NUTRITION KNOWLEDGE IN ASSOCIATION WITH HEALTH STATUS OF PROSTATE CANCER (PCa) PATIENTS IN SELECTED HOSPITALS OF BATHINDA CITY

A

Thesis

Submitted to

LOVELY PROFESSIONAL UNIVERSITY

For the award of

DOCTOR OF PHILOSOPHY

in

Nutrition and Dietetics

By

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PUNJAB

2020

CANDIDATE'S DECALARATION

I hereby declare that this thesis or part

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Date:

CERTIFICATE

This is to certify that the thesis entitled **"Nutrition Knowledge in association with Health status of Prostate Cancer (PCa) Patients in selected Hospitals of Bathinda City"** submitted to the Faculty of Agriculture, Lovely Professional University, Phagwara, Punjab in partial fulfilment of the requirement for the award of degree of Doctor of Philosophy in Food Technology and Nutrition embodies the result of a piece of a bonafide research conducted by Ms. Piverjeet Kaur Dhillon under our guidance and supervision. No part of this thesis has been submitted for any other degree or diploma to any other institute. All the assistance and support received during the entire course of investigation and source of literature have been duly acknowledged by her. The research work report is suitable for Ph. D degree award submission in Nutrition and Dietetics.

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ABSTRACT

The present research was planned to investigate "Nutrition Knowledge in association with Health Status of Prostate Cancer (PCa) Patients in selected Hospitals of Bathinda City" and conducted through two different study designs i.e. i) a multistage systematic sampling was worked out on 150 males (aged >30 years) from different randomly selected households belonging to 50 wards of Bathinda City to see the prevalence of prostate cancer in Bathinda City and ii) a randomized controlled trial by employing cluster design was conducted on 200 post-operative males (aged between 40 to 90 years) with prostate cancer stage I and II from three different hospitals in Bathinda City to study the impact of nutrition counselling on knowledge, attitude, practices and health status of PCa patients. Besides, different types of value added nutraceutical products (biscuits, muffins and noodles) were developed and evaluated for their physicochemical characteristics for PCa prevention. Results showed that PCa was as prevalent as 4.67 percent among males in Bathinda City. As the demographic information was concerned, majority of the subjects i.e. 39 & 48 percent and 33 & 29 percent were in the age group of 60-70 years and 70-80 years in group I & group II, respectively. It was observed that the maximum number of subjects (65 and 53 percent in group I and group II, respectively) were from agricultural community. Regarding socioeconomic status, 40 and 82 percent; 44 and 18 percent and 16 and 00 percent were from middle income group; low income group and high income group in group I and group II, respectively. Findings on lifestyle related information of the subjects revealed that 78 and 75 percent of the subjects in group I and group II, respectively lived sedentary lifestyle. Furthermore, 51 and 35 percent of the subjects had treated source of drinking water in group I and II, whereas 49 and 65 percent were accessing drinking water from untreated sources, respectively. It was further reported that 18 & 08 percent and 10 & 06 percent of the subjects had family history of PCa & other types of cancer in group I and group II, respectively. As the dietary pattern was concerned, vegetarianism & non-vegetarianism eating pattern was observed in 49 & 43 percent and 43 & 51 percent of the respondents in group I and group II, respectively. Data regarding consumption of fats showed that most of the patients i.e. 78 & 62 percent and 61 & 67 percent in group I & group II consumed *dalda* and *desi ghee* whereas very few subjects (01 and 09 percent) consumed combination of fats and oils in group I and II, respectively. Average daily nutrient intake of energy, protein, β-carotene, vitamin C, iron and zinc was found inadequate while intake of fat and B-complex vitamins was more than adequate among the subjects in both the groups during beginning and end of the study when compared to the RDA (ICMR 2010). A significant ($p<0.05$) increase in calculated BMI of the subjects in group II was found at the end of the study. The mean serum PSA was 15.59 & 12.23 ng/ mL and 20.05 & 14.47 ng/ mL during beginning & end of the subjects in group I and group II, respectively and found 2 to 4 times higher than the reference range of 0 to 4 ng/ mL. The mean hemoglobin level was below the minimum reference value of 12 g/ dl in the subjects of group I whereas normal hemoglobin level was observed in the subjects of group II after imparting nutrition counselling. The mean serum zinc (57.9 and 57.12 µmol/ l in group I and II) was found lower than the reference range of 70 to 100 µmol/ l at the beginning of the study while it was observed as normal (76.30 µmol/ l) among group II patients after imparting nutrition counselling. The mean post intervention test scores for knowledge, attitude and practices of the respondents in group II were significantly ($p<0.05$) improved after nutrition intervention. The chemical analysis of raw ingredients indicated the highest crude protein content was found in defatted soya flour as 59.21 percent. The mean content of crude fat ranged from 0.75 to 7.73 percent. The mean fiber was recorded in tangerine peel powder as 11.14 percent followed by ginger (10.47 percent) and defatted soya flour (4.28 percent). The mean ash content was observed as 7.38, 4.48 and 3.75 percent in defatted soya flour, tangerine peel powder and ginger, respectively. *In vitro* protein digestibility of raw ingredients ranged from 18.58 to 67.28 percent with the highest value in defatted soya flour. Vitamin C content was 3.1, 26.1 and 28.7 mg in ginger, tomato and tangerine peel powder, respectively while this vitamin was absent in cereal and legume flours. The mean calcium and phosphorus content was ranged from 12.14 to 201.4 and 17.1 to 799.2 mg, respectively. Further, the mean iron content was 7.87, 6.7 and 4.15 mg in 100 g of tangerine peel powder, defatted soya flour and wheat bran, respectively. The mean zinc content in tangerine peel powder, defatted soya flour and wheat bran was 6.96, 2.1 and 1.85 mg, respectively. Data on anti nutritional factors depicted that the mean total phenol content was ranged from 10.8 to 1203.2 mg GAE/ 100 g and the highest content was observed in wheat bran. Findings revealed that there was a significantly ($p<0.05$) higher total antioxidant activity in tomato, tangerine peel powder and ginger when compared to other ingredients. Three variations of the biscuits (T_1, T_2 and T_3)

were standardized against T_0 control (100 % wheat flour) and on the basis of the most acceptable ratio of wheat flour (WF) and defatted soya flour (DSF) (60:40) i. e. T_3, two treatments of value added biscuits (T_4 and T_5) incorporated with blend of nutraceutical ingredients such as wheat bran, tomato, ginger, tangerine peel powder, turmeric and black pepper) were developed. The prepared products were standardized as T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper and T_5= 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper. Results of physical characteristics of control and value added biscuits reported that T_3 (8.8 g) followed by T_0 (8.7 g) had the highest whereas T_1 had the lowest (8.3 g) mean weight. The mean spread ratio was 8.77, 7.89, 8.47 and 8.51 in $T_{0,}$ T_1, T_2 and T_3, respectively. The mean weight of T_4 and T_5 was 10.5 and 10.9 g, respectively. The mean spread ratio was 7.37 and 6.66 in T_4 and T_5, respectively. Proximate composition of control and value added biscuits showed that crude protein, fat and ash content in T_0 was significantly ($p<0.05$) lower than other three variations (T_1, T_2 and T_3) whereas moisture and carbohydrate content was higher in T_0 as compared to other three samples. Further, the crude protein content in T_4 and T_5 was significantly ($p<0.05$) higher than control biscuits. The mean crude fat content of T_4 and T_5 was 22.49 and 22.31 percent. A significantly ($p<0.05$) higher fiber content was reported in T_5 as compared to T_4. The mean ash content was 5.39 and 5.42 percent in T_4 and T_5, respectively. It was observed that T_3 and T_5 had the highest (76.54 and 73.87 percent) *in vitro* protein digestibility. Further, vitamin C was not present in control treatments whereas T_4 and T_5 had 4.7 and 5.6 mg of vitamin C, respectively, due to presence of tomato and tangerine peel powder. Data regarding calcium content revealed that $T_{0,}$ T_1, T_2, T_3, T_4 and T_5 had 56.2, 474.7, 208.7, 174.8, 258.4 and 281.6 mg calcium, respectively. The higher mean iron content was in T_4 and T_5 when compared to control samples. The mean zinc content was 0.4, 1.68, 1.52, 1.24, 2.76 and 2.69 mg in $T_{0,}$ T_1, T_2, T_3, T_4 and T_5 samples. Higher content of minerals in value added biscuits was due to incorporation of wheat bran. Total phenol content was 105.7, 217.4, 152.8 and 124.5 mg GAE/ 100 g in T_0, T_1, T_2 and T_3 samples while it was 1541 and 1544 mg GAE/ 100 g in T_4 and T_5, because of addition of herbal nutraceuticals i.e. ginger and turmeric with better stability in products prepared at high baking temperature due to formation of maillard reaction products. A significant ($p<0.05$) difference was observed in total antioxidant activity of control and value added biscuit samples. Additionally, T_4 and T_5 had higher total antioxidant activity as compared to control samples due to addition of tomato, tangerine peel powder, ginger and turmeric in value added treatments and production of maillard reaction products during baking. Sensory characteristics depicted that among control treatments, T_3 was the most acceptable with 92.77 percent index of acceptability. For value added treatments, T_4 had higher index of acceptability i.e. 91.44 percent as compared to T_5 with index of acceptability of 78.22 percent due to its dark brown color produced by degradation of carotenoid pigment and maillard reaction during drying of tangerine peel and bitter after taste. Three types of muffins (T_1 (Control) = 100 % Wheat Flour, T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper and T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper) were developed. Proximate composition of the muffins reported the highest (16.2 percent) mean crude protein content in T_3. The crude fat content ranged from 20.75 to 23.55 percent. Presence of crude fiber content was reported in T_2 (13.5 percent) and T_3 (9.5 percent) due to value addition of wheat bran, tomato pulp, tangerine peel powder and ginger powder in experimental treatments. The total ash content of muffins ranged from 1.07 to 2.27 percent. *In vitro* protein digestibility of T_1, T_2 and T_3 was 69.6, 71.5, and 72.9 percent, respectively. Vitamin C content was reported as 9.6 and 7.8 mg in T_2 and T_3, respectively, whereas T_1 had no content of vitamin C. Calcium content of muffins ranged from 174.7 to 209.5 mg. T_2 has the highest content while the lowest amount was observed in T_1. Further, iron and zinc content of T_1, T_2 and T_3 were significantly ($p<0.05$) different. Data regarding anti nutritional factors revealed a lower content of total phenols in T_1 than T_2 and T_3, respectively. Results regarding total antioxidant activity of muffins showed a significant ($p<0.05$) difference between all the three samples. It was further reported that T_2 and T_3 had comparatively higher free radical scavenging capacity as compared to T_1. The findings of sensory parameters revealed that the percentages for Index of Acceptability were 82.44, 68.44 and 87.78 in T_1, T_2 and T_3, respectively. Four different ratios i.e. T_1, T_2 T_3 and T_4 (DSF: WF 00:100, 60: 40, 50: 50, 40: 60) of noodles using defatted soya flour and wheat flour were developed and evaluated. Results of proximate composition of noodles depicted that the crude protein content was 8.20, 32.05 29.15 and 27.65 percent in T_1, T_2, T_3 and T_4, respectively. Moreover, the mean crude fat was again maximum (1.01 percent) in T_2 while minimum (0.89 percent) in T_4. A significant ($p<0.05$) difference was observed in fiber content of all the noodle samples. T_2 was reported with higher (2.46 percent) fiber content as compared to $T_{3,}$ T_4 and T_1 (2.32, 2.03 and 1.65 percent), respectively. Higher percentage (4.22 and 3.91) of ash content was observed in T_2 and T_3, respectively. *In vitro* protein digestibility of T_1 and T_4 was 69.6 and 69.7 percent, respectively. It was observed as 78 and 76.6 percent in T_2 and T_3, respectively. The calcium content of noodles ranged from 90.6 to 124.3 mg with higher content in T_2 followed by T_3. Further, T_2 and T_3 had 4.69 and 4.50 mg iron. The zinc content ranged from 1.09 to 1.81 mg and higher amount of zinc was reported in T_2 while the lesser value was observed in T_1. Regarding anti nutritional factors, T_2 and T_3 had 234.5 and 100.8 mg GAE/ 100 g of total phenols, respectively. A significant ($p<0.05$) difference was observed in total antioxidant activity of control and value added samples. T_2 was reported with the highest value of total antioxidant activity due to higher proportion of defatted soya flour. Data regarding sensory characteristics of noodles showed the higher (69.58 and 69.48 percent) Index of Acceptability (I. A.) in T_4 and T_1, respectively. Three different noodle flavors i.e. T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %), T_2 = Tomato (60 %) + Ginger (20 %), T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %), Turmeric (7.5 %) + Salt (10 %) + Pepper (2.5 %) in each variation were developed. Fot proximate composition of three noodle flavors, T_1 presented the higher protein content as compared to T_2 and T_3, respectively. Further, the crude fat content ranged between 0.6 to 2.2 percent with highest content in T_1. A significantly ($p<0.05$) higher content of crude fiber was reported in T_1 than two other treatments, respectively. The mean ash content was 1.8, 1.0 and 1.0 percent in $T_{1,}$ T_2 and T_3, respectively. It was showed that $T_{1,}$ T_2 and T_3 had 82.74, 72.04 and 70.22 percent *in vitro* protein digestibility, respectively. Vitamin C was 1.0, 0.79 and 0.92 mg in T_1, T_2 and T_3, respectively. Results regarding mineral content of noodle flavors revealed that higher content of calcium and phosphorus was observed in T_1 and T_3 than T_2. The mean iron & zinc content was 0.06 & 0.03, 0.04 & 0.00 and 0.02 & 0.12 mg in T_1, T_2 and T_3, respectively. Data on anti nutritional content of noodle flavors depicted that the total phenol content was ranged from 6.72 to 7.04 mg GAE/ 100 g with highest value in T_1. The data showed that T_1 had significantly ($p<0.05$) higher total antioxidant activity as compared to other samples.

As the sensory characteristics were concerned, it was reported that T_2 was the most acceptable with an Index of Acceptability (I. A.) score of 65.88 percent. Thus, it can be concluded that nutrition education through individual dietary counselling and printed literature along with promotion of nutraceutical enriched food products should be imparted to the prostate cancer patients in order to maintain their nutritional and health status.

Keywords: Prostate Cancer, Recommended Dietary Allowances, Biochemical assessment, Knowledge Attitude and Practices, Physico-chemical evaluation, Sensory evaluation

ACKNOWLEDGEMENT

First, I would like to express my sincere gratitude to my Major Advisor, Dr. Beenu Tanwar and Co-Advisor Dr. Rasane Prasad Jayprakash, Assistant Professor, Food Technology for their enthusiastic support and knowledgeable guidance throughout my Ph. D study and research work. Without their mentorship with full of encouragement, I could not have imagined this research project successful as they helped me at every step of the study and writing of thesis.

Besides, I am thankful to Dr. Ramesh Kumar Sadawarti, Dean, School of Agriculture, and the expert panel of research review committee for their insightful comments and discussion regarding the betterment of this research study which directed me working on diverse aspects of this research project to attain more scientific conclusions.

I am also grateful to the entire faculty, dieticians and doctors, lab technicians and other staff at Lovely Professional University, Punjab Agricultural University and three hospitals of Bathinda City for their timely support by providing me required amenities available at these campuses throughout the whole research work.

Finally, my sincere thanks goes to God, my family members, the subjects of this research study and friends as without their continuous and timely motivation this task could not been accomplished.

Thanks to everybody who supported me during my research work.

Place: LPU, Phagwara

Date: 28.07.2020 (Piverjeet Kaur Dhillon)

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LIST OF ABBREVIATIONS USED

Kcal	: Kilocalorie
G	: gram
Mg	: milligram
Mg	: microgram
Ng	: nanogram
Cm	: centimetre
Kg	: kilogram
m^2	: meter square
ML	: millilitre
Dl	: decilitre
L	: liter
Mmol	: micromole
N	: Normal
N	: Newton
N content	: Nitrogen content
°C	: degree Celsius
Rpm	: revolution per minute
Hr	: hour
e.g.	: For example
i.e.	: that is
viz.	: videlicet (namely)
et al.	: and others

1. INTRODUCTION

Cancer, a chronic immune and metabolic disorder is becoming an increasingly important factor in the "global burden of disease" and "dominating challenge" being faced by the medical world both in developed and developing countries (Kowsalya et al. 2008). It accounts for about 23 and 7 percent deaths in USA and India, respectively and is responsible for maximum deaths after cardiovascular disease in the world (Jemal et al. 2007). Cancer induction, growth and progression are multistep events (Mukhopadyay 2010), leading to substantial damage to feasible and successful life, therefore, social and economic effects of cancer are immense in terms of both direct (medication, hospitalization, travel etc.) and indirect costs (loss of income, premature death etc.) (Anand et al. 2008).

Cancer is prevalent throughout India at the average rate of 800 cases/ million/ year. In Punjab, cancer becomes a burden in the age group of 35-65 years, and has over 90 cases per one lakh population. Maximum number of cancer cases were reported in southern districts of Punjab namely Bathinda (3099), Mansa (1258), Muktsar (1201), and Faridkot (1167) in a report issued by Indian Council of Medical Research, 2012-13. The Malwa region, comprising of Ludhiana, Rupnagar, Patiala, Sangrur, Bathinda, Mansa, Firozpur, Fazilka, Rajpura, Moga and Ajitgarh, also known as cancer belt, has the highest average cases of 136 patients per one lakh population (The Department of Health, Punjab 2010), and the prevalence was recorded as 1089 case/ million/ year when compared with Doaba (881 cases/ million/ year) and Majha (647 cases/ million/ year) regions (Department of Health and Family Welfare 2013). After the year 2011, the incidence of cancer has increased 2.8 times (from 485 in 2011 to 1367 cases in 2012) in Punjab and by the year 2014-15, the trend has remained continuous (Socio-Economic statistical information about India 2015). There are many attributes such as population growth, industrialization, lifestyle changes, urbanization and increased lifespan, primarily responsible for the cancer. Several other factors are related to environment such as the use of chemicals, heavy metals, insecticides and pesticides. As evident from the data, on an average, 18 people die of cancer every day during the last five years, and about 90 percent cancer is due to the environmental contaminants (The Department of Health, Punjab 2010).

The development of Prostate Cancer (PCa) is connected to andropause, when the estrogens to androgens circulating ratio may perhaps rise till 40 percent. The rise in estrogens are well-known to overwhelm the production of testosterone and compete for the androgen receptor with androgens. It was assumed that the rise in the concentration of estrogen can cause direct mutagenic effects and unprepared proliferation, to a certain extent, it is caused by the endogenous estrogens (E1-estrone and E2-estradiol) metabolism, by enzyme CYP - cytochrome P450, with successive formation of electrophilic intermediates and extra powerful estrogens (Hamilton-Reeves et al. 2007). In this cancer, cell usually loses the cell-cell recognition leading to uncontrolled cell growth and proliferation of malignant cells in lieu of the normal, healthy cells.

Globally, Prostate Cancer is the second prevalent type of cancer among men with large variations in occurrence and different mortality rate in different regions (Landberg et al. 2010) and the disease is presently ranked at sixth position of all the common cancers among the Asian population by the year 2012 (International Agency for Research on Cancer 2012). The rates for PCa incidence among East, South-Central Asian population were reported as 10.5 and 4.5 percent, respectively. Prostate Cancer is the common instinctive malignancy prevalent in men, with an average increase of 39 cases per year in Punjab (Socio-Economic statistical information about India 2015). Even though, its tumors are affected by hereditary factors to a large extent than numerous other type of tumors but at present known risk factors such as levels of androgen, age, dietary habits and race have been emerged (Tang et al. 2005). Studies have also been undertaken to study the risk assessment of dietary contamination through pesticide residues in the "Cotton belt of Punjab" (Battu et al. 2005) but the area wise etiology of prostate cancer and its correlation with environmental pollutants is not clear yet.

Vasanthamani and Banitha 2012 enlisted contaminated water supply, poor sanitation and hygiene, ignorance of good food habits and lack of consumption of fruits and vegetables as the causative agents of cancer. Among various contributory factors, diet and lifestyle have an edge over other factors (Kowsalya et al. 2008) and as reported by Saxena 2015, almost 35 percent of all types of cancers are interrelated with diet. Lowered consumption of protective foodstuffs such as vegetables and fruits in addition to increased per capita consumption of animal fat has played a significant role, in increasing the incidence of prostate cancer in developing countries like India, China and Japan (Zhang et al. 2012). There is simply no doubt to say that good nutritious diet plays an essential role to fight with the cancer and showed positive interrelation to each other.

Dietary pattern is possibly to affect both the frequency and advancement of prostate cancer. Nonetheless, the interactions to specific foodstuff and the bioavailability of its nutrition are not simple and are most likely improved by hereditary factors (Swami et al. 2007). Cancer cachexia becomes a reason for approximately 20 to 40 percent of related deaths is characterized by anorexia (loss of appetite), quick satiety, poor absorption of nutrients, muscle weakness, low immune status and increased risk of infections which ultimately leads to death. The diagnosis of cancer and its harsh treatment becomes a difficult task for patient to cope up. It may leave a patient exhausted physically and emotionally. As the disease assaults the body, it not only disrupts the regular metabolic and physiological processes but also adversely affects the normal metabolism and increase the body's energy requirements, resultant loss of lean body mass, because of augmented tissue protein and fat breakdown. It ultimately leads to negative nitrogen balance, loss in body weight, wasting and weakness in muscles and increased insulin resistance. Thus, the consideration of important factors related to diet and nutrition has been proven as modifying issues in the management of prostate cancer (Saxena 2015). No specific planned diet for all types of cancer patients is available and the nutritional goals are highly individualized and variable. Thus, dietary counseling and nutritional support early in the treatment can prevent the much-dreaded complication "cachexia" (Hemalatha and Prakash 2002).

Apart from the healthcare amenities and adequate nutrition, the need for additional health determinants in terms of the social and physical environment has also been recognized (Public Health Agency of Canada 2006; Ratzan 2001). Nutrition knowledge can be affected by various factors such as literacy, employment, socioeconomic status and so on. Poor nutrition knowledge can be associated with inadequate intake of functional foods, results in poor nutritional as well as the health status of the patient (Friedman and Hoffman-Goetz 2008). Nutritional and health status of an individual might be assessed through recording and analyzing the anthropometric and biochemical parameters viz. height, weight, body mass index and hemoglobin, serum minerals, antioxidant levels etc. and along with that, the degree of the disease can be determined by estimating serum prostate-specific antigen (PSA). Nutrition literacy is considered a most significant predictor of diet quality (Gibbs et al. 2018). To ensure good health status of the patient, it is pragmatic to upgrade nutrition knowledge, promote a positive attitude and inculcate good health practices among the patient and his next of kin's as well. For that reason, nutrition education should be imparted and strengthened via promoting awareness regarding the disease, its causative factors, and preventive measures, through different educational aids such as compact disc, booklets, pamphlets etc. (Bisht and Raghuvanshi 2007). With increased awareness about nutrition among consumers, the food related industries has also been laying emphasis on production of efficient functional foods exercising various type of nutraceuticals.

Nutraceuticals are helpful in enhancing food value with both special physical and psychological conditions (Ashwini et al. 2013). As being natural, these compounds have lesser side-effects as compared to traditional and modern medications. Nutraceuticals can be classified as nutrients, herbs or botanical extracts and dietary supplements (Sapkale Anita et al. 2012). The bioactive compounds, in the form of phytochemicals such as genistein present in soy, lycopene in tomatoes, curcumin in turmeric, gingerol in ginger etc. have been explored in relation to their disease targeting potential after numerous scientific investigations (Rai et al. 2012). Simultaneously, epidemiological studies revealed that wheat, soy, citrus peel and tomato based products had anti-prostate cancer activity (Zuniga et al. 2013). Disease-preventive efficacy of seven different compounds (epigallocatechin 3-gallate (EGCG), genistein, curcumin, apigenin, quercetin, baicalein and resveratrol) have been assessed and found four (genistein, curcumin, EGCG and resveratrol) of the above-said compounds as antagonists to Hedgehog signaling transduction pathway which hyper-activates during prostate cancer progression. Furthermore, during *in vivo* (transgenic adenocarcinoma of mouse prostate - TRAMP) and *in vitro* (prostate cancer cell lines) trials, 58 percent reduction and 81 percent prolongation in prostate cancer were recorded, on the mixed feeding of all the seven compounds (Slusarz et al. 2010).

Nutraceuticals possess bioactive components which potentially promote health and well-being by reducing the progression of diseases (Aryee 2015). An ample number of clinical trials affirmed an inverse relationship between dietary consumption of nutraceuticals and delayed growth of malignant prostatic cells. Lycopene present in tomatoes has proven chemo-preventive ability in the inhibition of malignant cells during androgen-independent prostate cancer (Tang et al. 2005). Total five plasma carotenoids including α and β-carotene, β-cryptoxanthin, lutein and lycopene vis-à-vis risk of prostate cancer in men had been assessed during a nested case-control study and observed that odd ratio to prostate cancer was significantly ($p<0.05$) reduced with the increased level of lycopene supplementation in prostate cancer patients. It has been emphasized that increased consumption of tomatoes and its products might contribute towards the decline in both the occurrence and the progression of prostate cancer (Gann et al. 1999). Further, its role in inhibition of proliferating cancer cells was explained through several mechanisms such as protection of cellular biomolecules i.e. lipoproteins and DNA (Rao and Agarwal 1998) and it has antioxidant properties identical to statins (Fuhrman et al. 1997). Moreover, it also decreases the lipid oxidation and inhibits malignant cell proliferation at the G0-G1 cell cycle transition (Wei and Giovannucci 2012). Lycopene interferes with growth factor receptor signaling, up-regulates the gene (connexin 43) expression and permits direct intercellular gap junctional communication which is responsible for the decreased proliferation of malignant tumors (Heber and Lu 2002). During clinical trials, administration of tomato sauce providing 30 mg lycopene/ day for 21 days was carried out to study its impact on Prostate-specific antigen. Increased serum lycopene whereas decreased PSA levels were reported in the experimental subjects. On the contrary, control subjects were assessed with vice-versa trend (van Breemen et al. 2011; Kucuk et al. 2002). Simultaneously, the form of lycopene while ingestion is also important as the pigment is highly bioavailable when in cis form after its processing which induces isomerization and converts trans form of lycopene to absorbable cis form (Rao and Agarwal 2000). In addition, Etminan et al. 2004 meta-analyzed 11 case-control and 10 cohort studies determining Log relative risks (RRs). While comparing the non-frequent consumers of tomato products with raw tomato consumers and cooked tomato products consumers, the researchers scrutinized that the values for RR were significantly lower in regular consumers of cooked tomato products.

Further, both *in vitro* and *in vivo* experiments were reviewed to check out the viability of functional compounds such as soy based isoflavones for prostate cancer prevention and treatment and scrutinized that the bioactive compound had shown a great efficacy against malignant cells during cell cycle regulation and induction of apoptosis (Perabo et al. 2008). Similarly, results obtained from animal trials were found satisfactory in regard to the potential chemopreventive action exhibited by genistein present in soy products. Genistein inhibits the activity of steroid metabolizing enzyme 5α-reductase in human genital skin fibroblast and prostatic tissues (Ibitwar et al. 2006). Isoflavones bind to estrogen receptors and positively effect estrogen-regulated gene products. Besides, soya bean is the richest dietary source of tocopherols (Vitamin E), B-group vitamins and protein of high nutritional and therapeutic value (Venkatachalam et al. 2004), occupies a unique status among pulses and oilseeds. This wonder bean is becoming popular in India (Kaveri and Uma Bindu 2004) as the country has a prime position in pulse production in the global scenario, and has turned out to be one of the largest exporters of soy meal (Chauhan et al. 2012). Also, its availability throughout the year and high water absorption capacity (WAC) could be useful, to be transformed into a number of traditional local recipes (Sood et al. 2012; Chauhan et al. 2012).

Likewise, whole grains, for instance, wheat and rye are not only concentrated sources of dietary fiber but also a significant source of phytoestrogens with hormonal effects that may help to protect against prostate cancer (Landberg et al. 2010). Clinical studies suggest that higher amount of alkylresorcinols (AR) and phenolic lipids found entirely in the outer surface of grains viz. rye and wheat and also show positive and valuable effects on the progression of prostate cancer via reducing the concentrations of prostate-specific antigen (PSA) (Landberg et al. 2009). Besides, peels of citrus fruits possess methylated flavones which have an efficacy to prevent as well as treat the hormone-dependent cancers (Mukhopadyay 2010). Additionally, curcumin has been found helpful in the treatment of prostate cancer when administered to the subjects during several clinical studies as AR expression and its binding ability to androgen response were down-regulated with end outcome of lowered prostate-specific antigen level (Dorai et al. 2000; Nakamura et al. 2002; Tsui et al. 2008). It possesses a compound called as diferuloylmethane extracted from rhizomes of the plant which down-regulates the androgen receptors and epidermal growth receptors, therefore, inhibits the metastasis development during prostate cancer (Teiten et al. 2010). Further, inhibition of proliferation and angiogenesis via inhibiting molecular pathways such as tyrosine kinase and protein kinase- C and improvement in anticarcinogenic activity were evidenced during dietary intervention through feeding the nude mice on diet consisted of 2 percent curcumin for six weeks (Gopalakrishna and Gundimeda 2002). Thus, in epidemiological studies, whole wheat, soy and tomato based products revealed anti-prostate cancer effect (Zuniga et al. 2013). Hence, nutraceuticals from dietary components may be proposed for designing prostate cancer preventive food products (Slusarz et al. 2010).

Additionally, carcinoma prostate is considering perfect for chemoprevention therapy due to its elongated latency time (Ansari 2002). Interference of dietary ingredients such as replacement of refined wheat flour to whole wheat flour and defatted soy is valuable to slowdown the progress of prostate cancer and also for minimizing the side effects of cancer treatments such as chemotherapy. In addition to this, it might be executed for common population due to its less economic burden (Mukhopadyay 2010). Several new trends have been emerging to the food industry in the contemporary era, with major emphasis on nutraceutical enriched food production, since a constant increase in awareness level of the consumers was found in relation with nutrition security in the course of normal health condition and diseased circumstances. The most commonly consumed food products are biscuits and breads the world over, especially in developing countries like India and have become inevitable parts for interims between main meals and make available the key nutrients i.e. proteins, vitamins and carbohydrates. Among these two, relying on biscuits, particularly, is attributed to its extended shelf life, presenting it as the most fittest alternative for nutritional upgrading via fortification. Furthermore, biscuits occupy a noticeable position among all bakery products that accounts 30 percent, with 28 percent as highest record of consumption in Northern India followed by Western (25 %), Eastern (24 %) and Southern (23 %) regions of the country, respectively (Ahmad and Ahmed 2014). The incorporation of combination of whole grains and other bioactive compounds make sure the improved nutritional profile of final product as these are concentrated and significant sources of dietary fiber and phytoestrogens that have proven mechanism against prostate malignancy (Landberg et al. 2010). Though, there is robust evidences of improvement regarding nutrient profile, on inclusion of defatted soy flour to whole wheat flour while developing cookies/ biscuits (Farzana and Mohajan 2015; Olapade and Adeyemo 2014; Zaker et al. 2012; Youssef and Mousa 2012) but products available in the market are consisting fewer amounts of bioactive components (only one or two), with no multiple nutraceuticals blending approaches.

Simultaneously, the muffin is a type of baked bread, without icing and less sweet than a cupcake. It can be served both during breakfast and mid-evening meal (Limbachiya and Amin 2015). Few efforts have been made by some scientists via incorporating various foods viz. bengal gram flour, carrot powder, date, soybean, wheat bran etc. to wheat flour so as to improve protein, vitamin and fiber contents of final products (Romjaun and Prakash 2013; Limbachiya and Amin 2015; Yaseen et al. 2012). With changing food habits in both rural and urban setups, noodles are consumed among masses, belong to all type of socioeconomic strata's as being a cheaper source of energy as well as its instant cooking quality (Taneya et al. 2014). This food product is also suitable to enhance its nutritive potential in form of vitamins and minerals. Taking all these evidence into consideration, regularly consumable snacks i. e. biscuits, muffins, and noodles can be modified, through working out a mixture of bioactive compounds, present in numerous locally available foods, possessing the potency to delay the progression or prevention of PCa. Moreover, this approach may add variations to patients' diet which is the most desirable facet of therapeutic nutrition.

As a whole, PCa is a multi-factorial disease, requiring a multidimensional approach, for its prevention and treatment. Hence, it was thought of interest to find out the prevalence and causative factors of PCa, to impart nutritional counselling to PCa patients and to develop different types of nutraceutical products incorporating a varied array of bioactive phytochemicals, each separately at modest concentrations, can be more efficacious for prevention of prostate cancer rather than using specific individual constituent.

Objectives/ Scope of the study:

Numerous literatures reports indicated a positive impact of nutrition counseling in management of different degenerative diseases and development of antioxidant mix for certain types of cancer. Research evidences on role of nutrition counselling in altering Prostate Specific Antigen (PSA) level and Bone Mineral Density (BMD) in prostate cancer patients is not appropriately available. Hence, the present study is planned with the following objectives:

1. To study the prevalence and causative factors of prostate cancer (PCa) by determining the nutritional knowledge and health status of PCa patients.
2. To impart nutrition counselling to selected subjects by administering PCa specific extension aids and study its impact on knowledge, attitude, practices and health status of PCa patients.
3. To develop, evaluate and compare the physico-chemical properties of different types of nutraceutical products for PCa prevention.

2. REVIEW OF LITERATURE

An effort has been made to review researches available to provide a glimpse of work done in the area of prostate cancer, in regard to patients' nutritional status, anthropometric measurements, morbidity status and nutrition knowledge in association with their health status and the development of value added nutraceutical products for disease prevention.

The available researches are reviewed under following sub-heads:

2.1 Prevalence and Causes of Cancer

- 2.1.1 World Prostate Cancer rates and its Causes
- 2.1.2 Prostate Cancer in India: Prevalence and causative factors
- 2.1.3 Prevalence and Causes of Cancer and PCa in Punjab

2.2 Food consumption pattern and nutrient intake of cancer patients in India

2.3 Effectiveness of Nutrition education through audio-visual aids in curing the diseases

2.4 Prostate Cancer and Chemoprevention

2.5 Development of value added nutraceutical products

2.1 Prevalence and Causes of Cancer

2.1.1 World Prostate Cancer rates and Causes

According to International Agency for Research on Cancer Data (2012), prostate cancer ranks as second most common cancer with prevalence of 1.1 million the world over after lung cancer (1.8 million) in men. Furthermore, Torre et al. 2015 reported that prostate cancer is the most frequently diagnosed cancer in male worldwide. The incidence rates vary worldwide as Australia, New Zealand (111.6 cases/ 1,00,000 population), North America (97.2 cases/ 1,00,000 population), Northern and Western Europe (94.9 cases/ 1,00,000 population) and some Caribbean nations (79.8 cases/ 1,00,000 population), and lowest (11.2 cases/ 1,00,000 population) in South-Eastern Asia.

Tang et al. 2005 reported prostate cancer as second most common cancer after lung cancer, in the United States alone prostate cancer accounts for 33 percent all cancer incidences in male and 10 percent of mortality caused by all types of male cancer. The American Cancer Society (2004) estimated 2,30,110 and 29,500 fresh cases and deaths due to prostate cancer, respectively, in 2004. Risk factors for prostate cancer were reported to be age, androgen levels, dietary habits and race. Also, The American Cancer Society (2015) has considered prostate cancer as elderly men disease. It was further affirmed that about 6 out of every 10 cases are diagnosed at the age of 65 years or later and rarely happens before 40 years. Similar results were reported in a study done by Li et al. 2012, wherein they investigated the prostate cancer incidence trends in United States through National Program of Cancer Registries and Surveillance, Epidemiology and End Results Program data (2001 to 2007). They found that overall prostate cancer incidence rate was stable, it increased noticeably among males aged between 40-49 years and decreased in the age group of 70 to 79 or older. Berkow et al. 2007 observed in their findings that the rate of prostate cancer was 111.2/ 1,00,000 men in Atlanta, Ga, USA. Also, the authors established a significant negative association between dietary fat intake and prostate cancer risk. On the contrary, consumption of tomato, soy products and cruciferous vegetables were reported to have inverse relationship with prostate cancer risk.

Adeloye et al. 2016 abstracted from the meta-analysis of numerous studies available (inclusive of registry-based African hospital population and exclusive of non-human studies) that on the whole, incidence of prostate cancer in Africa was 21.95 cases/ 1,00,000 population. As far as the age group was concerned, the utmost rate was pragmatic among the people aged 70 years and above. Dickinson et al. 2016 observed the trends in incidence and mortality in Canadian males due to prostate cancer and found that prostate cancer incidence increased piercingly at the rate of 12.8 percent/ year even after the PSA screening was introduced in early 1990s. During 1993-96, it decreased in all age groups while the incidence remained somewhat similar in the people aged up to 70 years. Further, age-oriented mortality was found constant from 1969 to 1977. It increased as 1.4 percent/ year to crest in 1995 and later declined to 3.3 percent/ year in 1987 in masses aged less than 60 years.

Bray and Kiemeney 2017 affirmed prostate cancer as a substantial public health problem around the world. It is considered as third most common cause of demise in males in Europe with about 4,00,000 cases and above 92,000 deaths. Earlier, its occurrence rate was higher among the population residing in higher-income countries of Northern, Southern and Western Europe. However, now incidence rate is on the rise ranging from 3 to 10 percent/ annum crosswise all European regions. Yu et al. 2015 composed the figures from New South Wales Central Cancer Registry so as to congregate the data regarding incidence of prostate cancer among Australian men. In New South Wales, it was anticipated (on the basis of data collected from 1996 to 2007) that there will be a climb of 14 percent from 2007 to 2017 in prostate cancer cases.

Baade et al. 2013 reviewed the data on incidence and mortality in the Asia-Pacific region and Country-wise statistics were cited from World Health Organization Information system Mortality Database. Around 14 percent of all incidences diagnosed globally were within Asia-Pacific region; three out of every four of these occurred in Australia (15 percent), China (28 percent) or Japan (32 percent). Moreover, about 42,000 deaths caused by prostate cancer were recorded in that region. Nagata et al. 2007 also revealed that age-specific occurrence rate of prostate cancer was lesser in Japan i.e. 9.2/ 1,00,000 than other countries (50.7/ 1,00,000 in Germany and 44.4/ 1,00,000 in England) reason being the higher dietary intake of soy and fish in Japanese diet.

Bashir and Malik 2015 abstracted in their review that diets loaded with fatty food items could raise the risk of onset of prostate cancer. The authors stated that the consumption of whole milk and red meat was positively related to progression of prostate cancer. Ragin et al. 2013 conducted a meta-analysis to study the effect of pesticide use on development of prostate cancer and found that the farmers involved in spraying pesticides had almost four times increased risk to develop prostate cancer when compared to the controls.

Wong et al. 2016 collected data from the GLOBOCAN database and correlations were evaluated between incidence and socioeconomic indicators in terms of Human Development Index (HDI) and Gross Domestic Product (GDP). The findings reported that PCa incidence rates varied as high as 25 folds throughout the world with maximum rates noticed in the USA and European nations while mortality rates were highest in Africa. Thus, it was concluded that nations with higher HDI and GDP reported higher incidence rates of PCa.

