



# Lipid profile of hyperlipidemic males after supplementation of multigrain bread containing sunflower (*Helianthus annuus*) seed flour

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**Abstract** Sunflower seeds are produced and consumed worldwide due to their richness in heart friendly nutrients in the bakery products. Its effect on reducing the incidences of cardio vascular diseases have not been studied, through supplementary studies therefore present supplementation study was planned in which five samples of bread were prepared by using various multigrain flours and sunflower seed flour at five different levels of 2.5, 5, 7.5, 10 and 12.5%. The bread sample supplemented with 7.5% sunflower seed flour was found to be highly acceptable. Sunflower seeds were roasted at 60 °C for 10–15 min. Chemical analysis of sunflower seed flour (raw and roasted) and highly acceptable bread pertaining to proximate composition, in vitro protein digestibility, antioxidant activity, bioactive compounds, essential fatty acids and minerals was done using standard methods. Roasting of seeds resulted in significant increase in carbohydrates, protein digestibility, phosphorus, magnesium and copper contents. The most acceptable sample had good amount of energy (324.48 kcal), protein (10.61 g), fat (2.92 g), fibre (11.36 g), ash (3.29 g), antioxidant activity (12.13%), total phenols (31.54 mg), flavonoids (17.53 mg), omega 3 fatty acids (186.16 mg), omega 6 fatty acids (13,701.40 mg), phosphorus (68.74 mg), magnesium (28.13 mg) and copper (0.12 mg) per 100 g. Sixty hyperlipidemic males aged 30–50 years were supplemented with highly acceptable bread for four months. After the intervention period,

mean daily intake of and nuts and oilseeds increased significantly ( $p < 0.01$ ) and a significant increase ( $p < 0.01$ ) in the intake of total fat, dietary fibre, PUFA, linoleic acid, ascorbic acid, alpha tocopherols and phosphorus was observed. The weight, BMI, total cholesterol, LDL cholesterol and triglycerides significantly reduced after the supplementation.

**Keywords** Sunflower seed flour · Multigrain bread · Organoleptic evaluation · Chemical analysis · Hyperlipidemics · Supplementation

## Introduction

Cardiovascular diseases (CVDs) have annihilated the whole world with higher manifestation in middle and low income countries. In Western countries, where only 22% people (aged < 70 years) die of Coronary Heart Diseases, India suffers 50% deaths from them. As claimed by Global Burden of Diseases report, 64 million cases of cardiovascular diseases were reported in India in the year 2015 causing demise of 3.4 million people out of which 61 million cases accounted for coronary heart diseases. Indians are more susceptible to hyperlipidemia and cardiovascular diseases that too at juvenescence because of the unsound nutritional habits like greater intake of unhealthy foods, an upsurge in sedentary lifestyle and increase in smoking and tobacco use (Aslesh et al. 2016). In recent years, the propitious nutrient composition of oilseeds has fostered their utilization in development of multifarious products for prevention of metabolic diseases as well as for providing enhanced organoleptic properties to foods. Moreover, steady consumption of a single food such as wheat is liable to cause lysine deficiency and allergic

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reaction (due to gluten) in sensitive people. Here comes the role of multigrain food products that contribute to healthy gut, cardiac health, diabetic control cancer prevention and weight loss mechanisms (Malik et al. 2015).

The sunflower seed which is produced and consumed globally provides a diversified array of nutritious components like proteins, fibre, unsaturated fats, vitamin E, copper, phosphorus, magnesium and phytochemicals. Sunflower seeds (per 100 g) provide 14 g of carbohydrates, 7 g of which comprise of fibre and approximately 20% proteins in the form of 11 s globulins and napin type 2 s albumins. They are a pronounced source of glutamic acid, aspartic acid, cysteine and arginine possessing a sound amino acid profile and low anti-nutritional content. The essential amino acids in the seed include phenylalanine, tyrosine, methionine, leucine and cysteine. The seeds accommodate 35–42% oil which has 55–70% linoleic acid as the predominant unsaturated fatty acid followed by 20–25% of oleic acid. Oleic acid helps to improve the HDL levels and lower the proportion of LDL and triglycerides. Both linoleic and oleic acids have been linked to have reverse association with coronary heart diseases. Alpha tocopherol accounts for 90% of the total tocopherols. A substantial amount of phosphorus, magnesium and copper is also present in the seeds. Magnesium promotes muscle, skeletal, respiratory and nervous function. Copper is a crucial part of various enzymes possessing antioxidant activity (Srivastava and Verma 2014). Sunflower seeds offer antimicrobial, antioxidant, antihypertensive, anti-inflammatory, wound healing and cardiovascular health benefits. One of the cardinal advantages of the seeds includes amelioration of lipid profile. The unsaturated fatty acids, high fibre, phytosterols and the vitamin E content of the seeds promote cardiovascular health. This essential vitamin acts as an antioxidant helping to neutralize free radicals and protect cell membranes from damage. The oil extracted from sunflower seeds also possess antioxidant characteristics and therefore, may assist in decreasing the LDL and total cholesterol (Bester et al. 2010).

