge between collagen and glycoprotein platelet receptors with which platelets continue joining. In this process, anthocyanins break the linkages of collagen with platelets at site of injury via the action of proline hydroxylase enzyme. Furthermore, anthocyanins have the ability to suppress the expression of epinephrine gene involved in platelet aggregation and that of P-Selection; inflammatory agent. Apart from this, catechins and quercetin could inhibit NADPH oxidase in platelets; suppressing superoxide anion generation, improving NO and regulating glycoprotein platelet receptors hence down-regulating platelets activation & adherence ultimately thrombosis.

Now, it is clear that red cabbage and its aqueous extract are the safe therapeutic approaches to suppress lipid peroxidation induced via the over-consumption of hypercholesterolemic diet ultimately restored the fenestrations of various organs from injury. Alongside, the inclusion of exogenous antioxidants not only control lipid peroxidation reactions but also strengthen the endogenous antioxidants hence fight against free radical as a team, securing inherent stores of SOD & CAT activities and prevent their leakage as well. Conclusively, red cabbage based designer food has proven efficacious against hyperlipidemia induced liver compromised conditions or heart malfunctions, whereas red cabbage extract showed an upper hand in delaying renal stress by negating the harms associated with junk foods, either due to high absorptivity of extract in renal tissues or presence of low sodium content in extract as compared to cabbage or it might be due to absence of some moieties or presence of others during extraction.
CHAPTER 5

SUMMARY

The nutritional transition towards hypercaloric diets and sedentariness has raised lifestyle related disorders, reduced work productivity and escalated health care cost. Thus, it is necessitated to tackle this epidemiological shift by re-introducing health benefits of wholesome, natural foods i.e. safer and easily convincing for the health-aware and -active individuals. In this context, the concept of designer foods is the first step to bring back the people from empty caloric conventional diets to such replenished processed versions that are enriched with such ingredients that contribute health and prevent the onset of modern lifestyle ailments. Currently, oxidant-antioxidant status of the junk food seekers has imbalanced in such a way that free radicals are increasing ultimately exhausting free radical trapping agents. This scenario has compromised various organs of the body such as liver; the non-alcoholic fatty liver diseases have covered 3-30% of the globe. Besides, heart ailments have encountered up to 27-48% of the Asian Pacific and 40-58% of the western countries. Alongside, chronic kidney diseases have resulted in 1.5% of the deaths, world widely. Hence, to cope up with the situation, the masses should switch to healthy, affordable foods to up-regulate endogenous antioxidant status.

In this regard, cruciferous vegetables should be considered owing to their rich phytochemistry based on anthocyanins, phenolic acids and flavonoids. Furthermore, such vegetables are hypocaloric in nature and economically feasible besides high nutritive value in terms of minerals, antioxidant vitamins and dietary fiber. Resultantly, sulfur containing ingredients in cabbage are negatively associated with oncogenic events however, cabbage juice is considered as a remedy against gastric ulcer. Besides, the health boosting ingredients are related to anti-inflammation and anti-oxidative stress activities. Previous researchers have been focusing on solvent extraction and phytochemistry analyses of various types of cabbage from different geographical regions of the world. There is a bulk of review articles available discussing the role of cabbage in preventing various ailments however, scientific researches are missing to endorse these points. Besides, as we are consuming the vegetable as a whole so there is a need to assess the vegetable in the same format to validate the health implications associated with it because plant matrix matters a lot and various ingredients may undergo degradation during
extraction procedures. Additionally, there are numerous antioxidants, exist either bound to plant matrix or showing their effectiveness in-combination with the matrix.

Purposely, the commonly available green and red cabbages in Pakistan were tested. The study was basically divided into three parts; 1ˢᵗ part based on compositional and antioxidant status of green and red cabbage, whereas 2ⁿᵈ part was explaining the product development & analyses, employing different types formulations and cooking methods. In 3ʳᵈ part, the selected cabbage and extract, based on antioxidant capacity and sensory response, were assessed against hyperlipidemia induced oxidative stress.