2.1.2 Prostate Cancer in India: Prevalence and causative factors

Ali et al. 2011 reported that cancer, the second most common disease in India is responsible for maximum mortality with about 0.3 million deaths per year. The total cancer cases reported in the year 2004 and 2010 were 8,19,354 and 9,79,786, respectively. Ansari 2002 reviewed the rate of types of cancer in India and found that cancer of lung and oropharyngeal malignancies followed by prostate carcinoma were the predominant cause of oncological mortality. Jain et al. 2014 obtained data from national cancer registries and found that prostate cancer is the second leading cause of all male cancers and documented a statistically significant rising trend in incidence rates in four metropolitan cities; Bangalore, Chennai, Delhi and Mumbai with annual percentage change of 3.4, 4.2, 3.3 and 0.9, respectively. Chen et al. 2014 reported the figures for PCa mortality and incidence ratio as >60 percent in India which represents the high magnitude of this deadly disease in the country. In another study, it has been revealed that in India, the estimated cancer and prostate cancer cases has increased from 28,19,457 to 30,16,628 and 31,311 to 37,055 and deaths caused by cancer and prostate cancer has been increased from 4,52,541 to 4,91,597 and 13,146 to 15,562 in year 2011 to 2014, respectively (Socio-Economic statistical information about India 2015). Furthermore, Aulakh 2013 stated that prostate cancer incidence is increasing in India at the rate of one percent per year.

Yeole 2008 reported that trends in five (Mumbai, Chennai, Bangalore, Delhi, and Bhopal) population-based cancer registries in India showed the significantly increased incidence of prostate cancer (ranked 8th in 1988 and 4th in 2003 registry) as compared to all cancers. Ramadas and Somepalli 2002 carried out a survey on nutritional profile of selected patients in Hyderabad and observed that the highest incidence (62 percent) of cancer was among the subjects in the age range of 41 to 60 years of which 12 percent were affected by lung cancer, 10 percent by oral cancer (both males and females) and 62.5 percent males by prostate cancer. Swaminathan et al. 2011 observed during their study that prostate cancer was the ninth most common cancer among males in Tamil Nadu as the average annual age-standardized rate for prostate cancer had significantly increased by 47 percent during 2002-2006 in Chennai when compared to the previous years. Mathew et al. 2017 abstracted the data related to cancer incidence (crude and age-adjusted rates) and mortality from Trivandrum District cancer registry. Researchers reported the highest incidence of breast cancer (43.9 and 35.5 percent) trailed by prostate (11.5 and 10.2 percent), colorectal (10.7 and 11.2 percent) in males, corpus-uteri (6.6 and 5.5 percent) and urinary bladder cancers (6.0 and 5.3 percent), respectively. Tyagi et al. 2010 reported that the prostate cancer incidence was higher among North Indians as compared to South Indians. The authors found a significant (p=0.001) association between personal habits (alcohol consumption and past history of smoking); dietary factors (excessive egg and fish intake) and increased risk of developing prostate cancer. The mean age of prostate cancer patients was 69.7 years. Further, as the specific age-group wise prevalence was analyzed, it was observed that it was as high as 22.8 percent among the subjects with age between 70 to 74 years. Moreover, 21.1 and 15.2 percent of the cases were from the age between 65 to 69 years and 75 to 79 years, respectively.

Nemesure et al. 2013 investigated the association between development of prostate cancer and family history and observed that males with first degree relatives such as fathers or brothers with prostate cancer had three-fold higher risk of developing PCa as compared to those without family history. Usharani et al. 2001 reported the various risk factors associated with cancer as smoking (100 percent), alcoholism (75 percent) and tobacco chewing (66.67 percent). Further, the authors also reported that the serum levels of β–carotene and vitamin C were found significantly lower in cancer patients as compared to the normal subjects.

2.1.3 Prevalence and Causes of Cancer and PCa in Punjab

Bathinda district has been reported to be the leading site of cancer occurrence (8.0 percent) in Punjab (Department of Health and Family Welfare 2013). A study conducted by PPCB-PGIMER (2007) in Talwandi Sabo Block reported 125 cancer cases per lakh population in the area. The expert committee reported that in Bathinda District, there were 1574 cancer patients in the entire population of 12.55 lakh and in 4 districts namely Bathinda, Faridkot, Muktsar and Mansa with a total population of 32 lakh, there were 4012 patients of cancer who needed urgent treatment. A survey was performed by The Department of Health, Punjab (2010) during 2005 to 2009 in 4 districts of Punjab i.e. Muktsar, Bathinda, Faridkot, and Mansa to find out the prevalence of cancer in these districts. The findings of the survey were:

S. No.	District	Population	No .of cancer patients	*
1	Muktsar	8, 27,906	453	54.7
2	Bathinda	12, 00, 736	711	59.2
3	Faridkot	5,85, 500	164	28.0
4	Mansa	7, 31, 535	420	57.4

*No. of cancer patients/ lakh population

A maximum increment (231) in the cases of cancer was reported in 2009 as the total number of cancer cases went up to 942 in Bathinda district. Blaurock-Busch et al. 2014 highlighted that cancer prevalence in Malwa region of Punjab and reported it as high as 1089/ million/ year when compared to the 800/ million/ year national cancer prevalence average. The authors further compiled the data on metal concentration in 50 healthy individuals and 49 cancer patients living in the Malwa region. They observed that the mean concentration of toxic metals such as uranium in breast cancer patients was double the figure found in the hair sample of the healthy patients. It was also reported that the metal was present more than six times (0.63 µg/ g) than the reference range (0.1µg Uranium/ g of water). It was observed that mortality due to cancer is directly correlated to gender and occupation in Malwa region. The higher incidence was reported in farming community and prominent cause for the same was considered the arbitrary use of pesticides which in turn resulted in poor groundwater quality in Malwa belt (Singh 2008). Numerous studies have observed that approximately 90 percent of total dietary intake of toxic metals (cadmium and lead) is contributed by foods viz. fruits and vegetables grown in contaminated soil and irrigated with contaminated water in Malwa region which may be correlated with the higher incidence of cancer in the area (Singh 2008; Mittal et al. 2014 and Sharma 2012).

A PPCB-PGIMER (2007) Report, also revealed that there was contamination of arsenic, cadmium, chromium, selenium and mercury in surface water of Bathinda and the partially or untreated industrial waste water was frequently drained in the local drains, which had led to this problem. The findings revealed that quantities of pesticides (chlorpyrifos, ethion and heptachlor) found in the samples of drinking water, vegetables, and human blood in the cotton belt of Punjab was quite higher. A study conducted on "Analysis of pesticide residues in blood samples from villages of Punjab" revealed high pesticide residues levels in the blood samples collected from residents of villages (Mahi, Nangal, Jajjal and Balloh) and Dher in Bathinda and Ropar districts, respectively. Report further indicated that there were six to twelve types of pesticide residues in human blood samples (Centre for Science and Environment (CSE), New Delhi 2005). The average levels of monocrotophos (0.095 µg/ g) were four times higher than the short-term exposure limit for humans set by the World Health Organization and the Food and Agriculture Organization. Besides, the Department of Entomology and Soil Sciences, PAU, Ludhiana (Battu et al. 2005) carried out an investigation to assess the status of pesticide residues in Jajjal and Giana villages, District Bathinda. A total number of 15 samples including soil, water, and vegetable (okra) were collected from Giana (7 samples) and Jajjal (8 samples). Findings depicted that the residues of insecticides viz. organochlorine, organophosphate and synthetic pyrethroid in all water and soil samples (except one) found lower than detection limits of 0.01 ppm and 0.1 ppb, respectively. Though, residues of ethion in okra at village Jajjal was 1.42 µg/ g and was beyond the maximum residue limit (MRL) (1 µg/ g) recommended for vegetables under Prevention of Food Adulteration Act, 1954. This investigation concluded that the arsenic and chromium was present in water, soil and crops owed to multiple pesticides applications for crop protection.

2.2 Food consumption pattern and nutrient intake of cancer patients in India

Ramadas and Somepalli 2002 reviewed the nutritional profile of hundred subjects (both male and female) suffering from different types of cancer in Hyderabad and observed that fresh fruits and vegetables were consumed in meager amounts by the subjects and consumption of cereals and green leafy vegetables were also found deficient in their diets. Usharani et al. 2005 reported that the energy and protein intake of hospitalized cancer patients was very less and adequacy of iron intake was only 15 to 28 percent. Further, intake of dietary fiber was also very low whereas calcium and fat intakes were adequate. Usharani et al. 2001 studied the nutritional status of cancer patients in relation to antioxidant vitamins at Hyderabad and reported that all the subjects were smokers and seventy-five percent of them consumed alcohol. The percentage of tobacco chewers was as high as 66.67. Moreover, the mean serum antioxidant level in terms of β-carotene and vitamin C was significantly low in all types of cancer patients due to lesser fruits and vegetables consumption. Vasanthamani and Banitha 2012 investigated the causative agents of cancer in selected coastal villages of Tamil Nadu and reported that all the subjects were non-vegetarian and consumed fish in higher quantities whereas the consumption of fruits (apple, sapota, grapes, guava, and orange) were very low. It was also reported that onion, potato, and tomato were consumed frequently. Sumathi et al. 2009 conducted a study on the patients with carcinoma stomach in southern part of India and observed their dietary intake. It was found that the intake of preserved foods in form of pickles was higher as compared to the protective foods such as fresh fruits, vegetables, and pulses in the subjects. Rajaram and Sabate 2000 stated that large population of Indians (particularly Hindus) is vegetarian and at lower risks of prostate cancer. Ganesh et al. 2009 while conducting a case-control study at Tata Memorial Hospital, Mumbai, India collected the data on dietary habits of 203 patients with colorectal cancer and 1628 controls. The investigators observed that the subjects ate cabbage and sprouts had 50 and 30-50 percent lower risk of developing cancer. Additionally, 40 to 70 percent reduction was found among fresh fish eater population as compared to dry fish eaters who were monitored with 1.6 fold risk augmentation.

Vasanthamani and Banitha 2012 carried out a survey on identification of causative agents of cancer and found that 64 percent of the subjects had the habit of chewing tobacco and 52 percent were smokers and 61 percent were consuming alcohol. Gupta et al. 2017 during a study targeting squamous cell carcinomas of the upper aerodigestive tract patients revealed that tobacco and alcohol consumption elevated the risk of UASTSCC by twelve folds on comparison with non-consumers, moreover, it had been observed that the early exposure to this had shown a positive association with cancer incidence. Sofi et al. 2018 enrolled 100 newly diagnosed breast cancer female patients for determining their nutritional status in relation to breast cancer risk. Data regarding food intake was collected through 24-hour recall method and frequency of food consumed by using food frequency questionnaire

(FFQ). The findings indicated a significant ($p<0.05$) positive association between saturated fat intake (>20 g/ day) with risk of developing breast cancer. Similarly, the subjects with rare or negligible fruit intake had too increased risk of this type of cancer.

2.3 Effectiveness of Nutrition education through audio-visual aids in curing the diseases

Nutrition education is a process to modify behavior and attitude through upgrading the existing nutrition knowledge and practices of an individual. It is vital to navigate the masses towards appropriate dietary habits in order to cease the disease progression, thus, maintains their adequate nutritional as well as health status.

Aggarwal et al. 2007 studied the impact of nutrition counselling on lipid profile and blood glucose level of female subjects (suffering from Non-insulin Dependent Diabetes Mellitus) from Punjab Agricultural University Hospital, Ludhiana and reported that the blood glucose, triglycerides, and total cholesterol significantly reduced after imparting nutrition education regarding diabetes, its causes, symptoms, complications and dietary management by delivering lectures and giving demonstrations to the subjects. Banga et al. 2005 studied the efficacy of nutrition counselling on anthropometric measurements of respondents with non-insulin dependent diabetes mellitus and reported a significant ($p<0.01$) decrease in the waist-hip ratio in nutrition counselling group as compared to control group. Bisht and Raghuvanshi 2007 reported that through comic book, 47 percent increase in knowledge was observed in school children (10 to 15 years) and 37.13 percent through audio cassette during nutritional intervention. Deshpande and Bargale 2006 investigated the impact of nutrition education on intake of soy-based food in rural women and found that after completing the nutrition education program, soy-based food intake had increased by 27 percent in the daily diets of the respondents, thus exhibiting substantial impact of nutrition education program. Dhindsa et al. 2008 compared three groups (Group I (medical prescription only); Group II (medical prescription + nutrition counselling) and Group III (medical prescription + nutrition counselling + 1 g stevia supplementation)) of non-insulin dependent diabetic subjects. The investigators observed that blood glucose levels (fasting & postprandial) were significantly reduced 17.1 & 32.5 percent and 24.8 & 33.8 percent by nutrition education and supplementation of 1 g stevia leaf powder in Group II and III, respectively. Gowri et al. 2010 carried out a study to see the efficacy of nutrition education and food safety practices in self-help group women and reported a statistically significant increase in knowledge on food safety and hygiene practices among the respondents with different levels of education. Joshi and Singh 2002 developed the educational material for nutrition education and evaluated those on different groups of women i.e. illiterate, women with reading skills and community based workers and found that all the women gained knowledge after nutrition education. Kalpana et al. 2007 also revealed in their findings that nutrition education had significantly improved the haemoglobin levels of anaemic adolescent girls from high schools in Chennai. Kaur and Chawla 2006 stated that nutrition counselling based on dietary modification can be imparted as the basis of prevention and as a general strategy to overcome deadly disease. The authors observed a significant improvement in the scores of knowledge (from 0 to 11), attitude (5 to 20) and practices (5 to 20) in practice test. Narayanan and Ramaraj 2013 assessed the nutritional status of alcoholics before and after imparting nutrition education and found that nutrition education helped in improving the dietary intake and body weights of the subjects. Prakash and Prakash 2012 conducted a study on the food behavior and impact of nutrition education on nutrition knowledge of children and their parents. The findings revealed the significant improvement (51.58 and 35.58 percent) in food behavior and nutrition knowledge of both children and their parents, respectively. Sanmukha Priya and Kowsalya 2013 carried out a survey in Devarayapuram village of Coimbatore district and imparted nutritional awareness by promoting kitchen garden and delivering lectures on micronutrients to adolescents and revealed that nutrition education can be implemented effectively to achieve favorable and significant changes in the dietary pattern of general masses. Srivastava et al. 2007 studied the impact of nutrition counselling on blood profile and knowledge of juvenile diabetics and found a significant decrease in blood glucose levels (both fasting and postprandial) by 12.47 and 8.9 percent, respectively, in the subjects. Tiwari and Yegammai 2011 explored in their study that nutrition education and stress management training along with positive therapy can help to effectively improve nutritional status, increase physical activity and minimize stress and anxiety in adolescents. Ammerman et al. 2002 performed the meta-analysis of 33 articles to investigate the efficacy of nutrition counselling with reference to food intake of the primary care patients. The authors concluded that nutrition counselling influenced the dietary behaviors through decreased consumption of total and saturated fat and increased intake of fruits and vegetables with more intensive counselling.

2.4 Prostate Cancer and Chemoprevention

Shukla and Gupta 2005 suggested that the long latency time of prostate cancer propose plenty of time to pursue chemopreventive strategies to relapse the disease.

The authors highlighted the impact of plant-based food components such as genistein from soy and lycopene from tomatoes in curtailing the progression of malignant prostatic cells. Kowsalya et al. 2008 developed an antioxidant mix from whole wheat flour, roasted bengal gram flour, oats powder, amaranths' powder, *amla* powder and soy flour (55: 10: 15: 10: 5: 5) and supplemented that mix to breast cancer patients. The investigator observed a significant improvement in plasma vitamin C, super-oxidase dismutase (SOD) and total antioxidant activity in the patients.

Fruits and vegetables are considered as protective foods in our diet. These are known to have mechanisms such as bioactive nutrient effects (antioxidants and electrolytes) and some functional properties (vitamins, fiber, minerals, phytochemicals etc.), effective in protecting against chronic diseases and also protect against radiation-induced cancer (Hayes 2005). Berkow et al. 2007 reviewed the findings of eight observational studies and suggested that fruits (watermelon, orange, and grapes) and vegetables (particularly cruciferous and tomato) confer protection against prostate cancer by decreasing serum PSA levels.

Cruciferous group includes variety of vegetables from Brassicaceae family such as broccoli, brussels' sprouts, cauliflower and so on. Crucifers metabolize into glucosinolates, indole-3-carbinol and sulforaphane which interrupts cell signaling pathways during prostate cancer cells, results in epigenetic modulations by blocking initiation and suppressing prostate cancer progression. Frequent intake of cruciferous vegetables, including broccoli and cauliflower may decrease the risk of aggressive prostate cancer, mainly extraprostatic disease (Kirsh 2007).

Lycopene, predominantly found in tomatoes are excellent source of vitamin C and also contain important phytochemicals including polyphenols and vitamin E. Lycopene is also present in tomato-based products and is reportedly one of the most effective antioxidants possessing a singlet-oxygen generating capacity (two folds higher as that of carotenoids). The compound significantly reduces the growth of androgen-sensitive LNCaP cell lining. Moreover, its uptake and absorption in human body is dependent on its preparation method being enhanced if processed or heat treated as compared to be in the raw or unprocessed state (Von Low et al. 2007).

Mein et al. 2008 focused on carotenoids (lycopene) particularly with reference to its association with reduced risk of cancer. The authors explained that lycopene metabolites have specific biological activities which have an impact on a number of essential cellular signaling pathways such as insulin-like growth factors (IGFs) that play a central role in cellular proliferation, differentiation, and apoptosis. Tang et al. 2005 tested the effect of lycopene on mouse prostate cancer model and concluded that lycopene inhibits the growth of androgen-independent DU 145 and PC-3 cells than androgen-dependent LNCaP cells. The apoptosis rate was up to 42.4 percent higher in control cells as compared to DU145 cells treated with 32 mol/ L lycopene. The authors concluded that growth of androgen-independent malignancy of prostate was hindered by lycopene. van Breemen et al. 2011 conducted a clinical trial wherein tomato sauce which contained 30 mg lycopene was administered on per day basis to African American males for 21 days. Their PSA level, as well as serum lycopene levels, were assessed at the beginning and end of the study. The investigators found the elevated level of serum lycopene in the patients and declined concentration in PSA whereas decreased level of serum lycopene and increased level of PSA were reported among the subjects in control group. Zu et al. 2014 studied the dietary intake by administering food frequency questionnaire and prostate-specific antigen level was also assessed. The study reported a significant inverse association of lycopene content with PSA level. Several other studies (Bunker et al. 2007; Schwarz et al. 2008) have also emphasized on increasing concentration of lycopene in the body for lowering the PSA level on one-month intervention.

Isoflavone administration in male with carcinoma prostate was linked with decrease in the rate of increase of PSA relative to the previous rate of increase (de Souza et al. 2010). Krishnan et al. 2007 revealed that genistein, a predominant component of soy, shows anti-proliferative effects in human prostate cancer cells via stabilizing serum Prostate Specific Antigen (PSA). Nagata et al. 2007 carried out a survey on two hundred prostate cancer patients from three geographic areas (Ibaraki, Nara and Hokkaido) in Japan to see the effect of dietary isoflavones on prostate cancer patients and reported that family history of prostate cancer, occupation, medical history, BMI and physical activity were not associated with prostate cancer risk. On the other hand, isoflavones and their aglycones (genistein and daidzein) significantly lowered prostate cancer progression, despite alteration made by magnesium, n-6 fatty acids or PUFA.

Von Low et al. 2007 reviewed the pre-clinical data on notable phytochemicals viz. genistein, lycopene and resveratrol with respect to their tendency of treating prostate cancer. The authors observed that the aforesaid bioactive compounds have outstanding efficacy against prostate cancer cells, targeting cell cycle regulation from initiation to apoptosis through regulation of both androgen and estrogen receptors during *in vitro* trials. Swami et al. 2007 concluded in their study that a main component of soy i.e. genistein was a strong inhibitor of CYP24 and also has capacity to inhibit the prostaglandin pathway. Mukhopadyay 2010 stated that genistein, a hydroxyisoflavone present in soyabean, inhibits tyrosine kinase, arrests cell cycle at G2/ M stage, inhibits cell growth with inhibiting MEK4 kinase activity possibly by binding to the site, lowers MMP-2 transcript level significantly in prostate epithelial cells, which are target cells for chemoprevention. Thus, it is an effective therapeutic alternative to manage prostate cancer. Hamilton-Reeves et al. 2007 studied the effect of supplementation of soy protein isolates on prostate cancer patients by providing them two doses daily as partial meal replacement and compared their pre and post intervention serum hormone and prostate biopsy samples for androgen receptor (AR) and estrogen receptor-β expression. The data showed that the soy protein isolate consumption suppressed AR expression significantly ($P=0.09$), may be helpful in prevention of prostate cancer. Kumar et al. 2004 carried out a trial on supplementation of 60 mg soy isoflavones for 12 weeks and observed a significant decrease in PSA level by two points and more. Hamilton-Reeves et al. 2007 conducted a study on fifty-eight prostate cancer patients aged between 50 to 85 years at Minneapolis Veteran's Administration Medical Centre. The habitual diets of experimental group were supplemented with three different protein isolates viz. i. Isoflavone-rich soya protein isolate (SPI+) (containing 107 mg isoflavones/ d) ii. Alcohol washed soya protein isolate (SPI-) (containing <6 mg isoflavones/ d) iii. Milk protein isolate (MPI), with each providing 40 g protein/ d). After supplementation at 0, 3rd and 6th months; urinary estrogen metabolite profile was assessed and found first two groups with higher estradiol (E2) excretion than third group at 3rd and 6th month and significantly higher urinary 2: 16 OH-E1 ratio was found in SPI+ group than MPI group. The study suggested that soy intake affects endogenous estrogen metabolism which may be advantageous for men with progressing to advanced prostate cancer.

Whole grains possessing plenty of dietary fiber and bioactive compounds exert health benefits. The most appropriate instances are, whole grain wheat and rye containing alkylresorcinols which are validated as important inhibitors of prostate cancer progression. Landberg et al. 2010 studied the impact of consuming high amount of rye (whole grain and bran) on prostate cancer progression (through PSA assessment) in 17 males with prostate malignancy. The subjects consumed 485 g rye (whole grain and bran) products (RP), whereas the subjects from other group were fed on refined wheat products (with added cellulose) (WP). The results showed that the urinary C-Peptide excretion and plasma PSA were significantly ($P<0.001$) lower in the 1st group as compared to the later group. This effect leads to inhibition of prostate cancer progression occurred due to declined contact to insulin, as pointed out through plasma insulin and urinary C-Peptide excretion. Ansari 2002 observed in their study that rye bran and soy-phytoestrogens intake through diet decreased prostate weight and testosterone level with no alteration in luteinizing hormone (LH). The study was performed on Lobund-Wistar (L-W) rats which share most of their characteristics with normal history of prostate cancer in human males comprising innate tendency, elevated production of testosterone and ageing risk factor. It was reported that early detected prostate cancer was reversed by producing testosterone scarcity through dietary changes (by replacing L-485 diet with soy protein isolate/ isoflavone diet), thus averting fatal ailment.

Vitamin E has been proven to have chemopreventive effect for prostate cancer as it regulates the malignant cell growth, initiating apoptosis and ceasing metastatic. Major et al. 2014 reported the association of serum vitamin-E concentrations with cancer risk and the findings suggested a significant ($P<0.003$) lower prostate cancer risk in males with higher vitamin E genotype.

Inedible portion of fruits such as citrus peels contain polymethoxyflavones which has shown anti-carcinogenic effect during clinical trials. Among all, tangerine peels have been used as medicinal ingredient in South-East Asia from the ages. Lai et al. 2013 investigated the impact of citrus peel extract in immuno-deficient mice having human prostate cancer cell line PC-3 tumor xenografts. Two groups of animals were injected citrus peel extract in two dosage i. e. 25 L and 50 L, five times per week for 23 days while a control group was also there. The researchers explored a clear inhibition in PC-3 xenograft tumor growth both the groups. The tumor weight was reduced by 57 percent in first group when compared to the control group. Tumor was almost disappeared in the animals treated with higher dose of citrus peel extract.

Ginger (*Zingiber officinale*) has been widely used spice, worldwide. It possesses various bioactive compounds such as gingerol, shogaols, paradols etc. The ginger phenolic group includes 6- gingerol, 8- gingerol, 10- gingerol and 6- shogaols. Bioactive compounds present in ginger have efficacy to inhibit malignant tumor growth. Karna et al. 2012 investigated the relationship between ginger extract and inhibition in rise of prostate cancer (PC-3) xenografts. The investigators established that 100 mg/ kg body weight of ginger extract was helpful to decrease tumor volume by around 56 percent in nude mice. Mukkavilli et al. 2018 administrated ginger extract orally (250 mg per kg of body weight) in mice for 28 days; then recorded the tumor growth and compared to control model. Findings revealed 68 percent lesser growth in experimental animals fed on whole ginger extract than controls. The researchers also compared these findings with quasi mixture of phenolic components

(6- gingerol, 8- gingerol, 10- gingerol and 6- shogaols) and ginger extract without these components, obtained only 28 and 35 percent inhibitions, in tumor growth as compared to the controls.

Turmeric has antioxidant properties (curcumin) which prevents the activation of oncogens. It bears 40 percent homology to tumor necrosis factor TNF-α, thereby exhibiting anti-carcinogenic effect (Srinivas and Chethankumar 2006). Dinesha and Srinivas 2010 isolated and performed preliminary purification of BGS-Haridrin from turmeric. The authors reported that this compound possesses 82 percent inhibition in hydroxyl radical scavenging assay.

2.5 Development of value added nutraceutical products

Soy, wheat, tomato, fruit waste, ginger, and turmeric possess an excellent nutritional profile in terms of digestible protein, carbohydrates, dietary fiber, polyphenols, vitamins, and minerals. Reedy et al. 2014 emphasized on dietary pattern of cancer patients rather than on single nutrient or food group and reflected in their findings that DASH (Dietary Approaches to Stop Hypertension) diet (whole grain, legumes, fruits, nuts, vegetables and low-fat dairy) showed lower the risk of mortality outcomes. Therefore, various investigators have worked on these foods with prior emphasis on their functional components in order to develop value added products.

Awasthi et al. 2012 developed the three variations T_1, T_2 and T_3 combining wheat flour and soya flour as 80: 20, 85: 15 and 90:10 along with T_0 (control), respectively in order to develop high protein, high calories supplementary biscuits. The investigators found the highest content of protein in T_1 (9.17 percent) followed by T_2 (8.44 percent) and T_3 (7.6 percent). Sangwan and Dahiya 2012 reviewed in their study that wheat and soybean in the ratio of 40 and 60 showed highest calcium as 150.45 mg, iron as 8.28 mg and copper as 0.98 mg and zinc as 1.93 mg/ 100 g.

Venkateswari and Parmeshwari 2016 evaluated the chemical characteristics of soya flour and wheat flour enriched biscuits at two (10 and 15 percent) incorporation levels of soya flour and established that there was an increase of almost two fold (from 13 to 37.5 percent) in the protein content alongside fiber which was 3.5 percent after supplementation of soya flour at 15 percent to wheat flour. Vyas and Diwedi 2016 developed two treatments of cereal pulse based flour mixtures viz. wheat moong blend (WMU) and wheat moth blend (WMO) via roasting method and reported that WMU possessed higher content of ash (3.3 g), protein (14.23 g) and fiber (5.48 g) as compared to WMO. Further, on organoleptic basis, muffins developed using WMU were found highly acceptable. Zaker et al. 2012 standardized the wheat and defatted soya flour (DSF) biscuits at 10, 20 and 30 percent levels of incorporation and concluded that adding DSF at the rate of 20 percent to wheat resulted in high quality biscuits in terms of both physical as well as chemical characteristics reported in stipulations of overall acceptability (8.37), protein (13.53 %) and ash (1.75 %) contents, respectively.

Kumar et al. 2015 devised a multigrain premix (MGP) using defatted soya flour, chickpea, pea, whole barley and sorghum each at 20 percent level. Further, level of MGP and wheat flour was optimized in order to develop multigrain biscuits and obtained 40 g and 60 g blend as highly acceptable both in view of nutrition (16.61 percent and 2.57 percent soluble dietary fiber) and overall acceptability (8.0) which were significantly ($p<0.05$) higher than the control. Mishra and Chandra 2012 formulated the functional biscuits with addition of soya flour and rice bran to wheat flour. The analysts observed an increase of 1.4 in thickness on maximum substitution (25 %) of wheat flour with soya flour and rice bran. In contrast to this, width and spread ratio were found to be decreased. Rathi and Mogra 2013 developed three diverse variations of biscuits superseding wheat flour with flaxseed at the rate of 20, 30 and 40 percent, respectively to prepare 100 g flour mixture and analyzed the developed products regarding their sensory parameters via 9 point hedonic scale. The observations made by the investigators clearly depicted that the treatment standardized at 30 percent incorporation level reflected the most acceptable results in terms of overall acceptability i.e. 8.0 in contrast to the lowest score (6.4) attained by 40 percent incorporation level. Further, it was also noticed that the former variation was almost equal up to the standard (8.6) as that of the control.

Chaudhary et al. 2011 evaluated the soy blended *mathari* with lotus stem powder and recorded the highest (7.7) acceptability scores of 20 percent defatted soy flour + 5 percent lotus stem powder + 75 percent refined wheat flour and higher protein and iron content i. e. 18.8 and 5.2 percent as compared to refined wheat flour *mathari*. Lakshmi and Gomathi 2011 observed in their study that non-edible portion of grapes, mango, sweet lime (*masaumbi*) and watermelon were 42, 42 and 68 percent, respectively. The authors reported high β-carotene, protein, calcium, iron and vitamin C content in peels and seeds of these fruits. Sindhu et al. 2016 formulated biscuits incorporated with defatted soya flour (DSF) and carrot pomace powder (CPP) in order to enhance the nutritive value of the final product by utilization of by-products. DSF was added at 5, 8, 11, 14 and 17 percent levels whereas addition of CPP was at 0, 3, 6, 9 and 12 percent, respectively. On chemical analysis of the biscuits, it was observed that the protein, fiber, and β-carotene contents significantly increased with the increment in DSF and CPP amounts, respectively. Youssef and

Mousa 2012 revealed in their study that citrus peel powder incorporated at 10 percent level into wheat flour while making biscuits results in improved sensory characteristics and may be recommended to obese and diabetic persons.

Limbachiya and Amin 2015 formulated multigrain muffins by incorporating soyabean, finger millet (*ragi*) and maize at three different ratios i.e. 50: 20: 30, 40: 20: 20 and 30: 20: 50, respectively. The latter ratio was most acceptable in sensory aspect. That formulation was taken as basic composite flour (control) and further, developed three more treatments namely sundry, roasted and oven dry as the ingredients were treated under sun drying, roasting and oven drying conditions. The investigators compared all the variations and found the control sample as most nutritious with higher amount of ash, calcium, phosphorus, and iron in it. Further, Singh and Bose 2016 developed muffins for diabetic patients through addition of flaxseed to whole wheat flour. Sugar was also replaced with orange pulp and honey. Researchers observed that the intervention was beneficial in terms of both sensory and physicochemical evaluation. The contents of crude protein, fiber, calcium, phosphorus, and iron were significantly increased and carbohydrate got decreased in experimental variation as compared to control product. Nandkule et al. 2015 developed and evaluated functional noodle enriched with jackfruit seeds at different levels along with soya flour while the major flour was refined wheat flour. Six variations incorporating refined wheat flour, jackfruit seed flour, and soya flour were standardized as 90: 5: 5, 80: 10: 10, 70: 15: 15, 60: 20: 20, 70:10: 20 and 70: 20: 10, respectively. It was concluded that the first ratio was most acceptable among all during sensory evaluation. Dhull and Sandhu 2018 developed noodles from wheat fenugreek based composite flour. The researchers added fenugreek at 2, 5, 7 and 10 percent to wheat flour and evaluated the products organoleptically. The overall acceptability scores showed that the fenugreek supplementation was acceptable up to 7 percent in wheat flour noodles.

It is staunchly reviewed that nutritional interventions have a significant positive impact on ceasing the progression of different diseases. Hence, considering above evidences, the current research has been carried out in order to curb the metastatic during prostate cancer through implicating nutrition education with improved health status of prostate cancer patients.

3. MATERIALS AND METHODS

The current study was planned to examine "Nutrition Knowledge in association with Health Status of Prostate Cancer (PCa) Patients in selected Hospitals of Bathinda City" using dietary survey, anthropometric measurements, biochemical assessment and knowledge, attitude and practices (KAP) questionnaire. The materials and methods worked out for present investigations have been discussed under the following heads:

3.1 Location/ Place of work

- 3.1.1 Selection of subjects to study the prevalence of Prostate Cancer
  - a) Sampling method
  - b) Study population
- 3.1.2 Selection of the subjects for nutrition intervention

3.2 Development of Questionnaire cum Interview schedule

3.3 Collection of data

- 3.3.1 Demographic, Lifestyle and Health related information
- 3.3.2 Pre-intervention test of knowledge, attitude and practices of PCa patients
- 3.3.3 Dietary Survey
- 3.3.4 Anthropometric measurements
- 3.4.5 Biochemical assessment

3.4 Development of PCa specific extension aids

3.5 Nutrition counselling

3.6 Post-intervention test of PCa patients

3.7 Impact of Nutrition Counselling on knowledge, attitude, practices and health status of PCa patients

3.8 Development and evaluation of different types of value added nutraceutical products

3.9 Statistical Analysis

3.1 Location/ Place of work:

3.1.1 Selection of subjects to study the prevalence of Prostate Cancer

In Bathinda City, there were 50 wards under municipal corporation, Bathinda where the survey to find out the prevalence of prostate cancer was taken place.

a) Sampling method

Multistage systematic sampling was worked out to study the prevalence of prostate cancer among males residing in different wards of Bathinda City.

b) Study population

Sample size to identify the prevalence of prostate cancer was calculated through working out the formula given below:

$$N = \frac{z^2 P (1-P)}{e^2}$$

Where N = Sample size

z = Statistics for α error

P = Estimated prevalence of the disease during last review

e = Margin of Error

Assumption: If we set alpha error at 5 %, z would be 1.96.

According to NCRP (2013), prevalence of prostate cancer in males was 8 percent in Bathinda District.

$$N = \frac{1.96^2 \times 0.08 (1-0.08)}{0.05^2}$$

Therefore, N was calculated as 114 according to formula mentioned above. A total number of 150 subjects (from randomly selected households in each ward) who fulfilled the inclusion criteria were invited to participate in the prevalence survey to ensure the prevalence rate of prostate cancer.

Inclusion criteria: Males above the age of 30 years.

3.1.2 Selection of the subjects for nutrition intervention

A randomized controlled (clinical) trial employing cluster design was undertaken on a set of 200 patients who fulfilled the inclusion criteria were selected from the Department of Urology of three different hospitals namely Adesh Institute of Medical Sciences and Research, Bucho Mandi, Bathinda, Max Hospital, Bathinda and Punjab Cancer Care, Bathinda. These were divided into two groups viz. Group I and

Group II with no significant difference in their baseline characteristics viz. age group, education level etc. Nutrition counselling was imparted to the subjects in Group II while the subjects in Group I were treated as control.

Inclusion criteria: Outdoor, Post operative males with prostate cancer.

3.2 Development of Questionnaire cum interview schedule

An open ended questionnaire cum interview schedule, consisting of three parts i.e. i) Demographic, lifestyle and health related information pertaining to age, education, occupation, socioeconomic status, marital status, type of family, sleeping span, physical activity level, family history of PCa, clinical signs and symptoms of the disease etc. (Appendix I), ii) A food questionnaire to record dietary habits viz. eating pattern, meal pattern, type of fat consumed and daily food intake of the subjects (Appendix II) and iii) A KAP questionnaire with multiple choice questions to assess the scores under knowledge, attitude and practices (KAP) of the subjects regarding PCa and its management (Appendix III) was developed. The face validity of the questionnaire cum interview schedule was carried out by obtaining the comments of the expert panel consisting of Assistant Scientist, Food and Nutrition, Punjab Agricultural University, Ludhiana and Dietitians of concerned hospitals. Along with that, ten subjects were selected randomly to check the feasibility and reliability of the prepared questionnaire. Depending upon the reply received through review process and pre-testing, necessary alterations were made in schedule. Further, subjects involved in the pretesting were excluded from the actual study sample. Therefore, the modified questionnaire was exercised to gather ultimate records for research.

3.3 Collection of data

Personal interaction was made to collect the detailed data related to subjects in order to ensure first hand authenticated information.

3.3.1 Demographic, Lifestyle and Health related information

Data concerning demographic information such as age, religion, educational level, occupation, socioeconomic status, marital status, type and composition of family, presence of allied diseases etc. was collected. Further, lifestyle related information in terms of sleeping span, physical activity level (PAL), household practices (food storage containers, source of drinking water, method for cleaning fruits and vegetables, practice of kitchen garden, source of fruits and vegetables) and health related information in reference to family history of disease, clinical signs and symptoms of prostate cancer, other complications, onset of the disease and aggravating factors was elicited. Besides, clinical signs and symptoms of nutritional deficiencies such as anemia and Vitamin-C deficiency in terms of pallor of eyes and skin, spoon-shaped nails, breathlessness etc. and spongy bleeding gums and teeth mottled enamel were also recorded.

Physical Activity Level

The record of time spent on different activities viz. farming, walk, cycling, watching television etc. using Physical Activity Diary Method (PADM) was taken for three consecutive days, simultaneously with the on-going dietary survey. The detailed information related to time allocated to individual task performed under physical activity by each respondent was recorded. Then, the obtained data was multiplied with energy cost defined for each activity and then, the total sum attained was divided by 24 to get Mean PAL (Physical Activity Level) value which was used to categorize the respondents into three different categories i.e. sedentary, moderate and active. Depending on the obtained value of PAL the selected subjects were classified into three categories according to different age groups (FAO/WHO/UNU 2004).

Category	Type of activity	**Physical Activity Level**
Sedentary	Office worker/ Little or no exercise	1.40-1.69
Active	Construction worker/ Running for one hour daily	1.70-1.99
Vigorous	Agricultural worker (non-mechanized)	2.00-2.40

3.3.2 Pre-intervention test of knowledge, attitude and practices of PCa patients

Three parameters i.e. knowledge, attitude and practices regarding PCa and its management were assessed among all the subjects, through administering the developed KAP questionnaire containing a set of multiple choice questions under each parameter head. As far as the allocation of marks was concerned, one (1) mark was awarded for every correct answer whereas zero (0) point was allotted for each wrong answer. The responses recorded as don't know were expelled from the evaluation process.

3.3.3 Dietary survey

Information regarding dietary habits viz. eating pattern, meal pattern, fat consumption using standard procedure were recorded. Daily food intake for three consecutive days was recorded both at the beginning and at the end of the study using 24 hour recall method. Daily average of nutrients in terms of energy, protein, fat, vitamins and minerals was also calculated using software "Dietcal - A Tool for Dietary Assessment and Planning" (Kaur 2014). Further, daily intake of nutrients was compared to Recommended Dietary Allowances suggested for Indian males (ICMR 2010).

3.3.4 Anthropometric measurements

Information regarding height and weight was recorded both at the beginning and at the end of the study. Standard methods (Jelliffe 1966) were followed while recording these two measurements. The height of the subjects was measured with a rod placed on the platform in vertical position. The subjects were requested to put off their shoes and stand parallel to the height measuring scale with touching their heels to the wall. The height was recorded by holding the head securely at erect position, lower head piece, measured in centimeters (cm) and noted with an accuracy of at least 0.5 cm. The subjects were weighed on a digital weighing balance. The subjects were asked to weigh themselves with minimum clothing and without shoes in order to record actual body weight and was noted in kilograms with an accuracy of at least 0.1 kg. The following formulae was used to calculate the Body Mass Index (BMI) (WHO 2004) to assess the proportionality of physique of the subjects.

Body Mass Index (Kg/ m^2) = Weight (Kg)/ Height $(m)^2$

Classification of body mass index (WHO 2004)

Classification	BMI(Kg/m^2) Principal cut-off points
Underweight	<18.50
Normal	18.50-24.99
Overweight	≥25.00
Pre-obese	25.00-29.99
Obese	≥30.00

3.3.5 Biochemical assessment

Values for prostate cancer biomarker i.e. prostate-specific antigen (PSA) and bone mineral density (BMD) were noted from the medical records of the subjects. Biochemical parameters viz. haemoglobin, serum albumin, serum globulin, serum vitamin-A, vitamin-E, vitamin-C, iron, selenium and zinc were also noted both at the beginning and the end of the study to find out the relationship of these parameters to nutrition counselling.