The nutritional quality of bakery products prepared from refined wheat flour is poor as it lacks some essential amino acids and inadequately meets the requirements of macro and micro nutrients. The amino acids lysine, threonine and valine are deficient in wheat. The utilization of bakery products as a medium for supplementation of various nutrients is augmenting gradually in a progressive manner. Bread and cookies occupy the foremost position acting as supplementation vehicles. Sunflower seeds can be supplemented in conventional recipes like *ladoo*, *biscuit* and *missi roti*. It is an ideal low carbohydrate and high protein raw or roasted snack option. It can also be used as a confectionary

nut (Nadeem et al. 2010). Even though sunflower seeds have a plethora of nutrients but no study is available on their utilization in bakery products and supplementation in hyperlipidemic patients. Therefore, taking into consideration the advantageous effects of sunflower seeds, the present study was framed with the objective to supplement sunflower seeds in multigrain bread and to gauge its effect in lowering the risk of menacing cardiovascular disease.

## Materials and methods

### Procurement of sample

Sunflower (*Helianthus annuus*) seeds (hybrid PSH 1962) were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Other ingredients like refined wheat flour, whole wheat flour, barley flour, oats flour, rice flour, maize flour, compressed yeast, powdered sugar and refined vegetable oil were purchased from the local market.

### Processing of sunflower seeds

Sunflower seeds were washed thoroughly under running water to eliminate any foreign particles and dust. After sun drying the clean seeds, they were roasted at 60 °C for 10–15 min and then ground in an electric grinder to obtain fine flour. Sunflower seed flour (in raw form) was used in the development of multigrain bread.

### Preparation of bread

Five experimental samples of multigrain bread were developed by incorporating sunflower seed flour at different levels i.e. 2.5, 5, 7.5, 10 and 12.5% (Fig. 1). Samples of multigrain bread prepared with supplementation of sunflower seed flour) whereas control sample of bread was developed using 100% refined wheat flour. The percentages of wheat flour, barley flour, oats flour, rice flour and maize flour were kept constant i.e. 15, 15, 10, 5 and 5% respectively while the amount of sunflower seed flour was replaced with the corresponding amount of refined wheat flour. Sample 'a' (Fig. 1) contained 100% refined wheat flour without any supplementation. Samples 'b', 'c', 'd', 'e' and 'f' contained 47.5, 45, 42.5, 40 and 37.5% refined wheat flour and 2.5, 5, 7.5, 10 and 12.5% sunflower seed flour respectively. The breads were prepared using standardized recipes in the bakery laboratory of Department of Food and Nutrition, College of Community Science, Punjab Agricultural University, Ludhiana.

**Fig. 1** Samples of multigrain bread prepared with supplementation of sunflower seed flour



### Organoleptic evaluation of developed multigrain bread

The developed samples were subjected to organoleptic evaluation by a semi-trained panel of ten judges from Department of Food and Nutrition, College of Community Science, Punjab Agricultural University, Ludhiana. Sensory evaluation was done to determine the most suitable percentage of sunflower seed flour incorporated in multigrain bread.

### Nutritional evaluation of sunflower seed flour (raw and roasted) and highly acceptable developed product

Sunflower seed flour (raw and roasted) and highly acceptable developed product were analyzed for proximate composition (AOAC 2000), *in vitro* protein digestibility (Akeson and Stachman 1964), Total antioxidant activity using DPPH method (Dehshahri et al. 2012), Flavonoids (Zhishen et al. 1999), Total Phenols (Singleton et al. 1999), Omega 3 and omega 6 fatty acids (Appelqvist 1968) and mineral content of copper, magnesium and phosphorus (AOAC 2000).

### Selection of subjects

Sixty hyperlipidemic males free from serious complications aged 30–50 years were selected from Delta Heart Center, Ludhiana. The complete study was divided into two periods i.e. pre and post intervention period. Pre-intervention period pursued for 4 weeks (February, 2019) where the subjects consumed regular diet devoid of any supplementation. Post intervention period accounted for 16 weeks (May to August, 2019) when the selected subjects were provided with highly acceptable sunflower seed flour supplemented multigrain bread daily. Weight per piece of bread was 25 g and the subjects were asked to have 4 pieces in breakfast. The record of dietary intake, anthropometric parameters and biochemical profile of the subjects was taken during both the periods and compared to analyze the efficacy of supplementation of sunflower supplemented multigrain bread in reducing hyperlipidemia.

### Ethical approval

The research was approved by the ethical committee of Punjab Agricultural University, Ludhiana (No. DR-8323-32-19-4-19).