Compositional analyses of green and red cabbage indicated significantly higher amounts of crude protein, fat, fiber and ash content in red cabbage as compared to green counterparts. Besides, the mineral contents were also on the higher side in red cabbage except Mg, Zn and Cu. However, potassium was the most abundant mineral in both cabbage samples varying between 53.42±1.87 to 96.44±3.38 mg/100g F.W. Among antioxidant vitamins, vitamin C was the major moiety differing from 121.46±3.28 to 139.07±2.23 mg/100g F.W. along with vitamin E and \( \beta \)-carotene however, all antioxidants were momentously higher in red cabbage.

The HPLC quantification of phenolic acids and flavonoids presented significant variations in both types of cabbage. The green cabbage extract showed higher proportion of chlorogenic acid 17.47±0.63 mg/100g F.W., whereas vanillic acid was the major phenolic acid found in red cabbage i.e. 13.20±0.33 mg/100g F.W. Furthermore, caffeic acid was quantified in green cabbage only however, ferulic, \( p \)-coumeric, cinnamic and syringic acids were merely detected in red cabbage. The flavonoids like kaempferol, epigallocatechin gallate and catechins showed more abundance in red cabbage especially kaempferol i.e. 171.10±5.99 mg/100g F.W. On the other hand, quercetin was more in green cabbage than red equivalent. The total polyphenols and flavonoids in red cabbage were momentously in greater amount as compared to green cabbage, ranged from 58.41±3.01 to 224.37±6.96 and 34.04±1.06 to 219.15±10.30 mg/100g F.W., respectively. As expected from color, the anthocyanins were detected in red cabbage only i.e. 69.86±4.12 mg/100g F.W.

The antioxidant assays including free radical scavenging potential using DPPH, ABTS and hydrogen peroxide reagents explicated significantly higher quenching potential of red cabbage 87.79±3.69%, 6.04±0.21 μM Trolox/g F.W. & 63.45±3.05% in contrast to green cabbage.
31.22±1.65%, 1.51±0.07 μM Trolox/g F.W. & 48.03±1.68%, accordingly. Moreover, reducing power; ability to donate electron to free radicals was measured by FRAP & PFRAP assays that varied remarkably, higher in red cabbage than green cabbage, ranging from 0.95±0.04 to 1.16±0.04 μM Fe^{2+}/g F.W. and 41.16±2.10 to 59.32±2.14%, correspondingly.

In 2\textsuperscript{nd} part; the product development module, designer croquettes were developed containing four different types of formulations; green & red cabbage stuffed and their respective extract enriched croquettes along with control. Besides, two types of cooking procedures; baking and frying were employed to assess their impact on antioxidant activity as well as hedonic characteristics. The proximate composition explicated significant difference amongst all macro-components with higher moisture content in green cabbage stuffed baked croquette 61.85±2.47% and minimum in control (baked prototype) i.e. 54.09±2.54%. Furthermore, fat content portrayed as the most varied component in both cooking procedures. As expected, it was far higher in fried samples 11.20±0.27% (green cabbage stuffed) to 17.78±0.43% (green cabbage extract enriched) as compared to baked ones; maximum in red cabbage extract enriched sample (3.96±0.29%) and minimum in green cabbage stuffed prototype; 3.07±0.25%.

In parallel, the caloric count was higher in fried croquettes (199.80±8.19 to 247.21±8.16 kcal/100g) than baked (155.65±10.82 to 191.64±10.8 kcal/100g) and significantly varying with respect to treatments, minimum in cabbage stuffed croquettes followed by extract based, whereas maximum calories were reported in control samples.