3.4 Development of PCa specific extension aids

An information booklet containing information such as the prostate, its functions, prostate-specific antigen, difference between benign prostate hyperplasia and prostate cancer, causative factors, signs and symptoms, complications, timely detection of disease, treatments, prevention and control, dietary requirements, important foods to help preventing PCa, different methods of cooking, household practices to minimize the pesticide residues, importance of reverse osmosis in water purification, promoting organic farming etc. was developed in both English and Punjabi languages to impart awareness among masses. The contents of information booklet were formulated reviewing the most appropriate literature available in regard to prostate cancer. Further, the text was supplemented with illustrations and pictures in order to make the same more understandable to the readers.

3.5 Nutrition counselling

Nutrition counselling focused on balanced diet and important anti cancer foods was imparted to the subjects and their attendants in Group II for 90 days with an interval of one week in order to control the progression of the disease. Information booklets were distributed among subjects of Group II to strengthen their knowledge.

3.6 Post-intervention test of PCa patients

The post-intervention test was conducted at the end of the study using pre-structured KAP questionnaire. The questions and evaluation process remained same as during the pre-intervention test.

3.7 Impact of Nutrition Counselling on knowledge, attitude, practices and health status of PCa patients

Impact of nutrition counselling and administration of PCa specific extension aids was studied by comparing the pre and post intervention test scores obtained by the subjects as per the formulae mentioned below:

Gain in knowledge = Score of post test - Score of pre test

Quantum of improvement = Post test score/ Pre test score

Furthermore, the anthropometric and biochemical parameters of the subjects were also recorded at the end of the study and compared to the initial values. Thereafter, both the obtained values were compared to the reference values.

3.8 Development and evaluation of different types of value added nutraceutical products

Three types of value added products viz. biscuits, muffins, and noodles were developed using a diverse array of ingredients (whole wheat flour, defatted soya flour, tomato, powder of tangerine peel, ginger (powder), black pepper, sugar, turmeric etc.)

in standard ratio to obtain a beneficial and safe effect. The procedures involved in preparation of the aforementioned value added products were as follows:

3.8.1 Procurement of raw material

Ingredients such as wheat flour, wheat bran, powdered sugar, ammonium bi-carbonate and baking powder were procured from Raj *Karyana* Store, Ludhiana, India. Defatted soya flour (toasted, Taj Mahal Allegro brand, containing 0.5 percent fat and 50 percent protein) was bought from Super Foods, Chandigarh, India. Fresh ginger and tangerine peels were collected from local vegetable market, Phagwara, India and both the ingredients were finely grated and dried at 60°C to maintain moisture content to 8 percent. The dried ingredients were crushed in the grinder and sieved through 1mm sieve-shaker; fine ginger and tangerine peel powders so obtained were kept in dry place and stored in food grade, air-tight containers prior to use. Perishable foods i.e. tomatoes and butter (Amul Delicious with 0 percent trans fat and cholesterol) were purchased from WH Smith General Store, Lovely Professional University, Phagwara, India, at the time of preparation.

3.8.2 Preparation of the biscuits

The ingredients used for both control and value added biscuits are enlisted below in Table 3.1 and Table 3.2, respectively.

3.8.2.1 Development of Biscuits (Control)

Three treatments of the biscuits (control) viz. T_1, T_2 and T_3 were standardized in different ratio of wheat flour and defatted soya flour (40:60), (50:50) and (60:40) against T_0 (consisting of 100 % wheat flour) to obtain the most acceptable combination for value added biscuits as stated in Table 3.1.

Table 3.1 Ingredients for biscuits (Control)

S. No.	Ingredients	T_0 (g)	T_1 (g)	T_2 (g)	T_3 (g)
1.	Defatted Soya Flour	---	150	125	100
2.	Wheat Flour	250	100	125	150
3.	Butter	125	125	125	125
4.	Sugar (Powdered)	125	125	125	125
5.	Ammonium bi-carbonate	3.5	3.5	3.5	3.5

Where T_0 = 100 % Wheat Flour,

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour,

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour,

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

3.8.2.2 Development of Biscuits (Value added)

The ratio (40:60) i. e. T_3 was finalized for value added biscuits according to obtained scores for different sensory parameters for control treatments. Then, half of the portion (20 %) of defatted soya flour was replaced with nutraceutical ingredients (in the form of wheat bran, tomato pulp, ginger powder, tangerine peel powder, turmeric and black pepper) were incorporated as per the details mentioned in Table 3.2.

Table 3.2 Ingredients for biscuits (Value added)

S. No.	Ingredients	T_4 (g)	T_5 (g)
1.	Defatted Soya Flour	50	50
2.	Wheat Flour	150	150
3.	Butter	125	125
4.	Sugar (Powdered)	125	125
5.	Ginger (Powder)	5	--
6.	Wheat Bran	17.5	17.5
7.	Tomato pulp	25	25
8.	Tangerine peel (Powder)	--	5
9.	Black pepper	1.25	1.25
10.	Turmeric	1.25	1.25
11.	Ammonium bi-carbonate	3.5	3.5

Where T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5= 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

3.8.2.3 Method for preparation

To develop the biscuits, traditional creamery method was practiced (Meena and Meena 2004). All the raw ingredients were weighed and then, creaming of butter was done by cut and fold method along with mixing of sugar in it. Further, other ingredients were added gradually in this mixture with continuous stirring to obtain a uniform smooth dough. The dough was refrigerated and after 45 minutes, it was kneaded back and spreaded to a 2.5 mm thick sheet. Then, the sheet was cut into different shapes and baked at 180°C for 12 minutes.

3.8.3 Preparation of Muffins

The ingredients used for control and value added variations of muffins are depicted below in Table 3.3.

Table 3.3 Ingredients for muffins

S. No.	Ingredients	T_1 (g)	T_2 (g)	T_3 (g)
1.	Defatted Soya flour	---	50	50
2.	Wheat flour	250	150	150
3.	Sugar (Powdered)	125	125	125
4.	Butter	125	125	125
5.	Tomato pulp	---	25	15
6.	Wheat Bran	---	17.5	17.5
7.	Tangerine peel (Powder)	---	5	---
8.	Ginger (Powder)	---	---	5
9.	Turmeric	---	1.25	1.25
10.	Black pepper	---	1.25	1.25
11.	Baking powder	5	5	5

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

3.8.3.1 Method for preparation

Butter was creamed, along with consistent addition of sugar. All the raw ingredients were added and mix to attain a uniform batter. After greasing the muffin moulds, the better was poured into the moulds and baked at the temperature of 170°C for 25 minutes.

3.8.4 Development of noodles

The preparation of noodles and noodle flavors has been shown in Figure 3.1 below:

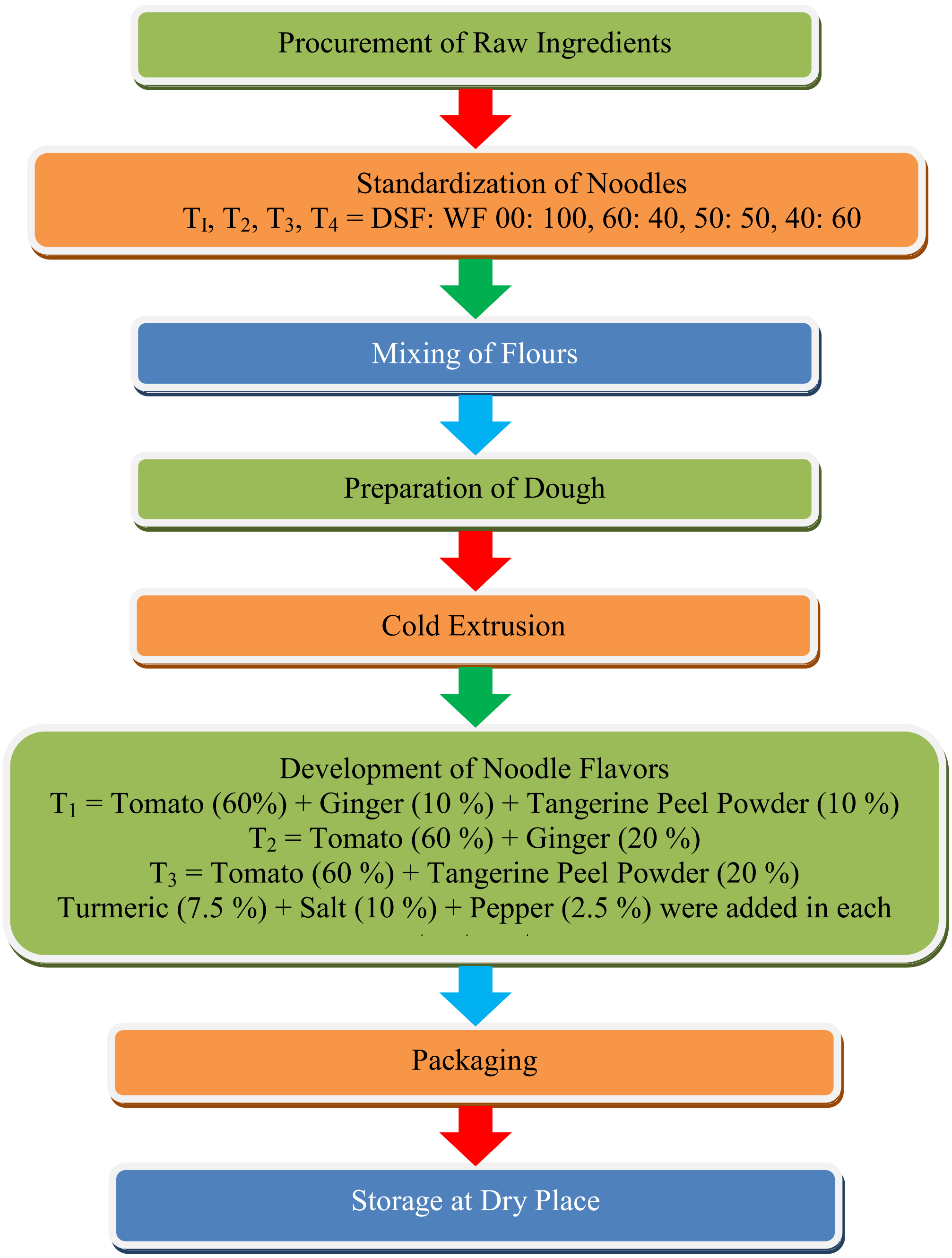


Figure 3.1 Development of noodles and noodle flavors

3.8.4.1 Method for preparation

After a smooth dough was prepared, balls weighed 50 g each were made and spread to sheets of 2 mm thickness. A mesh was adjusted to extruder and inserted the sheets one by one in between two rollers fixed in the equipment and dried at 60°C for 1 hour in hot air oven.

3.8.5 Sensory evaluation

The sensory evaluation including five different attributes i.e. appearance, color, texture, flavor (aroma/ taste) and overall acceptability was done using 9 point hedonic scale (Appendix II) from a panel of 10 semi-trained judges and scores of each treatment under three value added nutraceutical products were recorded.

3.8.6 Physical characteristics

Randomly selected biscuits were evaluated in terms of their weight, diameter, thickness and spread ratio following standard procedure (Hussain et al. 2006). The samples were weighed on analytical weighing balance. Diameter was measured by placing four samples next to one another before and after baking using a vernier caliper with 0.01 mm accuracy. Final diameter was obtained by measuring all the biscuits and then the average of two measurements divided by four. Thickness was measured by stacking the biscuit one above the other and restacking four times. The spread ratio was calculated using the formula: diameter of biscuits divided by thickness of biscuits. Hardness was measured on texture analyzer (TMS-PRO, Food Technology Corporation, USA). After placing on the loading platform, the biscuits were compressed. The compression platform of 25 mm was used as a probe. To calculate the accurate figures, each sample was undergone through this process for four times.

3.8.7 Chemical analysis

The raw materials and value added nutraceutical products (biscuits, muffins and noodles) were analyzed for proximate composition, amino acids, *in-vitro* protein digestibility, vitamins and minerals, in triplicates, on wet weight basis to determine their nutritional quality.

3.8.7.1 Proximate composition

The samples were analyzed in terms of ash, fat, fiber, moisture and protein in triplicates on wet weight basis (AOAC 2000).

a) Moisture (AOAC 2000)

All the samples were finely ground and weighed as 5 g each. The samples were dried for 8 hours at 105°C in hot air oven. In case of wet samples, constant weight was achieved. The dried material along with china crucibles were transferred instantly to desiccators, cooled and weighed.

Calculations

$$\text{Moisture \%} = \frac{\text{Loss in weight (g)}}{\text{Sample weight (g)}} \times 100$$

b) Crude protein (AOAC 2000)

The determination of nitrogen by macro-kjeldahl method and crude protein was calculated by using a factor of 6.25 for conversion of nitrogen into crude protein.

Reagents

1. Conc. H_2SO_4
2. Digestion mixture: $CuSo_4$ (1 part) and K_2SO_4 (9 parts) were taken and mixed together.
3. Boric acid (4 percent)
4. NaOH (40 percent)
5. Mixed indicator: Methyl red and bromocresol green (0.1 and 0.5 g) were dissolved in ethanol (95 percent).
6. Standardized H_2SO_4 (0.1N)

Procedure

Weighed samples (500 mg each) were mixed with 5 g of digestion mixture in a kjeldahl flask. Then, it was digested with conc. H_2SO_4 (25 ml) till it turned clear bluish green and nitrogen was converted to $(NH_4)_2SO_4$. Volume was prepared till 100 ml and used for distillation as single sample. Further, 50 ml of 40 percent NaOH and 100 ml of H_2O were added to neutralize excess amount of acid as well as to generate strong alkaline pH. After adding NaOH, flask was directly attached to a condenser with another flask (250 ml) comprising 4 percent boric acid (25 ml) with mixed indicator. Then, process of distillation was started; when solution became almost dual, distillation was stopped and it was titrated with 0.1N H_2SO_4 till the end point color (pink-red) was appeared. Similarly, the sample blank was run.

Calculations

$$N\% = \frac{\text{Volume of 0.1N } H_2SO_4 \text{ used x 0.0014}}{\text{Sample Weight}} \times 100$$

% crude protein = % N x 6.25

Note : After subtracting blank sample, volume of 0.1N H_2SO_4 used was taken.

Most proteins contain 16 % N and thus 100/16 = 6.25, therefore, factor of 6.25 was based on that fact. But for specific proteins where N content of protein was known with certainty, different factors were used e.g. 5.7 for wheat proteins.

c) Total ash (AOAC 2000)

Samples (5 g each) were shifted to pre-weighed crucibles and were weighed again. The muffle furnace was ignited at 550°C and samples were placed in it for 4 hours. The residues remained in china crucible was weighed up again.

Calculations

$$\text{Ash } \% = \frac{\text{Weight of ash (g)}}{\text{Sample Weight (g)}} \times 100$$

d) Crude fat or ether extract (AOAC 2000)

Whatman No. 1 sheet, thread and test tube with diameter of 2 cm was used to prepare the thimble. Then, 5 g of moisture free samples were shifted to already prepared thimble and its mouth was plugged with absorbent cotton (fat free). Then, these thimbles were placed in the soxlet assembly apparatus (Sox Plus). The flask (having 1.5 times capacity of soxlet apparatus) was filled with Petroleum ether (40-60°C). Afterwards, it was sat at 60°C along with ensured supply of tap water. Finally, remained ether in the flask was evaporated and the substances with small quantities of petroleum ether were transferred to pre-weighed crucibles and kept in the water bath to evaporate excess amount of ether and then weight of crucibles were taken.

Calculations

$$\text{Crude fat } \% = \frac{\text{Weight of fat (g)}}{\text{Sample weight (g)}} \times 100$$

e) Crude fiber (AOAC 2000)

Reagents

1. H_2SO_4 (1.25 percent)
2. NaOH (1.25 percent)

Procedure

Moisture and fat free samples (5 g each) were weighed and transferred in 500 ml beakers and 1.25 percent H_2SO_4 was added to each sample. Boiling of contents was done for 30 minutes and then those were filtration was done with muslin cloth, Buchner funnels and filtration flasks. Washing of residues was performed by hot water till those became acid free and moved to beakers. Further, 1.25 percent NaOH (200 ml) was poured in each spoutless beaker, again boiled for half an hour and similarly, the filtered contents were washed by hot water. Residues shifted to pre-weighed crucibles were kept in hot air oven at 100°C for dried to a constant weight. Afterwards, residues were kept in muffle furnace for ashing at 550°C temperature and weight was taken.

Calculations

$$\text{Crude fiber, \%} = 100 \times \frac{\text{Weight of residue - Weight of ash after ignition}}{\text{Sample Weight}}$$

f) Carbohydrates (AOAC 2000)

Values of moisture, crude protein, crude fat, crude fiber and ash contents were added and subtracted from 100. Difference was accepted as the value of available carbohydrates.

3.8.7.2 Amino acid composition

i) Lysine estimation (Carpenter 1960)

Reagents

1. $NaHCO_3$ (8 percent)
2. Ethyl alcohol
3. 1 Flouro-2-4-dinitrobenzene (FDNB)
4. HCl, 8.1 N HCl. conc. HCl.AR (1 N)
5. Ethyl ether
6. Buffer pH 8.5 (19 parts 8 percent $NaHCO_3$ and 1 part 8.1 percent Na_2CO_3)
7. NaOH (2 N)
8. Methoxy carbonyl chloride (methyl chloroformate)

Procedure

Stage I

In $NaHCO_3$ (8 percent, 8 ml), defatted samples (0.5 g each) and ethyl alcohol (12 ml) containing FDNB (0.3 ml) were mixed in 100 ml conical flasks. The contents were kept in water bath-cum-shaker at 50ºC for one hour. Ethanol in all flasks was vaporized in hot water bath and then 8.1N HCl (24 ml) was added to each flask. The refluxing of contents for 16 hours were done gently. Volume was prepared to 100 ml for every sample and then filtration of contents was done.

Stage II

From all samples, filtrate (2 ml) was added in three tubes viz. A, B and C; and each marked till 10 ml capacity. The filtrate volume of 2 ml in all the tubes were extracted with ethyl ether (5 ml) for two times and layer of ether was thrown away. For the removal of excess ether layer, these test tubes were kept immersing in bath of hot water. Then, the amount of tube A was raised with 1N HCl till 10 ml for each sample and kept to obtain final reading.

Stage III

An indicator (phenolphthalein-1 drop) was added to test tube C, and titration was done with NaOH (2 N). After that, the contents of tube C were discarded. Then, same amounts of alkali was poured to tube B with addition of buffer (pH 8.5 - 2 ml). Methoxy carbonyl chloride (0.05 ml) was also mixed and precipitates formed due to its addition, were dissolved by vigorous shaking. After 10 minutes, drop wise concentrated HCl (0.75 ml) was added. The extraction of contents were done twice, with ethyl ether (5 ml) and excess layer of ethyl ether was thrown away (same as Tube A) and kept in hot water for excess removal of layer. The tubes were cooled and volume for each tube was made with 1 N HCl (10 ml).

Stage IV

The measurement of OD (Optical density) of the available contents in A and B tubes was taken at 430 nm. OD difference of test tubes i.e. A and B was considered as final optical density and was matched with obtained corresponding values with prepared solution of standard E-DNP lysine (2 ml).

Standard E-DNP solution

E-DNP lysine (Standard, 12.5 mg) was dissolved in 1 N HCl (250 ml).

Standard curve

For the preparation of standard curve, 20 µg to 100 µg per ml (0.4, 0.8, 1.2, 1.6 and 2.0 ml) concentration of E-DNP lysine HCl was taken and it was raised with distilled water till the volume of 10 ml. The measurement of optical densities were taken at 430 nm. Similarly, the blank sample was also run.

Calculations

Available lysine: g available lysine/ 100 g protein =

$$\frac{0.851 \times 0.4862 \times \text{dil. factor} \times 100 \times 100 \times \text{conc. of E-DNP lysine } HCl.H_2O}{\text{Sample Weight} \times \text{percent protein}}$$

ii) Methionine estimation (Horn et al. 1946)

Hydrolysis of samples

In the conical flask, fat and moisture free samples (2 g each) and 2.5 N HCl (25 ml) was taken. It was covered with beaker and placed in autoclave (at 15 lb pressure) for 6 hours. Volume of samples was raised till 25 ml after hydrolysis, a pinch of activated charcoal was added in the samples and filtered. Aliquots thus obtained were used for the determination of methionine.

Reagents

1. Sodium hydroxide (5 N)
2. Sodium nitroprusside (10 percent) (prepared fresh)
3. Glycine (3 percent)
4. Orthophosphoric acid (85 percent)
5. Standard methionine solution: Methionine (500 mg) in 2.5 N HCl was dissolved and made the volume to 500 ml.

Procedure

Firstly, 5 N NaOH (1 ml) and 10 percent solution of sodium nitroprusside (0.1 ml) was added to distilled water (3 ml). This solution was added in a test tube containing protein hydrolysate filtrate (2 ml) and kept in a shaker for ten minutes. Then, add the 3 percent solution of glycine (2 ml) and mixture was kept at room temperature for 10 minutes. Then, addition of 85 percent orthophosphoric acid (2 ml) was completed drop by drop with continuous shaking. After 10 minutes, it was measured at 540 nm. Similarly, blank and standard were also ran.

The concentration for standard curve was 200 to 1000 µg/ ml methionine.

3.8.7.3 *In vitro* protein digestibility (Akeson and Stachman 1964)

Reagents

1. Pepsin solution: In 1 liter of 0.1 N HCl, pepsin (5 g) was dissolved.
2. Pancreatin solution: Pancreatin (4 g) was dissolved in 1 liter of phosphate buffer.
3. NaOH (0.2 N)

4. HCl (0.1 N)
5. Acid phosphate (0.2 M): Potassium phosphate (27.32 g) was dissolved in distilled water and diluted to 1 L.
6. Phosphate buffer (pH 8.0) - BaOH was made as 0.2 N and then 46.80 ml of it was added to 0.2 M acid phosphate (50 ml) and diluted till 200 ml.
7. Toluene

Procedure

Dry samples (0.5 g each) in conical flask (250 ml) were taken and pepsin solution (50 ml) was added to each sample. Samples were kept in incubator at 37° C for 24 hours. The solutions were neutralized with 0.2 N NaOH (30 ml), then pancreatin solution (50 ml) was added and were again kept for 24 hours incubation. A blank was also ran. Toluene (few drops) was added to preserve aseptic environment and were configured at high speed. Filter was done through Whatman No. 44 filter paper. Analysis of residues for N content was done via macro-kjeldahl method. Digestibility coefficients were calculated by subtraction of the residual protein from initial protein at 100 g of sample basis.

3.8.7.4 Vitamin A (AOAC 1980)

Reagents

1. Absorbent- Neutral aluminium oxide (Moisture content was controlled before use)
2. Chloroform- Chloroform was purified by distillation. Distilled chloroform was discarded after one week.
3. Hexane- Hexane was free from alcohols, esters and ketones.
4. Alcohol (95 percent)
5. Potassium hydroxide solution - Reagent grade KOH (70 g) was dissolved in 70 ml water, mixed and cooled. It was prepared fresh each time.
6. Antimony trichloride reagent (20 percent): Antimony trichloride crystals were taken from unopened tightly sealed bottle. Antimony trichloride crystals (100 g) were added to chloroform and diluted to 500 ml. The mixture was heated enough to get warm and mixed the contents to dissolve. The solution was cooled and 15 ml acetic anhydride was added in it. It was stored in tight brown bottle.
7. Vitamin A standard solution (1 µg/ ml): Vitamin A standard solution (25 mg) was dissolved in chloroform and the volume was raised to 500 ml. Then, 10 ml of this solution was diluted to 500 ml with chloroform (1 µg/ ml).

8. β-carotene standard solution: β-carotene standard (25 g) was dissolved in hexane and volume was made to 100 ml in hexane. After that, 5 ml from this stock solution was diluted to 100 ml (5 µg/ ml).

Procedure

Extraction

Samples (10 g each) were taken and added into 40 ml of alcohol. These samples were mixed with added 10 ml of potassium hydroxide solution. Solutions were refluxed for 30 minutes with continuous stirring. Then cooled rapidly and alcohol water solution (3+1) up to 20-30 ml was added. Solutions were shook and washed with hexane in separating funnel and the hexane washings were collected. The hexane extracts so pooled were condensed by evaporating hexane in water bath at 60ºC in the presence of nitrogen gas to 5 ml.

The chromatography tubes (18 x 200 mm with 12 mm width sealed to tube 5 x 100 mm each) with absorbent by tapping lightly to 5-6 cm were packed. Tops of chromatography tubes were covered with 0.5 cm anhydrous sodium sulphate and were used, immediately. The columns were washed with hexane. The extracts were added in the column. Carotene was eluted with 4 percent acetone in hexane. After that, vitamin A was eluted with 15 percent acetone in hexane.

β-carotene elute was collected volumetric flask (25 ml) and more of 4 percent acetone was added in hexane and made volume to 25 ml and transmittances were read at 465 nm. Vitamin A elutes were dried in the presence of nitrogen to 1 ml and the volume was made to 10 ml with chloroform. Then, 1 ml of above solutions were taken in cuvettes and 4 ml of antimony chloride was added and the transmittances were noted at 620 nm, within 4 seconds.

Standard curve for β-carotene was prepared with 1 to 7 ml of stock solution in 25 ml volumetric flask in duplicate. Volume was made with 4 percent acetone in hexane. Standard curve for vitamin A was prepared with 1 to 7 ml standard in 25 ml volumetric flasks and volume was made with chloroform. The concentrations of vitamin A and β-carotene were calculated from the standard curve.

3.8.7.5 Vitamin C (AOVC 1996)

Reagents

1. Acetate Buffer (pH 4.0): Anhydrous sodium acetate (300 g) was dissolved in water (700 ml) and glacial acetic acid (1000 ml) was added in it.
2. 2,6-dichlorophenyl indophenol dye solution: In distilled water, sodium salt of 2,6-dichlorophenyl indophenol (25 mg) was dissolved. The volume was raised to 200 ml.

3. Metaphosphoric acid (HPO_3, 6 percent): Metaphosphoric acid (6 g) was dissolved in distilled water (100 ml)
4. Ascorbic acid standard (1 mg/ ml): Pure ascorbic acid (100 g) was dissolved in 6 percent metaphosphoric acid (100 ml).
5. Xylene

Procedure

Each sample (5 g) was taken in a mortar and pestle and then, after adding six percent HPO_3 (20 ml), gently made into a slurry. The filtration of contents with filter paper (Whatman no. 1) was completed. Further, 30 ml HPO_3 was mixed with remained material of each sample and again filtration was done. The separating funnels of 50 ml (3 in number) were set up and marked (sample in A, dye blank in B and standard in C). Then, filtrate (5 ml) was pipetted in separating funnel A. Then, standard ascorbic acid (0.1 ml) solution was pipetted into funnels C. The addition of acetate buffer (5 ml) to each separating funnel was done and then 2 ml of the dye solution was added in every sample. After that, xylene solution (10 ml) was added rapidly and were shaken for 5-10 seconds. The mixtures were kept for some time to allow to separate the two layers. The lowest water layer was thrown away from each funnel, xylene layer transferred into tubes and the absorbance for each sample were read at 500 nm in a photoelectric colorimeter.

Calculations

$$X \text{ mg} \quad \frac{0.1\ (b-a)}{(b-c)}$$

OD of blank (b)

OD of sample (a)

OD of standard (c)

$$\text{Filtrate (5 ml) contains} = \frac{50 \times X}{5} = 1 \text{ mg}$$

Sample (5 g) contains = Y mg

$$\text{Sample (100 g) contains} = \frac{Y \times 100}{5} = \text{mg}$$

3.8.7.6 Vitamin E (Emmerie and Engel 1938)

Reagents

1. Absolute alcohol
2. Xylene

3. α-α-dipyridyl (Fischer Scientific Co.): In 100 ml of n-propanol, α-α-dipyridyl (120 mg) was dissolved.
4. Ferric chloride hexahydrate: In 100 ml of absolute ethanol, ferric chloride hexahydrate (120 mg) was dissolved and was stored into a dark brown bottle.
5. Vitamin E standard: DL- α-tocopherol was used as a reference standard. It was dissolved in absolute ethanol to yield a solution containing 1 mg/ 100 ml ethanol. DL- α-tocopherol (91 mg) was equivalent to 100 mg of DL- α-tocopherol acetate. (The latter was International reference standard for vitamin E).

Procedure

Sample

Ethyl alcohol (1.5 ml) was mixed to food sample (1.5 ml) in centrifuge tubes, then the tubes were stoppered and mixed the solutions, thoroughly.

Standard

H_2O (1.5 ml) was added to 1.5 ml of vitamin E standard (1 mg/ dl) in a centrifuge tube, and mixed well.

Blank

Distilled water (1.5 ml) was mixed to alcohol (1.5 ml) was mixed in a centrifuge tube.

Procedure

Xylene (1.5 ml) was poured in all tubes, stoppered and mixed (vortexed) for 21 minutes. All the tubes were centrifuged for 10 minutes. Xylene layer (1 ml) was pipetted into clear glass stoppered tubes. Only xylene layer was taken but not the alcohol. Then, 1 ml of α-α-dipyridyl reagent was mixed to all tubes. Mixtures were transferred to spectrophotometer cuvettes individually. Zero setting of instrument was done with distilled water at 460 nm and optical density was noted for samples and standards. Ferric chloride reagent (0.33 ml) was added to the blank and mixed for 30 seconds. Then, 1.5 minutes after the addition of ferric chloride, zero setting was done at 520 nm with a blank. The ferric chloride addition was repeated for the standard and samples to read the absorbance exactly 1.5 minutes after addition of ferric chloride reagent at 520 nm.

Calculations

$$\text{mg \% vit. E in Std.} \times \frac{\text{Sample } OD_{520} - (0.29 \times \text{Sample } OD_{460})}{\text{Standard } OD_{520}} = \text{mg \% vit. E in sample}$$

3.8.7.7 Determination of minerals by Inductively Coupled Plasma Atomic Emission Spectroscopy (Dilek et al. 2011)

The ICP technology has been advantageous for mineral estimation in cereals and legumes as minerals (Ca, Cd, Co, Cu, Fe, Mg, Mn, Mo, Ni, P, S and Zn) can be accurately identified and computed in composite flours (Nelms 2005). Samples were analyzed through operating ICP-OES Perkin-Elmer; model Optima™ 2000 DV, using winLab32 software.

Apparatus decontamination

All types of glassware and centrifuge tubes were washed with teepol and water which is essential for estimation of minerals. Then, those were dipped in hydrochloric acid (5 %) for overnight, rinsed with deionised water, dried and labeled.

Reagents

Diacid mixture comprising 1: 3 ratio of perchloric and nitric acid was prepared for digesting the food sample. It was used fresh.

Procedure

The weighed samples (0.5 g each) put in 100 ml conical flasks and diacid mixture (10 ml) was used to digest by keeping it overnight. Then digested samples were heated by keeping those on a hot plate at a low temperature till those becomes clear, cooled and shifted to volumetric flasks (25 ml) and volumes were raised with deionised water. The filtration of digests were made and stored in centrifuge tubes for determination of minerals by Inductively Coupled Plasma Atomic Emission Spectroscopy. In case of blank, only mixture of diacids (10 ml) was used for digestion and finally the volume was raised with deionized water (25 ml).

3.8.7.8 Total phenols (Singleton et al. 1999)

Extraction of Bioactive components

Known quantity of weighed samples were taken in 100 ml conical flasks. Then,15 ml of 80 percent methanol (acidified) was added to pH 2.0 with 6 N hydrochloric acid by shaking at room temperature for 30 minutes. Supernatants were decanted and re-extracted the residues for complete removal of phenolic and antioxidant compounds. This procedure was repeated twice. The three supernatants were pooled from each sample, centrifuged at 6000 rpm for 15 minutes and filtered (through Whatman No.1 filter paper). The volumes were raised with the solvent up to

50 ml. The samples were transferred to micro centrifuge tubes and stored at -20°C for total phenolic content (TPC) determination.

Reagents

1. Gallic acid (GA) standard solution (100 percent)

   Stock solution: GA (100 mg) was dissolved distilled water (100 ml).

   Working solution: Stock solution (1 ml) was raised till 20 ml with distilled water.
2. Folin-Ciocalteu (FC) reagent (50 percent): Folin-Ciocalteu (1 part) and distilled water (1 part).
3. Sodium carbonate (7.5 percent): Na_2CO_3 (7.5 g) was dissolved in distilled water (100 ml).

Procedure

Sample

Known quantity of aliquot of samples were taken and volumes were prepared to 1.5 ml by adding distilled water. To those, FC reagent (0.5 ml) was added; 10 ml of 7.5 percent Na_2CO_3 was mixed and incubated at 37˚C for 60 minutes. A blue coloured complex was obtained and it was read at 750 nm.

Standard

Standard series of known concentration of Gallic acid (5 μg to 20 μg) was made. For that 0.1, 0.2, 0.3, 0.4 ml of aliquots treated in the same manner as samples.

Blank

Distilled water (0.5 ml) was treated in same way as sample.

Calculations

$$(\text{mg GAE/100 g}) = \frac{\text{Total Phenol}}{\text{Std. O.D.}} \times \frac{\text{Std. Conc.}}{\text{Aliquot taken}} \times \frac{\text{Sample O.D.}}{\text{Sample taken}} \times \frac{\text{Vol. made up to 100}}{1000} \times \text{Dilution factor}$$

3.8.7.9 Trypsin inhibitor (Roy and Rao 1971)

Reagents

1. Phosphate buffer (0.01 M, pH 7.6): Took 16 g sodium dihydrogen phosphate (NaH_2PO_4) (0.02 M) and 84 g disodium hydrogen phosphate and were mixed in distilled water (200 ml) and adjust the pH to 7.6.
2. Phosphate buffer (0.02 M) – pH 7.0: The above solution of phosphate buffer (50 ml - 0.01 M) was diluted with H_2O to 100 ml and adjusted the pH to 7.0.

3. Solution of Casein (20 percent): In phosphate buffer (pH 7.6), casein (2 g) was dissolved by keeping it in water bath for 10 minutes and then cooled. Then, volume was raised till 100 ml with phosphate buffer and placed in refrigerator.
4. Solution of Trypsin (5 mg/ ml): In 25 ml phosphate buffer (pH 7.6), trypsin (125 mg) was dissolved.
5. 0.001 Normal solution of HCl
6. TCA- Trichloroacetic acid (5 percent): An amount of 5 g was mixed in H_2O to raise the volume till 100 ml.

Extraction

Samples (5 g each) with phosphate buffer, pH 7.0 of 25 ml were mixed in conical flasks and shaken on mechanical shaker for three hours. Then centrifuged for 20 minutes at 10,000 rpm.

The sets of incubations prepared were mentioned as under:

	Sample (ml)	Control (ml)	Blank (ml)
Phosphate buffer (0.01 M, pH 7.6)	0.1	1.1	1.1
Trypsin (5 mg/ ml)	0.5	0.4	0.5
HCl	0.4	0.4	0.4
TCA (5 percent)	--	--	--
Casein (2 percent)	2.0	2.0	2.0
Extract incubated at 37ºC for 20 minutes	1.0	--	--
TCA	6.0	6.0	6.0

Lowry's procedure (Lowry et al. 1951)

Reagent

1. Na_2CO_3 (4 percent)
2. $CuSO_4.5H_2O$ (0.5 percent) was added in potassium-sodium tartarate (1 percent).
3. Solution of alkaline copper: Reagent 1 (50 ml) was mixed with reagent 2 (2 ml) (prepared on daily basis)
4. 0.1 Normal solution of NaOH
5. Folin's reagent (Diluted): Reagent Folin-ciocalteau and 0.1 N NaOH in ratio of 1:1.
6. BSA (Bovine serum albumin) was prepared 100 µg/ ml for Standard protein solution.

Procedure

To test sample (0.5 ml), NaOH (0.1 N, 1.0 ml) was added. Then, after aadding alkaline copper reagent (1.5 ml), the mixture was kept it aside for 10 minutes. After that, mixing of Folin's reagent (diluted, 0.15 ml) was performed by continuous shaking. Tubes were allowed to stand for 30 minutes and OD was measured at 750 nm. Standard curve was prepared with concentration of BSA till 150 µg. A blank was also ran. The standard curve was used to read the test sample concentration and the value of protein was calculated using dilution factors.

Protein determination

The above incubated contents after the addition of tricholoroacetic acid were centrifuged at 10,000 rpm for 10 minutes. Supernatant (0.5 ml) was determined to analyze the TCA soluble protein. Standard curve was prepared using casein solution containing 400 µg/ ml (0.1 to 0.5 ml).

TIU

Quantity of enzyme which changed one mg casein to trichloroacetic acid solution is defined as TIU. Constituents were incubated at 37ºC (pH 7.6) for 20 minutes. Under the assay conditions, one unit of TIA reduces the trypsin activity by one unit.

Calculations

0.5 ml contains 10 mg casein

$$\text{TIU per mg protein} = \frac{\text{O.D. of test}}{\text{O.D. of Std.}} \times \frac{100}{\text{Conc. of Std.}} \times \frac{\text{Dil. factor}}{\text{Wt. of Sample}} \times \frac{1}{10}$$

3.8.7.9 Determination of Total antioxidant capacity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity (Brand-Williams et al. 1995)

Reagents

1. Trolox standard solution (10 percent): Trolox (10 mg) was dissolved in distilled water (100 ml).
2. DPPH solution: DPPH (15.77 mg) was dissolved in methanol (200 ml) and followed by setting the O.D. 1.0 at 517 nm.
3. Methanol

Procedure

Sample

Different sample aliquots were taken and volume was prepared to 1 ml by adding methanol in it. Subsequently, 3 ml of DPPH reagent was mixed in it properly. Solutions were incubated for 20 min at 37°C. The absorbance of the resulting oxidized solution was read at 517 nm against methanol as blank.

Control

Methanol (1.0 ml) was treated same as samples.

Standard

Standard series of known concentration of Trolox (5-20 μg) was made. For that 0.05, 0.1, 0.15, 0.2 ml aliquots were taken and made volumes to 1.0 ml with methanol and treated same as samples.

Calculations

$$\textbf{Percent inhibition} = \frac{(Ac - Ae)}{Ac} \times 100$$

(Where, Ac = Absorbance of control; Ae = Absorbance of extract)

$$\text{TAC (mg TE/100 g)} = \frac{\text{Std. Conc.}}{\text{Std. \% Inhi.}} \times \frac{\text{Sample \% Inhi.}}{\text{Aliquot taken}} \times \frac{\text{Vol. made up}}{\text{Sample taken}} \times \frac{100}{1000} \times \text{Dilution factor}$$

3.9 Statistical analysis

The collected information was subjected to Tukey's test, One-way ANOVA (analysis of variance) to analyze the significant difference ($p<0.05$) between all the parameters recorded throughout the investigation using the software GraphPad Prism (version 5.01).

4. RESULTS AND DISCUSSION

It is projected that worlds' population will increase to 7.5 billion and about 15 million new cancer cases will be detected with approximately 12 million deaths (Bray and Moller 2006). Lifestyle as well as environment attribute to 90-95 percent of developing chronic illnesses instead of human genes. Cancer is one of these that persists despite the abundance of research in medical science since past decade, therefore provides lot of opportunities for its prevention and cure through dietary and lifestyle modifications (Anand et al. 2008). Moreover, 30-35 percent of cancer deaths are due to poor dietary habits and there are specific chemo-preventive foods which help preventing cancer through specific mechanisms can be utilized for both prevention and management of the disease. Simultaneously, numerous studies have been conducted to manage nutritional status of the patient during cancer implementing dietary and lifestyle changes reflected positive improvements in health status of the subjects (Greenwald 2005; Divisi et al. 2006). Prostate cancer is an appropriate target for its primary prevention because of its long latency time and potential to cure through dietary agents (Krishna Moorthy and Venugopal 2008). Thus, the present study entitled "Nutrition knowledge in association with Health status of Prostate Cancer (PCa) patient in selected hospitals of Bathinda City" was conducted on a statistically adequate samples of 150 and 200 patients with prostate cancer in order to study the prevalence of prostate cancer in Bathinda City and efficacy of nutrition education during the disease, respectively.