## Collection of data

General information (age, religion, education, occupation, marital status, family size, family type, monthly income, occupation and lifestyle related information and physical activity pattern), medical information (relating to family history of disease, etiology related to diseases, treatment of diseases and intake of supplements), smoking, tobacco and alcohol related information was collected via interview schedule. Dietary information (food likes and dislikes, consumption of junk food and oilseeds, usage of cooking oils, meal pattern, information about home remedies, dietary intake by 24 h recall method and food frequency questionnaire) and anthropometric measurements of height and weight (Jelliffe 1966), Body Mass Index (Garrow 1981) and body fat percentage (using Omron body composition monitor BF-214) were also recorded. The average daily intake of nutrients was calculated using Diet Cal (Kaur 2017) and the mean daily nutrient intake was compared with the dietary intakes of balanced diet and recommended dietary allowances by ICMR (2010). Blood analysis was done for Total cholesterol (Richmond 1973), HDL cholesterol (Lopes et al. 1977), Triglycerides (Fossati and Principe 1982), LDL and VLDL cholesterol (Friedwald et al. 1972) and fasting blood glucose (Oser 1976).

## Statistical analysis

Mean values, standard deviation and percentage distribution for various parameters were calculated. Paired *t* test was used for the parameters compared during pre and post intervention period and the statistical significance discerned using computer software SPSS 23 (Statistical Package for the Social Sciences).

## Results and discussions

### Effect of processing on the nutritional value of sunflower seed flour

Sunflower seed flour in its raw and roasted form was chemically analyzed for proximate composition, in vitro protein digestibility, total antioxidant activity, bioactive compounds, omega 3 and omega 6 fatty acids and mineral content with the help of standardized methods. The values have been estimated per 100 g of raw and roasted sunflower seed flour on dry weight basis (Table 1). The moisture, crude fat and crude fibre content in the flour significantly ( $p \leq 0.01$ ) decreased after roasting of sunflower seeds from 3.40%, 41.98% and 19.58% to 1.27%, 29.45% and 17.52%. The results were in accordance with Soleimanieh et al. (2015), the moisture content reduced

from 4% in raw sunflower seed kernels to 0.75–1.66% in sunflower seed kernels roasted for different time durations in microwave. Gashaw (2010) stated that the reduction in crude fibre may be attributed to the removal of the indigestible carbohydrates and some water-soluble oligosaccharides (raffinose family) during thermal treatment. The crude fat contains not only fat but also fat soluble vitamins, free fats, cholesterol, lecithin, chlorophyll, resins and volatile oils which may be destructed during heat treatment. Roasting non-significantly increased the crude protein (from 14.52% to 14.54%) and decreased the total ash content (from 3.63% to 3.59%). Abdulsalami and Sheriff (2010) reported that the heat treatments may increase crude protein content because of degradation of anti-nutrients and release of nutrients. Similar findings were discovered by Adesina (2018). The effects of different processing techniques like roasting, boiling, mechanical extraction and solvent extraction were studied on the proximate parameters of sunflower seed meals. The moisture and ash contents reduced from 10.29 to 6.45% and 5.46 to 4.54% while crude protein increased from 27.02 to 37.02% on roasting. The crude fibre also reduced by 10% while the crude fat varied to a little extent (from 12.40% to 12.41%). The carbohydrate content of roasted sunflower seed flour (34.70%) was 71.02% more than the raw sunflower seed flour (20.29%). The energy content of raw sunflower seed flour was 517.02 kcal per 100 g while that of roasted sunflower seed flour was 462.37 kcal per 100 g. Enyeribe et al. (2016) analyzed the proximate composition of powdered sunflower (*Helianthus annuus*) seeds and found that the carbohydrates, proteins and lipids contributing the major portion were 39.40, 28.28 and 19.80% respectively. Other constituents present in smaller amounts were moisture (5.03%), crude fibre (3.50%) and total ash (4.02%). Anjum et al. (2010) reported 5.50% moisture, 6.11% carbohydrates, 18.72% protein, 37.47% crude fat, 28.30% crude fibre and 3.49% ash in whole sunflower seeds. In vitro protein digestibility of roasted sunflower seed flour (24.57%) was 28.33% more than the raw sunflower seed flour (17.61%). Embaby (2011) observed that the heat treatment increases flexibility of protein chains making them more accessible to protease enzyme action and thus improving the protein digestibility. In vitro protein digestibility of raw defatted sunflower seed flour, protein isolate and fibrous concentrate obtained from the defatted flour were found to be above 90% for all samples (Alexandrino et al. 2017). No significant difference was observed in the total antioxidant activity of raw sunflower seed flour ( $61.91 \pm 0.24\%$ ) and roasted sunflower seed flour ( $61.43 \pm 0.41\%$ ). Sunarharum et al. (2019) reported that the antioxidant activity increases on roasting up to 95 °C due to the increase in availability or release of antioxidant components. At roasting temperature of 125 °C