The color tonality in terms of L, a*, b*, chroma and hue angle showed significant impact of treatments and storage in both cooking methods except hue angle of fried croquettes that showed non-significant impact of storage. The L value indicates lightness that was minimum in red cabbage based treatments, while a* value showed negative bars for green cabbage stuffed treatments, whereas positive bars for red cabbage based prototypes because a* value is negative for green hue and positive for red coloration. Additionally, more the b* value, more the yellowness, that was maximum in control treatments and minimum in red cabbage containing croquettes. The similar trend was followed by chroma indicating saturation. Besides, hue angle depicts region from 0 to 360°; the control and red cabbage based samples were lying within the region of 0-90° (red to yellow), whereas green cabbage prototypes were falling in the range of 90-180° (green to blue hue). Apart from this, 30 days frozen storage presented decline in L, a* & b* values, showing lesser color acceptability.
The hardness and water activity was varying significantly as a function of treatments and storage. The hardness was higher in control treatments especially in baked 23.11±0.84 N as compared to fried 14.70±0.29 N however during storage, baked croquettes showed maximum value 14.51±0.40 N at 15th day, whereas fried treatments presented linear increment over storage intervals from 9.10±0.30 to 11.13±0.29 N. The water activity was varying from 0.89±0.04 to 0.70±0.02 in baked and 0.82±0.02 to 0.67±0.02 in fried samples during storage.

The antioxidant indices of baked and fried croquettes differed significantly with respect to treatments however, storage imparted momentously on total polyphenol content and ABTS assay for baked prototypes. Whilst considering fried croquettes, only ABTS assay showed significant variation over the storage. Though, total polyphenol content in both cooking methods were comparable, still the TPC in fried croquettes indicated an upper hand 125.82±6.09 mg/100g F.W. as compared to baked treatments 121.61±5.85 mg/100g F.W. i.e. maximum in red cabbage stuffed prototypes. It might be due to frying in olive oil or the possibility of synthetic antioxidants existence in oil as preservative. Apart from this, maximum flavonoids were found in baked red cabbage extract enriched treatments 92.83±4.13 mg/100g F.W. in contrast to fried red cabbage stuffed samples 78.17±3.77 mg/100g F.W. might be due to leeching of lipophilic flavonoids in frying oil. With respect to treatments, the red cabbage and its aqueous extract based treatment presented higher antioxidant potential as compared to green cabbage based designer croquettes, whereas with respect to cooking methods, the maximum antioxidant potential was noted for fried treatments owing to their linearly higher total polyphenols. The antioxidant assays of fried red cabbage croquettes depicted 32.00±1.75% & 3.05±0.15 µM Trolox/g F.W. (DPPH & ABTS scavenging potential, accordingly) and 1.37±0.05 µM Fe^{2+}/g F.W. reducing power via FRAP assay.

Considering sensory response, the color, taste, odor, juiciness and overall acceptability of designer baked & fried croquettes were varying significantly regarding treatments however, tenderness of baked croquettes showed momentous impact with respect to storage. The trend of sensory attributes was almost similar in both types of cooking methods. The color scores were more for extract enriched treatments and control, whereas minimum scores were assigned to cabbage stuffed croquettes. Furthermore, declining trend was viewed in color score with progression in storage. Regarding taste, the maximum scores were attained by extract enriched followed by cabbage based croquettes. The taste scores for cabbage based treatments were
improving over storage except extract enriched and control samples. The odor of cabbage stuffed croquettes was more prominent due to the presence of sulfurous compounds and declining trend in scores was achieved during storage. The tenderness was minimum in cabbage based treatments at day 1 followed by improvement in the said trait scores at day 30, whereas inverse trend was viewed for extract based treatments and control. Furthermore, similar trend was noted for juiciness as that of tenderness. Hedonic rating for overall acceptability depicted maximum scores for fried treatments at initiation. Amongst treatments, the overall acceptability showed maximum scores attainment by control samples; 7.14±0.59 (baked croquettes) and 7.27±0.56 (fried croquettes) followed by red cabbage extract (7.23±0.47 via frying) and green cabbage extract (7.13±0.42 via baking) while minimum scores were allotted to cabbage stuffed croquettes. Conclusively, designer croquettes remained freezer friendly for one month without employing any preservative. Furthermore, red cabbage has proven its nutraceutic worth for the development of healthy and cost-effective designer food products.