The results of the study have been discussed under the following sub-heads:

4.1 Prevalence of prostate cancer in Bathinda City

4.2 Causative factors of prostate cancer

- 4.2.1 Demographic information of prostate cancer patients
- 4.2.2 Lifestyle related information of prostate cancer patients
- 4.2.3 Health related information of prostate cancer patients
- 4.2.4 Dietary habits and Nutrient intake of prostate cancer patients
- 4.2.5 Anthropometric measurements of prostate cancer patients
- 4.2.6 Biochemical assessment of prostate cancer patients

4.3 Impact of Nutrition Counselling on knowledge, attitude, practices and health status of PCa patients

4.4 Development, physico-chemical and sensory evaluation of different types of value added nutraceutical products for PCa prevention

4.1 Prevalence of prostate cancer in Bathinda City

To study the prevalence of prostate cancer (PCa), a total number of 150 males from different randomly selected households belonging to 50 wards of Bathinda City were invited for the screening of prostate cancer. The findings signify that there were 7 subjects who were suffering from PCa. Therefore, the incidence of PCa was observed as 4.67 percent in Bathinda City. Hebert et al. 2006 reported the range from 5.0 to 9.1/ 1,00,000 annual prevalence rate of prostate cancer in Indian males. According to NCRP (2013), prostate cancer has been reported to be the leading malignancy among all types of cancers in males with the prevalence of 8 percent in Bathinda District.

4.2 Causative factors of prostate cancer

A total number of 200 patients who were visiting three different hospitals at Bathinda City for their post-operative follow up treatment were studied regarding below-mentioned information to study the causative factors of prostate cancer and impact of nutrition counselling on their health status.

4.2.1 Demographic information of prostate cancer patients

Demographic information pertaining to age, education level, occupation and socioeconomic status of prostate cancer patients has been described in Figures 4.1, 4.2, 4.3 (a) & (b) and 4.4. To start with very important and uncontrollable variable i.e. aging process, it was observed that the incidence of the disease was found highest (39 & 48 percent) among the individuals between the age group of 60-70 years followed by 70-80 years (33 & 29 percent); 50-60 years (17 & 09 percent) and 80-90 years (11 & 06 percent) in group I & group II, respectively (Figure 4.1).

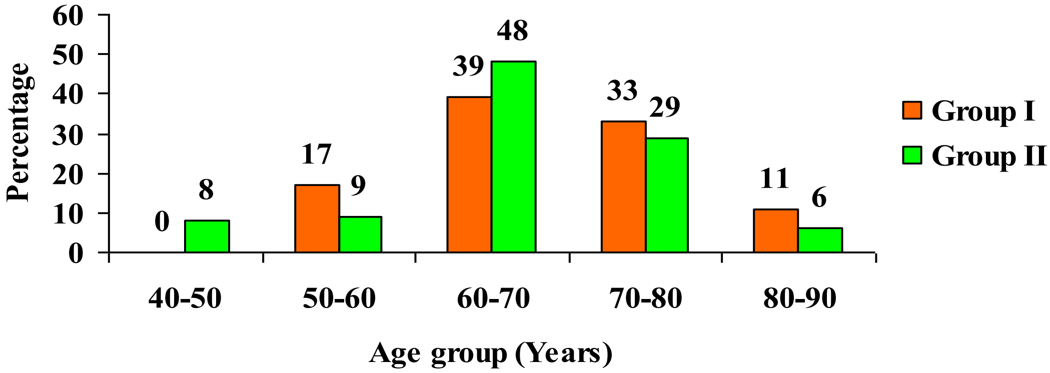


Figure 4.1 Age wise distribution of prostate cancer patients

Moreover, it has been reported that the peak incidence of prostate cancer was observed in the age group above 65 years, and thus, considered as the disease of elderly (Lalitha et al. 2012).

With regard to education level, most (37 and 34 percent) of the respondents were illiterate in both the groups whereas only twelve (12) percent continued their studies after matriculation (Figure 4.2). Similarly, Shahmoradi et al. 2009 also observed during their study that majority (82 percent) of the respondents were educated up to secondary level. Bidoli et al. 2009 too reported the maximum percentage of prostate cancer patients below middle (49.6) and matric (29.9) levels of education.

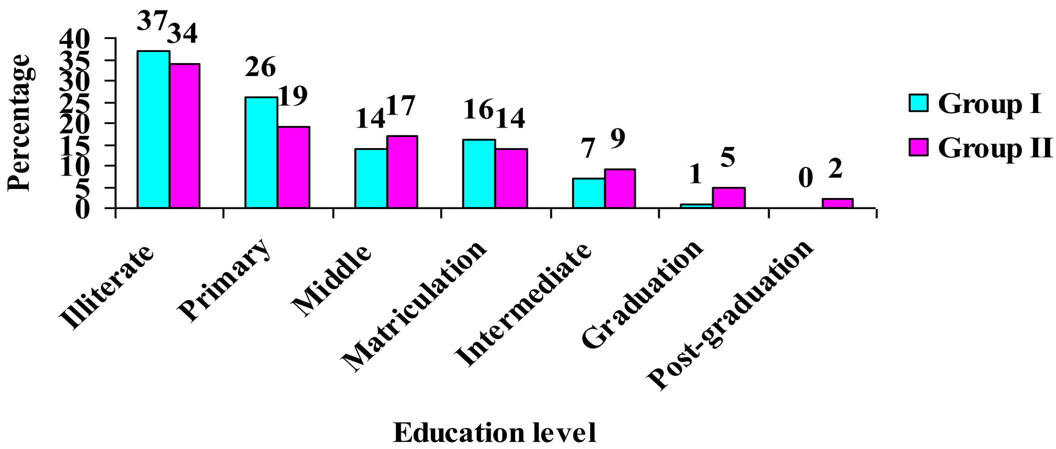


Figure 4.2 Education level of prostate cancer patients

It can be evidently seen from Figures 4.3 (a) and 4.3 (b) that majority (65 and 53 percent) of the subjects belonged to farm community. Sharma-Wagner et al. 2000 reported that farmers and other agriculture workers have 7 to 12 percent increased risk of developing cancer.

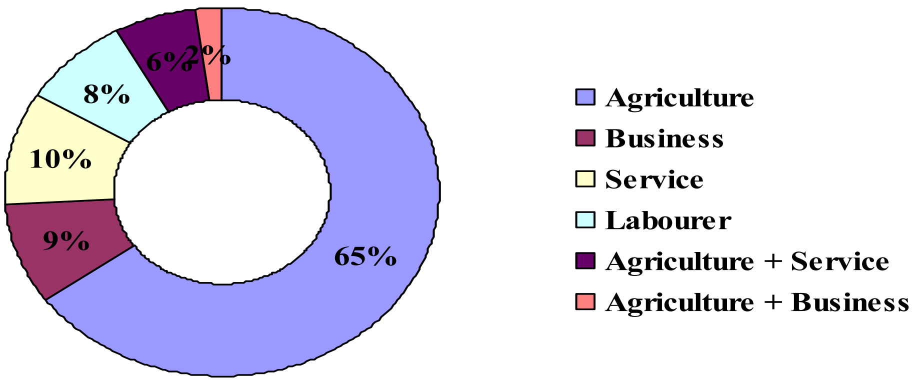


Figure 4.3 (a) Occupation of prostate cancer patients in Group I

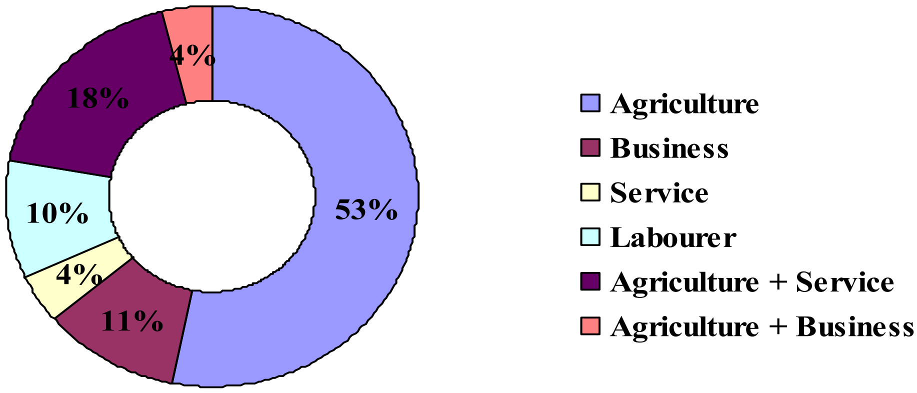


Figure 4.3 (b) Occupation of prostate cancer patients in Group II

Further, additional information such as employment status, religion, marital status, type and composition of family, has been described in Table 4.1. As far as the employment status was concerned, the percentage of part time workers and retirees was quite high (68 & 29 in group I and 22 & 51 in group II) as compared to the full-time workers (03 and 27 percent in two groups, respectively). A survey carried out in Malaysia too reflected this factor as the findings have shown the percentage of retired respondents was 47.5 (Shahmoradi et al. 2009).

The natives of Punjab predominantly belong to two religious communities i.e. Sikhs and Hindus. Most (87 and 93 percent in group I and group II) of the subjects were Sikhs in the present study. Brar (2015) also reported that the majority of the respondents were Sikhs (66 percent) during her survey on breast cancer patients.

Most of the subjects i.e. 67 and 71 percent in group I and group II, respectively, were based in rural households. In contrast to this, 24 and 19 percent of the subjects were residing in urban settings. Hariharan and Venugopal 2016 after scrutinizing the MEDLINE database from 1990 to 2014 with respect to demographic information of PCa patients in India, reported that majority (68.84 percent) of the respondents belonged to rural areas.

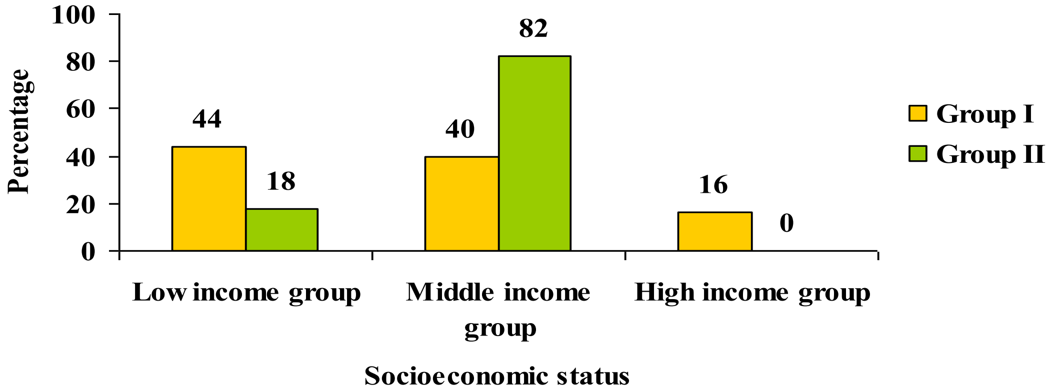
The data showed that greater than $3/4^{th}$ of the total participants (88 and 96 percent) were married in both the groups whereas, only 12 and 04 percent were widowers. Data regarding the familial system of the patients demonstrated that joint family set up was widespread in both the groups as the corresponding values had been recorded as 87 and 93 percent along with majority of the family size reported from 5 to 7 (61 and 49 percent) and 8 to 10 (28 and 38 percent) members in two groups, respectively.

Table 4.1 Demographic Information of Prostate Cancer Patients (N= 200)

Particulars	No. of Patients	
	Group I (N=100)	**Group II (N=100)**
Employment status		
Full time	03	27
Part time	68	22
Retired	29	51
Religion		
Hindu	13	07
Sikh	87	93
Locality		
Rural	67	71
Urban	24	19
Semi-urban	09	10
Marital status		
Married	88	96
Widower	12	04
Type of family		
Joint	87	93
Nuclear	13	07
Family size		
2-4	10	05
5-7	61	49
8-10	28	38
>10	01	08

Original figures represent the percentages

Socioeconomic status is considered as an important factor in determining the nutritional as well as health status of an individual. It is the measure of classification of socio-economic status, based on per capita income of family (Ankitha et al. 2016). Results have been recorded and compared with Kuppuswamy socioeconomic classification (2016) according to which it was shown that 40 and 82 percent of the respondents were categorized under middle income strata having per capita income between INR 9794-19605, preceded by low income group with per capita income between INR 1964-9793 (44 and 18 percent) in both the groups, respectively. In contrast to this, only 16 percent of the total subjects were categorized under high income group with per capita income of INR 19606- 39377 and higher (Figure 4.4).



Figures 4.4 Socioeconomic status of prostate cancer patients

Niclis et al. 2015 conducted a study on 147 males with prostate cancer in Cordoba, Argentina during the year 2008 to 2012 and revealed that majority of the respondents i.e. 41.50 and 34.01 percent were belonging to low and middle income groups, respectively whereas a small percentage (24.49) was reported with high socioeconomic group. Cheng et al. 2009 scrutinized the data focused on relationship between socioeconomic status and prostate cancer from California Cancer Registry (A population based surveillance, epidemiology and end results [SEER] registry) and concluded that the socio-economic status (SES) portrays itself as a strong determinant in incidence of the disease as the highest quintile had a 28 percent higher rate of incidence as compared to the lowest one. Hayes et al. 1999 carried out a survey on socio-demographic background of American-Black and American-White men and reported that the prevalence of PCa was found higher among the patients belonging to low income group and lower in moderate income strata in American-Black population while an opposite trend was found among American-Whites.

4.2.2 Lifestyle related information of prostate cancer patients

Simultaneously, it is likely to be an interplay between several other factors such as social, environmental and genetic etc. Further, incidence of prostate cancer may be affected by lifestyle practices as well as awareness level and accessibility to health care service (Cheng et al. 2009). Lifestyle related information of the subjects is summarized in Figure 4.5 and Table 4.2 (a & b).

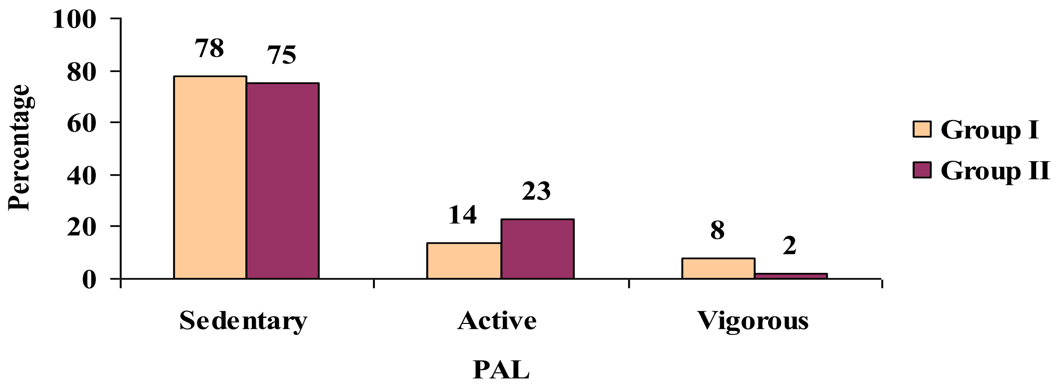


Figure 4.5 Physical Activity Level (PAL) of prostate cancer patients

Sedentary lifestyle (with PAL value of 1.40- 1.69) was found in more than three quarters (78 and 75 percent) of the subjects in group I and group II, respectively (Figure 4.5) while only 14 and 23 percent in group I and group II, respectively, were active (with PAL value of 1.70- 1.99), according to the different activities performed as well as time spent on the particular activity. Furthermore, only 08 and 02 percent of the subjects in group I and group II, were vigorously active (with PAL value of 2.00- 2.40). Similar findings were observed during the study conducted by Hayes et al. 1999 and Niclis et al. 2015. Further, Clarke and Whittemore 2000 studied the relationship of physical activity and prostate cancer risk and revealed that low levels of physical activity was associated with increased prostate cancer risk among African-American males as compared to highly active subjects. It was reported that prostate cancer incidence significantly ($p<0.05$) increased approximately three folds between the age 45 to 54 years among Malaysian men (Shahar et al. 2011). Increased physical activity reduces obesity, improves immunity by declining the levels of free testosterone in the body and thus, reduced risk of developing prostate cancer (Lee et al. 2003).

Data regarding sleeping span of the subjects showed that most (91 and 83 percent) of the respondents had normal sleeping span (6- 8 hours) and rest (09 and 17 percent) used to have their sleep time as more than 8 hours a day (Table 4.2 (a)). Cancer patients usually face the nutritional deficiencies during the disease as the maintenance of good nutritional status is quite a tedious task to perform. Thus, symptoms of three major micronutrients deficiencies such as iron and vitamin-C were recorded (Table 4.2 (a)) and it was observed that fatigue (47 & 33 percent), breathlessness (46 & 20 percent), pale conjunctiva (22 & 44 percent), paleness of skin (25 & 33 percent) and headache (32 & 24 percent) were found the prominent features of anemia. Pallor of skin was found only in 06 percent population in group I as compared to 32 per cent in group II. Further, higher (21) percentage of lethargy was present among the subjects in group I than in group II (05 percent). Very few (02 and 04 percent) cases of spoon shaped nails were detected. Apart from these, vitamin-C deficiency was noticed in the form of spongy bleeding gums (15 & 09 percent) and mottled teeth enamel (53 & 31 percent) in the subjects of group I & II, respectively.

Table 4.2 (a) Lifestyle related Information of Prostate Cancer Patients (N= 200)

	No. of Patients	
i. Sleeping span/day (hrs)	**Group I (N=100)**	**Group II (N=100)**
<6	00	00
6-8	91	83
>8	09	17
ii Signs and symptoms of Nutritional deficiencies		
a. Symptoms of Anaemia		
Paleness of skin	25	33
Pallor of tongue	06	32
Pale conjunctiva	22	44
Spoon shaped nails	02	04
Headache	32	24
Feeling of lethargy	21	05
Feeling of fatigue	47	33
Breathlessness	46	20
b. Symptoms of Vitamin-C deficiency		
Spongy bleeding gums	15	09
Teeth mottled enamel	53	31
iii. History of allied diseases		
Diabetes Mellitus	11	09
Renal Disease	00	04
Cardio-vascular Diseases	04	00
Respiratory Diseases	02	02
iv. Presence of other disorders		
Hypertension	08	04
Uric acid	01	01

Original figures represent the percentages

Other major lifestyle related diseases such as diabetes were observed among 11 and 09 percent of the subjects in group I and group II, respectively. Further, renal, cardio-vascular and respiratory diseases were observed as 00 & 04, 04 & 00 and 02 & 02 percent in two groups, respectively. Other disorders i.e. hypertension and uric acid were observed among 08 & 04 percent and 01 & 01 percent of the respondents in group I & II, respectively. Ganesh et al. 2011 reviewed the prostate cancer cases from registries available at Mumbai and found that history of diabetes and hypertension was three to four folds among the affected population when compared to the control group. Further, it was also revealed during a study on Spanish prostate cancer patients that the percentage of prostate cancer patients with diabetes and hypertension were 16.48 and 45.03, respectively (Gomez-Acebo et al. 2017).

Among household practices, as the storage containers for food commodities were observed, it was seen that practice of food grade containers for procurement of food grains was as low as 16 and 10 percent in group I and group II, respectively (Table 4.2 (b)). Steel and ordinary plastic containers were in style as much as 32 & 32 and 18 & 14 percent in group I and group II, respectively. Further, 16 and 42 percent of the respondents were using both above-mentioned containers in both the groups. It was too noted that 03 and 05 percent of subjects in group I and group II, respectively. stocked up their food materials in empty pesticide containers and steel containers, combined. Combination of food grade plastic and steel containers was into practice only among 01 and 11 percent of the subjects in both the groups. Storage of food commodities in plastic containers causes release of carcinogenic agents such as bisphenol from plastic containers which can result in prostate cancer (Ho et al. 2006).

Source of drinking water was also documented in Table 4.2 and the finding of the survey reported that 51 and 35 percent of the populations in both the groups had the reverse osmosis water purifiers installed, while accessibility of water from other untreated sources were observed as municipal water supply, submersible, hand pump among 49 & 65 percent of the subjects in both the groups, respectively. Simultaneously, washing was found the common method to clean the fruits and vegetables. Mittal et al. 2014 reviewed that poor ground water quality in Malwa belt is one of the prominent reasons for incidence of many diseases including cancer. Blaurock-Busch et al. 2014 analyzed and compared the hair samples of 50 healthy individuals and 49 cancer patients in Malwa region of Punjab and reported that higher concentrations of lead, uranium and barium were found in the hair samples of breast cancer patients. Furthermore, the mean concentration of uranium among cancer patients was six times higher than the reference range of 0.1 μg/ g and was also present as more than double in control group. This could be due to increased metal burden in soil and water resulted from over use of phosphate fertilizers.

Table 4.2 (b) Lifestyle related Information of Prostate Cancer Patients (N= 200)

	No. of Patients	
Household practices	**Group I (N=100)**	**Group II (N=100)**
i. Storage containers for food commodities		
Empty pesticide containers (EPC)	00	00
Food grade plastic (FGP)	16	10
Steel containers (SC)	32	18
Ordinary plastic (OP)	32	14
SC + OP	16	42
EPC + SC	03	05
EPC + OP	00	00
FGP + SC	01	11
ii. Source of drinking water		
Treated source	51	35
Untreated source	49	65
iii. Method for cleaning of fruits and vegetables (Washing)	100	100
iv. Kitchen garden		
Practised	20	24
Not practised	80	76
v. Source of fruits and vegetables		
Kitchen garden	20	24
Farm	19	25
Organized Food Outlets	00	00
Vendors	61	51
vi. Source of milk		
Livestock	55	60
Commercial Dairy	45	40

Original figures represent the percentages

With respect to organic farming practices, only 20 and 24 percent of the subjects were having kitchen gardens in both the groups. Hence, vendors (61 & 51 percent) and farms (19 & 25 percent) were the main sources to avail fruits and vegetables for human consumption. Thus, presence of pesticide residues in foods could be one of the causative factors for development of prostate cancer. Alavanja et al. 2003 carried out a study on male farm workers (more than 50 years of age) spraying chlorinated pesticides and methyl bromide and the results revealed a significant association between use of pesticides and prostate cancer.

Apart from this, livestock rearing was observed among more than a half (55 and 60 percent) of the total population. Rest of the subjects relied on commercial dairy produce (45 and 40 percent) to meet their daily requirements of milk.

4.2.3 Health related information of prostate cancer patients

Health related information of the subjects was observed through recording family history of PCa in specific and other types of cancer, signs and symptoms (general and PCa specific), onset of the disease, other complications and aggravating factors (Table 4.3).

General symptoms viz. weight loss, hair loss, weakness, weight gain, fatigue, loss of appetite were assessed and found their percentage as 15 & 13, 04 & 02, 83 & 69, 00 & 00, 77 & 71 and 99 & 61 in the subjects of group I & II, respectively.

Among disease characteristics, lesser urine output and exertion during urination were experienced by all (100 percent) the patients in both the groups while symptoms such as pelvic pain and enlargement of prostate were common in most (82 & 94 percent and 95 & 87 percent) of the subjects of group I & II, respectively.

Constipation was also reported as a major complication by the subjects in group I (44 percent) and group II (31 percent), respectively. History of piles was also noted in 06 and 04 percent of the subjects in two groups. Frequent urination and off odor during urination were experienced by few (04 & 02 percent and 01 & 07 percent) subjects in group I and group II.

Onset of the disease as explained by the subjects was gradual (81 and 63 percent) in nature as most common irrespective of some exceptions. In contrast to this, some (14 and 19 percent) patients experienced the same as off and on.

Further, both exertion (39 & 81 percent) and diet (48 & 12 percent) had been reported as aggravating factors in disease progression. In addition, 13 and 07 percent of the subjects in group I and group II, were experiencing both (exertion and diet) the factors.

Table 4.3 Health related Information of Prostate Cancer Patients (N=200)

	No. of Patients	
	Group I (N=100)	**Group II (N=100)**
a. Family history of the disease		
Prostate Cancer	18	08
Other Cancers	10	06
b. Clinical signs and symptoms of Prostate Cancer		
i. General symptoms		
Weight loss	15	13
Hair loss	04	02
Weakness	83	69
Weight gain	00	00
Fatigue	77	71
Loss of appetite	99	61
ii. PCa specific symptoms		
Pelvic pain	82	94
Lesser urine output	100	100
Exertion during urine	100	100
Enlargement of prostate	95	87
c. Other complications		
Constipation	88	62
Piles	06	04
Frequent urination	04	02
Off odour during urination	01	07
d. Onset of the disease		
Gradual	81	63
Sudden	05	18
Off and on	14	19
e. Aggravating factor		
Exertion	39	81
Diet	48	12
Both	13	07

Original figures represent the percentages

4.2.4 Dietary habits and Nutrient intake of prostate cancer patients

i) Dietary habits: As far as the dietary pattern of the subjects was concerned, an opposite distribution of vegetarianism & non-vegetarianism as 49 & 43 percent and 43 & 51 percent was observed in the respondents of group I and group II, respectively (Table 4.4). Only 08 and 06 percent of the total population were ova-vegetarians.

Table 4.4 Dietary habits of Prostate Cancer patients (N=200)

	No. of Patients	
i. Dietary pattern	**Group I (N=100)**	**Group II (N=100)**
Vegetarian	49	43
Non-vegetarian	43	51
Ova-vegetarian	08	06
ii. Meal pattern		
Twice a day	39	35
Thrice a day	61	65
iii. Type of fat used to cook food		
Butter	27	29
Desi ghee	61	67
Mustard oil	80	66
Hydrogenated fat (*Dalda*)	78	62
Refined oil	31	23
All in combination	01	09

Original figures represent the percentages

During present investigation, it was found that 39 and 35 percent of population in both the groups were skipping breakfast in their major meals which was a notable risk factor for inadequate nutrient intake. It has also been shown in Table 4.4 that *dalda* (78 & 62 percent) and *desi ghee* (61 & 67 percent) were the saturated fats mainly consumed in group I & II, respectively. Consumptions of butter and refined oil were recorded only in 27 & 29 percent and 31 & 23 percent of the subjects in Group I and Group II. Moreover, combination of fats was practiced as unusual as 01 and 09 percent among the subjects of two groups, respectively. Walker et al. 2005 stated that dietary pattern had a positive correlation with increased risk of developing prostate cancer. The authors concluded that there was more than 2.5 fold increased risk of prostate cancer in subjects with high intake of fat. Richman et al. 2013 also reported a significant relationship between saturated fat intake and PCa mortality. Higher intake of saturated fat may increase the risk of progression of prostate tumor due to increased heterocyclic amines with carcinogenic properties produced during cooking of saturated fat at high temperature and human prostate is capable of activating these heterocyclic amines and binds to DNA (Strom et al. 2008).

ii) Nutrient intake: The average daily nutrient intake (Table 4.5) was calculated on the basis of average daily consumption of food recorded by 24 hour recall method and compared with the recommended dietary intake (ICMR 2010) are under:

a) Energy

The average daily intake of energy was recorded as 1556 & 1630 Kcal and 1779 & 1929 Kcal during initial & final phase of the study in the subjects of group I and group II, respectively which was deficient while compared to the RDA (2320 Kcal/ d). Whittemore et al. 1995 concluded that the energy consumption of American-Blacks was the highest (2455.8 Kcal) whereas the same was found as the lowest (2162.1 Kcal) among Asian-Americans. Further, Van Hoang et al. 2018 reported the mean intake of energy as low as 1712 Kcal/ d among PCa patients in Vietnam.

b) Carbohydrate

The mean intake of carbohydrate was 286.1 & 292.1 g/ d and 339.6 & 379.2 g/ d at the beginning & at the end of the study in group I and group II, respectively. Similarly, Ohno et al. 1988 reported the mean intake of carbohydrate as 291 g/ d among prostate cancer patients in Kyoto, Japan. Traditional diets with carbohydrates pattern were associated with PCa progression (Niclis et al. 2015) since higher consumption of carbohydrates promotes the insulin release which leads to boost PCa progression (Marvopoulos et al. 2009; Drake et al. 2012; Lin et al. 2015).

c) Proteins

The average daily intake of protein by the subjects in group I and group II was 41.5 & 42.0 g/ d and 44.2 & 48.5 g/ d at the beginning & at the end of the study, respectively and was found inadequate when compared to recommended intake of 60 g/ d (ICMR 2010). It was reported that the males above the age of 65 years with inadequate protein intake had higher risk for developing prostate cancer (Levine et al. 2014) and moreover, consumption of protein from animal and dairy sources had more risk in aggravating the disease by increasing serum IGF-1 (Insulin-like growth factor) whereas plant sources of protein due to presence of phytoestrogens have the potential to reduce the disease symptoms (Young et al. 2012).

d) Fat

The mean intakes of fat were 27.3 & 26.9 g/ d and 27.1 & 25.8 g/ d during the beginning & the end of the study in the subjects belonging to group I and group II, respectively which were reported as more than the recommended level (25 g/d). More than adequate fat intake (31.7 g/ d) was reported by Brar 2015 while conducting dietary survey on breast cancer patients. Ohno et al. 1988 too found a higher mean (46 g/ d) intake of fat among males suffering from prostate cancer in Japan. Bassett et al. 2013 conducted a cohort study on plasma saturated fatty acid level of PCa patients and

found that elevated levels were associated with PCa development in males. In a study, it was observed that risk of mortality due to prostate cancer was increased by 2.8 fold in males on 5 percent increase in total calories per day by saturated fat intake (Van Blarigan et al. 2015).

Table 4.5 Average daily nutrient intake of Prostate Cancer patients (N=200)

Nutrients	Mean±SD*				RDA**
	Group I (Initial) (N=100)	Group I (Final) (N=100)	Group II (Initial) (N=100)	Group II (Final) (N=100)	
Energy (Kcal/ d)	$1556±266.5^{d}$	$1630±202^{c}$	$1779±359.3^{b}$	$1929±304.4^{a}$	2320
Carbohydrate (g/ d)	$286.1±73^{d}$	$292.1±65^{c}$	$339.6±86^{b}$	$379.2±71^{a}$	----
Protein (g/ d)	$41.5±7.83^{d}$	$42.0±9.15^{c}$	$44.2±10.92^{b}$	$48.5±1.56^{a}$	60
Fat (g/ d)	$27.3±3.42^{a}$	$26.9±3.23^{c}$	$27.1±3.37^{b}$	$25.8±2.5^{d}$	25
β-carotene (µg/ d)	$2975±766^{c}$	$2643±842.5^{d}$	$3624±745.6^{b}$	$3871±652.4^{a}$	4800
Vitamin C (mg/ d)	$33.8±7.19^{c}$	$29.45±5.35^{d}$	$35.1±2.22^{b}$	$39.4±4.3^{a}$	40
Thiamine (mg/ d)	$1.26±0.15^{c}$	$1.02±0.33^{d}$	$1.43±0.25^{b}$	$1.61±0.41^{a}$	1.2
Riboflavin (mg/ d)	$1.49±0.41^{c}$	$0.99±0.23^{d}$	$1.81±0.21^{b}$	$1.90±0.3^{a}$	1.4
Niacin (mg/ d)	$16.3±3.33^{c}$	$14.0±2.21^{d}$	$16.8±3.01^{b}$	$17.4±1.29^{a}$	16
Calcium (mg/ d)	$619.1±77.1^{b}$	$596.5±89.5^{d}$	$605.5±83.2^{c}$	$641.1±79.9^{a}$	600
Phosphorus (mg/ d)	$569.6±134.2^{c}$	$557.2±114.1^{d}$	$584.4±131.9^{b}$	$621.1±123.4^{a}$	600
Iron (mg/ d)	$8.2±2.24^{c}$	$7.7±1.30^{d}$	$10.4±2.32^{b}$	$12.1±2.1^{a}$	17
Zinc (mg/ d)	$8.7±2.47^{c}$	$7.3±1.14^{d}$	$9.6±1.83^{b}$	$10.8 ±1.51^{a}$	12

*Values are Mean ± SD from hundred determinations; different superscripts in the same row are significantly different ($p<0.05$)

Initial and Final figures correspond to pre intervention and post intervention assessment.

****Recommended Dietary Allowances for Indian Males (ICMR 2010)**

e) β-carotene

The average daily intakes of β-carotene (μg/ d) were calculated as 2975 & 2643 and 3624 & 3871 μg/ d at the beginning & at the end of the study in two groups which were inadequate while compared to recommended intake of 4800 μg/ d. Sosanya et al. 2014 also reported the inadequate mean intake of β-carotene (13.4 mg) among prostate cancer patients in Nigeria. Van Hoang et al. 2018 reported the mean intake of β-carotene 2841.3 μg/ d among PCa patients in Vietnam. Moreover, it was observed that inadequate dietary intake of β-carotene among patients significantly lead to higher risk of prostate cancer while carrying out a study on 100 Japanese males suffering from benign hyperplasia (Ohno et al. 1988).

f) Vitamin C

The mean intake of vitamin-C was too found inadequate (33.8 & 29.45 mg/ d and 35.1 & 39.4 mg/ d) during initial & final phase of the study in the subjects of group I and group II, respectively when compared to the RDA (ICMR 2010). Ali et al. 2011 also stated insufficient intake of fresh fruits and use of high cooking temperature in preparation of Indian foods leading to low intake of vitamin C.

g) Thiamine

The consumption of thiamine was 1.26 & 1.02 mg/ d and 1.43 & 1.61 mg/ d at initial & final phase, in the subject of group I and II, respectively and was more than adequate in comparison with recommended intake of 1.2 g/ d (ICMR 2010).

h) Riboflavin

The average intake of riboflavin (1.49 & 0.99 mg/ d and 1.81 & 1.90 mg/ d at the beginning & at the end of the study in group I and group II, respectively) were too found beyond adequate intake levels of 1.4 mg/ d (ICMR 2010).

i) Niacin

The values for niacin intake were 16.3 & 14 mg/ d and 16.8 & 17.4 mg/ d for the subjects in group I and group II, respectively.

j) Calcium

The average calcium intake was 619.1 & 596.5 mg/ d and 605.5 & 641.1 mg/ d during initial & final phase of the intervention among the subjects in group I and group II, respectively and found inadequate in group I at the end of the study while compared with the RDA (600 mg/ d). Chan et al. 2001 reported that higher (>600 mg/ d) intake of calcium from skimmed milk was associated with higher (32 percent) risk of prostate cancer as the investigator observed the lower plasma levels of vitamin-D in the subjects. Vitamin-D hinders the cell proliferation through endorsement of prostatic cell differentiation (Esquenet et al. 1996), with the end result of reduced growth of malignant tumors (Schwartz et al. 1995; Williams et al. 2012).

k) Phosphorus

The phosphorus intake was observed as 569.6 & 557.2 mg/ d and 584.4 & 621.1 mg/ d during initial & final phase of the intervention among the subjects in group I and group II, respectively. The mean intake was found adequate in group II during final phase o the study on comparison with the recommended intake of 600 mg/ d.

l) Iron

Iron intake was observed as low as 8.2 & 7.7 mg/ d and 10.4 & 12.1 mg/ d at the beginning & at the end of the study among the subjects in Group I and Group II, respectively when compared with the recommended intake of 17 mg/ d. Lane et al. 2017 also reported an inadequate intake i.e. 13 mg/ d among prostate cancer patients in the United Kingdom.

m) Zinc

The mean zinc intake was 8.7 & 7.3 mg/ d and 9.6 & 10.8 mg/ d during initial & final phase of the intervention in the subjects of group I and group II, respectively and was below the recommended intake (12 mg/ d) for this nutrient. Gallus et al. 2007 found the mean zinc intake as low as 9.93 mg/ d among prostate cancer patients in Italy. Similar findings were reported by Lane et al. 2017. Besides, Epstein et al. 2011 reported that high dietary zinc intake was associated with lower prostate cancer mortality. The researchers revealed in their study that the group with inadequate intake of zinc (9.0 to 12.8 mg/ d) had higher number of deaths (60) as compared to the second group (46) with adequate zinc intake i.e. 12.8 to 14.1 mg/ d.

4.2.5 Anthropometric measurements of prostate cancer patients

The anthropometric measurements of the subjects in both the groups has been shown in Table 4.6. The average height measured for the subjects in two groups was 175.5 and 178.2 cm, respectively. A significant ($p<0.05$) difference was observed in the body weight of the subjects both at the beginning (70.13 & 70.53 kg) and at the end (67.12 & 72.60 kg) of the study in group I & group II, respectively. Further, a significant ($p<0.05$) increase was recorded in body mass index (BMI) of the patients belonging to group II (0.65 kg/ m^2) whereas in group I, a decline of 0.98 kg/ m^2 was noted. Both height and weight were found beyond the reference ranges (ICMR 2010). The average calculated BMI was found within the reference range (WHO 2004).

Table 4.6 Anthropometric measurements of Prostate Cancer patients (N=200)

Parameters	Mean±SD*				Reference value**
	Group I (N=100)		Group II (N=100)		
	Initial	Final	Initial	Final	
Height (cm)	175.5±7.42[a]		178.25±5.53[b]		161.4 - 172.3
Weight (kg)	70.13±6.75[a]	67.12±7.92[a]	70.53±8.15[ab]	72.60±6.96[bc]	48.9 - 66.6
BMI (kg/ m^2)	22.77±1.63[a]	21.79±1.59[a]	22.20±2.12[ab]	22.85±1.98[bc]	18.5 - 24.9***

*Values are Mean ± SD from hundred determinations; different superscripts in the same row are significantly different ($p<0.05$)

Initial and Final figures correspond to pre intervention and post intervention assessment.

** ICMR 2010 *** WHO 2004

Singh et al. 2013 revealed no significant ($p>0.05$) association between Body Mass Index (BMI) and Prostate-Specific Antigen (PSA) levels in prostate cancer patients of North India.

4.2.6 Biochemical assessment of prostate cancer patients

The average figures for biochemical parameters of the subjects have been presented in Table 4.7.

a) Prostate specific antigen (PSA): PSA has been extensively used as a reliable diagnostic tool in the screening for PCa. During present study, the mean scores for serum PSA were found 15.59 & 12.23 ng/ mL and 20.05 & 14.47 ng/ mL during initial & final assessment of the subjects in group I and group II, respectively which were 2 to 4 times higher than the reference range (0 to 4 ng/ mL). Amin et al. 2008 reported the mean serum PSA level as 13.4 µg/ L in males with prostate cancer. Serum prostate specific antigen decreased significantly ($p<0.05$) (from 20.05 to 14.47 ng/ mL) after the nutrition intervention in the subjects of group II. Ornish et al. 2005 while conducting nutritional intervention in the form of dietary and lifestyle changes in prostate cancer patients monitored a significant decrease of 4 percent in PSA level of experimental group whereas an increase of 6 percent was found in the average PSA levels of control group. Paur et al. 2017 carried out a nutrition intervention through supplementation of tomato products to the prostate cancer patients and observed a decrease in PSA level by 1 percent.