**Table 1** Nutritional evaluation of raw and roasted sunflower seed flour (on dry weight basis)

Nutrient composition	Raw	Roasted	t test
Moisture (%)	3.40 ± 0.06	1.27 ± 0.12	27.54**
Crude protein (%)	14.52 ± 0.19	14.54 ± 0.22	0.50 <sup>NS</sup>
Crude fat (%)	41.98 ± 0.27	29.45 ± 0.25	58.46**
Crude fibre (%)	19.58 ± 0.40	17.52 ± 0.45	5.95**
Total ash (%)	3.63 ± 0.17	3.59 ± 0.16	0.45 <sup>NS</sup>
Carbohydrate (%)	20.29 ± 0.73	34.70 ± 0.21	32.79**
Energy(Kcal/100 g)	517.02 ± 0.93	462.37 ± 3.38	26.97**
In vitro protein digestibility (%)	17.61 ± 0.23	24.57 ± 0.46	23.37**
DPPH activity (% inhibition)	61.91 ± 0.24	61.43 ± 0.41	1.73 <sup>NS</sup>
Total phenols (mg GAE/100 g)	104.63 ± 7.95	88.50 ± 3.95	3.15*
Flavonoids (mg QE/100 g)	86.15 ± 19.12	123.98 ± 3.07	3.38 <sup>NS</sup>
Omega 3 fatty acid (mg/100 g)	30.23 ± 0.31	20.41 ± 0.33	28.60**
Omega 6 fatty acid (mg/100 g)	25,519.90 ± 1.97	20,417.80 ± 3.01	1566.44**
Phosphorus (mg/100 g)	129.94 ± 0.25	188.73 ± 0.21	313.20**
Magnesium (mg/100 g)	59.76 ± 0.31	85.66 ± 0.27	109.49**
Copper (mg/100 g)	0.27 ± 0.02	0.41 ± 0.01	11.58**

Values are Mean ± S.D.

\* Values are significant at 5% level

\*\* Values are significant at 1% level

NS – Non significant

and 165 °C, the antioxidant activity declines be due to deterioration of phenolic components or their inability to react with DPPH free radical. The result of total antioxidant activity is a product of temperature dependent destruction of some naturally occurring antioxidant components and development of some new compounds after maillard reaction which exhibit antioxidant activity (Chandrasekara and Shahidi 2011). Islam et al. (2016) reported total antioxidant potential of sunflower seed extract (51.57%) was found to be more than the standard ascorbic acid (46.66%) and was believed to be mainly due to the high content of polyphenols present in the seeds. The data revealed that the total phenolic content significantly declined ( $p \leq 0.05$ ) from 104.63 mg per 100 g in raw sunflower seed flour to 88.50 mg per 100 g in roasted sunflower seed flour. Similar to the results of present study, Smith et al. (2016) observed that the amount of phenols were found to be inversely related to temperature and so the content of phenols reduced as the temperature elevated. A non-significant increase was observed in the total flavonoid content (from 86.15 to 123.98 mg per 100 g) on roasting sunflower seeds. Elhamirad and Zamanipoor (2012) discovered that quercetin (a type of flavonoid) was more thermally stable than gallic acid (a kind of phenolic acid). Therefore, the flavonoid content enhanced after roasting sunflower seeds while the phenolic content

declined. There was a non-significant increase by 25.8% in the total phenolic content after roasting sesame seeds which may be attributed to partial cell structure destruction causing easy extraction of bound phenolics. A similar significant increase in the total flavonoid content of sesame seeds was observed after roasting (Kamalaja et al. 2018). There was a significant reduction in the amount of both the fatty acids ( $p \leq 0.01$ ) after roasting treatment. The omega 3 fatty acid decreased from 30.23 to 20.41 mg per 100 g while the omega 6 fatty acid reduced from 25,519.90 to 20,417.80 mg per 100 g after the roasting process. This may due to the oxidative decomposition of monounsaturated and polyunsaturated fatty acids in seed oils at a temperature ranging from 100 °C to 600 °C thus, reducing their contents after heat treatment (Raba et al. 2018). The minerals increased significantly after roasting treatment of sunflower seeds ( $p \leq 0.01$ ). The phosphorus, magnesium and copper contents (mg per 100 g) in raw sunflower seed flour were 129.94, 59.76 and 0.27 which significantly increased to 188.73, 85.66 and 0.41 by 45, 43 and 52% respectively. Similarly, Makinde et al. (2016) reported that roasting enhanced the levels of calcium, potassium, magnesium and zinc in pumpkin seeds which may be due to improvement in digestibility and release of minerals from the matrix.

### Nutritional evaluation of highly acceptable multigrain bread containing sunflower seed flour

The most acceptable sunflower seed flour supplemented multigrain bread (with 7.5% sunflower seed flour) was analyzed chemically for proximate composition, in vitro protein digestibility, total antioxidant activity, bioactive compounds, omega 3 and omega 6 fatty acids and mineral content with the help of standardized methods. The values have been calculated per 100 g of developed bread sample on dry weight basis and compared with control bread sample made from refined flour (Table 2).