Thus, red cabbage owing to its higher antioxidant potential and its respective extract, being highly rated and acceptable prototype amongst the designed formulations were screened to relate their efficacy in biological system. In bioefficacy assessment trial (12 weeks), there were two dietary patterns; normal and hypercholesterolemic diet based regimens (induction source; 2% cholesterol for 1st week followed by 1% cholesterol for rest of three weeks). There were six groups in total, each carrying 10 animals; first three groups were following normal dietary pattern in addition to the inclusion of red cabbage leaves @ 20% (NRC) and its aqueous extract in equivalence to cabbage amount (NRCE) along with normal diet based group (N). The similar trend was followed in the hypercholesterolemic rabbit groups; H; hypercholesterolemic diet based group, HRC; hypercholesterolemic diet+red cabbage leaves @ 20% and HRCE; hypercholesterolemic diet+red cabbage extract in equivalence to cabbage amount.

The FER (Feed Efficiency Ratio) expounded momentous impact of diet in both normal (0.52±0.02 to 0.78±0.01%) as well as hypercholesterolemic diet (1.11±0.04 to 1.20±0.05%) based regimens. Based on treatments, lipidemic profile especially cholesterol, LDL and n-HDL demonstrated significant variations in normal and hypercholesterolemic diet fed rabbits, whereas triacylglycerol and VLDL levels presented obvious differences in hypercholesterolemic animals. On the other hand, HDL showed non-momentous response in
both types of dietary patterns. The red cabbage fed animals showed better control on hyperlipidemia than extract administered groups, additional impact might be related to dietary fiber. The cholesterol level down-regulated from 73.02±3.69 (N) & 146.31±7.29 (H) to 69.12±3.56 (NRC) & 124.09±5.56 (HRC) and 70.09±3.16 (NRCE) & 130.52±6.94 (HRCE) mg/dL. The percent reduction in cholesterol was noted up to 5.34 & 4.01 (normal animals) and 15.19 & 10.79 (hypercholesterolemic animals) in red cabbage & its extract supplemented groups, correspondingly. Besides, the decrement in triacylglycerol was ranged between 5.72 to 9.42% in hypercholesterolemic rabbits, whereas the decline in normal study was minor. In both dietary modules, the reduction in LDL-c (mg/dL) was from 20.09±1.13 (N) & 92.99±4.36 (H) to 18.47±1.41 (NRC) & 72.01±3.87 (HRC) and 18.92±1.40 (NRCE) & 77.16±4.21 (HRCE) with percent reduction up to 8.07 & 18.09 (red cabbage) and 6.46 & 12.24% (red cabbage extract) in normal & hypercholesterolemic animals, correspondingly. The similar trend was viewed in case of n-HDL & VLDL-c. Furthermore, the calculated values of AI and CRR were higher for hypercholesterolemic control group and minimum for red cabbage fed groups and inverse effects were observed in HTR and AAI thus depicted higher cardiac risk reduction via supplementation of red cabbage based treatment over its aqueous extract. The serum CAT and MDA levels presented significant modulation with respect to diets in both feeding trials, whereas serum SOD depicted momentous increment in hypercholesterolemic animals. The SOD (U/mL) and CAT (U/mL) in normal control rabbits were 187.39±9.64 and 98.02±4.39 that decreased to 78.53±3.78 and 61.24±2.77 in hypercholesterolemic control that later raised to 90.06±3.02 & 86.79±4.48 and 74.35±4.26 & 70.83±3.11 in HRC & HRCE groups, correspondingly. Thus, red cabbage supplementation showed enhancement of SOD & CAT up to 13.29 & 17.63% due to decrement in MDA by 27.86% in hypercholesterolemic diet fed groups, whereas red cabbage extract administered group showed relatively poor amelioration in lipid peroxidation i.e. 21.42%.