Table 4.7 Biochemical assessment of Prostate Cancer patients (N=200)

Parameters	Mean±SD*				Reference range**
	Group I		Group II		
	Initial	Final	Initial	Final	
Serum PSA (ng/ mL)	15.59±22.63[b]	12.23±18.6[d]	20.05±15.25[a]	14.47±9.03[c]	0.0 to 4.0
Bone Mineral Density	0.34±0.13[d]	0.48±0.23[c]	0.52±0.19[b]	0.57±0.16[a]	0 to -1.0
Serum albumin (g/ dl)	3.73±0.45[d]	4.15±0.55[b]	3.77±0.39[c]	4.40±0.28[a]	3.5 to 5.5
Serum Globulin (g/dl)	2.59±0.46[c]	2.50±0.43[d]	2.81±0.35[b]	3.30±0.28[a]	2.0 to 3.5
Hemoglobin (g/ dl)	11.18±1.75[c]	11.27±1.29[b]	9.79±1.39[d]	12.1±1.31[a]	12.5 to 17
Serum Vitamin-A (µg/ dL)	42.4±8.12[c]	29.2±6.24[d]	47.1±7.49[a]	42.9±7.87b	30 to 65
Serum Vitamin-C (mg/ dL)	0.76±0.26[b]	0.56±.21[d]	0.64±0.27[c]	0.85±0.22[a]	0.4 to 1.5
Serum Vitamin-E (µg/ mL)	13.95±3.67[b]	11.40±2.67[c]	14.10±3.11[a]	9.93±2.18[d]	5.5 to 17.0
Serum Iron (µg/ dl)	47.3±7.52[c]	54.1±10.6[a]	46.8±7.21[d]	52.4±6.53[b]	55 to 160
Serum Selenium (µmol/ l)	0.99±0.41[d]	1.00±0.39[c]	1.08±0.21[b]	1.12±0.13[a]	1.0 to 1.5
Serum Zinc (µmol/ l)	57.9±12.14[c]	61.53±11.23[b]	57.12±7.78[d]	76.30±5.71[a]	70 to 100

*Values are Mean ± SD from hundred determinations; different superscripts in the same row are significantly different ($p<0.05$)

Initial and Final figures correspond to pre intervention and post intervention assessment.

** Adesh Institute of Medical Sciences & Research, Bathinda.

b) Bone mineral Density (BMD): Furthermore, bone mineral density was assessed and the average values were reported within the normal range among both the stages in two groups. Further, this biochemical parameter was maintained with positive swell of 0.05 in group II. It has been explained that the lower BMD level and higher PSA levels were correlated since prostate cancer cells stimulate cytokines release which help in activating osteoclasts formation and bone resorption (Kim et al. 2009). Millar and Davison 2012 noted in their systematic review that adequate nutrition knowledge is imperative during the treatment in order to avert serious complication such as osteoporosis which may occur as a common side effect after androgen deprivation therapy (ADT).

c) Serum albumin and Serum globulin: It was evidently seen that the serum albumin and globulin diminished from 4.15 to 3.73 g/ dl and from 2.59 to 2.50 g/ dl among the subjects of first group while raised from 3.77 to 4.40 g/ dl and from 2.81 to 3.30 g/ dl, respectively in the patients of the latter group. Though, minimum acceptable ranges for these two parameters were maintained. Decreased albumin levels in the patients indicated the presence of inflammation and some inflammatory mediators such as interleukin-1 (IL-1), IL-6, necrosis factor α and acute phase reactants release which increase the transcapillary escape rate of albumin and decrease albumin synthesis by hepatocytes and recurrence of the tumor by increasing Gleason score thereby progression of the disease (Mantovaani et al. 2008; Sejima et al. 2013; Wang et al. 2017).

d) Hemoglobin: The average hemoglobin (Hb) levels of the subjects were 11.18 & 11.27 g/ dl and 9.79 & 12.1 g/ dl at the beginning and end of the study in group I and group II, respectively. Regarding Hb levels, observed values were found below the minimum reference value (12 g/ dl) whereas this figure was achieved by the subjects in group II after imparting nutrition counselling.

e) Serum Antioxidant status: Scores obtained for serum vitamin-A were at a significant ($p<0.05$) decline among respondents in group I at the end of the study. Conversely, the scores obtained for serum vitamin-C (0.76 & 0.56 mg/ dl and 0.64 & 0.85 mg/ dl) and serum vitamin-E (13.95 & 11.40 µg/ mL and 14.10 & 9.93 µg/ mL) at initial & final phases of the investigation in group I and group II, respectively were within the normal ranges. Berndt et al. 2005 concluded that there is no association between normal intake of vitamin C and development of prostate cancer. In addition to these, serum iron stores were reduced by approximately 6.8 µg/ dl in group I and it had been augmented by 5.6 µg/ dl in group II at the end of the study. Sarafanov et al. 2011 affirmed after analyzing post-prostatectomy tissue samples of 40 males that decreased concentration of both iron and zinc was inversely correlated to increased serum PSA level. The mean serum selenium level (0.99 µmol/ l) was found below the minimum reference value initially in group I patients while it was recorded within the reference range (1.00 µmol/ l) during final phase of the study. The average initial (1.08 µmol/ l) and final (1.12 µmol/ l) values for the above mentioned parameter were within the reference range (1.0 to 1.5 µmol/ l) among the subjects of group II. Brinkman et al. 2006 met-analyzed 20 studies to investigate the correlation of selenium intake with prostate cancer status and found that lower intake of the nutrient was associated with increasing rate of PCa since, selenium restrains cell proliferation during the disease hence induces apoptosis (Chan et al. 2005). Further, Adedapo et al. 2012 reported the mean selenium, iron, zinc and vitamin-E levels as 58.6±12.8 µg/ dl, 60.6±12.8 µg/ dl, 119.8±25.2 µg/ ml and 10.9±1.0 mg/ dl, respectively among prostate cancer patients and found no significant ($p>0.05$) difference between serum PSA and vitamin- E levels.

The average figures for serum zinc were recorded quite lower (57.9 and 57.12 µmol/ l in group I and II) at the beginning of the study when compared to the reference range (70 to 100 µmol/ l). A significant ($p<0.05$) increase in the mean serum zinc level was evidenced in both the groups but still below (61.53 µmol/ l) the normal (70 to 100 µmol/ l) range in group I. This figure returned to normal (76.30 µmol/ l) level in case of the mean score for group II patients subsequent to the nutritional intervention through providing individual nutrition counselling and information booklet. This could be the reason for improving serum PSA level of the respondents in group II at the end of the study. The relationship of zinc and Gleason score in North Indian prostate cancer patients had been investigated to find out the therapeutic implication of the nutrient to prolong the disease. The researchers observed that low level of serum zinc was associated with higher Gleason score (Zn: r = - 0.68 and G score: 7). These results suggested that improved zinc status may contribute to decrease tumor burden in stipulations of lesser Gleason score vis-à-vis sustain the prostate cell integrity (Abhishek et al. 2017).

4.3 Impact of Nutrition Counselling on knowledge, attitude, practices and health status of PCa patients

Poor health knowledge is associated with increased hospital rates as well as late cancer diagnosis (Baker et al. 2002; Wolf et al. 2005; Lindau et al. 2006; Bennett et al. 1998). Thus, health literacy is vital for helping the masses navigate the appropriate health care system leading towards maintenance of their health status. Health literacy through imparting nutrition counselling has gained advocacy as this type of education practice specifically emphasizes to motivate the sufferers' towards gaining understanding of the disease and using that information in the promotion and maintenance of good health (Nutbeam 1998; Friedman and Hoffman-Goetz 2008).

In present study, the impact of nutrition counselling was studied through pre and post intervention tests of the subjects with respect to their knowledge, attitude and practices during the disease (Table 4.8 (i)).

Table 4.8 (i). Distribution of the subjects according to KAP score (N=200)

Parameters	No. of patients			
	Group I (N=100)		Group II (N=100)	
	Pre test Score	Post test Score	Pre test Score	Post test Score
Knowledge				
1-8	100	100	100	22
9-17	00	00	00	78
18-25	00	00	00	00
Attitude				
1-5	100	85	97	00
6-10	00	15	03	66
11-15	00	00	00	34
Practice				
1-5	100	100	100	01
6-10	00	00	00	78
11-15	00	00	00	11

Original figures represent the percentages

Initial and Final figures correspond to pre intervention and post intervention assessment.

With the help of nutritional guidance highlighting consumption of diet rich in whole grains, fruits and vegetables and low in saturated fat, the overall health status of PCa patients can be improved (Demark-Wahnefried 2008). Gibbs et al. 2018 validated nutrition literacy assessment instrument and identified the relationship between nutrition education and diet quality. The scientists concluded that nutrition education had a significant ($p<0.01$) positive impact on diet quality. A significant decline in prostate cancer progression on raise in education level was observed during an interventional study (Mills et al. 1989). At the beginning of the study, all the subjects obtained lower scores for all the three parameters i.e. knowledge, attitude and practices in both the groups. The corresponding mean pre & post intervention test scores for knowledge, attitude and practices were observed as 4.3 & 6.2 and 7.4 & 19.8; 3.1 & 3.7 and 3.3 & 10.2 and 2.5 & 2.8 and 2.7 & 9.1 of the respondents in group I and group II, respectively (Table 4.8 (ii)). Bisht and Raghuvanshi 2007 reported in their study that gain in knowledge was found 47 percent through comic book and 37.13 percent though audio cassette during nutritional intervention. Further, a significant ($p<0.05$) difference was observed under gain in score in two groups during present study.

Table 4.8 (ii). Gain in KAP score after nutrition counselling

Parameters	Mean±SD*				Gain in Score**	
	Group I (N=100)		Group II (N=100)			
	Pre test Score	Post test Score	Pre test Score	Post test Score	Group I	Group II
Knowledge	4.3 ± 1.78^{d}	6.2 ± 2.04^{c}	7.4 ± 3.64^{b}	19.8 ± 2.33^{a}	1.9	12.4
Attitude	3.1 ± 1.35^{d}	3.7 ± 1.71^{b}	3.3 ± 1.51^{c}	10.2 ± 2.23^{a}	0.6	6.9
Practices	2.5 ± 1.04^{d}	2.8 ± 1.43^{b}	2.7 ± 1.02^{c}	9.1 ± 1.70^{a}	0.3	6.4
Overall Mean ± S. D.	3.3 ± 1.39^{d}	4.2 ± 1.73^{c}	4.5 ± 2.10^{b}	13.0 ± 2.16^{a}	1.1	8.5

*Values are Mean ± SD from hundred determinations; different superscripts in the same row are significantly different ($p<0.05$)

Gain in Score = Post test score - Pre test score

Evidently, there was almost two fold increment in quantum of improvement in nutrition knowledge of the respondents in group II as compared to the subjects in group I (Figure 4.6). During a pilot study on twenty males with prostate cancer from New Zealand, the effect of Mediterranean diet was studied for three months and compared the biochemical parameters i.e. prostate specific antigen (PSA) of experimental group with the control group. It was observed that the patients without medical treatment were benefitted with their PSA doubling time. It has been recommended that PSA level can be decreased on three month of dietary shift of plant based diet (Erdrich et al. 2015). Further, Baguley et al. 2017 reviewed that dietary intervention through suggesting healthy eating guidelines can improve efficacy of nutrition management and alleviate cancer-related fatigue.

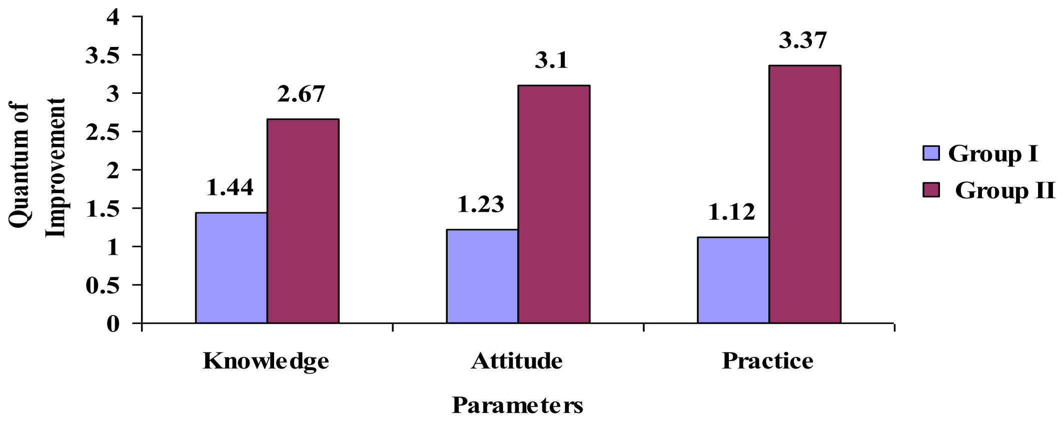


Figure 4.6 Quantum of Improvement in KAP scores of prostate cancer patients

***Quantum of Improvement = Post test score / Pre test score**

It is also concluded that a healthy lifestyle by adopting an appropriate dietary pattern exerts a protective role against development and progression of prostate cancer (Capurso and Vendemiale 2017).

4.4 Development, physico-chemical and sensory evaluation of different types of value added nutraceutical products for PCa prevention

Refined wheat flour is main ingredient for variety of baked food products but lacks in dietary fiber; amino acid particularly lysine and other micronutrients such as vitamins and minerals. Till now, numerous cereal-pulse flour blends based value added products are developed and evaluated by various researchers and wheat soya based products are found suitable due to their cost effective trait and recommended for improving the nutritional profile of the developed products in order to obtain their therapeutic aspect. Apart from this, combination of these two food stuffs have been considered as an effective remedy for their total antioxidant activity in human body during deadly diseases such as cancer. Besides, incorporation of both whole fruits and vegetables and their by-products (apple pomace powder, mango peel, orange peel etc.) as functional and bioactive ingredients become a latest trend in the food industry targeting the increased health knowledge and concern among consumers (Pakhare et al. 2018). Numerous scientists scrutinized the effect of bioactive compounds present in various plant based foods and herbal nutraceuticals such as soy (Krishna Moorthy and Venugopal 2008), tomato (Wei and Giovannucci 2012; Zu et al. 2014), ginger (Rashid and Umamaheswari 2017; Karna et al. 2012; Brahmbhatt et al. 2013), citrus peels (Rawson et al. 2014; Rafiq et al. 2018; Wang et al. 2014) and curcumin (Cimino et al. 2012) for their potential to target prostate malignancy. Thus, three types of nutraceutical products i.e. two types of baked products (biscuits and muffins) and an extruded product (noodles with three different flavors) using whole wheat flour and defatted soya flour as composite flour supplemented with fruit and vegetable produce and their by-products have been developed and evaluated in order to prevent prostate cancer in future generations. The detailed results are given as follow:

4.4.1 Chemical analysis of raw ingredients

i) Proximate composition of raw ingredients

The proximate composition of raw ingredients used, during preparation of biscuits, muffins and noodles has been presented in Table 4.9. Amount of moisture present in the flour is dependent of, both atmospheric and processing conditions (Reddy et al. 2015).

Table 4.9 Proximate composition of raw ingredients

Sample	Moisture (%)	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Carbohy--drate (%)
Defatted Soya Flour	7.79±0.41^{e}	59.21±0.91^{a}	0.75±0.11^{e}	4.28±0.18^{c}	7.38±0.39^{a}	20.59±0.41^{e}
Wheat Flour	11.4±0.36^{b}	9.26±0.28^{c}	1.15±0.13^{d}	0.00±0.00^{f}	0.60±0.06^{e}	77.59±0.59^{a}
Wheat Bran	7.27±0.35^{f}	25.59±0.88^{b}	4.62±0.27^{b}	1.97±0.21^{d}	2.12±0.12^{d}	58.43±0.44^{c}
Tomato	93.96±0.26^{a}	1.57±0.35^{f}	0.28±0.04^{f}	0.85±0.11^{e}	0.47±0.16^{f}	2.87±0.34^{f}
Tangerine Peel	7.9±0.35^{d}	5.3±0.11^{e}	2.2±0.30^{c}	11.14±0.22^{a}	4.48±0.23^{c}	68.98±0.42^{b}
Ginger	8.2±0.36^{c}	12.4±0.25^{d}	7.73±0.25^{a}	10.47±0.28^{b}	4.75±0.14^{b}	56.45±1.04^{d}

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

The moisture content of key flours viz. defatted soya flour, refined wheat flour and whole wheat flour ranged from 7.79 to 11.4 percent. Suriya et al. 2017 reported the moisture range from 9.53 to 11.34 percent in while developing composite flour from refined wheat flour and elephant foot yam flour, for development of functional cookies. The corresponding values for moisture content in nutraceuticals such as wheat bran, tomato, tangerine peel powder and ginger were 7.27, 93.96, 7.9 and 8.2 percent, respectively, in present investigation. The crude protein content was estimated as 59.21 and 9.26 percent in two flours i.e. defatted soya flour and wheat flour. It was recorded that wheat bran, tomato, tangerine peel powder and ginger were containing 25.59, 1.57, 5.3 and 12.4 percent protein. Among above-mentioned ingredients, defatted soya flour had the highest protein content, may be, due to, its high water holding capacity and low moisture content (Rababah et al. 2006; Ayele et al. 2017). Little amount of fat has been detected, in defatted soya flour (0.75 percent) and tomato (0.28 percent), among all the ingredients.

At the same time, tangerine peel powder was analyzed, with relatively higher (11.14 percent) amount of fiber, among all nutraceuticals, having fair amounts of pectin (Abou-Arab et al. 2017; Obafaye and Omoba 2018). These findings were in agreement with USDA National nutrient database (10.68 percent) (USDA Food Composition databases 2018). Notable amount of minerals, in the form of ash content was observed in defatted soya flour (7.38 percent), and might be attributed to higher amount of total dry solids as well as emulsifying properties when compared with other ingredients (Amin et al. 2017), followed by tangerine peel powder (4.48 percent), and ginger (3.75 percent), particularly, due to presence of good amounts of potassium in it (Gopalan et al. 2004). Furthermore, the computed values for carbohydrate were reported as 20.59, 77.59, 58.43, 2.87, 68.98 and 56.45 percent in defatted soya flour, wheat flour, wheat bran, tomato, tangerine peel powder and ginger, respectively. The observed values for above-mentioned nutrients were close to the reference values reported by Gopalan et al. 2004.

ii) *In vitro* protein digestibility and Amino acid composition

Results regarding *in vitro* protein digestibility and amino acid content in various raw ingredients have been presented in Table 4.10.

a) Lysine

Lysine content ranged from 0.0 to 3.71 g/ 100 g protein. Defatted soya flour and wheat bran contained 3.71 and 3.64 g lysine/ 100 g protein, respectively. Further, no amounts were recorded in ginger and tangerine peel powder. The observed values were in accordance to the reference values reported by Gopalan et al. 2004.

Table 4.10 *In vitro* protein digestibility and Amino acid composition of raw ingredients

Sample	In vitro protein digestibility	Lysine (g/ 100 g protein)	Methionine (g/ 100 g protein)
Defatted Soya Flour	67.28±1.74[a]	3.71±0.22[a]	0.71±0.05[c]
Wheat Flour	51.94±1.61[b]	1.06±0.05[d]	0.83±0.02[b]
Wheat Bran	28.03±0.90[e]	3.64±0.21[b]	0.97±0.05[a]
Tomato	50.31±1.46[c]	1.44±0.12[c]	0.42±0.08[d]
Tangerine Peel	18.58±0.49[f]	0.0±0.0	0.0±0.0
Ginger	35.83±0.64[d]	0.0±0.0	0.0±0.0

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

b) Methionine

During present study, wheat bran was observed with 0.97 g/ 100 g protein) content of methionine. Similarly, in wheat flour, defatted soya flour and tomato, it was 0.83, 0.71 and 0.42 g/ 100 g protein, respectively. Negligible amounts of this essential amino acid were recorded in tangerine peel powder and ginger. These figures were in agreement with the reference values reported by Gopalan et al. 2004.

iii) Vitamins

Vitamin content of different ingredients has been presented in Table 4.11 and the detailed findings are discussed as under:

Table 4.11 Vitamin content of raw ingredients

Sample	Vitamin-A (µg/ 100 g)	Vitamin-C (mg/ 100 g)	Vitamin-E (mg/ 100 g)
Defatted Soya Flour	402±1.98[b]	0.0±0.0	0.07±0.01[d]
Wheat Flour	19.7±0.2[e]	0.0±0.0	0.0±0.0
Wheat Bran	0.0±0.0	0.0±0.0	0.71±0.03[b]
Tomato	144±1.23[c]	26.1±0.05[b]	0.91±0.06[a]
Tangerine Peel	554.2±4.14[a]	28.7±1.24[a]	0.26±0.02[c]
Ginger	24.2±0.37[d]	3.1±0.12[c]	0.0±0.0

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

a) Vitamin A

Vitamin A content ranged from 19.7 to 554.2 μg/ 100 g of the raw ingredients. Lesser (24.2 and 19.7 μg) contents have been reported in ginger and wheat flour whereas wheat bran was lacking in vitamin A content. These results were in accordance to the reference nutritive values for Indian foods (Gopalan et al. 2004).

b) Vitamin C

Cereal and legume flours contains no vitamin C (Gopalan et al. 2004). Sufficient amounts of this vitamin were reported in tangerine peel powder (28.7 mg) and tomato (26.1 mg), respectively. Furthermore, this figure was noted as 3.1 mg in case of ginger.

c) Vitamin E

It was observed that tomato and wheat bran possessed good amounts of vitamin E as the corresponding value were 0.91 and 0.71 mg per 100 g of each ingredient, respectively. In contrast to this, both refined wheat flour and ginger had no vitamin E content. The observed values for above-mentioned nutrients were close to the reference values reported by Gopalan et al. 2004.

iv) Minerals

The contents of important minerals in terms of calcium, phosphorus, iron and zinc were estimated in all the raw ingredients which are shown in Table 4.12. It was reported that highest (201.4 and 157.3 mg) calcium content have been found in defatted soya flour and tangerine peel powder whereas, lowest (18.9 and 12.14 mg) were observed in wheat flour and tomato, respectively.

Table 4.12 Mineral content of raw ingredients

Sample	Calcium (mg/ 100 g)	Phosphorus (mg/ 100 g)	Iron (mg/ 100 g)	Zinc (mg/ 100 g)
Defatted Soya Flour	201.4 ± 1.2^{a}	663.4 ± 1.56^{b}	6.7 ± 0.8^{b}	2.1 ± 0.12^{b}
Wheat Flour	18.9 ± 0.61^{e}	108.9 ± 0.33^{c}	1.9 ± 0.2^{e}	0.45 ± 0.07^{e}
Wheat Bran	34.6 ± 0.45^{d}	799.2 ± 0.66^{a}	4.15 ± 0.21^{c}	1.85 ± 0.1^{c}
Tomato	12.14 ± 0.56^{f}	31.6 ± 0.17^{e}	1.1 ± 0.04^{f}	0.0 ± 0.0^{f}
Tangerine Peel	157.3 ± 1.16^{b}	17.1 ± 0.31^{f}	7.87 ± 0.4^{a}	6.96 ± 0.34^{a}
Ginger	61.2 ± 0.32^{c}	53.8 ± 0.42^{d}	2.7 ± 0.3^{d}	0.67 ± 0.03^{d}

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

As the level of phosphorus is concerned, wheat bran and defatted soya flour were loaded with this mineral possessing 799.2 and 663.4 mg of it, respectively. Further, iron was present in fairly good amounts in tangerine peel powder, defatted soya flour and wheat bran, as the corresponding values were 7.87, 6.7 and 4.15 mg/ 100 g of above-mentioned ingredients. Besides, figures for zinc content were recorded as 6.96, 2.1 and 1.85 mg in these ingredients, respectively. The observed values for above-mentioned nutrients were close to the reference values reported by Gopalan et al. 2004. Further, it is reported that 44.45 percent of the total vitamins and minerals are present in germ and bran parts of grains (Majzoobi et al. 2014). Similarly, in present study, a significantly ($p<0.05$) higher content of vitamins and minerals was recorded in wheat flour and wheat bran as compared to refined wheat flour.

v) Anti nutritional factors

Findings regarding anti nutritional factors i. e. total phenols (mg GAE/ 100g) and trypsin inhibitor (mg/ g) have been shown in Table 4.13. The maximum (1203.2 mg GAE/ 100 g) total phenol content was reported in wheat bran followed by tomato (221.6 mg GAE/ 100 g) and wheat flour (164.3 mg GAE/ 100 g), respectively.

Table 4.13 Anti nutritional content of raw ingredients

Sample	Total Phenols (mg GAE/ 100 g)	Trypsin Inhibitor (mg/ g)
Defatted Soya Flour	10.8 ± 0.51^{f}	4.7 ± 0.4^{a}
Wheat Flour	164.3 ± 2.49^{c}	0.0±0.0
Wheat Bran	1203.2 ± 6.48^{a}	0.0±0.0
Tomato	221.6 ± 2.54^{b}	0.0±0.0
Tangerine Peel	31.72 ± 1.02^{d}	0.0±0.0
Ginger	12.4 ± 0.74^{e}	0.0±0.0

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Simultaneously, trypsin inhibitor was present as 4.7 mg/ g in defatted soya flour while it was absent in all other ingredients. Toasted defatted soya flour was used during present study and it was reported that trypsin inhibitor residues were present in the flour after toasting (Sessa and Bietz 1986).

vi) Total Antioxidant activity

Total antioxidant activity of raw ingredients has been presented in Figure 4.7. In present study, three ingredients i.e. tomato, tangerine peel powder and ginger have shown more effective total antioxidant activity (>50 mg TE/ 100 mg) to quench free radicals as compared to other ingredients.

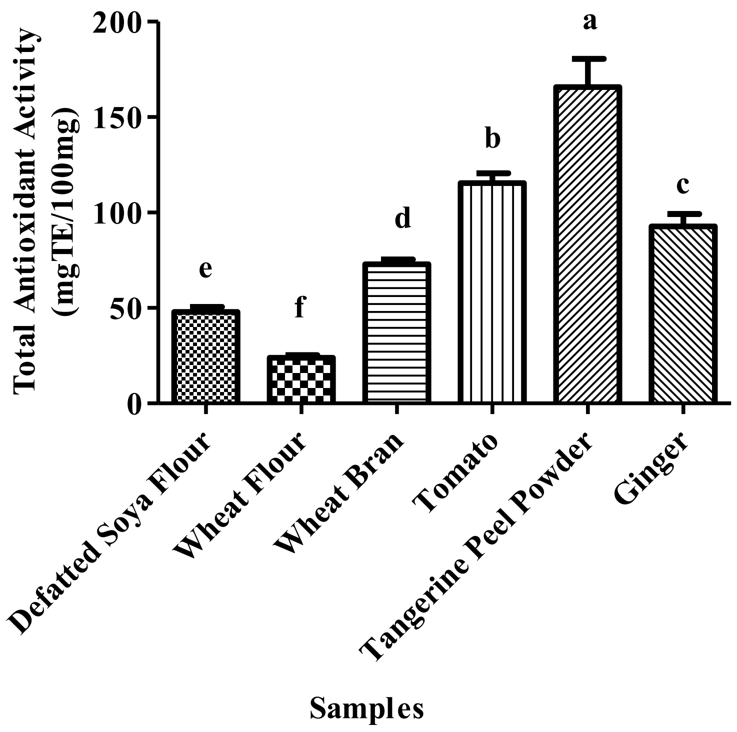


Figure 4.7 Total Antioxidant Activity of raw ingredients

Whole grains and other plant materials such as fruits, vegetables and spices & herbs are the good sources of phenolic acids which is known for its antioxidant potential to scavenge free radicals through inhibition of lipid peroxidation in human body. Thus, these functional ingredients can be beneficial for prostate cancer prevention (Dziki et al. 2014).

4.4.2 Development of biscuits

Biscuits were standardized as per the methods discussed in Table 3.1 and 3.2 under chapter 3: Materials and methods.

4.4.2.1 Physical characteristics of biscuits

a) Physical characteristics of control biscuits

The physical characteristics of control biscuits have been shown in Table 4.14. The mean weight of T_3 (8.8 g) was found significantly ($p<0.05$) higher when compared to the mean weight of T_1 (8.3 g). Further, diameters of developed products ranged from 4.19 to 4.3 cm. A significant ($p<0.05$) difference was observed between diameters of all the three treatments. Increase or decrease in the diameter of the product particularly depends on rate of melting of fats during baking. The higher rate makes bound water present in dough mixture available to dissolve the sugar which facilitates the increase in diameter of the final product (Lai and Lin 2006).

Table 4.14 Physical evaluation of control biscuits

Parameters	T_0	T_1	T_2	T_3
Weight (g)	8.7 ± 0.06^b	8.30 ± 0.15^d	8.53 ± 0.15^c	8.80 ± 0.08^a
Diameter (cm)	4.3 ± 0.04^a	4.19 ± 0.08^d	4.24 ± 0.12^c	4.29 ± 0.09^b
Thickness (cm)	0.49 ± 0.05^d	0.53 ± 0.07^a	0.51 ± 0.10^b	0.50 ± 0.04^c
Spread ratio (D/T)	8.77 ± 0.75^a	7.89 ± 0.90^d	8.47 ± 1.52^c	8.51 ± 0.84^b
Hardness (N)	37.2 ± 0.15^d	46.3 ± 0.26^a	42.4 ± 0.21^b	38.4 ± 0.35^c

*Values are Mean± SD from four determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

Similarly, in present research, butter was used as biscuit shortening rather than hydrogenated fat or other vegetable oils, could be beneficial, in dissolving a major part of sugar, prior to baking. Further, higher thickness was recorded in T_3 (0.53 cm) as compared to T_0 (0.49 cm), T_1 (0.50 cm) and T_2 (0.51 cm), respectively. Spread ratio is an ability of cookies to raise, hence the lower the value, the better the ability. The figures for spread ratio were observed as 8.77, 7.89, 8.47 and 8.51 in T_0, T_1, T_2 and T_3, respectively, during present study. Lower proportion of legume flour added to higher proportion of wheat flour results in absorption of more water during baking process and attributes towards increment in the spread ratio (Lai and Lin 2006).

b) Physical characteristics of value added biscuits

A significant difference ($p<0.05$) in weight was observed among both the value added treatments (Table 4.15). The mean weight of T_4 and T_5 was 10.5 g was 10.9 g, respectively. Diameters of the developed products have shown no significant difference ($p>0.05$) as the mean scores for T_4 and T_5 were found to be 4.61 cm and 4.48 cm, respectively. Though, possessing similar amount of sucrose as in control treatments but addition of various nutraceuticals, containing higher amounts of fiber, contributed towards increments in diameters of value added treatments. Diameter of the cookies or biscuits is dependent on sugar dissolved before and during baking process. The dough mixture with additional fiber content along with sucrose, results in final baked product with higher diameter as compared to the product developed from the dough with only sucrose in it because sugars with higher fiber content dissolve completely prior to baking whereas sucrose continues to be dissolved during baking (Handa et al. 2012).

Table 4.15 Physical evaluation of value added biscuits

Parameters	**T_4**	**T_5**
Weight (g)	10.5 ± 0.12^b	10.9 ± 0.21^a
Diameter (cm)	4.61 ± 0.09^a	4.48 ± 0.10^b
Thickness (cm)	0.62 ± 0.03^b	0.67 ± 0.04^a
Spread ratio (D/T)	7.37 ± 0.34^a	6.66 ± 0.55^b
Hardness (N)	40.8 ± 0.80^b	44.4 ± 0.39^a

*Values are Mean± SD from four determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5= 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper .

The values for thickness of T_4 and T_5, were recorded as 0.62 cm and 0.67 cm and showed a significant ($p<0.05$) difference with each other, similarly, these figures were found significantly higher ($p<0.05$), when compared to control treatments. Simultaneously, the corresponding values for spread ratio were 7.37 and 6.66 in T_4 and T_5, respectively. Saleh et al. 2012 observed that as the thickness of biscuits was increased, the value for spread ratio decreased. The authors explained that the thickness had been increased, with supplementation of defatted soya flour to wheat flour during biscuit making process. Moreover, biscuits' potential for spread ratio rely upon three factors; the spread ability of flour, activity of leavening agent and amount of heat during baking. Besides, composite flour mixture enhances the dough viscosity as hydrophilic sites increase which leads to rapid partitioning of free water during dough making process and ultimately limits the cookie spread and top grain formation during baking of the product (Rababah et al. 2006). Therefore, a significant difference ($p<0.05$) between the level of hardness was too observed among two value added treatments, may be, for the reason that T_4 was incorporated with tomato pulp and T_5 with tangerine peel powder, maintaining the product softness in T_4, provided with higher level of moisture in it.

4.4.2.2 Chemical analysis of biscuits

i) Proximate composition of control biscuits

Table 4.16 depicts results of proximate composition of control biscuits.

a) Moisture

The moisture content of three treatments ranged between 8.10 and 9.60 percent. A significant decrease ($p<0.05$) was recorded in moisture content of biscuits, on every 10 percent increase in level of defatted soya flour incorporation. Moisture content gradually decreases, with the increase in soy flour proportion, due to high emulsifying properties and low moisture content of soy flour as compared to wheat flour (Farzana and Mohajan 2015).

Table 4.16 Proximate composition of control biscuits

Parameters	T_0	T_1	T_2	T_3
Moisture (%)	9.60±0.13^a	8.10±0.13^d	8.29±0.16^c	8.32±0.11^b
Protein (%)	8.78±0.15^d	31.78±0.23^a	29.23±0.24^b	27.6±0.36^c
Fat (%)	15.7±0.23^d	17.59±0.39^c	18.94±0.38^b	20.36±0.30^a
Fiber (%)	0.00±0.0	2.84±0.08^a	2.50±0.13^b	2.14±0.11^c
Ash (%)	0.8±0.2^d	4.19±0.21^a	3.71±0.16^b	3.32±0.18^c
Carbohydrate (%)	65.12±0.52^a	35.50±0.49^d	37.48±0.37^c	38.29±0.11^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

b) Crude protein

The highest (31.78 percent) crude protein content was found in T_1 and the lowest (27.6 percent) was observed in T_3. Hasker et al. 2016 found a significant ($p<0.05$) increase in the protein content, on incorporation of soya flour to wheat flour during formulating functional biscuits. An approximate increase of 5 percent was recorded on 10 percent incorporation level of defatted soya flour to wheat flour (Amin et al. 2017).

c) Crude fat

The crude fat content varied from 17.59 to 20.36 percent. Similarly, an approximate increase of 3 percent was recorded, while comparing composite flour cookies to the control (Suriya et al. 2017). Ranjitha et al. 2018 reported 21.56 percent fat content in cookies prepared using defatted soya flour to refined wheat flour at 30 percent level.

d) Crude fiber

T_1 possesses significantly ($p<0.05$) higher (2.84 percent) amounts of crude fiber content as compared to other samples. The lowest content was 2.14 percent found in T_3. It was not detected in T_0.

e) Total Ash

The values recorded for ash content, depict the mineral content present in a particular food product. Maximum percentage (4.19) of ash was found in T_1 followed by T_2 (3.71) and T_3 (3.32), respectively. An increase in ash content was reported, on increase in defatted soy flour level which has been shown in accordance with the results obtained by Ayo et al. 2014. High (4.23 percent) ash content was reported in bread developed combining wheat flour and soya flour in the ratio of 70:30 (Ayele et al. 2017).

f) Carbohydrate

With regard to carbohydrate content, figures were recorded as 65.12, 38.29, 37.48 and 35.50 percent in $T_{0,}$ T_3, T_2 and T_1, respectively. It is evidenced that incorporation of legume flour to cereal flour, results in diluting the carbohydrate content in the final product (Olapade and Adeyemo 2014; Masur et al. 2009).

ii) Proximate composition of value added biscuits

Proximate composition is considered to be highly important as it portrays the effect of processing on nutrient content of the final product. The value added products developed by combining wheat flour and defatted soy flour, found to have significantly ($p<0.05$) higher protein and fiber contents than control treatments (Table 4.17).

a) Moisture

The moisture content of T_4 and T_5 was 10.42 and 9.38 percent, respectively.

b) Crude protein

The crude protein content in T_4 and T_5 was 34.96 and 33.6 percent, respectively. These figures were significantly higher ($p<0.05$) when compared to control treatments. Likewise, higher amounts of protein content in defatted soy flour and wheat flour based biscuits were reported when compared to samples with refined wheat flour (Zaker et al. 2012). Further, Salim et al. 2017 reported the range of protein from 17.10 to 19.25 percent in wheat soybean biscuits with soybean incorporation levels from 10 to 30 percent, respectively.

Table 4.17 Proximate composition of value added biscuits

Parameters	**T_4**	**T_5**
Moisture (%)	10.42 ± 0.21^a	9.38 ± 0.18^b
Protein (%)	34.96 ± 0.46^a	33.6 ± 0.13^b
Fat (%)	22.49 ± 0.18^a	22.31 ± 0.17^b
Fiber (%)	4.09 ± 0.08^b	4.46 ± 0.15^a
Ash (%)	5.39 ± 0.21^b	5.42 ± 0.20^a
Carbohydrate (%)	22.65 ± 0.24^b	24.83 ± 0.21^a

*Values are Mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

c) Crude fat

Crude fat content of two value added treatments was estimated as 22.49 and 22.31 percent, correspondingly. A significant ($p<0.05$) difference was observed, in the fat content of two value added treatments. These results were supported by various investigators (Farzana and Mohajan 2015; Akubor and Ukwuru 2005; Ayo et al. 2014). Further, Adekunle et al. 2007 reported the increased fat content from 14.80 to 24.01 percent in wheat soybean supplemented biscuits in the ratio of 70: 30. The range of fat content was reported from 18.29 to 21.81 percent by Adegoke et al. 2017 while preparing value added biscuits from wheat and soya flours.

d) Crude fiber

Further, enrichment of value added biscuits with bioactive compounds/ nutraceuticals (in the form of value addition of wheat bran and tangerine peel powder) contributed towards increased fiber contents. It can be evident from Table 4.17 that T_5 (4.46 percent) had significantly ($p<0.05$) higher amounts of fiber, as compared to T_4 (4.09 percent). Simultaneously, significant ($p<0.05$) increase in crude fiber content, on 10 percent addition of tangerine peel powder to wheat flour (Youssef and Mousa 2012) and every 5 percent increase of tangerine peel incorporation to pearl millet, was observed (Obafaye and Omoba 2018). Similarly, the cookies incorporated with composite flour (wheat, cassava and cowpea) at four different levels i.e. 30/ 50 / 20, 35/ 35/ 30, 20/ 70/ 10 and 00/ 80/ 20, respectively, were developed. Developed products were analyzed for their nutritional composition and observed that substitution of cassava flour with cowpea flour increased the fiber content from 0.63 per cent to 0.80 percent in the cookies, in view of the fact that cowpea possesses more fiber as compared to cassava (Olapade and Adeyemo 2014). Ahmed et al. 2016 reported the fiber content range between 1.14 to 2.03 percent in sorghum, maize and chickpea flour biscuit formulations at 10, 20 and 30 percent incorporation levels, respectively.

e) Total Ash

The mean values for ash content were recorded as 5.39 and 5.42 percent in T_4 and T_5, respectively, establishing the fact of significantly ($p<0.05$) higher mineral content in value added treatments than controls. Saleh et al. 2012 evaluated the effect of partial substitution of wheat flour with defatted soya flour and chickpea flour at 5, 10 and 15 percent levels while preparing dough for biscuit preparation. The researchers found that there was an ascending increase in both fiber and ash contents, on each increased level of incorporation in defatted soya flour and chickpea flour supplementation. Bashir et al. 2015 observed ash content as 1.77 percent on wheat soybean ratio of 85:15 during biscuit preparation in their study. A significantly ($p<0.05$) higher ash content in value added biscuits was reported on 10 percent incorporation of tangerine peel powder to wheat flour as compared to control biscuits (Youssef and Mousa 2012).

f) Carbohydrate

The carbohydrate content of T_4 and T_5 was 22.65 and 24.83 percent, respectively. Nutraceutical fortification in value added biscuits attributed towards the decreased carbohydrate content. Similar findings have been reported in the work of several researchers (Farzana and Mohajan 2015; Olapade and Adeyemo 2014; Masur et al. 2009).

ii) *In vitro* protein digestibility and Amino acid composition

Results regarding *in vitro* protein digestibility and amino acid composition of control and value added biscuits have been presented in Table 4.18. A significant ($p<0.05$) difference was observed in *in vitro* protein digestibility of all treatments of biscuits.