It was observed that except the carbohydrate content, all other parameters of proximate composition were higher in the developed sample containing 7.5% sunflower seed flour than the control sample. The moisture, crude fat, crude fibre and energy contents of the highly acceptable developed product (31.73%, 2.92%, 11.36% and 324.48 kcal/100 g) were significantly higher ( $p \leq 0.01$ ) than the control sample (29.41%, 1.26%, 2.52% and 268.58 kcal/100 g) respectively. There was a significant increase ( $p \leq 0.05$ ) in the amount of crude protein from 9.13% in control to 10.61% in most acceptable sample. Malik et al. (2015) reported that the crude protein content of barley flour (11.65%), oats flour (9.60%) and maize flour (9.78%) was higher than wheat flour (9.55%). The crude fat and

crude fibre content of these flours was also more than the wheat flour. So the multigrain bread developed by using combination of different flours had higher proximate parameters of protein, fat and fibre than the control sample. The total ash content of the highly acceptable developed product (3.29%) was reported to be significantly ( $p \leq 0.05$ ) more in comparison to the control (2.50%). Nadeem et al. (2010) found that addition of sunflower seeds (at 6, 10 and 14% levels) increased the crude fat, crude fibre and total ash contents of breads. The carbohydrate content of highly accepted sample (21.46%) showed a significant reduction ( $p \leq 0.01$ ) than the control (55.18%). In vitro protein digestibility of control (19.51%) was significantly higher ( $p \leq 0.01$ ) than the highly acceptable developed product (16.43%). The multigrain bread supplemented with 7.5% sunflower seed flour had significantly high ( $p \leq 0.01$ ) DPPH scavenging potential or antioxidant activity ( $12.13 \pm 0.37\%$ ) than the control sample ( $7.61 \pm 0.23\%$ ). Harris and Kris (2010) reported that thermal processing of grains enhances the availability of antioxidants. Additionally, the acceptable bread sample had more amount of total phenolic and total flavonoid content than the control sample. The total phenols (in gallic acid equivalent) and total flavonoids (in quercetin equivalent) in control sample were 27.06 mg/100 g and 2.82 mg/100 g while that in organoleptically most liked bread sample were 31.54 mg/100 g and 17.53 mg/100 g. Ge

**Table 2** Nutritional evaluation of highly acceptable multigrain bread containing sunflower seed flour (on dry weight basis)

Proximate composition	Control	Experimental	t test
Moisture (%)	29.41 ± 0.52	31.73 ± 0.23	19.20**
Crude protein (%)	9.13 ± 0.90	10.61 ± 0.28	2.71*
Crude fat (%)	1.26 ± 0.14	2.92 ± 0.22	4.44**
Crude fibre (%)	2.52 ± 0.27	11.36 ± 0.48	27.51**
Total ash (%)	2.50 ± 0.30	3.29 ± 0.22	3.71*
Carbohydrate (%)	55.18 ± 1.34	21.46 ± 1.33	25.39**
Energy (Kcal/100 g)	268.58 ± 2.78	324.48 ± 3.53	32.75**
In vitro protein digestibility (%)	19.51 ± 0.19	16.43 ± 0.40	12.03**
DPPH activity (% inhibition)	7.61 ± 0.23	12.13 ± 0.37	9.42**
Total phenols (mg GAE/100 g)	27.06 ± 3.22	31.54 ± 3.94	6.68**
Flavonoids (mg QE/100 g)	2.82 ± 1.28	17.53 ± 5.15	4.80**
Omega 3 fatty acid (mg/100 g)	148.69 ± 1.26	186.16 ± 0.86	45.33**
Omega 6 fatty acid (mg/100 g)	7104.94 ± 4.75	13,701.40 ± 2.28	1855.99**
Phosphorus (mg/100 g)	37.28 ± 0.10	68.74 ± 0.19	257.78**
Magnesium (mg/100 g)	22.83 ± 15.84	28.13 ± 0.07	0.58 <sup>NS</sup>
Copper (mg/100 g)	0.08 ± 0.01	0.12 ± 0.03	2.29 <sup>NS</sup>

Values are Mean ± S.D.