In liver oxidative stress markers, ALT, γ-GT and TB, DB & IDB in sera of hypercholesterolemic rabbits showed significant impact, whereas ALP explicated momentous impact in both types of dietary regimes. The red cabbage showed more ameliorative potential than red cabbage extract and ALT, ALP, γ-GT and TB reduced from 89.06±4.73 to 75.14±3.75 U/L (15.63%), 165.08±7.40 to 142.17±6.37 U/L (13.88%), 9.18±0.50 to 0.58±0.04 U/L (12.96%), 0.65±0.03 to 0.23±0.02 mg/dL (10.77%) via red cabbage supplementation in
hypercholesterolemic animals, respectively. The hepatic MDA elucidated significant differences in both normal and oxidative stressed animals, whereas increment in SOD and CAT was obvious in oxidative stressed animals. In hypercholesterolemic animals, red cabbage showed better reduction of lipid peroxidation (28.69±1.26 nM/g wet weight) followed by extract based diet (31.12±1.46 nM/g wet weight) as compared to control (40.75±1.88 nM/g wet weight). However, increment in hepatic SOD and CAT was observed up to 215.07±9.69 and 939.24±42.09 U/g wet weight via red cabbage supplementation from 176.43±7.74 and 710.12±29.77 U/g wet weight in hypercholesterolemic control. The red cabbage & its extract increased SOD and CAT by 17.97 & 15.92 and 24.39 & 20.98%, whereas reduction in MDA was up to 29.60 & 23.63% in contrast to hypercholesterolemic animals. The hepatosomatic index expounded significant impact of treatments on hypercholesterolemic diet fed rabbits, the difference was from 2.86±0.15 to 3.13±0.14%. The histomorphological examination of liver tissues of rabbit indicated normal hepatic parenchyma without any histological alterations in normal groups. On the other hand, hypercholesterolemic rabbits depicted mild degree of fibrotic and necrotic changes that were restored by the incorporation of red cabbage, whereas red cabbage extract based diet showed poor modulatory response due to the presence of fibrotic and inflammatory zones.

The dietary interventions depicted momentous decrease in serum cardiac indicators including CK, CK-MB and LDH in hypercholesterolemic animals, whereas decrease in AST was observed in normal as well as hypercholesterolemic animals. The maximum reduction was observed in red cabbage fed animals; CK decreased from hypercholesterolemic control 980.15±61.73 to 914.07±52.72, CK-MB 106.04±6.19 to 95.63±6.02, LDH 547.09±16.32 to 491.64±21.96 and AST 67.12±2.96 to 58.04±2.44 U/L. In hypercholesterolemic animals, the red cabbage showed significant reduction in lipid peroxidation 16.44% that resulted in momentous increment in SOD 10.47% and CAT 10.62%. The cardiosomatic index explicated non-significant impact of treatments on both types of dietary patterns; normal & hypercholesterolemic. The histomorphological examination of normal rabbit myocardium depicted mild degree of alterations that were controlled in cabbage fed group as compared to extract supplemented group. In hypercholesterolemic animals, mild degree of necrotic changes and congestion was modulated by red cabbage however, the extract administered group explicated mild to moderate degree of congestion and hemorrhage along with pyknotic nuclei.
Amongst kidney indicators, the creatinine and urea levels (mg/dL) explicated significant variations in hypercholesterolemic diet fed animals 1.03±0.05 and 53.97±3.72 with maximum reduction in extract based group 0.88±0.04 and 49.11±3.40. The percent decrement in creatinine and urea was ranged from 12.62 (RC) to 14.45 (RCE) and 7.28 (RC) to 9.01 (RCE).

Furthermore, renal SOD and MDA levels behaved significantly in both dietary regimens, whereas CAT activity in kidney tissues was varying significantly in hypercholesterolemic diet fed group. The maximum decrement in MDA was 24.07% in red cabbage extract supplemented oxidative stressed group, resulted in increment in SOD 11.38% and CAT 16.72%.

The nephrosomatic index affected significantly in hypercholesterolemic diet fed rabbits, varied from 7.26±0.24 (extract based group) to 8.24±0.39% (control). The histological features of animals explicated mild degree of congestion in normal animal nevertheless, normal renal parenchyma was viewed in cabbage and its extract supplemented groups. The hypercholesterolemic diet fed rabbits presented severe necrotic changes in renal parenchyma along with pyknotic nuclei and mild to moderate degree of congestion however, the severity was reversed via extract based groups more effectively as compared to cabbage fed group.