It was reported that T_3 had the highest (76.54 percent) protein digestibility among control samples. Further, these values were 73.87 and 71.48 percent in T_5 and T_4, respectively when value added biscuit samples were detected for the same (Table 4.18). Kumar et al. 2018 developed multigrain premix from wheat and soybean blended biscuits and compared those with wheat biscuits. The researchers revealed that multigrain biscuits up to 40 percent level of pulses supplementation had highest (71.73 percent) *in vitro* protein digestibility on comparison with control biscuits (38.13 percent).

Table 4.18 *In vitro* protein digestibility and Amino acid composition of biscuits

Sample	*In vitro* protein digestibility	Lysine (g/ 100 g protein)	Methionine (g/ 100 g protein)
T_0	55.62 ± 0.55^{f}	1.04 ± 0.05^{f}	0.77 ± 0.03^{f}
T_1	74.23 ± 0.55^{c}	2.43 ± 0.12^{c}	1.61 ± 0.15^{c}
T_2	75.43 ± 0.26^{b}	2.21 ± 0.13^{d}	1.41 ± 0.26^{d}
T_3	76.54 ± 0.79^{a}	1.98 ± 0.19^{e}	1.27 ± 0.23^{e}
T_4	71.48 ± 0.59^{e}	4.24 ± 0.14^{a}	2.18 ± 0.33^{a}
T_5	73.87 ± 0.56^{d}	4.01 ± 0.15^{b}	2.07 ± 0.5^{b}

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5= 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

a) Lysine

The lowest lysine content was found in T_0 (Table 4.18). The mean lysine contents were 2.43, 2.21 and 1.98 g/ 100 g protein in T_1, T_2 and T_3 samples. In contrast to these, T_4 and T_5 had increased contents of lysine as the reported figures were 4.24 and 4.01 g/ 100 g protein in these value added samples. Okoye et al. 2016 reported the value for lysine content as 4.47 g/ 100 g protein in wheat soybean (70:30) composite flour.

b) Methionine

As the methionine content was concerned, T_2 had the highest (1.61 g/ 100 g protein) amount of this amino acid as compared to T_1 (1.41 g/ 100 g protein) and T_3 (1.27 g/ 100 g protein), respectively. Among value added biscuit samples, T_4 contained slightly higher (2.18 g/ 100 g protein) methionine content in comparison to T_5 (2.07 g/ 100 g protein). Olagunju et al. 2018 formulated nutritious crackers using wheat flour and pigeon pea in the ratio of 70: 30 and observed 2.88 g methionine content per 100 g of protein in the final product.

iii) Vitamins

Vitamin content of both control and value added biscuit samples has been presented in Table 4.19.

a) Vitamin A

A significant ($p<0.05$) difference was observed in vitamin A content of all the treatments. It was found that among control biscuit samples, T_1 had the highest (1110.4 µg) vitamin A content as compared to T_2 (1067.1 µg) and T_3 (1028.9 µg), respectively. In value added treatments, vitamin A was reported as 1119.2 and 1331.5 µg in T_4 and T_5, respectively. Higher content of vitamin A in T_5 attributed to defatted soya flour and tangerine peel powder combination.

b) Vitamin C

Vitamin C was not present in all the control treatments whereas treatments developed after value addition i.e. T_4 and T_5 possessed 4.7 and 5.6 mg of vitamin C in these samples. It was due to incorporation of vitamin C rich ingredients such as tomato and tangerine peel powder in value added biscuits though cereal and pulse flours do not contain vitamin C.

Table 4.19 Vitamin content of biscuits

Sample	Vitamin-A (µg/ 100 g)	Vitamin-C (mg/ 100 g)	Vitamin-E (mg/ 100 g)
T_0	115.2 ± 6.2^f	0.0±0.0	0.0±0.0
T_1	1110.4 ± 6.2^c	0.0±0.0	0.04 ± 0.0^c
T_2	1067.1 ± 4.2^d	0.0±0.0	0.03 ± 0.0^d
T_3	1028.9 ± 8.6^e	0.0±0.0	0.02 ± 0.0^e
T_4	1119.2 ± 4.9^b	4.7 ± 0.42^b	1.30 ± 0.2^b
T_5	1331.5 ± 7.6^a	5.6 ± 0.30^a	1.32 ± 0.31^a

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5= 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

c) Vitamin E

Very less amounts of vitamin E were found in control biscuits as the figures were 0.04, 0.03 and 0.02 mg in T_1, T_2 and T_3, respectively. In T_4 and T_5, vitamin E was found as 1.30 and 1.32 mg per 100 g samples being supplemented with whole wheat flour and wheat bran.

iv) Minerals

Data regarding various minerals viz. calcium, phosphorus, iron and zinc has been presented in Table 4.20. The findings revealed that calcium content was highest (474.7 mg) in T_1 followed by T_2 (208.7 mg) and T_3 (174.8 mg), respectively. Further, it was present as 258.4 and 281.6 mg in T_4 and T_5, respectively. Higher (390 mg) amount of phosphorus was also observed in T_1 as compared to rest of the two (377.6 and 364.1 mg in T_2 and T_3) control samples. Phosphorus was detected as 726.4 and 717.2 mg in T_4 and T_5, respectively.

Table 4.20 Mineral content of biscuits

Sample	Calcium (mg/ 100 g)	Phosphorus (mg/ 100 g)	Iron (mg/ 100 g)	Zinc (mg/ 100 g)
T_0	56.2±2.85^f	209.35±1.95^f	1.46±0.0^f	0.4±0.0^f
T_1	474.7±4.30^a	390±4.2^c	4.08±0.07^c	1.68±0.43^c
T_2	208.7±1.25^d	377.6±4.6^d	3.1±0.02^e	1.52±0.15^d
T_3	174.8±2.10^e	364.1±3.1^e	3.92±0.06^d	1.24±0.30^e
T_4	258.4±5.00^c	726.4±7.2^a	6.0±0.23^b	2.76±0.53^a
T_5	281.6±3.15^b	717.2±5.0^b	6.7±0.15^a	2.69±0.11^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

The iron content was 4.08, 3.1 and 3.92 mg in per 100 g of control samples whereas it was found quite higher (6.0 and 6.7 mg) in value added biscuit samples i.e. T_4 and T_5, respectively. Similar findings were reported for iron content by Kumar et al. 2018. Farzana and Mohajan 2015 found 1.99 mg iron content in wheat: soybean: mushroom: milk powder fortified biscuits at 65: 20: 5: 10 level.During present study, zinc content was present as 1.68, 1.52 and 1.24 mg in T_1, T_2 and T_3 samples. These figures were higher (2.76 mg in T_4 and 2.69 mg in T_5) in value added samples. Wheat bran separated during refining of wheat flour is richest source of minerals such as calcium, iron and zinc (Oghbaei and Prakash 2013). Therefore, it is concluded that supplementing whole wheat flour with soya flour contributed to increased mineral content in value added biscuits. Further, Atobatele and Afolabi 2016 reported the zinc content between 3.25 to 5.35 mg/ kg of cookies developed using maize and soya flour.

v) Anti nutritional factors

Anti nutritional factors have been presented under Table 4.21. A significant ($p<0.05$) difference was observed with regard to total phenol and trypsin inhibitor contents between all the treatments.

Table 4.21 Anti nutritional content of biscuits

Sample	Total Phenols (mg GAE/ 100 g)	Trypsin Inhibitor (mg/ g)
T_0	105.7 ± 3.79^f	0.0 ± 0.0
T_1	217.4 ± 4.23^c	0.47 ± 0.05^a
T_2	152.8 ± 4.05^d	0.39 ± 0.4^b
T_3	124.5 ± 3.15^e	0.36 ± 0.2^c
T_4	1541 ± 9.60^b	0.33 ± 0.2^e
T_5	1544 ± 6.92^a	0.34 ± 0.3^d

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5= 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

a) Total phenols

It was reported in Table 4.21 that total phenols were present in amounts of 105.7, 217.4, 152.8, 124.5 and mg GAE/ 100 g in $T_{0,}$ T_1, T_2 and T_3 samples while these amounts were higher in value added samples (1541 and 1544 mg GAE/ 100 g in T_4 and T_5) attributed to addition of herbal nutraceuticals i.e. ginger and turmeric.

Adegoke et al. 2017 reported that the total phenolic content was significantly ($p<0.05$) increased in functional biscuits developed by combining wheat flour (70 percent), soya flour (29.5 percent) and turmeric powder (0.5 percent) as compared to control biscuits since the phenolic content was observed as 0.77 and 0.13 mg GAE/ g in functional and control samples, respectively. Total phenolic content increases in baked products due to formation of maillard reaction products when baked with addition of baking powder (Segev et al. 2012).

b) Trypsin inhibitor

Trypsin inhibitor ranged from 0.0 to 0.47 mg/ g. It was observed that T_4 contained the lowest amount of this anti nutritional factor whereas T_1 was reported with the highest content followed by T_2. Trypsin inhibitors are heat labile and heating foods at the temperature of 120°C for 15 to 30 minutes reduces almost whole amount of trypsin inhibitor present in legumes which also helps in improving protein digestibility. Though higher temperature treatment is required in case of some legumes such as soya bean to reduce this anti nutritional factor (Gopalan et al. 2004). A significant ($p<0.05$) reduction in trypsin inhibitor was reported after baking of dough mixture containing defatted soya flour at 180°C for 12 minutes during present investigation.

vi) Total Antioxidant activity

A significant ($p<0.05$) difference was observed in total antioxidant activity of control biscuit and value added biscuit samples (Figure 4.8). The total antioxidant activity level was increased, at every 10 percent increased incorporation of defatted soya flour, in case of control variations. Legume flours are full of antioxidants and considered responsible for increasing free radical scavenging activity of the developed product when combined with wheat flour (Thongram et al. 2016). Similarly, T_4 and T_5 both have been reported with higher total antioxidant activity when compared to control samples of biscuits. Besides, increased total antioxidant activity might be attributed to inclusion of nutraceutical blend as well in value added treatments as well as maillard reaction products produced during baking (Chang et al. 2011; Rao et al. 2011).

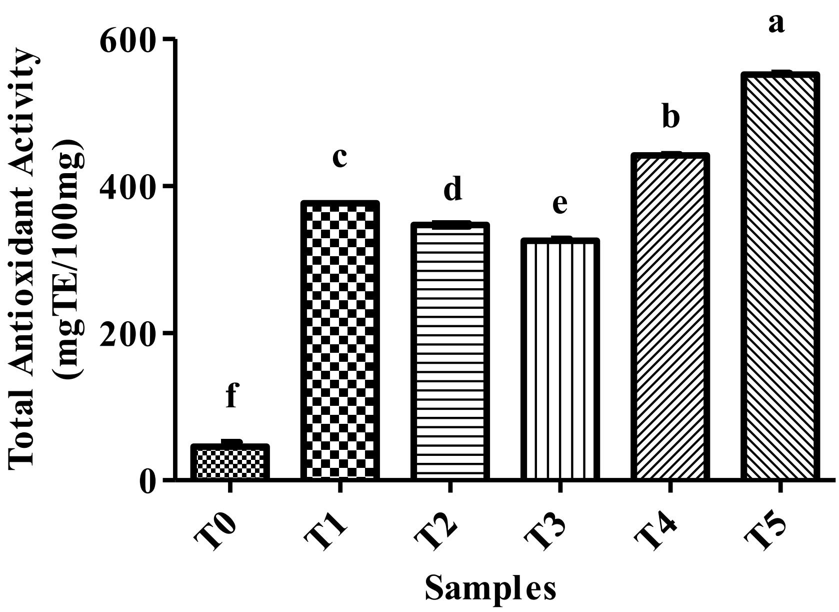


Figure 4.8 Total Antioxidant Activity of biscuits

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

Further, tangerine peels have a great potential to quench free radicals, though being underutilized, can be included as beneficial nutraceutical component while producing biscuits. Hence, in present study, T_5 showed a remarkable total antioxidant activity as compared to control treatments. A significant increase in antioxidant activity (ranged from 1.17 to 2.19 mmol/ TEAC/ g), on elevated level (from 5 to 20 percent) of orange peel supplementation to pearl millet was recorded, during production of pearl millet biscuits (Obafaye and Omoba 2018). Adegoke et al. 2017 reported highest positive impact on antioxidant properties i.e. 48.77 percent DPPH due to addition of turmeric powder in wheat soya based biscuits.

4.4.2.3 Sensory evaluation

a) Sensory evaluation of control biscuits

Sensory characteristics are the most important attributes of a food product. Sensory evaluation can be defined, as a scientific method, used to analyze and interpret the responses recorded by seeing, smelling, touching, tasting and hearing. In contemporary world, sensory evaluation is considered as essential tool for judging the quality of food products and consumer's preferences in terms of its level of acceptability. Results have shown that there is a significant difference ($p<0.05$) in appearance, color, texture and flavor between all the three treatments (Table 4.22).

Table 4.22 Sensory evaluation of control biscuits

Parameters	T_0	T_1	T_2	T_3
Appearance	8.4 ± 0.42^a	7.1 ± 0.39^d	7.7 ± 0.48^c	8.3 ± 0.48^b
Color	8.5 ± 0.34^a	6.9 ± 0.32^d	7.55 ± 0.44^c	8.35 ± 0.53^b
Texture	8.5 ± 0.36^a	6.8 ± 0.63^d	7.55 ± 0.68^c	8.4 ± 0.66^b
Flavor (Aroma/ Taste)	8.5 ± 0.60^a	6.4 ± 0.70^d	7.3 ± 0.67^c	8.4 ± 0.70^b
O. A.	8.4 ± 0.23^a	6.65 ± 0.47^d	7.4 ± 0.57^c	8.3 ± 0.63^b
I. A. (%)	93.54	75.22	83.33	92.77

*Values are Mean ± SD from ten determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_0 = 100 % Wheat Flour

T_1 = 60 % Defatted Soya Flour and 40 % Wheat Flour

T_2 = 50 % Defatted Soya Flour and 50 % Wheat Flour

T_3 = 40 % Defatted Soya Flour and 60 % Wheat Flour

O. A.= Overall Acceptability I. A.= Index of Acceptability

Among value added treatments, the maximum (8.3) mean score for appearance was observed in T_3 followed by T_2 (7.7) whereas T_1 was at last position with the score of 7.1. The scores for color ranged from 6.9 to 8.35 and the value for color of T_3 was superior to all which was close to T_1. Zaker et al. 2012 reported that drastic reduction was found in appearance, color, texture and flavor with every 10 percent increase of soy flour in the basic ratio of flour mix, could be due to heat liable browning and beany flavour of baked products containing higher protein. Similar findings were observed in the present study. Apart from these, it is worth to note that the brown color of the products was due to the maillard reaction took place while baking. Furthermore, close figure of texture was recorded in T_3 as of T_0.

Taste is an important sensory attribute of any food because of its influence on the acceptability. The mean score for flavor of the biscuits was ranged from 6.4 to 8.5. Lower scores as 6.4 and 7.3 were recorded in T_2 and T_3, respectively, while the highest value was observed as 8.5 in $T_{1,}$ again. Furthermore, T_3 obtained highest overall acceptability scores as 8.4. Patil et al. 2014 also observed the highest overall acceptability score (8.26) of composition A2 (refined wheat flour: soya flour: *jamun* powder as (60: 32: 8) during their investigation. Finally, superior (92.77 percent) index of acceptability was recorded in T_3, trailing behind the two value added treatments i.e. T_2 (83.33 percent) and T_1 (75.22 percent), respectively. Lower proportion of defatted soya flour blended with higher amounts of wheat flour results into higher overall acceptability score of the baked products (Udofia et al. 2013; Ayele et al. 2017).

b) Sensory evaluation of value added biscuits

Table 4.23 represents the detailed sensory attribute scores for value added treatments.

Table 4.23 Sensory evaluation of value added biscuits

Parameters	T_4	T_5
Appearance	8.4 ± 0.46^a	7.3 ± 0.48^b
Color	8.3 ± 0.42^a	7.3 ± 0.42^b
Texture	8.2 ± 0.63^a	7.0 ± 0.82^b
Flavor (Aroma/ Taste)	8.0 ± 0.53^a	6.6 ± 0.52^b
O. A.	8.25 ± 0.42^a	7.0 ± 0.53^b
I. A. (%)	91.44	78.22

*Values are Mean ± SD from ten determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_4 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_5 = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

A significant difference ($p<0.05$) in appearance, color, texture, flavor and overall acceptability was found between T_4 and T_5. The mean scores of flavor were 8.0 and 6.6 in both the treatments, subsequently. Lower score obtained by T_5, since it gave bitter after taste, as per the remarks of the panelists. Further, it can be observed from figure 4.16 that higher percentage (91.44) of index of acceptability was reported for T_4 as compared to T_5 (78.22).

The deviations observed in the appearance and taste between two treatments showed that there is a significant role of combination of two different nutraceuticals in determining the sensory aspects of the final product.

4.4.3 Development, chemical and sensory evaluation of muffins

4.4.3.1 Development of muffins

Muffins are sweet baked products with spongy and porous texture with high volume. These are made up of complex emulsion containing milk, sugar, fat and water in continuous phase, with air bubbles as discontinuous phase in which above-mentioned particles are dispersed (Martinez-Cervera et al. 2012). During present study, muffins were standardized as per the method discussed in Table 3.3 under chapter 3: Materials and methods.

4.3.3.2 Chemical evaluation of muffins:

i) Proximate composition of muffins

The Proximate composition of the muffins has been presented in Table 4.24.

a) Moisture

A significant difference ($p<0.05$) between the moisture content in all the three variations of muffins was observed. The moisture content was ranged between 26.42 and 33.46 percent. It was evidently seen that T_3 was found to have higher (33.46 percent) moisture content as compared to T_1 (27.64 percent) and T_2 (26.42 percent), respectively. Alamu et al. 2017 revealed the moisture content as 21.22 percent in 20 percent of soya flour supplementation to wheat flour during muffin preparation which is corresponding to the values obtained during present study. Ndife et al. 2011 reported the moisture content as 33, 36 and 37.50 percent in 10, 20 and 30 percent defatted soya flour supplementation in whole wheat flour bread during their study.

Table 4.24 Proximate composition of muffins

Parameters	T_1	T_2	T_3
Moisture (%)	27.64±0.13[b]	26.42±0.11[c]	33.46±0.16[a]
Protein (%)	7.8±0.23[c]	15.75±0.36[b]	16.2±0.24[a]
Fat (%)	20.75±0.23[c]	23.55±0.36[a]	21.70±0.24[b]
Fiber (%)	0.0±0.0	13.5±0.11[a]	9.5±0.13[b]
Ash (%)	1.07±0.21[c]	2.27±0.16[a]	2.18±0.18[b]
Carbohydrate (%)	42.74±0.49[a]	18.6±0.11[b]	16.87±0.37[c]

*Values are Mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20% Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20% Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

b) Crude Protein

The highest crude protein content was found in T_2 and T_3 as 15.75 and 16.2 percent due to addition of bioactive compounds (wheat bran, tomato, tangerine, ginger etc. as compared to the figure (7.8) observed in T_1. Similar results were reported by Alamu et al. 2017. Ugwuona et al. 2012 observed during their investigation that the protein content (11.23 percent) was lesser and carbohydrate content was higher (47.13 percent) in control sample whereas on enrichment with 20 percent soybean and cassava flours, protein had increased to 13.08 percent with decreased carbohydrate content (23.11 percent).

c) Crude fat

The crude fat content ranged from 20.75 to 23.55 percent. Although, same amount of shortening was incorporated in all the variations. Higher value was observed in T_3, might be due to oil present in tangerine peel powder. Alamu et al. 2017 has also reported 23.36 percent fat content in muffins developed from wheat flour and defatted soya flour incorporated in the ratio of 5: 1.

d) Crude fiber

The maximum crude fiber content was noted in T_2 as 13.5 percent followed by T_3 as 9.5 percent. Presence of fiber content in value added samples was because of value addition of wheat bran, tomato pulp, tangerine peel powder and ginger powder. Ugwuona et al. 2012 further reported the fiber content in 10 percent, 15 percent and 20 percent variations as 9.63, 11.03 and 13.14 percent, respectively. The increased protein and fiber contents might be associated with fermentation process which takes place during batter preparation. (Akhtar et al. 2008 and Maneju et al. 2011). Similar trend was observed in T_2 and T_3 variations when the fiber was incorporated in experimental samples in the form of wheat bran.

e) Total Ash

Total ash content of muffins ranged from 1.70 to 2.27 percent. The highest value was obtained by T_2 whereas the lowest value was scored by $T_{1.}$ Maximum ash content was observed in wheat-soybean flour (60: 40) muffins as compared to controls (Rehman et al. 2016). Close values 2.30 and 2.50 percent of ash content were reported by Ndife et al. 2011 for bread samples developed using wheat flour and soya flour in 80: 20 and 70: 30 ratios, respectively.

f) Carbohydrate

Further, the carbohydrate content ranged from 16.87 to 42.74 percent which was less than half in experimental variations due to presence of complex carbohydrates in the form of fiber in higher amounts as compared to the control. These results were in agreement with Naik and Sekhon 2014.

ii) *In vitro* protein digestibility and Amino acid composition

In vitro protein digestibility and amino acid contents of muffins has been given in Table 4.25. The figures for *in vitro* protein digestibility of control sample i. e. T_1 and value added samples i. e. T_2 and T_3 have been recorded as 69.6, 71.5 and 72.9 percent, respectively. *In vitro* protein digestibility significantly increases in fermented wheat products after baking rather than normal products (Abdel-Aal 2008). Jisha et al. 2010 reported higher percentage (48.70) of *in vitro* protein digestibility on supplementation of whole wheat flour and chickpea flour to cassava flour as compared to cassava- rice flour blended muffins (26.20).

Table 4.25 ***In vitro*** **protein digestibility and Amino acid composition of muffins**

Sample	***In vitro*** **protein digestibility**	**Lysine (g/ 100 g protein)**	**Methionine (g/ 100 g protein)**
T_1	69.6±0.85^c	1.46±0.23^c	0.71±0.0^c
T_2	71.5±1.1^b	1.39±0.16^a	0.82±0.01^b
T_3	72.9±1.3^a	1.61±0.14^b	1.01±0.0^a

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different (p<0.05)

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20% Defatted Soya Flour, 60% Wheat Flour, 2% Tangerine peel (Powder), 7% Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

a) Lysine

The lysine content was higher (1.39 and 1.61 g/ 100 g protein) in T_2 and T_3 as compared to control sample (1.46 g/ 100 g protein). Higher amounts of lysine was attributed to incorporation of defatted soya flour and other nutracetical ingredients in value added samples. Dhingra and Jood 2002 reported a significant (p<0.05) increase in lysine content on every 5 to 10 percent defatted soya flour supplementation to wheat flour.

b) Methionine

Methionine contents in value added muffin samples were 1.01 and 1.01 g/ 100 g protein in T_2 and T_3, respectively. In contrast to this, T_1 contained only 0.71 g/ 100 g protein. Similar findings were reported by Csapo and Schobert 2017.

iii) Vitamins

Vitamin content of control and value added muffin samples has been presented under Table 4.26.

a) Vitamin A

Vitamin A content ranged from 1335 to 1608 μg. The highest figure was reported in T_2 which attributed to the higher content of vitamin A in tomato and tangerine peel powder. The lowest content was found in T_1 whereas T_3 was observed with 1417 μg of vitamin A in it.

b) Vitamin C

No (0.0 mg) amount of vitamin C was found in T_1 as flours from cereals and pulses contain no amount of vitamin C. On the other hand, 9.6 and 7.8 mg of vitamin C have been reported in T_2 and T_3, respectively which was due to the presence of tomato pulp, tangerine peel powder and ginger in value added products.

Table 4.26 Vitamin content of muffins

Sample	Vitamin-A (μg/ 100 g)	Vitamin-C (mg/ 100 g)	Vitamin-E (mg/ 100 g)
T_1	1335 ± 8.90^c	0.0 ± 0.0^c	0.02 ± 0.0^c
T_2	1608 ± 6.36^a	9.6 ± 0.20^a	0.64 ± 0.03^a
T_3	1417 ± 5.17^b	7.8 ± 0.34^b	0.55 ± 0.06^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

c) Vitamin E

The highest (0.64 mg) value of vitamin E was recorded in T_2 followed by T_3 (0.55 mg), respectively. Control sample (T_1) was observed with as low amount as 0.02 mg in it.

iv) Minerals

Mineral content of muffins has been presented in Table 4.27. Calcium content of muffins ranged from 174.7 to 209.5 mg. T_2 has the highest content of this mineral while the lowest amount was present in T_1.

Values for phosphorus were 413.2, 793.1 and 778.0 mg in T_1, T_2 and T_3, respectively. Increased phosphorus content was also observed in wheat bran supplemented muffins by Romjaun and Prakash 2013.

Table 4.27 Mineral content of muffins

Sample	Calcium (mg/ 100 g)	Phosphorus (mg/ 100 g)	Iron (mg/ 100 g)	Zinc (mg/ 100 g)
T_1	174.7±4.35^c	413.2±6.1^c	1.9±0.5^c	1.60±0.3^c
T_2	209.5±5.25^a	793.1±10.1^a	5.6±0.3^a	4.1±0.6^b
T_3	183.5±7.25^b	778±8.1^b	5.3±0.22^b	4.3±0.1^a

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

Further, iron & zinc contents were observed as 1.9 & 1.6, 5.6 & 4.1 and 5.3 & 4.3 mg in T_1, T_2 and T_3, respectively. Naik and Sekhon 2014 also revealed the close figures for iron and zinc content while developing wheat soybean bread.

v) Anti nutritional factors

Anti nutritional factors were presented in Table 4.28.

Table 4.28 Anti nutritional content of muffins

Sample	Total Phenols (mg GAE/ 100 g)	Trypsin Inhibitor (mg/ g)
T_1	186.6±8.35^c	0.21±0.03^c
T_2	2189±12.01^a	0.23±0.02^b
T_3	2186±14.19^b	0.24±0.04^a

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

a) Total phenols

The lowest (186.6 mg GAE/ 100 g) content of total phenols was observed in control sample. In value added muffins, it was present as 2189 and 2186 mg GAE/ 100 g in T_2 and T_3, respectively (Table 4.28). These findings were in agreement with Dhingra and Jood 2002. Total phenolic content was significantly ($p<0.05$) increased during preparation of bread incorporated with turmeric powder and ginger powder as these herbs have better stability to heat (Lim et al. 2011; Balestra et al. 2011).

b) Trypsin inhibitor

It was ranged between 0.21 to 0.24 mg/ g of the sample. A significant ($p<0.05$) difference was observed in three treatments of muffin samples might be due to equal proportion of soya flour. Otegbayo et al. 2018 reported trypsin inhibitor activity from 0.53 to 2.43 mg/ 100 g sample at 05, 10, 15 and 20 percent soybean supplementation to wheat while developing bread.

vi) Total Antioxidant activity

Total antioxidant activity of muffins has been presented in Figure 4.9.

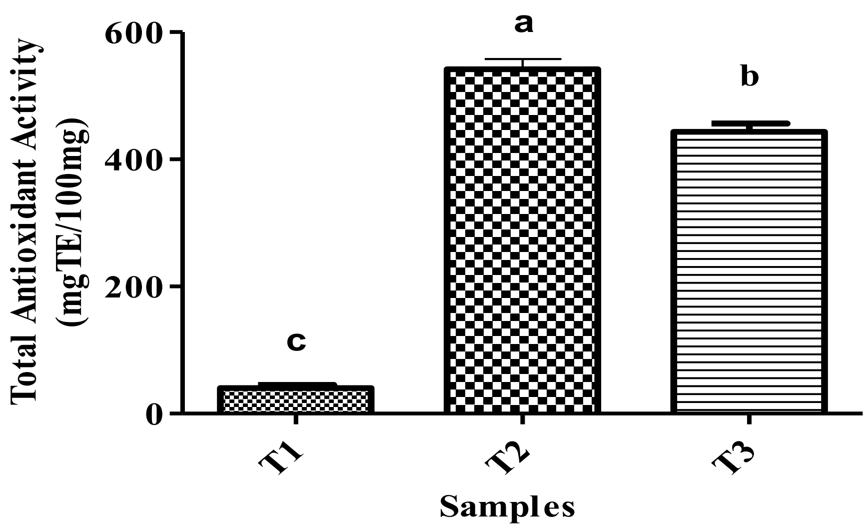


Figure 4.9 Total Antioxidant Activity of muffins

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

A significant ($p<0.05$) difference was observed in total antioxidant activity of all the three samples. It was reported that T_2 and T_3 had excellent potential to quench free radicals as compared to control sample. It is further explained that whole wheat flour had significantly ($p<0.05$) higher antioxidant capacity as compared to refined wheat flour. Moreover, higher total phenolic content of whole wheat and implication of baking increase the antioxidant properties by 1.8 times in whole wheat baked products as compared to refined wheat products due to 17 percent increase in ferulic acid after baking (Yu et al. 2013).

4.3.3.3 Sensory evaluation of muffins

The scores obtained under various sensory parameters of muffins were shown in Table 4.29. Results have revealed that there is a significant difference ($p<0.05$) in appearance, color, texture and flavor between both control and experimental variations.

Table 4.29 Sensory evaluation of muffins

Parameters	T_1	T_2	T_3
Appearance	$7.4±0.52^b$	$6.3±0.67^c$	$8.1±0.74^a$
Colour	$7.4±0.52^b$	$6.2±0.63^c$	$7.7±0.67^a$
Texture	$7.3±0.42^b$	$6.0±0.16^c$	$7.7±0.48^a$
Flavour (Aroma/ Taste)	$7.5±0.74^b$	$6.2±0.63^c$	$8..0±0.62^a$
O. A.	$7.5±0.58^b$	$6.1±0.52^c$	$8.0±0.47^a$
I. A. (%)	82.44	68.44	87.78

*Values are Mean ± SD from ten determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_1 (Control) = 100 % Wheat Flour

T_2 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Tangerine peel (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

T_3 (Value added) = 20 % Defatted Soya Flour, 60 % Wheat Flour, 2 % Ginger (Powder), 7 % Wheat Bran, 10 % Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

The highest (8.1) mean score for appearance was observed in T_3 followed by T_1 (7.4) and T_2 (6.3), respectively. Wheat possesses unique baking properties bestowed to the presence of glutein and glutenin in it. Sanful et al. 2010 concluded that soybean flour supplementation was acceptable only up to 30 percent level during preparation of batter for muffins. Vyas and Diwedi 2016 have developed muffins from two types of flour blends i. e. wheat-moong blend, wheat-moth blend and compared the acceptability scores of both the products. The researchers observed that the first flour blend and later flour blend had lower scores as 3.8 and 3.6, respectively, for the same parameter.

Furthermore, the mean score for color of the muffins was ranged from 6.2 to 7.7. The lowest score was recorded in T_2 while the highest value was observed in T_3. T_1 was found at second position with the mean score of 7.4. Various investigations have concluded that addition of soya flour and other ingredients such as basil leaves in the batter prepared for the muffin development were responsible for making the final product of darker color due to maillard reaction in presence of reducing sugars, amino acids, moisture content of the dough and time and temperature of baking (Bazrafshan et al. 2015; Ronda et al. 2005). At the same time, increased soybean supplementation to wheat flour resulted in enhance browning in muffins, was attributed to higher content of amino acids (Udofia et al. 2013). Similar observations were recorded regarding incorporation of tangerine peel powder in T_2 during the present study.

As far as the texture was concerned, a significant difference ($p<0.05$) was observed among all the three variations. It was seen that T_3 was again superior among all the variations. Ndife et al. 2011 developed four variations of functional bread along with wheat bread as control and reported that the variations with 10 percent and 30 percent substitution of wheat flour with soy flour were found more acceptable with average scores of texture as 5.2 and 5.4 on comparison to 20 percent and 40 percent substitutions having 4.9 and 5.0, respectively.

Moreover, the values for flavor of the muffins were found as high as 8.0 and 7.5 in T_3 and T_1, respectively. It was evidently reported that incorporation of defatted soy flour with bitter tangerine peel powder into wheat flour was associated with roasted flavor or aroma (Serrem et al. 2011).

Both T_3 and T_1 obtained highest overall acceptability scores as 8.0 and 7.5, respectively, whereas T_2 scored least (6.1) points for the same. Ugwuona et al. 2012 stated that higher acceptability scores can be achieved by incorporating soya flour at 10 and 20 percent levels. The percentages for Index of Acceptability were 82.44, 68.44 and 87.78 in T_1, T_2 and T_3, respectively during present study.

4.3.4 Development, chemical and sensory evaluation of noodles

4.3.4.1 Development of noodles

Noodle is one of the most affordable and easily accessible food product, consists of wheat as its main ingredient (Ginting and Yulifianti 2015). It can be developed from single or variety of dough, formulated into stripes, thin ribbons, curl-cues, pipes, waves etc. and is cooking with addition of fat and water (Shah and Jetwat 2012). In contemporary era, due to lifestyle changes most of the population prefer ready to cook foods such as instant noodles etc. but these products are loaded with saturated fats and artificial flavors. These products provide energy dense nutrients such as carbohydrates and fat lacking in micronutrients causes obesity with micronutrient deficiencies in human body. Unfit diet low in dietary fiber and antioxidants leads to risk for developing chronic diseases like cancer. Despite poor health concerns, world consumption of instant noodles was recorded 101.514 million servings per year during the years 2011 to 2015 (World Instant Noodles Association 2015). India has also become the second largest market for noodles since 2014 with number of brands (Kanteti 2015). Usually instant noodles are prepared from refined wheat flour lacking in important nutrients. Further, noodles and pasta products are recognized as important carriers of nutrient by World Health Organization and U. S. Food and Drug Administration (Chillo et al. 2009). Nutraceutical ingredients provide health benefits preventing diseases by increasing antioxidant activity. Considering these aspects, there is a need to develop a cost effective as well as nutrient efficient product supplemented with natural ingredients to provide some amount of micro nutrients and non-nutritive components (Saharan and Jood 2017). In present study, different types of noodles were standardized as per the methods discussed in Fig. 3.1 under chapter 3: Materials and methods.

4.3.4.2 Chemical evaluation of noodles:

i) Proximate composition of noodles

Proximate composition of noodles has been presented in Table 4.30.

a) Moisture

It has been analyzed that the moisture content of T_2 (9.40 percent) was the highest as compared to other value added variations viz. T_3 (9.01 percent), T_4 (9.23 percent) as well as control i. e. T_1 (9.34 percent), respectively. Pakhare et al. 2016 reported 8.43 percent moisture content in defatted soya flour incorporated at 10 percent level in refined wheat flour.

b) Crude protein

The percentage of crude protein was observed as 32.05 in T_2 followed by 29.15 in T_3, 27.65 in T_4 and 8.20 in T_1, respectively. Omeire et al. 2014 observed 13.38 percent crude protein in noodles prepared from composite flours using wheat and soya (75: 25). Bhise et al. 2015 reported protein content as 23.77 percent in wheat soya noodles prepared in the ratio of 60:40. Further, Shogren et al. 2006 observed the higher protein content (33.5 percent) at 50 percent addition of soy flour to wheat flour as compared to control (15.4 percent) sample.

c) Crude fat

Crude fat was found maximum (1.01 percent) again in T_2 while recorded minimum (0.89 percent) in T_4. Beniwal and Jood 2015 too reported the increased percentage of fat in cereal pulse based noodles as compared to control sample.

Table 4.30 Proximate composition of noodles

Parameters	T_1	T_2	T_3	T_4
Moisture (%)	9.34 ± 0.30^b	9.40 ± 0.20^a	9.01 ± 0.16^d	9.23 ± 0.21^c
Protein (%)	8.20 ± 0.13^d	32.05 ± 0.26^a	29.15 ± 0.32^b	27.65 ± 0.20^c
Fat (%)	0.96 ± 0.10^b	1.01 ± 0.15^a	0.93 ± 0.25^c	0.89 ± 0.21^d
Fibre (%)	1.65 ± 0.20^d	2.46 ± 0.15^a	2.32 ± 0.15^b	2.03 ± 0.31^c
Ash (%)	1.02 ± 0.15^d	4.22 ± 0.10^a	3.91 ± 0.20^b	3.47 ± 0.25^c
Carbohydrate (%)	78.83 ± 0.19^a	50.86 ± 0.16^d	54.69 ± 0.28^c	56.72 ± 0.21^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

d) Crude fiber

Furthermore, there was a significant ($p<0.05$) difference among all the variations with reference to fiber composition. The highest (2.46 percent) content of fiber was observed in T_2 as compared to other samples; T_1, T_3 and T_4 (1.65, 2.32 and 2.03 percent), respectively. Similar results were reported by Beniwal and Jood 2015. Similarly, an increase of 0.65 g/ 100 g of sample was noted in functional noodles developed with 15 percent replacement of wheat flour with black gram flour (Anjali and Rani 2018).

e) Total ash

Regarding ash content, T_2 was having the maximum ash content i. e. 4.22 percent preceded by T_3 with the value of 3.91 percent. Simultaneously, T_4 and T_1 were reported with the figures as 3.47 and 1.02 percent, respectively. It was reported that the ash content was increased by almost double amounts when replaced the refined wheat flour with bengal gram and broken rice flour at the rate of 20 percent each during development of functional noodles (Beniwal and Jood 2015). Pakhare et al. 2016 observed 1.54 percent ash on 10 percent replacement of refined wheat flour with defatted soya flour.

f) Carbohydrate

Maximum (78.83 percent) carbohydrate content was computed in T_1 variation whereas T_2 was found with 50.86 percent only. A significant ($p<0.05$) decrease in carbohydrate content was reported by Omeire et al. 2014 on increased incorporation level of soya flour to wheat flour.

ii) *In vitro* protein digestibility and Amino acid composition

In vitro protein digestibility and amino acid content have been presented in Table 4.31. *In vitro* protein digestibility of noodles ranged between 69.6 to 78 percent. The outstanding digestibility percentages (78 and 76.6) were observed in T_2 and T_3 whereas T_1 and T_4 possessed almost same (69.6 and 69.7) figures. Bhise et al. 2015 observed the highest (56.33 percent) protein digestibility in wheat soybean textured flour noodles (60: 40) on comparison with the incorporation of other ingredients i. e. sunflower (32.54 percent) and fenugreek (43.77 percent), respectively.

Table 4.31 *In vitro* protein digestibility and Amino acid composition of noodles

Sample	*In vitro* protein digestibility	Lysine (g/ 100 g protein)	Methionine (g/ 100 g protein)
T_1	69.6 ± 5.8^d	1.29 ± 0.11^d	1.03 ± 0.01^a
T_2	78 ± 2.8^a	3.56 ± 0.06^a	0.72 ± 0.02^d
T_3	76.6 ± 4.05^b	2.31 ± 0.09^b	0.75 ± 0.03^c
T_4	69.7 ± 2.05^c	1.92 ± 0.08^c	0.77 ± 0.01^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

a) Lysine

The maximum content of lysine was recorded in T_2 (3.56 g/ 100 g protein), followed by T_3 (2.31 g/ 100 g protein) and T_4 (1.92 g/ 100 g protein), respectively. The minimum (1.29 g/ 100 g protein) content was observed in T_1. Highest content of lysine was attributed to additional amount of defatted soya flour in T_2. More than double increase in lysine content of developed pasta from incorporation of defatted soya flour into sweet potato flour as compared to control sample was observed by Gopalakrishanan et al. 2011 during preparation of protein fortified pasta. Moreover, lysine content was increased by 1.34 percent in wheat soy flour spaghetti up to 50 percent incorporation level as compared to control (Shogren et al. 2006).

b) Methionine

All the samples were significantly ($p<0.05$) different in their methionine content. Control sample (T_1) has been reported with highest (1.03 g/ 100 g protein) amount of this amino acid as compared to three value added (0.72 g/ 100 g protein in T_2, 0.75 g/ 100 g protein in T_3 and 0.77 g/ 100 g protein in T_4) samples consisting of 60, 50 and 40 percent portion of defatted soya flour. It can be explained as pulses contain lesser amount of methionine. If the proportion of pulse flour to cereal flour is increased in composite flour mixture, the final product results into decreased methionine content. Mahmoud et al. 2012 too reported decreased methionine content in high quality protein noodles supplemented with lupine flour to wheat flour.

iii) Vitamins

The vitamin content of noodles has been presented in Table 4.32.

a) Vitamin A

The highest amount of vitamin A has been observed as 219.6 μg/ 100 g in T_2, followed by T_3 (186.2 μg/ 100 g), T_4 (179.4 μg/ 100 g) and T_1 (161.3 μg/ 100 g), respectively. It is important to mention that defatted soya flour contained significantly higher content of vitamin A as compared to wheat flour.