\* Values are significant at 5% level

\*\* Values are significant at 1% level

NS – Non significant

Experimental – multigrain bread supplemented with 7.5 percent sunflower seed flour

et al. (2020) reported barley contained 10.67 and 983.42 µg/g of phenols and flavonoids respectively, which was one of the major ingredient in the multigrain bread and significantly contributed to the enhanced antioxidant activity. The addition of sunflower seeds in multigrain bread enhanced the contents of essential fatty acids. The amount of omega 3 fatty acid in control sample was 148.69 mg per 100 g which significantly increased ( $p \leq 0.01$ ) to 186.16 mg per 100 g in highly acceptable developed sample. Similarly, a significant elevation ( $p \leq 0.01$ ) in the amount of omega 6 fatty acid by 93% was observed from 7104.94 mg per 100 g (in control) to 13,701.40 mg per 100 g (in the sample supplemented with 7.5% sunflower seed flour). Skrbic and Filipcev (2008) reported that addition of high oleic acid sunflower seeds at 16% level non-significantly improved the contents of omega 3 and omega 6 fatty acids in refined flour bread. The amount of phosphorus in highly acceptable product (68.74 mg/100 g) was significantly ( $p \leq 0.01$ ) higher than the control (37.28 mg/100 g). A non-significant increase was observed in magnesium and copper contents of the most acceptable formulated sample from 22.83 mg/100 g and 0.08 mg/100 g to 28.13 mg/100 g and 0.12 mg/100 g after addition of sunflower seed flour in multigrain bread.

**General information of the selected subjects**

The data pertaining to general information revealed that majority of the subjects (63%) were in the age group of 40–50 years and belonged to Sikh religion (75%). Most of the subjects (60%) were businessmen while 40% belonged to service class. The monthly income of 60% subjects was above Rs. 40,000. Majority of the subjects (82%) belonged to nuclear family while only 18% had joint family.

**Physical activity and lifestyle pattern of the subjects**

The physical activity and lifestyle pattern of the subjects (Table 3) reported that only 35% individuals were engaged in one or the other form of physical activity out of which 15% each performed the physical activity for twice or thrice a week and only 5% subjects did it in their daily routine. Out of the total activity doers, 27% subjects carried out the activity for less than 30 min. The incidence of CVD reduces with hike in occupational physical activity pattern as physical activity has a protective health outcome. Sedentary people are more prone to heart diseases. Moderate intensity exercise for 30 min a day for at least 5 days a week or high intensity exercise for 20 min a day for at least 3 days a week has been recommended (Carnethon 2009). Walking was the most preferred type of physical activity (20%) followed by yoga (13%). It was observed that 62% subjects had stressful environment at

**Table 3** Physical activity and lifestyle pattern of the subjects (n = 60)

Parameters	Number of subjects	Percentage
Work involved in job <sup>a</sup>		
Desk	43	71.67
Supervision	39	65.00
Touring	17	28.33
Physical work	8	13.33
Mode of transport		
Two wheeler	31	51.67
Car 23 (38.33)	23	38.33
Walk	4	6.67
Public transport	2	3.33
Physical activity		
Yes	21	35.00
No	39	65.00
Frequency of exercise		
Daily	3	5.00
Thrice a week	9	15.00
Twice a week	9	15.00
Time spent on exercise (min/day)		
< 30	16	26.67
30–60	5	8.33
Type of physical activity preferred <sup>a</sup>		
Walking	12	20.00
Yoga	8	13.33
Jogging	5	8.33
Gymming	5	8.33
Cycling	2	3.33
Home/work atmosphere		
Stressed	37	61.67
Peaceful	23	38.33
Sound sleep		
Yes	30	50.00
No	30	50.00
Sleep hours		
6–7	36	60.00
7–8	15	25.00
> 8	9	15.00

\*Multiple responses

their home or work. Dar et al. (2019) observed link of inciting stressors like marital discord, death of a loved one and low socioeconomic with coronary artery disease, myocardial infarction and congestive heart failure, respectively. Majority of the subjects (60%) slept for 6–7 h a day followed by 25 and 15% subjects who obtained 7–8 h and more than 8 h of sleep. Nagai et al. (2010) stated that sleeping for less than 5 and 6 h has a direct link with

Hypertension and Coronary Heart Diseases. Dearth of sleep causes salt retention, vasoconstriction, increases the heart rate and working of sympathetic nervous system. Not merely this, getting lesser sleep hikes cortisol levels and inhibits pancreatic function causing insulin resistance and thus Diabetes Mellitus too. The leptin and ghrelin levels are also disrupted due to exiguous sleep consequently causing obesity.

### **Dietary habits of the subjects**

Data on dietary pattern of the subjects revealed that 53% subjects were non vegetarian while 40% were vegetarians. A small proportion of subjects (7%) were consumers of egg and its products in their diet. Majority of the subjects (70%) took five to six meals a day while 30% had about three to four meals per day. Skipping meals was not very frequent among the subjects. Only 35% subjects skipped meal where skipping breakfast was most common. A high%age of subjects (85%) had a fervent desire of consuming fast food and most of them (47%) consumed it once a week followed by 24% subjects who consumed it fortnightly. Azemati et al. (2020) observed that consumption of junk food had undesirable effects on cardiometabolic risk factors among children and adolescents as the consumption of these foods were found to be associated with the increase odds of obesity or overweight. A small proportion of subjects (4%) took fast food three times a week. The subjects were not much careful about their diet and 65% of them had the same diet as their family members. Barely 35% subjects ate the diet prescribed by their doctor or dietician. It was observed that intake of bakery items like biscuits, cookies, *mathi* etc. was the maximum accounting for 72% followed by beverages (55%). Roasted snacks and fruits were consumed by 50% subjects. Sweets were the least preferred (30%). Among the types of cooking oils used, desi ghee was very common (88%). The use of mustard oil and refined oil was equally 78% followed by butter (38%) and vanaspati (35%). Canola oil was likely to be used by only 15% subjects. The consumption pattern of oilseeds as perceived uncovered the fact that lesser proportion of subjects consumed oilseeds (17%) and the major oilseeds consumed were flaxseeds (100%) and chia seeds (10%). Pumpkin seeds and sunflower seeds did not occupy any position in the diet of the selected subjects. Most subjects (80%) consumed the oilseeds in their unprocessed (raw) form and 20% of them roasted the seeds before consuming.