Lastly, the treated diets influenced on hematological aspects; Hb, Hct, MCV, MCHC, WBC, LYM, platelets and ESR in hypercholesterolemic dietary pattern. The rabbit is an important model for the study of human atherosclerosis and lipoprotein metabolism. Besides, it gives useful information regarding non-alcoholic fatty liver disorder. However, the negative impacts noticed on heart via chronic ailments were minor, this might be due to stronger heart muscles means to pump blood throughout the body. Still, one species could not be a suitable model or representative for all the metabolic disorders.

Conclusively, the vegetables grown in Pakistan have splendid nutritional and nutraceutical worth hence their incorporation in already existing conventional edibles could replenish them, supporting the concept of designer foods. In this context, nutritious, approachable and cost-effective vegetables should be encouraged. Furthermore, quantitative analyses of the current study could be used to update the dietary guidelines. For future works, it is suggested that the resultant prototypes should be assessed for bioavailability and nutrikinetic studies. Additionally, it offers an opportunity to food manufacturers to improve the conventional edibles on the basis of sensory acceptability as well as freezer friendly nature. Domestically,
such studies would go a long way in guiding vegetables growers of the country to prefer red cabbage production over green equivalent to promote health and prevent disease incidences.
RECOMMENDATIONS

1. Vegetables like red cabbage should be encouraged in diet based therapies to optimize liver and renal health

2. Food professionals should focus on healthy dietary pattern by incorporating red cabbage to address oxidative stress mediated malfunctions

3. Information of the current study need to be disseminated at community levels to shift the consumer preference towards red cabbage

4. Cohort studies are necessitated with special reference to cabbage polyphenols bioactivity, nutrikinetics and drug interactions

5. Consumer awareness campaigns should be launched nurturing nutritive value of cabbage based foods to overcome poor dietary behaviors

6. Pakistani vegetable growers ought to be educated to enhance the cultivation of red cabbage for easy accessibility and affordability

7. Policy makers should devise some strategies to limit fast food density & proximity and shape out effective reforms to promote vegetable based designer foods

8. In Pakistan, nutrition should be introduced in school based programs as a mandatory subject to encourage healthy eating and improve quality of life

9. Mass media campaigns ought to be launched to combat the issues of malnutrition and lifestyle related disorders
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Appendix-I
SENSORY EVALUATION PERFORMA
For baked designer croquettes

Name of Judge: ……………………
Designation: …………………….…….
Gender: …………………………
Age: …………………………..

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Signature: ……………………
Date: ………………………..

Instructions:
1. Take a sample of croquette and score for sensory characteristics using 9-point hedonic scale.
2. Before proceeding to the next sample, rinse mouth with water or clean the palate via unsalted crackers
3. Make inter-comparison of the samples and record the score.
4. Don’t disturb the order of samples.

9-point hedonic scale system:
- 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
Appendix-II
SENSORY EVALUATION PERFORMA
For fried designer croquettes

Name of Judge: ……………………..
Designation: ………………………
Gender: ………………………..
Age: …………………………..

<table>
<thead>
<tr>
<th>Sensory characters</th>
<th>C₀F</th>
<th>C₁F</th>
<th>C₂F</th>
<th>C₃F</th>
<th>C₄F</th>
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</thead>
<tbody>
<tr>
<td>Color</td>
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<tr>
<td>Taste</td>
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<td>Odor</td>
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<td>Tenderness</td>
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<td>Juiciness</td>
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<tr>
<td>Overall acceptability</td>
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</tbody>
</table>

Signature: ……………………..
Date: ………………………

Instructions:
1. Take a sample of croquette and score for sensory characteristics using 9-point hedonic scale.
2. Before proceeding to the next sample, rinse mouth with water or clean the palate via unsalted crackers.
3. Make inter-comparison of the samples and record the score.
4. Don’t disturb the order of samples.

9-point hedonic scale system:
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