Table 4.32 Vitamin content of noodles

Sample	Vitamin-A (μg/ 100 g)	Vitamin-C (mg/ 100 g)	Vitamin-E (mg/ 100 g)
T_1	161.3 ± 1.20^d	0.0 ± 0.0	0.02 ± 0.0^d
T_2	219.6 ± 4.70^a	0.0 ± 0.0	0.07 ± 0.03^a
T_3	186.2 ± 2.20^b	0.0 ± 0.0	0.04 ± 0.0^b
T_4	179.4 ± 3.05^c	0.0 ± 0.0	0.03 ± 0.0^c

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

b) Vitamin C

All of the samples has been reported with no (0.0 mg/ 100 g) amounts of vitamin C in these as cereals and pulses contain no amount of vitamin C.

c) Vitamin E

Value added sample (T_2) possessed highest (0.07 mg/ 100 g) vitamin E content as it contained wheat flour and defatted soya flour while preparation of the composite flour as whole grains contain tocopherols and tocotrienols (Soukoulis and Aprea 2012). On the other hand, all the control samples were found lacking in vitamin E content being prepared from refined wheat flour.

iv) Minerals

The values for minerals such as calcium, phosphorus, iron and zinc were reported in Table 4.33. Calcium content was ranged from 90.6 to 124.3 mg. The maximum amount of this mineral was found in T_2 and the minimum was observed in T_1, respectively. On the other side, T_3 has been observed with good (120.6 mg) amounts of calcium. Anjali and Rani 2018 also reported a significant ($p<0.05$) increase in calcium and iron content of functional noodles developed with wheat and black gram flour blend (85: 15). As far as the phosphorus is concerned, it ranged from 296.4 to 409.2 mg. It was further reported that T_1 contained the highest amounts of phosphorus in it followed by T_2, T_3 and T_4, respectively.

Table 4.33 Mineral content of noodles

Sample	Calcium (mg/ 100 g)	Phosphorus (mg/ 100 g)	Iron (mg/ 100 g)	Zinc (mg/ 100 g)
T_1	90.6±1.9^d	409.2±8.00^a	3.87±0.21^d	1.09±0.03^d
T_2	124.3±2.0^a	398.4±5.15^b	4.69±0.13^a	1.81±0.3^a
T_3	120.6.±3.9^b	352.8±2.96^c	4.50±0.15^b	1.40±0.2^b
T_4	107.4±3.1^c	296.4±4.05^d	4.12±0.10^c	1.25±0.1^c

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

Furthermore, iron ranged from 3.87 to 4.69 mg/ 100 g of sample and T_2 and T_3 were observed with almost close figures i. e. 4.69 and 4.50 mg/ 100 g of sample. Kumari et al. 2018 observed a significant ($p<0.05$) increase in calcium and iron contents in value added noodles prepared from wheat flour and soya flour along with jackfruit and pearl millet flours. The values for zinc content ranged from 1.09 to 1.81 mg and higher amount of zinc was recorded in the value added sample i. e. T_2 while the lesser value was there in control sample i. e. T_1. Thilagavathi et al. 2015 reported increase in calcium, phosphorus, iron and zinc contents on 10 percent level of supplementation of soya flour to whole wheat and pearl millet flours.

v) Anti nutritional factors

The data for anti nutritional factors has been presented in Table 4.34.

Table 4.34 Anti-nutritional content of noodles

Sample	Total Phenols (mg GAE/ 100 g)	Trypsin Inhibitor (mg/ g)
T_1	70.25±4.1^d	0.0±0.0
T_2	234.5±7.10^a	2.72±0.16^a
T_3	100.8±3.05^b	2.32±0.01^c
T_4	85.42±2.15^c	1.70±0.02^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

a) Total phenols

Total phenol content ranged from 70.25 to 234.5 mg GAE/ 100 g (Table 4.34). Among value added samples, T_2 was reported with the highest (234.5 mg GAE/ 100 g) content as compared to two other value added samples. On the contrary, control noodle sample (T_1) contained lowest amount of total phenols in it. Total phenolic content of the final product is increased when composite flour mixture contains whole grain flours instead of refined flours (Scalbert and Williamson 2000). A significant ($p<0.05$) increase was noted in polyphenol content of wheat-black gram flour blended noodles by Anjali and Rani 2018.

b) Trypsin inhibitor

Two (T_2 and T_3) of the value added samples have shown more (2.72 and 2.32 mg/ g) amounts of trypsin inhibitor in those as compared to T_4 being with only 1.70 mg/ g of samples each. It is concluded that trypsin inhibitor activity decreases on decreased portion of legume flour addition and after pre-milling treatments such as soaking, fermentation, drying etc. in most of the cereal and legume flours (Xu and Chang 2008; Khattab and Arntfield 2009). No amount of trypsin inhibitor was detected in T_{1} (control) as it contained cereal flour only.

vi) Total Antioxidant activity

Total antioxidant activity of noodles (control and value added) has been shown in Figure 4.10. A significant ($p<0.05$) difference was observed in antioxidant activity of control and value added samples.

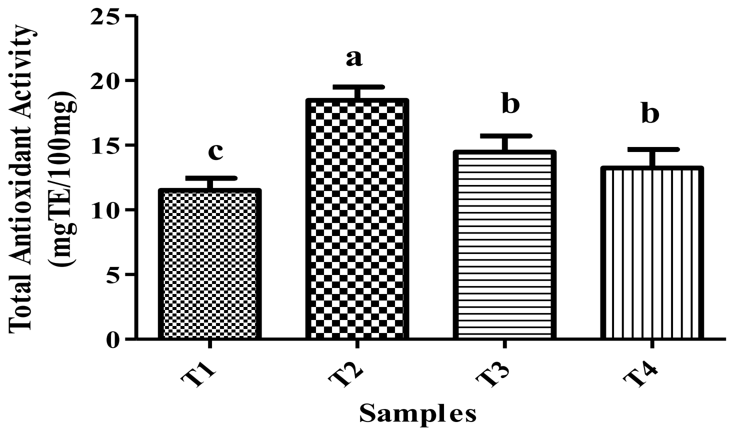


Figure 4.10 Total Antioxidant Activity of noodles

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

T_2 was reported with the highest value of free radical scavenging activity, may be due to higher amount of whole grain (defatted soya flour) consists of ferulic acid (a phenolic compound) present in outer layer of wheat has the capacity to quench free radicals (Nystrom et al. 2007). There was a continuous decrease in total antioxidant activity on every 10 percent increased amount of wheat flour, in case of value added variations.

4.3.4.3 Sensory evaluation of noodles

Sensory characteristics of noodles has been presented in Table 4.35. The mean score of appearance of T_1, T_2 and T_3 were 7.5, 6.5 and 7.0, respectively. On the contrary, value added noodles (T_4) were the most acceptable with score of 8.0 points with regard to their appearance. Omeire et al. 2014 concluded that wheat soya blended noodles in ratio of 75: 25 were most acceptable after pure wheat noodles in terms of texture, color, flavor, aroma and overall acceptability. The average color scores for T_1, T_2 and T_3 have been reported as 7.0, 7.0 and 7.5, respectively. The mean score for color and texture, T_4 scored highest (8.0 and 7.5) points as compared to all the other samples. Dark color of value added noodles might be due to high ash content in these (Ginting and Yulifianti 2015). The flavor of T_4 was proved better in value added samples with average score of 7.5. Besides, T_1 has been reported with close value (7.0). Shogren et al. 2006 revealed that 35 percent of soy flour incorporation to wheat flour was highly acceptable for spaghetti with no significant difference in flavor and texture of the final product when compared to control sample.

Table 4.35 Sensory evaluation of noodles

Parameters	**T_1**	**T_2**	**T_3**	**T_4**
Appearance	7.5 ± 0.15^{b}	6.5 ± 0.10^{d}	7.0 ± 0.20^{c}	8.0 ± 0.05^{a}
Colour	7.0 ± 0.10^{d}	7.0 ± 0.15^{c}	7.5 ± 0.10^{b}	8.0 ± 0.15^{a}
Texture	7.5 ± 0.50^{b}	6.5 ± 0.20^{d}	7.0 ± 0.15^{c}	7.5 ± 0.25^{a}
Flavour (Aroma/ Taste)	7.0 ± 0.25^{b}	6.0 ± 0.30^{d}	6.5 ± 0.20^{c}	7.5 ± 0.50^{a}
Overall Acceptability	7.2 ± 0.20^{b}	6.5 ± 0.50^{d}	7.0 ± 0.30^{c}	7.7 ± 0.25^{a}
I. A. (%)	68.48	59.04	65.34	69.58

*Values are Mean ± SD from ten determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_1 = 100 % Wheat Flour

T_2 = 60 % Defatted Soya Flour, 40 % Wheat Flour

T_3 = 50 % Defatted Soya Flour, 50 % Wheat Flour

T_4 = 40 % Defatted Soya Flour, 60 % Wheat Flour

Moreover, both T_2 and T_3 (6.5 and 7.0) were less acceptable in regard to their overall acceptability score while T_1 and T_4 were highly acceptable as the mean scores were 7.2 and 7.7. Similarly, T_1 and T_4 were most acceptable among all the treatments with an Index of Acceptability (I. A.) as 69.48 and 69.58 percent Bhise et al. 2015 reported the overall acceptability score of wheat soya (60: 40) noodles as 7.35 during their study.

4.3.5 Development, chemical and sensory evaluation of noodle flavors

4.3.5.1 Development of noodle flavors

Artificial noodle flavors are generally lacking in dietary fiber, vitamins and minerals. Natural spices are used in cooking in order to enhance flavor and physical appeal to the developed product. Besides, their antioxidant potential and ability to enhance nutrient absorption made them being used in traditional medicines around the globe (Li et al. 2014). Thus, these can be utilized as good vehicles for supply of micronutrients and bioactive compounds through value addition enhancing quality of the product vis-à-vis physical and mental well being of the consumer (Shere et al. 2018). Fruit and vegetable processing results into production of by-products, thus their utilization in value added products is of both economic and nutritional importance (Vania et al. 2010). Accompanying natural functional ingredients to fast food is an effective way to compensate nutrient losses as well as increase antioxidant activity during lifestyle diseases such as prostate cancer (Li et al. 2014). Therefore, noodle flavors were standardized as per the methods discussed under chapter III: Materials and methods.

4.3.5.2 Chemical evaluation of noodle flavors

i) Proximate composition of noodle flavors

Proximate composition of three noodle flavors i. e. T_1, T_2 and T_3 has been presented in Table 4.36.

a) Moisture

The moisture contents of three noodle flavors was ranged from 57.6 to 59.2 percent. The sample (T_1) containing tomato, ginger and tangerine peel powders was the moistest among all whereas T_2 and T_3 had less moistened, respectively, attributed to low level of water in single ingredient.

Table 4.36 Proximate Composition of noodle flavours

Parameters	T_1	T_2	T_3
Moisture (%)	59.2 ± 0.02^a	57.6 ± 0.08^b	57.8 ± 0.08^b
Protein (%)	4.4 ± 0.02^a	2.0 ± 0.01^c	2.4 ± 0.03^b
Fat (%)	2.2 ± 0.01^a	0.6 ± 0.0^c	1.6 ± 0.0^b
Fibre (%)	4.6 ± 0.03^a	2.6 ± 0.01^b	2.4 ± 0.01^c
Ash (%)	1.8 ± 0.01^a	1.0 ± 0.0^b	1.0 ± 0.01^b
Carbohydrate (%)	26.8 ± 0.13^c	36.2 ± 0.02^a	34.8 ± 0.04^b

*Values are Mean ± SD from triplicate determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

b) Crude protein

The protein content in T_1 was higher (4.4 percent) as compared to T_3 and T_2 i. e. 2.4 and 2.0 percent, respectively. It is concluded that vegetables and spices possess less amount of protein. It is reported that a significant ($p<0.05$) increase was there in final product after value addition of pasta with tomato (Yadav et al. 2014).

c) Crude fat

The crude fat content was ranged between 0.6 to 2.2 percent may be due to very less amount of fat present in fruit and vegetable preparations. Highest fat content in T_1 might be due to mixture of all three nutraceuticals i. e. tomato, ginger and tangerine peel powder, respectively.

d) Crude fiber

A significant ($p<0.05$) increase was noted in crude fiber content when combining all the raw ingredients together as in T_1 (4.6 percent) rather than excluding one of these in two other treatments (2.6 and 2.4 percent in T_2 and T_3), respectively. Results were in accordance to the values observed by Mishra and Bhatt 2016. Padalino et al. 2017 reported approximately 4 percent increase in dietary fiber at 10 and 15 percent incorporation level of tomato peel to durum wheat meal. Further, an increase of 0.2 g in fiber content for per kg pasta was noted while 400 g tomato puree was supplemented to 1 kg of flour by Rekha et al. 2013.

e) Total Ash

The mean ash content was almost double in T_1 (1.8 percent) as compared to 1.0 percent in T_2 and T_3, respectively. Close findings were reported by Mishra and Bhatt 2016. An increase of 0.24 percent has been observed in ash content of tomato blended pasta as compared to wheat pasta by Yadav at al. 2014.

f) Carbohydrate

The carbohydrate content ranged from 26.8 to 36.2 percent. Maximum amount of carbohydrate was reported in T_2 and minimum in T_1, respectively.

Shere et al. 2018 reported a decrease of 0.05 percent in fat content, a significant ($p<0.05$) decrease in carbohydrate content while a significant ($p<0.05$) increase in fiber and ash contents of wheat noodles on addition of spinach puree up to 40 percent level.

ii) *In vitro* protein digestibility and Amino acid composition

In vitro protein digestibility and amino acid contents of noodle flavours is presented in Table 4.37. Among three treatments, T_1 has shown the highest (82.74 percent) *in vitro* protein digestibility on comparison with T_2 and T_3 with figures 72.04 and 70.22 percent, respectively.

Table 4.37 *In vitro* protein digestibility and Amino acid composition of noodle flavors

Sample	*In vitro* protein digestibility	Lysine (g/ 100 g protein)	Methionine (g/ 100 g protein)
T_1	82.74 ± 0.41^a	0.07±0.0	0.02±0.0
T_2	72.04 ± 0.35^b	0.06±0.0	0.01±0.0
T_3	70.22 ± 0.76^c	0.06±0.0	0.01±0.0

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

a) **Lysine**

Lysine content was 0.07 g/ 100 g protein in T_1; 0.06 and 0.06 g/ 100 g protein in T_2 and T_3 noodle flavour samples, respectively.

b) **Methionine**

Methionine was present in negligible amounts (0.02, 0.01 and 0.01 g/ 100 g protein) in T_1, T_2 and T_3 treatments, respectively.

iii) **Vitamins**

The vitamin content of noodle flavour has been given in Table 4.38.

a) **Vitamin A**

Vitamin A content ranged from 4.5 to 13.4 µg. T_3 has been reported with the highest amounts while T_2 possessed least amounts. Further, 10.8 µg of vitamin A was reported in T_1. A significant ($p<0.05$) increase in β-carotene content was reported when tomato peel was added to durum wheat meal at 10 and 15 percent level (Padalino et al. 2017).

b) **Vitamin C**

Higher content of Vitamin C was observed in T_1 (1.0 mg) as compared to T_2 (0.79 mg) and T_3 (0.92 mg), respectively.

Table 4.38 Vitamin content of noodle flavors

Sample	Vitamin-A (µg/ 100 g)	Vitamin-C (mg/ 100 g)	Vitamin-E (mg/ 100 g)
T_1	10.8 ± 0.2^{b}	1.0 ± 0.05^{a}	0.03 ± 0.01^{a}
T_2	4.5 ± 0.1^{c}	0.79 ± 0.04^{c}	0.02 ± 0.0
T_3	13.4 ± 0.75^{a}	0.92 ± 0.08^{b}	0.03 ± 0.0

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

c) Vitamin E

Negligible amounts (0.03, 0.02 and 0.03 mg) were reported in T_1, T_2 and T_3, respectively. By incorporating natural flavors such as tomato and other fruit and vegetable by-products, the amount of vitamin A and C can be increased and the losses occur during milling process of flour can be compensated (Li et al. 2014).

iv) Minerals

Mineral content of noodle flavors was reported in Table 4.39. Calcium was ranged from 0.56 to 0.61 mg/ 100 g of the sample.

Maximum amounts of calcium (0.61 mg) and phosphorus (1.52 mg) was found in T_1 followed by T_3 (0.59 mg calcium and 1.26 mg phosphorus) and T_2 (0.56 mg calcium and 1.12 mg phosphorus), respectively.

The figures for iron & zinc contents were observed as 0.06 & 0.03, 0.04 & 0.00 and 0.02 & 0.12 mg in T_1, T_2 and T_3, respectively.

A significant ($p<0.05$) increase in phosphorus and iron content was noted during incorporation of tomato paste to wheat-pearl millet pasta by Yadav et al. 2014.

Table 4.39 Mineral content of noodle flavors

Sample	Calcium (mg/ 100 g)	Phosphorus (mg/ 100 g)	Iron (mg/ 100 g)	Zinc (mg/ 100 g)
T_1	$0.61±0.05^a$	$1.52±0.08^a$	$0.06±0.01^a$	$0.03±0.0^b$
T_2	$0.56±0.03^c$	$1.12±0.03^c$	$0.04±0.0^b$	0.0±0.0
T_3	$0.59±0.02^b$	$1.26±0.04^b$	$0.02±0.0^c$	$0.12±0.01^a$

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

v) Anti nutritional factors

Anti nutritional content of noodle flavors are presented in Table 4.40.

Table 4.40 Anti nutritional content of noodle flavors

Sample	Total Phenols (mg GAE/ 100 g)	Trypsin Inhibitor (mg/ g)
T_1	7.04±0.4[a]	0.0±0.0
T_2	6.72±0.35[c]	0.0±0.0
T_3	6.91±0.11[b]	0.0±0.0

*Values are Mean ± SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

a) Total phenols

The total phenol content of three noodle flavors ranged from 6.72 to 7.04 mg GAE/ 100 g. It was observed as 7.04, 6.72 and 6.91 mg GAE in 100 g of T_1, T_2 and T_3 samples, respectively.

b) Trypsin inhibitor

No amounts of trypsin inhibitor were observed in all the noodle flavor samples.

vi) Total Antioxidant activity

A significant ($p<0.05$) difference in total antioxidant activity of T_1, T_2 and T_3 was observed (Figure 4.11). It has been reported that T_1 had the maximum free radical scavenging potential as compared to other samples. It can be explained that dehydrating tomato and other functional ingredients with phenolic content have potential to enhance their total phenolic content by almost 30 times after drying process and further, mixing all the functional ingredients with bioactive compounds such as tomato, ginger, tangerine peel powder and turmeric combined, can help achieving the significantly ($p<0.05$) enhanced total antioxidant activity in the final product (Lutz et al. 2015).

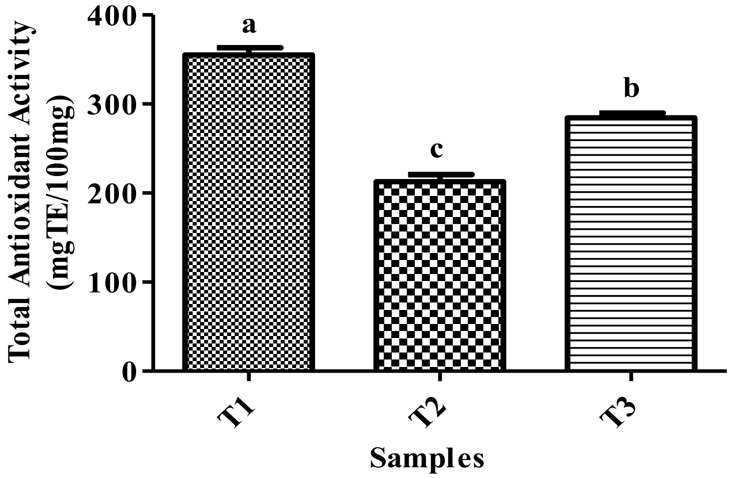


Figure 4.11 Total Antioxidant Activity of noodle flavors

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

4.3.5.3 Sensory evaluation of noodle flavors

Sensory characteristics of noodle flavors has been shown in Table 4.41. It has been reported that the mean score of appearance of three treatments i. e. T_1, T_2 and T_3 were found to be 6.7, 7.1 and 7.0, respectively. The average color scores for T_1, T_2 and T_3 have been reported as 6.3, 7.2 and 7.3, respectively. Sunil et al. 2019 formulated noodles (T_{90}, T_{80}, T_{70}, T_{60} and T_{50} with control i.e. T_{100}) by incorporating different proportions of soy bean flour, carrot flour, mushroom and apple pomace powders (2.5, 5, 7.5, 10 and 12.5 percent each, respectively) to wheat flour (90, 80, 70, 60 and 50 percent) and evaluated the products in their sensory aspects. It was revealed that mean color scores were 8.6, 8.5, 8.4, 8.4, 8.2 and 8.0 for T_{90}, T_{80}, T_{70}, T_{100}, T_{60} and T_{50}, respectively.

Considering the scores obtained under texture, T_2 again found scoring highest (7.2) points trailing behind the other two T_1 and T_3 by 1.1 and 0.1 points, respectively. Mishra and Bhatt 2016 developed pasta incorporated with ginger powder at 1, 3 and 5 percent level to wheat flour and reported the higher mean scores for color, flavor and texture, at 1 and 3 percent level as compared to 5 percent incorporation level.

As the flavor of the products was concerned, no significant ($p>0.05$) difference was found in the scores obtained by T_1 and T_3 during present study.

Table 4.41 Sensory evaluation of noodle flavours

Parameters	T_1	T_2	T_3
Appearance	6.7 ± 0.29^c	7.1 ± 0.30^a	7.0 ± 0.00^b
Colour	6.3 ± 0.15^c	7.2 ± 0.50^b	7.3 ± 0.10^a
Texture	6.1 ± 0.30^c	7.2 ± 0.50^a	7.1 ± 0.30^b
Flavour (Aroma/ Taste)	6.3 ± 0.15^c	7.3 ± 0.29^a	6.5 ± 0.50^b
Overall Acceptability	6.0 ± 0.00^c	7.8 ± 0.29^a	7.2 ± 0.30^b
I. A. (%)	56.52	65.88	63.18

*Values are Mean ± SD from ten determinations; different superscripts in the same row are significantly different ($p<0.05$)

Where T_1 = Tomato (60 %) + Ginger (10 %) + Tangerine Peel Powder (10 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5 %)

T_2 = Tomato (60 %) + Ginger (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

T_3 = Tomato (60 %) + Tangerine Peel Powder (20 %) + Turmeric (7.5 %) + Salt (10 %) + Black Pepper (2.5%)

Further, it was also observed that combining tomato with ginger (T_2) performed well with an overall acceptability score of 7.8 while T_1 and T_2 were as acceptable as 6.0 and 7.2, respectively. Similarly, highest overall acceptability score (7.85) in pasta sample fortified with ginger at 1 percent level was observed (Mishra and Bhatt 2016) which is found in agreement with the present study. Sunil et al. 2019 reported the range of overall acceptability score of noodles supplemented with soy bean flour and vegetable powders between 7.55 to 8.27. The highest mean score was obtained in T_{90} and lowest score was observed in T_{50}. Moreover, it is impertinent to mention that addition of all nutraceuticals (T_1) resulted into least (56.52 percent) acceptable final product. Furthermore, T_2 was most acceptable among all the variations with an Index of Acceptability (I. A.) as 65.88 percent (Table 4.41).

Shere et al. 2018 concluded that addition of functional ingredients such as spinach up to 40 percent to wheat flour noodle helps improving the overall acceptability score of the developed products. The present findings were in accordance to the results reported by Rekha et al. 2013 formulated bread wheat pasta with vegetable paste. Yadav et al. 2014 developed vegetable blended pasta through incorporating tomato at 25.5 g/ 100 g to wheat-pearl millet (90:10) flour and observed the overall acceptability score of 7.9 during sensory evaluation pasta.

It is summarized that nutritional and health status can be ameliorated through imparting nutrition knowledge among prostate cancer patients. Simultaneously, ready to eat, value added food products with combination of nutraceutical ingredients in those help to enhance the potential to maintain the nutritional as well as anti-oxidant status of both the healthy and affected individuals in order to prevent prostate cancer and cease its progression.

5. SUMMARY AND CONCLUSIONS

The present investigation was planned to study the prevalence of prostate cancer (PCa) in Bathinda City, its causative factors, to see the efficacy of nutrition education on the health status of prostate cancer patients and development of various food products to prevent PCa. The present research was conducted in three phases: 1. A statistically adequate sample of 150 individuals from different households (randomly selected) belonging to 50 wards of Bathinda City was selected for PCa screening, 2. another set of sample consisted of 200 post-operative males with PCa stage I and II (aged between 40-90 years) who were visiting three different hospitals at Bathinda City was taken for randomized controlled trial through implicating cluster design to carry out nutrition intervention. The latter sample was divided equally into two groups viz. Group I and Group II. Nutrition counselling followed by distributing an information booklet was imparted to the subjects in Group II while Group I was treated as control, and 3. three different types of nutraceutical products for PCa prevention were developed in order to prevent PCa.

An open ended questionnaire cum interview schedule was developed to collect information regarding three different aspects such as demographic (age, education, occupation, socioeconomic status, type of family etc.); lifestyle viz. physical activity level, sleeping span, signs and symptoms of nutritional deficiencies, presence of allied diseases, source of drinking water etc.; health related information viz. family history of the disease, general symptoms, PCa specific symptoms, etc. and nutritional and health status in terms of dietary survey, anthropometric measurements and biochemical assessment. The interview schedule was validated through incorporating the suggested changes by expert panel comprising of Assistant Scientist, Punjab Agricultural University, Ludhiana and Dietitians of concerned hospitals, followed by pre-testing on ten subjects. Detailed information regarding the dietary intake of the prostate cancer patients were collected for three consecutive days by '24 hour recall method'. Daily nutrient intake was calculated using Indian nutrition software DietCal - A Tool for Dietary Assessment and Planning. Anthropometric measurements viz. body weight, height were measured and BMI was calculated. The patients were assessed for Prostate Specific Antigen (PSA) level, Bone Mineral Density (BMD) score, hemoglobin level and antioxidant profile both at the beginning and at the end of the study. The developed KAP questionnaire containing a set of multiple-choice questions under three parameter heads i. e. knowledge, attitude and practices was administered to all the subjects. As far as the allocation of marks was concerned, one (1) mark was awarded for every correct answer whereas zero (0) point was allotted for each wrong answer. The responses recorded as don't know were excluded from the evaluation process. Then, nutrition education was imparted to the prostate cancer patients and their attendants in group II through an information booklet developed in Punjabi language. At the end, the efficacy of nutrition education was assessed by calculating gain in scores obtained by respondents and quantum of improvement. Further, three types of value added nutraceutical products (biscuits, muffins and noodles) were developed and evaluated for physico-chemical and sensory characteristics.

Data on demographic information showed that majority of the subjects i.e. 39 & 48 percent and 33 & 29 percent were between the age group of 60-70 years followed by 70-80 years in group I & group II, respectively. Higher percentage i.e. 37 and 34 in group I and group II, respectively were illiterates. As occupation was concerned, 65 and 53 percent in group I and group II, respectively belonged to agricultural community. According to socioeconomic status, 40 and 82 percent of the respondents were categorized under middle income group; 44 and 18 percent were in low income group and 16 and 00 percent were in high income group in group I and group II, respectively. It was seen that the highest number of prostate cancer patients i.e. 67 and 71 in group I and group II, respectively, were based in rural households whereas small number (24 and 19) of urban residents was recorded in both the groups. Further, it was reported that most of them (87 and 93 percent in group I and group II) were Sikhs. Besides, 87 and 93 percent subjects in group I and group II, respectively resided in joint family system.

Findings related to lifestyle information depicted that the most (91 and 83 percent) of the subjects showed normal sleeping span i. e. 6- 8 hours/ d. More than three quarters (78 and 75 percent in group I and group II, respectively) had sedentary lifestyle whereas only 14 and 23 percent were active in group I and group II, respectively. Nutritional deficiency symptoms such as iron and vitamin-C were observed in the subjects of both the groups. Among iron deficiency symptoms, fatigue (47 & 33 percent), breathlessness (46 & 20 percent), pale conjunctiva (22 & 44 percent), paleness of skin (25 & 33 percent) and headache (32 & 24 percent) were found in the patients of group I & group II. Pallor of skin was present in 06 and 32 percent in group I and group II, respectively. Higher (21) percentage of lethargy was observed among the subjects in group I than in group II (05 percent). Further, cases of spoon shaped nails were detected as 02 and 04 percent in two groups, respectively. It was noticed that vitamin C deficiency symptoms i.e. spongy bleeding gums (15 & 09 percent) and mottled teeth enamel (53 & 31 percent) were there in group I & II, respectively. Allied diseases such as diabetes, renal, cardio-vascular and respiratory diseases were observed among 11 & 09, 00 & 04, 04 & 00 and 02 & 02 percent subjects in group I and group II, respectively. It was further reported under house hold information that only 51 and 35 percent of the subjects in both the groups were having access to treated sources of drinking water, while untreated sources of drinking water were also observed among the subjects in both the groups, respectively. Furthermore, very less percentage (20 and 24) of the subjects practiced kitchen garden.

Health related information of the subjects revealed that 18 & 08 percent and 10 & 06 percent of the subjects had family history of PCa & other types of cancer in group I and group II, respectively. Among general symptoms, weakness, fatigue and loss of appetite were prominently recorded in 83 & 69, 77 & 71 and 99 & 61 percent subjects in group I & II, respectively. Among PCa specific symptoms, lesser urine output and exertion during urination were experienced by all (100 percent) the patients in both the groups.

Dietary pattern of the subjects depicted that vegetarianism & non-vegetarianism eating pattern was seen as 49 & 43 percent and 43 & 51 percent in the respondents in group I and group II, respectively. Only 08 and 06 percent of the total population were ova-vegetarians. Further, skipping of major meal i.e. breakfast was as common as in 39 and 35 percent of population in both the groups. *Dalda* (78 & 62 percent) and *desi ghee* (61 & 67 percent) were found popular among most of the patients whereas combination of fats was quite uncommon (01 and 09 percent) among the subjects of two groups, respectively. Average daily nutrient intake was inadequate in terms of energy, protein, β-carotene, vitamin-C, iron and zinc in both the groups when compared to the RDA suggested by ICMR 2010. In contrast to this, intake of fat and B-complex vitamins was more than adequate in both the groups during beginning and end of the study.

The average weight, height and calculated BMI was found within the reference ranges. The mean serum PSA was as high as 15.59 & 12.23 ng/ mL and

20.05 & 14.47 ng/ mL during initial & final assessment of the subjects in group I and group II, respectively which were 2 to 4 times higher than the reference range (0 to 4 ng/ mL). The average values for BMD were reported within the normal range in two groups. The observed values for hemoglobin were below the minimum reference value of 12 g/ dl in the subjects of group I whereas normal hemoglobin level was maintained by the subjects in group II after imparting nutrition counselling. It was noticed that serum vitamin-A was declined among respondents in group I at the end of the study. The mean scores for both serum vitamin-C and serum vitamin-E were within the normal ranges both at initial & final phases of the study in group I and group II, respectively. Serum zinc was quite lower (57.9 and 57.12 μmol/ l in group I and II) at the beginning of the study when compared to the reference range (70 to 100 μmol/ l). Normal (76.30 μmol/ l) level of the mean score for group II patients was obtained after nutrition intervention.

All the subjects in both the groups obtained lower scores for all the three parameters i.e. knowledge, attitude and practices at the beginning of the study. The mean post intervention test scores for knowledge, attitude and practices of the respondents in group II were significantly ($p<0.05$) improved. Further, a significant ($p<0.05$) difference was observed under gain in score in two groups and almost two fold increment in quantum of improvement in nutrition knowledge of the respondents in group II as compared to the subjects in group I. Furthermore, mean scores under attitude and practices were more than twice after imparting nutrition counselling at the end of the study on comparison with initial mean values in group II patients. Overall mean score under gain in score of the subjects in group I was quite lower (1.1) as compared to the mean gain in score (8.5) in group II. The overall mean scores under quantum of improvement were 1.27 and 2.89 in group I and group II, respectively. Therefore, it is concluded that nutrition counselling should be imparted to prostate cancer patients in order to enhance nutrition knowledge and incorporate positive lifestyle changes with regard to their attitude and practices towards improvement of their overall health status by correcting their biochemical parameters related to the disease and other nutrient deficiencies.

Three (two baked and one extruded) types of value added nutraceutical products viz. biscuits, muffins, and noodles with noodle flavors were developed with mixture of defatted soya flour, wheat flour, wheat bran, tomato, tangerine peel (powder), ginger (powder), black pepper, sugar and turmeric. Both raw ingredients and developed products have been analyzed for proximate composition, *in vitro* protein digestibility, amino acid composition (lysine and methionine), vitamin (vitamin-A, C and E) content, mineral (calcium, phosphorus, iron and zinc) content, antinutritional factors (total phenols and trypsin inhibitor) and total antioxidant activity. Besides, biscuits were analyzed in terms of physical characteristics i.e. weight, thickness, diameter and spread ratio.

The observations recorded during chemical analysis of raw ingredients indicated that the moisture content of raw ingredients ranged from 7.27 to 93.96 percent. Tomato was recorded with the highest moisture content while wheat bran had the lowest. Further, defatted soya flour, tangerine peel and ginger powder were detected with 7.79, 7.9 and 8.2 percent moisture, respectively. Crude protein content was 59.21 and 9.26 percent in defatted soya flour and wheat flour. Four nutraceuticals such as tomato, wheat bran, tangerine peel powder and ginger contained 25.59, 1.57, 5.3 and 12.4 percent crude protein, respectively. The mean content of crude fat ranged from 0.75 to 7.73 percent. Very less amount of crude fat was reported in defatted soya flour (0.75 percent) and tomato (0.28 percent). In contrast to this, wheat bran (4.62 percent) and ginger (7.73 percent) had higher content of crude fat. Highest (11.14 percent) amount of fiber was recorded in tangerine peel powder followed by ginger (10.47 percent) and defatted soya flour (4.28 percent). These results were closer to the fiber content (10.68 percent) reported in tangerine peel powder by USDA National nutrient database 2018. Further, the mean ash content was observed as 7.38, 4.48 and 3.75 percent in defatted soya flour, tangerine peel (powder) and ginger (powder), respectively. The average carbohydrate content was calculated as 20.59, 77.59, 58.43, 2.87, 68.98 and 56.45 percent in defatted soya flour, wheat flour, tomato, wheat bran, tangerine peel powder and ginger, respectively. These figures were close to the reference values reported by Gopalan et al. 2004.

Results showed range of *in vitro* protein digestibility in raw ingredients between 18.58 and 67.28 percent. Defatted soya flour was reported with the highest value (67.28 percent) followed by wheat flour (51.94 percent) and wheat bran (28.03 percent), respectively. Among nutraceuticals, tomato and ginger had 50.31 and 35.83 percent whereas tangerine peel powder had only 18.58 percent *in vitro* protein digestibility. Amino acid content i. e**.** lysine and methionine of raw ingredients ranged from 0.0 to 3.71 g and 0.0 to 0.97 g/ 100 g protein, respectively. Defatted soya flour and wheat bran contained higher amounts (3.71 and 3.64 g lysine/ 100 g protein, respectively) of lysine as compared to wheat flour (1.06 g lysine/ 100 g protein) and tomato (1.44 g lysine/ 100 g protein), respectively. Lysine was not present in ginger and tangerine peel powder. Observed values were in accordance to reference values reported by Gopalan et al. 2004. Besides, wheat bran had 0.97 g/ 100 g protein of methionine. It was reported as 0.83, 0.71 and 0.42 g/ 100 g protein in wheat flour, defatted soya flour and tomato, respectively. No methionine was present in tangerine peel powder and ginger.

Among vitamin content, it was reported that vitamin A content ranged from 19.7 to 554.2 μg/ 100 g of the raw ingredients. Defatted soya flour and tangerine peel powder (402 and 554.2 μg/ 100 g, respectively) had higher vitamin A than in ginger, and wheat flour (24.2 and 19.7 μg) whereas vitamin A content was absent in wheat bran. Vitamin C content was reported as 3.1, 26.1 and 28.7 mg in ginger, tomato and tangerine peel powder, respectively while this vitamin was not present in wheat flour, defatted soya flour and wheat bran. Vitamin E content was ranged from 0.0 to 0.91 in raw ingredients. Fair content (0.91 and 0.71 mg per 100 g) of vitamin E was observed in tomato and wheat bran, respectively. These results were close to the reference values by Gopalan et al. 2004.

Regarding mineral content of raw ingredients, the mean calcium and phosphorus content was ranged from 12.14 to 201.4 and 17.1 to 799.2 mg, respectively. In case of calcium, highest content was found in defatted soya flour and tangerine peel powder (201.4 and 157.3 mg) whereas refined wheat flour and tomato had lowest (18.9 and 12.14 mg) content, respectively. With regard to the mean phosphorus content, wheat bran and defatted soya flour had 799.2 and 663.4 mg, respectively. Further, iron content was ranged from 1.1 to 7.87 mg. The mean iron content was 7.87, 6.7, and 4.15 mg in 100 g of tangerine peel powder, defatted soya flour and wheat bran, respectively. The zinc content ranged between 0.0 and 6.96 mg in raw ingredients. The average reported content of zinc was 6.96, 2.1 and 1.85 mg in above-said ingredients, respectively.

Anti nutritional factors i. e. total phenols (mg GAE/ 100 g) and trypsin inhibitor (mg/ g) were estimated in raw ingredients. The mean total phenol content was ranged from 10.8 to 1203.2 mg GAE/ 100 g. The highest (1203.2 mg GAE/ 100 g) content was observed in wheat bran followed by tomato (221.6 mg GAE/ 100 g) and wheat flour (164.3 mg GAE/ 100 g), respectively. Trypsin inhibitor was present only in defatted soya flour (4.7 mg/ g) while absent in all other ingredients.

Findings regarding total antioxidant activity of raw ingredients revealed a significant ($p<0.05$) difference between all the raw ingredients. It was found that tomato, tangerine peel powder and ginger had higher total antioxidant activity (>50 mg TE/ 100 mg) to quench free radicals as compared to other ingredients. Whole grains and other plant materials possess sufficient amounts of phenolic acids and show excellent antioxidant potential to scavenge free radicals through inhibition of lipid peroxidation.