### **Smoking, tobacco and alcohol related information of the subjects**

The information about smoking, tobacco chewing and alcohol consumption of the subjects was also recorded during the present study. It was apprehended that most

subjects (80%) were non-smokers. Out of 20% smokers, 58% subjects consumed one cigarette per day while 42% took two cigarettes per day. Only 5% subjects chewed tobacco and all subjects were addicted to it daily. Cora et al. (2020) revealed that the mechanism of adrenal Barrestin1 upregulation caused by tobacco (nicotine) promotes cardio-toxic hyperaldosteronism which results in cardiac dysfunction. The data showed that 30% subjects were habitual of alcohol intake and most of them (56%) were continuing since 6–10 years. Valerio et al. (2019) reported that the smokers showed higher risk factors for cardiovascular diseases whereas drinkers showed higher incidences of dyslipidemia and obesity as compared to non-smokers and non-drinkers, respectively. The quantity of alcohol consumed per day preferable by 56% subjects was 60–120 ml. Some subjects (6%) were also heavy drinkers consuming about 120–180 ml alcohol per day.

### **Medical information of the subjects**

The data on medical information of the subjects stated that most subjects (70%) had family history of one or more diseases which were directly associated with heart disease. Family history of diabetes mellitus was the most common (57%) followed by that of hyperlipidemia (55%) and hypertension (50%). Julibert et al. (2019) suggested that the intake of dietary fat resulted in the higher risk of hyperglycemia. Hyperglycemia and atherosclerosis share similar mechanisms including mitochondrial oxidative stress, alterations in extracellular matrix compounds, disruption of cellular defense systems, endothelial inflammation and activation (La Sala et al. 2019). The incidence of obesity and liver diseases in their family was 36 and 12% respectively. Obesity and hereditary factors occupied nearly equal percentages of 55 and 53% being the causative risk factors of the diseases. Koliaki et al. (2019) discussed direct and indirect effects of obesity that can increase morbidity and mortality in CVD. Structural and functional adaptations of the cardiovascular system to accommodate excess body weight and the maladaptive adipose tissue expansion leading to deregulated adipokine secretion, hypoxia, inflammation, impaired mitochondrial function, abnormal lipid or glucose metabolism, hypertension and endothelial dysfunction, all of which provide linking mechanisms for the association between obesity and CVD. Forty five percent subjects could not deny the existence of stress as a cause of their diseases whereas 43% subjects considered poor diet at fault. Nearly all the individuals in the study (97%) opted for some sort of treatment for their diseases where half of the subjects (50%) took the help of allopathy while 31% subjects took homeopathic treatment. Ayurvedic remedies and dietary restriction as a source of disease treatment were chosen by 28 and 26% subjects



respectively. Some of the hyperlipidemic men (38%) also took the support of statins to lower their cholesterol levels while the others did not. The most preferable salts of medicines were Rosuvastatin and Atorvastatin as taken by 39 and 22% subjects.

**Impact of supplementation on mean daily nutrient intake of the subjects**

The data in Table 4 revealed that supplementation of multigrain bread developed by using sunflower seed flour resulted in a non-significant increase in the mean daily intake of energy, protein, monounsaturated fatty acids and alpha linolenic acid by 3.65, 4.7, 13.21 and 7.29% respectively. The average daily intake of total fat, dietary fibre, polyunsaturated fatty acids and linoleic acid significantly increased ( $p \leq 0.01$ ) from  $65.93 \pm 21.35$  g,  $27.94 \pm 8.87$  g,  $7490.29 \pm 2752.59$  mg and  $8009.52 \pm 3218.01$  mg to  $67.87 \pm 11.90$  g,  $39.22 \pm 5.74$  g,  $8501.30 \pm 3670.88$  mg and  $14,627.59 \pm 2725.70$  mg respectively while that of carbohydrates and saturated fatty acids significantly decreased ( $p \leq 0.01$ ) from  $237.68 \pm$

$48.05$  g and  $32,009.53 \pm 13,774.32$  mg to  $190.89 \pm 35.21$  g and  $22,574.09 \pm 9180.47$  mg after the post intervention period. There was a non-significant reduction in the average daily intake of trans fatty acids by 11.93%. Furthermore, the mean daily intake of micronutrients like ascorbic acid, alpha tocopherols, calcium, copper and phosphorus increased while that of  $\beta$  carotene, magnesium and sodium decreased after the period of intervention.