Data regarding physical characteristics of control and value added biscuits showed that the mean weight of biscuits ranged from 8.3 to 8.8. Further, T_3 followed by T_0 (8.7 g) was observed with the highest mean weight whereas T_1 had the lowest. Further, diameters of developed products ranged from 4.19 to 4.29 cm. There was a significant ($p<0.05$) difference in diameters of T_1 and T_2. The highest value of diameter was reported in T_0 followed by T_3 as compared to other two other samples. Further, the mean value of thickness was recorded in T_3 (0.53 cm) as compared to T_1 (0.50 cm) and T_2 (0.51 cm), respectively. Increase in the diameter of the product particularly depends on rate of melting of fats during baking. Use of butter as biscuit shortening was attributed to achieve diameter of the developed products in present study. The mean spread ratio were 8.77, 7.89, 8.47 and 8.51 in T_0, T_1, T_2 and T_3, respectively. Lesser amount of defatted soya flour in T_3 was resulted to increment in its spread ratio. The mean hardness was ranged from 38.4 to 46.3 N among control biscuits. It was reported that T_1 (46.3 N) had higher level of hardness as compared to T_2 (42.4 N) and T_3 (38.4 N), respectively due to higher defatted soya flour supplementation level in it as compared to later samples. Among value added treatments, the mean weight of T_4 and T_5 was 10.5 g was 10.9 g, respectively. Further, diameter of the developed products i.e. T_4 and T_5 was 4.61 cm and 4.48 cm, respectively. Nutraceuticals such as tangerine peel powder and ginger with higher amounts of fiber attributed to higher diameter of value added treatments as compared to control biscuit sample. A significant difference ($p<0.05$) was observed in thickness of T_4 and T_5, whereas the mean thickness of value added treatments was significantly ($p<0.05$) higher when compared to control treatments. The mean values for spread ratio in T_4 and T_5 were 7.37 and 6.66, respectively. Biscuits' thickness was increased and value for spread ratio was decreased with higher portion of defatted soya flour. Further, a significant difference ($p<0.05$) was there between the hardness of T_4 and T_5

incorporated with tomato pulp and tangerine peel powder, respectively, higher level of moisture content in T_4.

It was observed that the moisture content of T_0, T_1, T_2 and T_3 was 9.60, 8.10, 8.29 and 8.32 percent, respectively. A significant decrease ($p<0.05$) was recorded in moisture content of biscuits, on every 10 percent increased defatted soya flour incorporation level. The crude protein content was ranged from 8.78 to 31.78 percent with higher content found in T_1 due to higher portion of defatted soya flour in it as compared to T_2 (29.23 percent) and T_3 (27.6 percent), respectively. The crude fat content was significant ($p<0.05$) decreased on 10 percent decreased amount of defatted soya flour to wheat flour in three control biscuit samples. The percentage for crude fiber was T_0, T_1, T_2 and T_3 was 0.00, 2.84, 2.50 and 2.14, respectively. It was recorded that highest percentage (4.19) of ash was found in T_1 followed by T_2 (3.71) and T_3 (3.32), respectively. Highest content of fiber and ash was attributed to higher level of defatted soy flour level in T_1. As the range of carbohydrate content was concerned, it was reported between 35.50 and 65.12 percent. Decreased carbohydrate content was observed in $T_{1,}$ T_2 and T_3 due to incorporation level of defatted soya flour to wheat flour as compared to T_0. On the other hand, moisture content of value added biscuits i.e. T_4 and T_5 was 10.42 and 9.38 percent, respectively. A significantly ($p<0.05$) lesser amount of moisture was found in T_5 because it contained tangerine peel powder with lower amount of moisture as compared to ginger powder present in T_4. Further, the crude protein content in T_4 and T_5 was 34.96 and 33.6 percent, respectively which was significantly ($p<0.05$) higher on comparison with control biscuits. The mean crude fat content of T_4 and T_5 was 22.49 and 22.31 percent. It was reported that T_5 (4.46 percent) had significantly ($p<0.05$) higher fiber content than T_4 (4.09 percent) as T_5 was supplemented with tangerine peel powder possessed higher fiber content than ginger. The mean ash content was 5.39 and 5.42 percent in T_4 and T_5, respectively. An increase was noted in fiber and ash contents, on tangerine peel powder addition while preparing T_5. The average carbohydrate content of T_4 and T_5 was 22.65 and 24.83 percent, respectively. Value addition of nutraceuticals in value added biscuits attributed towards the decreased carbohydrate content.

Results regarding *in vitro* protein digestibility of control and value added biscuits showed a significant ($p<0.05$) difference between all treatments of biscuits. It was observed that T_3 had the highest (76.54 percent) *in vitro* protein digestibility while lowest (55.62 percent) recorded in T_0. Besides, T_5 was reported with 73.87

percent *in vitro* protein digestibility as compared to T_4 with 71.48 percent, respectively, in case of value added biscuit samples. Products developed using supplementation of pulse flour to wheat flour resulted into higher *in vitro* protein digestibility. Further, the average lysine content was 1.04, 2.43, 2.21 and 1.98 g/ 100 g protein in T_1, T_2 and T_3 samples. On the contrary, T_4 and T_5 had increased contents of lysine i.e. 4.24 and 4.01 g/ 100 g protein due to addition of defatted soya flour to wheat flour. With regard to methionine content, a significant ($p<0.05$) difference between control biscuits was there. T_2 had the highest (1.61 g/ 100 g protein) content when compared to T_1 (1.41 g/ 100 g protein) and T_3 (1.27 g/ 100 g protein), T_0 (1.04 g/ 100g protein), respectively. Simultaneously, T_4 contained slightly higher (2.18 g/ 100 g protein) content of methionine in comparison to T_5 (2.07 g/ 100 g protein) among value added biscuit samples.

Vitamin A content of control and value added biscuit samples was significantly ($p<0.05$) different. Among control biscuit samples, T_1 had the highest (1110.4 μg) vitamin A content as compared to T_2 (1067.1 μg) and T_3 (1028.9 μg), respectively. It was reported as 1119.2 and 1331.5 μg in T_4 and T_5, respectively whereas T_0 had lowest value of vitamin A as 115.2 μg . Higher content of vitamin A in T_5 attributed to combination of defatted soya flour and tangerine peel powder in it. Further, vitamin C was not present in all the control treatments whereas value added treatments i.e. T_4 and T_5 had 4.7 and 5.6 mg of vitamin C, respectively, due to presence of tomato and tangerine peel powder. The mean vitamin E was 0.04, 0.03 and 0.02 mg in T_1, T_2 and T_3, respectively, while it was 1.30 and 1.32 mg in T_4 and T_5, respectively. No amount of vitamin C and E was detected in T_0.

Data regarding calcium content revealed a significant ($p<0.05$) difference between all the treatments of biscuit. It was reported that T_1 had 474.7 mg of calcium whereas T_2, T_3 and T_0 was observed with 208.7 mg, 174.8 mg and 56.2 mg of calcium, respectively. The average calcium content was 258.4 and 281.6 mg in T_4 and T_5, respectively. Phosphorus was also observed as high as 390 mg in T_1 when compared to T_2 and T_3 (377.6 and 364.1 mg, respectively). It was found as 726.4 and 717.2 mg in T_4 and T_5, respectively. A significant ($p<0.05$) difference was found in the iron content of control and value added treatments. The mean iron content was 4.08, 3.1 and 3.92 mg in T_1, T_2 and T_3, respectively whereas higher (6.0 and 6.7 mg) iron content was reported in T_4 and T_5, respectively. The mean zinc content was 1.68, 1.52 and 1.24 mg in T_1, T_2 and T_3 samples. These values were higher (2.76 mg in T_4 and

2.69 mg in T_5) in value added samples. Nevertheless, iron and zinc content was found as low as 1.46 and 0.4 mg in T_0. Wheat bran is considered as the richest source of calcium, iron and zinc and supplementing wheat bran with soya flour contributed to increased mineral content in value added biscuits.

A significant ($p<0.05$) difference was found in total phenol and trypsin inhibitor content between all the treatments. Total phenol content was 105.7, 217.4, 152.8 and 124.5 mg GAE/ 100 g in T_0, T_1, T_2 and T_3 samples while it was 1541 and 1544 mg GAE/ 100 g in T_4 and T_5, because of addition of herbal nutraceuticals i.e. ginger and turmeric with better stability in products prepared at high baking temperature due to formation of maillard reaction products. Trypsin inhibitor ranged from 0.00 to 0.47 mg/ g. It was observed that T_4 contained the lowest amount of whereas T_1 was reported with the highest content followed by T_2 (0.39 mg/ g) and T_3 (0.36 mg/ g). It was concluded that trypsin inhibitor was significantly ($p<0.05$) reduced after baking due to its heat labile nature.

The total antioxidant activity level was increased, at every 10 percent increased incorporation of defatted soya flour, in case of control variations. A significant ($p<0.05$) difference in total antioxidant activity showed by control and value added biscuit samples was there. Value added biscuits i.e. T_4 and T_5 both showed higher total antioxidant activity when compared to control samples. Addition of nutraceuticals such as tomato, tangerine peel powder, ginger and turmeric in value added treatments and production of maillard reaction products during baking enhanced the total antioxidant activity of value added treatments.

Results of sensory characteristics depicted that there was a significant difference ($p<0.05$) in appearance, color, texture and flavor among three treatments of control biscuits. Among control samples, T_3 was most acceptable with mean overall acceptability score of 8.3 whereas T_1 as least acceptable with 6.65 points. Moreover, highest (93.54 and 92.77 percent) index of acceptability was reported in T_0 and T_3, respectively. For value added treatments, a significant difference ($p<0.05$) was found in appearance, color, texture, flavor and overall acceptability between T_4 and T_5. Dark brown color of T_5 due to degradation of carotenoid pigments and maillard reaction during drying of tangerine peel and bitter after taste were the factors for its lesser (7.0) overall acceptability scores as well as lower index of acceptability (78.22 percent) when compared to T_4 with higher overall acceptability score and index of acceptability i.e. 8.25 and 91.44 percent, respectively.

Findings regarding proximate composition of muffins reported that the moisture content ranged from 26.42 to 33.46 percent. It was further observed that T_3 had higher (33.46 percent) moisture content as compared to T_1 (27.64 percent) and T_2 (26.42 percent), respectively. The mean crude protein content in T_3 was 16.2 percent which was again higher than T_1 (7.8 percent) and T_2 (15.75 percent) due to addition of nutraceuticals i.e. wheat bran, tomato, tangerine, ginger etc. in the flour mixture for T_2 and T_3. The crude fat content ranged from 20.75 to 23.55 percent. Higher value was observed in T_3 might be due to oil present in tangerine peel powder. A significant ($p<0.05$) difference in fiber content of all muffin samples was found. More than double i.e. 13.5 percent in T_2 and 9.5 percent in T_3 while it was absent in T_1. The average total ash content of muffins was ranged from 1.70 to 2.27 percent. The values were 1.07, 2.27 and 2.18 percent in T_1, T_2 and T_3, respectively. Further, the carbohydrate content was decreased to more than half in case of value added variations than it was present in the control sample.

In vitro protein digestibility of T_1, T_2 and T_3 was 69.6, 71.5 and 72.9 percent, respectively. Further, amino acid contents of muffins showed that the mean higher (1.39 and 1.61 g/ 100 g protein) lysine content in T_2 and T_3 as compared to T_1 (1.46 g/ 100 g protein), attributed to inclusion of defatted soya flour to whole wheat flour in value added samples. Further, the average methionine content was 0.82 and 1.01 g/ 100 g protein in T_2 and T_3, respectively, whereas T_1 had 0.71 g methionine/ 100 g protein.

Vitamin A content of muffins ranged from 1335 to 1608 μg. The highest value was reported in T_2 and the lowest content was found in T_1. Further, T_3 had 1417 μg vitamin A. Higher vitamin A in value added muffins than in control sample could be due to tomato and tangerine peel powder present in those. A significant ($p<0.05$) difference was found between all treatments of developed muffins. T_1 had no content of vitamin C while T_2 and T_3 were reported with 9.6 and 7.8 mg of vitamin C, respectively, was due to the presence of tomato pulp, tangerine peel powder and ginger in value added muffins. Besides, T_1, T_2 and T_3 had 0.02, 0.64 and 0.55 mg of vitamin E, respectively. Wheat bran added to T_2 and T_3 muffin samples contributed towards increased content of vitamin E.

Calcium content of muffins ranged from 174.7 to 209.5 mg. T_2 has the highest content while the lowest amount was present in T_1. Increased phosphorus content was observed in wheat bran supplemented muffins i.e. T_2 and T_3 (793.1 and 778.0 mg) when compared to T_1 (413.2 mg), respectively. Further, iron & zinc content of T_1 and T_2 were significantly ($p<0.05$) different and higher than in T_1, respectively.

Anti nutritional factors i.e. total phenols and trypsin inhibitor data revealed that a lower (186.6 mg GAE/ 100 g) content of total phenols was observed in T_1 than T_2 (2189 mg GAE/ 100 g) and T_3 (2186 mg GAE/ 100 g), respectively. It was significantly ($p<0.05$) increased when turmeric and ginger powder were incorporated in value added muffins being stable to higher temperature. Trypsin inhibitor ranged from 0.21 to 0.24 mg/ g in muffin samples.

Total antioxidant activity of muffins has shown a significant ($p<0.05$) difference in all three samples. It was reported that T_2 and T_3 (wheat flour with nutraceutical ingredients) had comparatively higher free radical scavenging capacity as compared to T_1 (with only wheat flour).

The mean scores of sensory parameters reported that a significant difference ($p<0.05$) was there in appearance, color, texture and flavor between control and experimental variations. Moreover, T_3 scored highest (8.0) overall acceptability scores as compared to T_1 and T_2 i.e. 7.5 and 6.1, respectively. The percentages for Index of Acceptability were 82.44, 68.44 and 87.78 in T_1, T_2 and T_3, respectively.

Among noodles, it was observed that the mean moisture content of T_2 was 9.40 percent which was the highest when compared to T_1(9.34 percent), T_4 (9.23 percent) and T_3 (9.01 percent), respectively. It was further reported that T_2 had 32.05 percent crude protein content whereas T_1, T_3 and T_4 had 8.20, 29.15 and 27.65 percent of crude protein, respectively. Defatted soya flour supplementation to wheat flour resulted into increased crude protein content of value added noodles. Moreover, the mean crude fat was again maximum (1.01 percent) in T_2 while minimum (0.89 percent) in T_4. A significant ($p<0.05$) difference in fiber content of all the noodle samples was observed. T_2 was reported with higher (2.46 percent) fiber content as found in $T_{1,}$ T_2 and T_3 (1.65, 2.32 and 2.03 percent), respectively. As the percentage of ash content was concerned, T_2 and T_3 had higher values i.e. 4.22 and 3.91, respectively. On the other hand, a significant ($p<0.05$) decrease was reported in carbohydrate content on increased incorporation level of soya flour to wheat flour in T_2. The figures for ash content in T_4 and T_1 were 3.47 and 1.02 percent, respectively. The average carbohydrate content was 56.72 percent in T_4 whereas T_1 was observed with 78.83 percent.

In vitro protein digestibility of T_1 and T_4 was almost same i.e. 69.6 and 69.7 percent, respectively. In T_2 and T_4, this percentage was observed as high as 78 and 76.6, respectively. The mean lysine content was ranged from 1.29 to 3.56 g/ 100 g protein. A higher content of lysine i.e. 3.56 g/ 100 g protein was observed in T_2 due to additional amount of defatted soya flour in it as compared to T_1 (1.29 g/ 100 g protein) and T_2 (1.92 g/ 100 g protein) and T_3 (2.31 g/ 100 g protein), respectively. content was observed in T_3. Further, a significant ($p<0.05$) difference was found in methionine content detected in all noodle samples. T_1, T_2, T_3 and T_4 was reported with 1.03, 0.72, 0.75 and 0.77 g methionine/ 100 g protein, respectively. Pulses contain lower amount of methionine as compared to cereals. The developed product results into decreased methionine content when the proportion of pulse flour to cereal flour is increased.

The average vitamin A content was 161.3, 219.6, 186.2 and 179.4 µg/ 100 g in T_1, T_2, T_3 and T_4, respectively. All the samples has been reported with 0.0 mg/100 g of vitamin C as cereals and pulses are lacking in vitamin C. The highest (0.07 mg/ 100 g) vitamin E content was observed in T_2 being wheat flour and wheat bran preparation. In contrast to this, all the control samples were having significantly lower amounts of vitamin E content.

The calcium content ranged between 90.6 to 124.3 mg. T_2 contained higher content as compared to other noodle samples. Besides, T_3 had 120.6 mg calcium in it. Simultaneously, phosphorus content was ranged from 296.4 to 409.2 mg. It was reported that T_1 possessed the highest amount of phosphorus while T_4 had the lowest content. Furthermore, T_2 and T_3 had 4.69 and 4.50 mg iron / 100 g of noodle sample. The zinc content ranged from 1.09 to 1.81 mg and higher amount of zinc was reported in T_2 while the lesser value was T_1. The mean content of minerals increased on 10 percent higher supplementation level of soya flour to cereal flour.

Among anti nutritional factors, total phenol content ranged from 70.25 to 234.5 mg GAE/ 100 g. T_2 contained the highest amount of total phenols in it while T_1 was reported with the lowest (70.25 mg GAE/ 100 g). Trypsin inhibitor was 0.00, 2.72, 2.32 and 1.70 mg/ g in T_1, T_2, T_3 and T_4, respectively.

A significant ($p<0.05$) difference in total antioxidant activity of control and value added samples was found. Moreover, a rise was observed on every 10 percent increase in defatted soya flour among control samples. T_2 with higher amount of defatted soya flour was reported with the highest value.

Data regarding sensory characteristics of noodles depicted that T_1 and T_4 were equally acceptable as their overall acceptability score were 7.2 and 7.7, respectively, while the mean scores for T_2 and T_3 were 6.5 and 7.0, respectively. Furthermore, Index of Acceptability (I. A.) was 69.58 and 68.48 percent in T_4 and T_1, respectively when compared to T_2 (59.04 percent) and T_3 (65.34 percent).

Proximate composition of three noodle flavors i. e. T_1, T_2 and T_3 showed that the moisture content was ranged from 57.6 to 59.2 percent. T_1 with tomato, ginger and tangerine peel powders was found with the highest moisture content whereas T_2 had the lowest moisture in it. The average protein content of T_1 was 4.4 percent as compared to T_2 and T_3 2.0 and 2.4 percent, respectively. Further, the crude fat content ranged between 0.6 to 2.2 percent. Highest fat content was reported in T_1 might be due to mixture of tomato, ginger and tangerine peel powder. A significantly ($p<0.05$) higher amount of crude fiber was reported in T_1 (4.6 percent) than two other treatments (2.6 and 2.4 percent in T_2 and T_3), respectively. The mean ash content was 1.8, 1.0 and 1.0 percent in T_1, T_2 and T_3, respectively. The range of carbohydrate content was 26.8 to 36.2 percent. Maximum amount of carbohydrate was reported in T_2 and minimum in T_1, respectively.

Findings regarding *in vitro* protein digestibility of noodle flavors showed that T_1, T_2 and T_3 had 82.74, 72.04 and 70.22 percent *in vitro* protein digestibility, respectively. The average lysine content was 0.07 g/ 100 g protein in T_1; 0.06 and 0.06 g/ 100 g protein in T_2 and T_3 noodle flavour samples, respectively. Further, methionine was present as 0.02, 0.01 and 0.01 g/ 100 g protein) in T_1, T_2 and T_3, respectively.

It was observed that vitamin A content of noodle flavors ranged from 4.5 to 13.4 µg. T_3 had the highest value while T_2 was reported with lowest figure. Further, it was reported as 10.8 µg in T_1. Vitamin C was observed as 1.0, 0.79 and 0.92 mg in T_1, T_2 and T_3, respectively. The content of vitamin E was 0.03, 0.02 and 0.03 mg in T_1, T_2 and T_3, respectively.

Results regarding mineral content of noodle flavors reported that the mean calcium was ranged from 0.56 to 0.61 mg. Higher content of calcium (0.61 mg) and phosphorus (1.52 mg) was observed in T_1 and T_3 (0.59 mg calcium and 1.26 mg phosphorus) than T_2 (0.56 mg calcium and 1.12 mg phosphorus), respectively. The average iron & zinc content was 0.06 & 0.03, 0.04 & 0.00 and 0.02 & 0.12 mg in T_1, T_2 and T_3, respectively.

Findings of anti nutritional content of noodle flavors revealed the range of total phenol content in three noodle flavors from 6.72 to 7.04 mg GAE/ 100 g. The mean values were 7.04, 6.72 and 6.91 mg GAE in 100 g of T_1, T_2 and T_3 samples, respectively. Trypsin inhibitor was not present in any of the noodle flavor sample.

Total antioxidant activity of T_1, T_2 and T_3 was significantly ($p<0.05$) different. Data revealed that T_1 had the maximum free radical scavenging potential as compared to other samples, might be prepared with incorporation of all the functional ingredients such as tomato, ginger, tangerine peel powder and turmeric.

With regard to sensory characteristics, it was reported that combination of tomato with ginger in T_2 had higher overall acceptability score of 7.8 when compared to T_1 and T_2 (6.0 and 7.2), respectively. Moreover, addition of all nutraceuticals in T_1 resulted into lower (56.52 percent) acceptability of developed product whereas, T_2 was the most acceptable with an Index of Acceptability (I. A.) as 65.88 percent.

Taking all the findings into consideration, it is concluded that prostate cancer can be managed with the help of nutrition interventions such as by imparting nutrition counselling and consuming nutritious food products with high antioxidant activity. Therefore, it is recommended that:

- The individuals with prostate cancer as well as their family members should be counselled about important anti cancer foods and balanced dietary lifestyle to maintain the health status of the patient.
- Awareness regarding occurrence, signs and symptoms, prevention and dietary management of the disease should be imparted through making the prostate cancer specific educational aids available at village and block level camps and campaigns.

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1. Piverjeet Kaur, Beenu Tanwar and Vikas Kumar. 2015. Food Toxicity in Punjab: A Review of Concerns and Strategies. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 6 (5): 302-308 Impact factor: 0.35
2. Piverjeet Kaur Dhillon and Beenu Tanwar. 2018. Rice bean: A healthy and cost-effective alternative for crop and food diversity. Food Security (Springer Nature) 10 (3): 525–535 Impact factor: 2.97 NAAS Rating: 8.97
3. Piverjeet Kaur Dhillon and Beenu Tanwar. 2018. Muffins Incorporated with Multiple Blend Functional Ingredients: Development, Sensory Evaluation, Proximate Composition and Total Antioxidant Activity. Journal of Agricultural Engineering and Food Technology (Krishi Sanskriti) 5 (3): 122-126
4. Piverjeet Kaur Dhillon and Beenu Tanwar. 2019. Nutrition knowledge *vis-à-vis* Health Status of Indian Punjabi Males with Carcinoma Prostate. Studies on Ethno-Medicine (KRE Publishers) 13 (1): 8-16. DOI:10.31901/24566772.2018.13.1.582 NAAS Rating: 5.0
5. Piverjeet Kaur Dhillon and Beenu Tanwar. 2018. Physico-Chemical and Sensory Characterization of Biscuits Developed using Multiple Blend Nutraceuticals. A poster was presented and abstract has been published during National Conference on Sustainable Agriculture Food and Nutritional Security (SAFNS 2018), organized by School of Agriculture, Lovely Professional University, Phagwara. 16-17 February, 2018. Page No. 168-169.
6. Piverjeet Kaur Dhillon and Beenu Tanwar. 2018. Muffins Incorporated with Multiple Blend Functional Ingredients: Development, Sensory Evaluation, Proximate Composition and Total Antioxidant Activity. Oral presentation during International conference on Recent trends in agriculture, food science, forestry, horticulture, aquaculture, animal sciences, biodiversity and climate change (AFHABC-2018) held at Jawahar Lal Nehru University, New Delhi. 09 September, 2018. Second Best oral presentation award.
7. Prostate cancer : Prevention and treatments by Piverjeet Kaur Dhillon, Beenu Tanwar and Rasane Prasad Jayprakash. (Booklet published in English, 2015)
8. Prostate cancer : Bachaa ate Ilaaz by Piverjeet Kaur Dhillon, Beenu Tanwar and Rasane Prasad Jayprakash. (Booklet published in Punjabi, 2015)

Appendix I

LOVELY PROFESSIONAL UNIVERSITY

DEPARTMENT OF FOOD TECHNOLOGY AND NUTRITION

RESEARCH SUBJECT INFORMATION AND INFORMED CONSENT FORM

Chief Investigator **:** Dr. Beenu Tanwar and Dr. Rasane Prasad Jayprakash

Investigator **:** Piverjeet Kaur Dhillon

We expect your valuable participation in the clinical research entitled:

"Nutrition Knowledge in association with Health status of Prostate Cancer (PCa) Patients in Selected Hospitals of Bathinda City"

Before agreeing to participate in this research, we strongly encourage you to read the following information about the conduct of this study:

1. **Introduction:** Prostate cancer (PCa) is the second most common cancer among male in both India and Punjab. The state of Punjab is experiencing a rising burden of this disease in the age group of 35-65 years which leads to considerable loss in potentially productive years of life. Numerous researches has shown that higher incidence of cancer is primarily attributed to urbanization, industrialization, lifestyle changes, environmental factors such as pesticides, insecticides, chemicals and heavy metals etc.
2. **Purpose of the study:** The present study has been designed to investigate the relationship between nutrition knowledge and health status of prostate cancer patients. During the study, we will attempt to assess several other factors to establish a prostate-cancer specific etiology.
3. **Procedure involved in the study:** Your participation will be completely voluntary. You can withdraw from this study at any stage without giving reason. During this research, you read the information about the study and sign a consent form you will find a well-designed questionnaire containing some questions regarding your general information, dietary lifestyle, medical history etc. and you will have to spare one hour to fill it as per your convenience. Nutrition counseling will be given to some selected subjects. No changes will be made in your medication and treatment by us.
4. **Confidentiality of research records:** The information gained during the study will be published in research journals and accessible to the researchers but the individual information will remain confidential.
5. **Potential benefits and risks:** Results of this study will contribute to scientific understanding about prostate cancer and the key factors affecting it. These findings may help in further prevention and treatment of prostate cancer. There are no foreseeable risks associated with this study.

INFORMED CONSENT FORM

I, ________________________________ hereby consent to participate as requested in research subject information for the research project entitled "Nutrition Knowledge in association with Health status of Prostate Cancer (PCa) Patients in Selected Hospitals of Bathinda City".

Please tick the box below.

1. I have read and understand the research subject information provided above and have had the opportunity to ask the questions. □
2. Details of procedures and any risks have been explained to my satisfaction. □
3. I have had the opportunity to discuss taking part in this research with my family members or friends. □
4. I understand that my participation is voluntary and I am free to withdraw at any stage without giving a reason. □

Therefore, I hereby extend my participation for the above-said project.

____

Participant's signature

Date: ______________________

I certify that I have explained about the study to the participant and consider that he understands it in quite a fair manner. He freely consents to participation in this research study.

____

Researcher's signature

Date: _______________________

APPENDIX – I

GENERAL, LIFESTYLE AND HEALTH RELATED INFORMATION

S. No. Date:

A. GENERAL INFORMATION:

a) Name:

b) Age:

c) Gender:

d) Address:

e) Since, when living in this area:

f) Father's name:

g) Contact no.:

B. EDUCATIONAL INFORMATION:

a) Educational Level: Illiterate □ Primary □ Middle □ Matric □ Intermediate □
Graduate □ Post-graduate □ Doctorate □

C. OCCUPATIONAL INFORMATION:

a) Occupation: Agriculture □ Business □ Service □ Labourer □ Unemployed □
Any other (specify):

b) Employment status: Retired □ Full-time □ Part-time □

c) If Agriculturist, specify land holding:

D. FAMILY INFORMATION:

a) Religion:

b) Caste:

c) Locality: Rural □ Urban □ Semi-urban □

d) Marital Status: Single □ Married □ Divorced □ Widower □

e) Type of family: Joint □ Nuclear □

f) Number of family members:

g) Family income (per annum):

h) Socio-economic status: LIG □ MIG □ HIG □

E. LIFESTYLE HISTORY:

a) Sleeping span/ day: <6 hrs □ 6-8 hrs □ >8 hrs □

b) Physical activity: Yes □ No □

if yes, specify type and time for that activity: Walk □ Cycling □ Watching TV □

Any other(specify):

c) Household practices:

i) Storage containers for food commodities: Empty pesticide containers □

Food Grade plastic □ Steel containers □ Other metals (specify):

ii) Source of drinking water: Municipal water supply □ Submersible □

Hand pump □ Public RO system □ Personal RO □

iii) Cleaning of fruits and vegetables: Yes □ No □

If yes, specify method:

e) Agricultural and allied practices:

i) Kitchen garden at home: Yes □ No □

ii) Source of Fruits and vegetables: Kitchen garden □ Farm □

Organized food outlets □ Vendors □

iii) Source of milk: Livestock □ Milkman □ Dairy □ Any other (specify):

F. MEDICAL HISTORY:

a) Family history of the disease: Yes □ No □

If yes, specify the relationship to the carrier of the disease:

b) Time of the diagnosis of the disease:

c) Stage: I □ II □ III □

d) PSA level:

G. COMMON COMPLICATIONS/ CLINICAL SIGNS AND SYMPTOMS:

a) General symptoms: Weight loss □ Hair loss □ Weakness □

Weight gain □ Fatigue □ Loss of appetite □

b) Prostate cancer specific symptoms: Pelvic pain □ Constipation □

Lesser urine output □

Exertion during urination □

Enlargement of prostate □

c) Onset of the disease: Gradual □ Sudden □ Off & on □

d) Aggravating factors: Exertion □ Diet □ Any other □

e) Relieving factors:

H. ANHROPOMETRIC MEASUREMENTS: a) Height:____________cm

b) Weight:____________kg

c) BMI:______________kg/m^2

I. BIOCHEMICAL ASSESMENT: **Pre** **Post**

a) Serum PSA level: ____

b) Bone Mineral Density: ____

c) Serum albumin: ____

d) Serum Globulin: ____

e) Hemoglobin: ____

f) Serum Vitamin- A: ____

g) Serum Vitamin- C: ____

h) Serum Vitamin- E: ____

i) Serum Iron: ____

j) Serum Selenium: ____

k) Serum Zinc: ____

J. CLINICAL ASSESSMENT OF DEFICIENCY DISORDERS;

a) Symptoms of Anaemia:

i) Paleness of skin: Yes □ No □

ii) Pallor of Tongue: Yes □ No □

iii) Pale conjunctiva: Yes □ No □

iv) Spoon- shaped Nails: Yes □ No □

v) Headache: Yes □ No □

vi) Feeling of Lethargy: Yes □ No □

vii) Feeling of Fatigue: Yes □ No □

viii) Breathlessness: Yes □ No □

b) Vitamin - C deficiency symptoms:

i) Spongy bleeding gums: Yes □ No □

ii) Teeth mottled enamel: Yes □ No □

K. HEALTH STATUS:

a) Allied diseases: Specify the type

i) Diabetes Mellitus: Yes □ No □

ii) Renal Disorder: Yes □ No □

iii) Cardio-vascular Diseases: Yes □ No □

iv) Respiratory Infections: Yes □ No □

v) Any other:

b) Medication prescribed:

APPENDIX-II

FOOD QUESTIONNAIRE

I. DIETARY PATTERN:

Vegetarian □ Non-vegetarian □ Ova-vegetarian □ vegan □

II. MEAL PATTERN: Twice/ day □ Thrice/day □

III. GENERAL INFORMATION ABOUT DIET CONSUMED:

a) Type of fat used to cook food: Butter □ Desi ghee □

Mustard oil □ Hydrogenated fat □

Refined oil □ (specify) __________________

Do you add extra fat in dal/ vegetables: Yes □ No □

If yes, No. of teaspoon:

b) Size of bowl used for vegetables/ dal/ curd: small □ medium □ large □

c) Size of glass/ cup used for milk and tea: small □ medium □ large □

d) Size of parantha/ chapatti: small □ medium □ large □

e) No. of teaspoons of sugar added to each glass of milk or tea:________

f) Type of diet: Normal □ Spicy □ Bland □ Fried □ Boiled □

g) Water Intake**:** < 6 glass □ 6-8 glass □ >8 glass □

IV. Dietary intake of three consecutive days:

Day 1:

Meal Pattern (Time)	**Type of preparation & amount**	**Ingredients used**	**Amount (g)**
Early Morning ( )			
Breakfast ( )			
Mid Morning ( )			
Lunch ( )			
Evening Tea ( )			
Mid Evening ( )			
Dinner ( )			
Post Dinner ( )			

Day 2:

Meal Pattern (Time)	**Type of preparation & amount**	**Ingredients used**	**Amount (g)**
Early Morning ( )			
Breakfast ( )			
Mid Morning ( )			
Lunch ( )			
Evening Tea ( )			
Mid Evening ( )			
Dinner ( )			
Post Dinner ( )			

Day 3:

Meal Pattern (Time)	**Type of preparation & amount**	**Ingredients used**	**Amount (g)**
Early Morning ( )			
Breakfast ( )			
Mid Morning ( )			
Lunch ( )			
Evening Tea ( )			
Mid Evening ( )			
Dinner ( )			
Post Dinner ( )			

APPENDIX – III

KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) QUESTIONNAIRE

S. No. Name: Date:

Total score: Score obtained:

Tick the correct answer out of the different choices:

Knowledge:

1. What is cancer?
   a) Control growth of normal cells
   b) Uncontrolled growth of normal cells
   c) Don't know
2. Is cancer contagious?
   a) Yes b) No c) Don't know
3. Are all the tumors cancerous?
   a) Yes b) No c) Don't know
4. What is benign prostatic hyperplasia?
   a) It is a condition of non-cancerous proliferation of prostate.
   b) It is cancerous
   c) Don't know
5. Can prostate cancer spread from one part to another part of the body?
   a) Yes b) No c) Don't know
6. Do you know the different stages of prostate cancer?
   a) Yes b) No c) Don't know
7. Which one is the male dominating hormone?
   a) Estrogen b) Androgen c) Don't know
8. Is prostate cancer hereditary?
   a) Yes b) No c) Don't know
9. Do you know about PSA level?
   a) Yes b) No c) Don't know
10. Is there any digestive system related problem such as exertion during urination or constipation are signs of PCa?
    a) Yes b) No c) Don't know
11. Is increased use of pesticides and polluted water, a contributory factor for cancer?
    a) Yes b) No c) Don't know

12. Does excessive fat consumption increase PCa risk?

a) Yes b) No c) Don't know

13. Do you know about good/ bad fats?

a) Yes b) No c) Don't know

If yes, differentiate____

14. Do you know about fluoride/heavy metal toxicity?

a) Yes b) No c) Don't know

15. Is stress a contributory factor in progression of PCa?

a) Yes b) No c) Don't know

16. What is the importance of reverse osmosis?

a) To eliminate dirt and impurities of water

b) To eliminate heavy metals

c) Both A and B

d) Don't know

17. Does a diet to fight PCa exist?

a) Yes b) No c) Don't know

18. Do whole grains, pulses and fruits and vegetables reduce the risk of PCa?

a) Yes b) No c) Don't know

19. Does a high bran diet help mitigate fluoride/ heavy metal toxicity/PCa?

a) Yes b) No c) Don't know

20. Sources of fiber in diet?

a) Whole grains and legumes b) Refined cereal

c) Milk and milk products d) Don't know

21. What are the plant sources of good quality protein in diet?

a) Soybean, pulses and legumes b) fruits and vegetables c) Don't know

22. Which vegetables and fruits are helpful in protecting against PCa?

a) Tomato, cruciferous vegetables and citrus fruit

b) Potato and Apple

c) Don't know

23. Are condiments helpful in reducing PCa?

a) Yes b) No c) Don't know

If yes, specify the names____

24. Are alcohol/ tobacco/ carbonated beverages/ artificial sweeteners good for PCa patients?

a) Yes b) No c) Don't know

25. Do you know about the cancer awareness days celebrated to create awareness among masses?

a) Yes b) No c) Don't know

Attitude:

1. What to do if you experience exertion during urination?
   a) Consult your physician
   b) Take medicine at your own
   c) Increase your water intake
   d) Don't know
2. What you do if you feel constipated?
   a) Go to doctor
   b) Enema
   c) Increase your dietary fiber
   d) Don't know
3. What to do if you face difficulty in going upstairs and feeling fatigue?
   a) Go to doctor and get your Hb test done
   b) It happens sometimes, ignore it
   c) Take rest
   d) Don't know
4. Which is better among following for water purification?
   a) Boiling of water
   b) Reverse osmosis
   c) Chlorine or potassium meta-bisulphate
   d) Don't know
5. Should we go for organic farming for household consumption?
   a) Yes b) No c) Don't know
6. Pesticide residues can be removed by:
   a) Washing of foods
   b) Soaking and peeling
   c) Cooking
   d) Don't know

7. Does combining one food stuff with another benefit your health?
   a) Yes b) No c) Don’t know
8. Do you wash the vegetables and fruits thoroughly before cutting?
   a) Yes b) No c) Don’t know
9. Fruits and vegetables should be stored in dry form in order to avail them during off season?
   a) Yes b) No c) Don’t know
10. Which cooking method is good?
   a) Open pan cooking b) Pressure cooking c) Don’t know
11. Pressure cooker should be used instead of open pan to:
   a) To retain nutrients of food
   b) To cook food easily and in minimum time
   c) Don’t know
12. Chapattis can be made without sifting the flour?
   a) Yes b) No c) Don’t know
13. Having tea along with meals is good for health?
   a) Yes b) No c) Don’t know
14. Can you have your favorite snacks/ meals during the disease?
   a) Yes b) Sometimes c) Never
15. Is it better to eat 6-8 meals instead of 2-3 meals in a day?
   a) Yes b) No c) Don’t know

Practices:

1. Do you compare the later medication or drugs prescribed by the doctor to earlier ones?
   a) Yes b) No
2. Have you taken second opinion of the medical practitioner for your disease?
   a) Yes b) No
3. Do you wear mask when exposed to open crop fields or while spraying?
   a) Yes b) No
4. In which containers you store your dried food stuffs i. e. Grains/ pulses/ spices/ condiments?
   a) Food grade plastic b) empty pesticide containers
   c) Steel containers d) Metal/ iron containers
   g) Any other (specify) ____________________

5. Which type of fat do you use to sauté vegetables/ pulses?

a) Butter b) Desi ghee c) Mustard oil

d) Refined oil e) Dalda f) All or combination

g) Any other (specify) ____________________

6. For how many times you maximum use a single serving of oil?

a) Once only b) twice c) more than two times

7. Do you combine pulses, cereals, fruits and vegetables during cooking?

a) Yes b) No

8. Do you grow vegetables at home?

a) Yes b) No

9. When do you wash vegetables and fruits?

a) After cutting b) Before cutting

10. Do you include lemon/ amla/ steamed capsicum in salad?

a) Yes b) No

11. How many servings of salad and fruits you consume per day?

a) 1-2 times b) 3-4 times c) >4 times d) none

12. Do you prefer fresh foods over preserved ones?

a) Yes b) No

13. Do you include any soya product in your daily diet?

a) Yes b) No

14. Do you manage your diet according to the permitted and omitted foods?

a) Yes b) No

15. Have you ever consulted the dietician?

a) Yes b) No

Appendix II

SENSORY EVALUATION PROFORMA

Name of the Panelist: **Date:**

Name of the Product: **Time:**

S. No.	Appearance	Color	Texture	Flavour (Aroma/Taste)	Overall Acceptability	Comment (If any)

Hedonic Scale

Expression	**Points to be assigned**
Liked extremely	9
Liked very much	8
Liked moderately	7
Liked slightly	6
Neither liked nor disliked	5
Disliked slightly	4
Disliked moderately	3
Disliked very much	2
Disliked extremely	1

SIGNATURE OF THE PANELIST

Appendix III

Information booklet (English)

Appendix IV
Information booklet (Punjabi)