**Impact of supplementation on anthropometric profile of the subjects**

The essential parameters of anthropometric profile like height, weight, body mass index (BMI) and body fat are crucial for the diagnosis of obesity which is further a risk factor for various other diseases like hypertension, cardiovascular diseases and diabetes mellitus. Based on the data collected, supplementation had no effect on the parameter of height of the subjects. The average weight during pre-intervention period was 85.74 kg which significantly reduced ( $p < 0.01$ ) by about 2% to 83.83 kg after the supplementation of the developed product. Similar to

**Table 4** Mean daily nutrient intake of the subjects (n = 60)

Nutrients	Pre intervention	Post intervention	% change	Paired t value	RDA#
Energy (Kcal/day)	1919.32 ± 385.19 (82.73)	1989.37 ± 222.71 (85.75)	3.65	1.65 <sup>NS</sup>	2320
Carbohydrates (g/day)	237.68 ± 48.05 (55.11)	190.89 ± 35.21 (44.39)	− 46.79	6.48**	430
Protein (g/day)	55.13 ± 11.81 (91.88)	57.73 ± 9.04 (96.22)	4.7	1.96 <sup>NS</sup>	60
Total fat (g/day)	65.93 ± 21.35 (164.82)	67.87 ± 11.90 (169.67)	2.94	7.60**	40
Dietary fibre (g/day)	27.94 ± 8.87 (86.35)	39.22 ± 5.74 (96.80)	40.37	6.08**	40
SFA (mg/day)	32,009.53 ± 13,774.32	22,574.09 ± 9180.47	− 2.95	10.16**	−
MUFA (mg/day)	9449.48 ± 4240.03	10,697.29 ± 8062.32	13.21	1.22 <sup>NS</sup>	−
PUFA (mg/day)	7490.29 ± 2752.59	8501.30 ± 3670.88	11.89	2.52**	−
Trans fatty acids (mg/day)	0.31 ± 0.40	0.27 ± 0.36	− 11.93	1.32 <sup>NS</sup>	−
Linoleic acid (mg/day)	8009.52 ± 3218.01	14,627.59 ± 2725.70	82.63	6.33**	−
Alpha linolenic acid (mg/day)	1462.55 ± 1844.90	1569.19 ± 1870.23	7.29	0.44 <sup>NS</sup>	−
$\beta$ carotene ( $\mu$ g/day)	3778.27 ± 3833.14	3253.95 ± 4205.28	− 13.877	0.27 <sup>NS</sup>	4800
Ascorbic acid (mg/day)	97.70 ± 27.89	157.79 ± 54.37	61.50	5.34**	40
Alpha tocopherols (mg/day)	2.54 ± 0.91	14.69 ± 5.82	54.33	8.48**	10
Calcium (mg/day)	632.48 ± 125.89	658.24 ± 153.16	4.07	1.59 <sup>NS</sup>	600
Copper (mg/day)	1.66 ± 0.30	1.92 ± 0.32	15.67	1.84 <sup>NS</sup>	−
Magnesium (mg/day)	404.71 ± 71.67	403.93 ± 62.49	− 0.19	0.12 <sup>NS</sup>	340
Phosphorus (mg/day)	679.29 ± 158.52	894.13 ± 131.33	31.63	7.41**	600
Sodium (mg/day)	2604.49 ± 235.15	2175.04 ± 120.57	− 16.49	7.68**	2500

Values are Mean ± S.D.

Figures in parenthesis indicate percent adequacy

\*\* Values are significant at 1% level

NS – Non significant

# ICMR (2010)

the findings of present study, Leverrier et al. (2019) reported that supplementation of 250 mg sunflower seed extract reduced the body weight, body mass index and waist circumference of the subjects aged between 18 and 65 years by 6.90 kg, 2.6 kg/m<sup>2</sup> and 8.44 cm respectively. The element of weight loss was unswervingly associated with rectification in lipid parameters. Weight loss of about 3 kg instigated reduction in triglycerides close to 15 mg/dl whereas scaling down by 5 to 8 Kgs augmented HDL cholesterol by 2–3 mg/dl and decreased LDL cholesterol by 5 mg/dl (Ebbert et al. 2014). The information about body mass index revealed that the corresponding figure showed a significant cutback ( $p < 0.01$ ) of about 2% from  $28.61 \pm 2.41$  kg/m<sup>2</sup> to  $28.05 \pm 2.61$  kg/m<sup>2</sup> which may be due to the reduction in weight of the subjects after the study period. The body mass index during both the periods exceeded the standard value of 18.5–24.99 kg/m<sup>2</sup> (WHO 2003). The supplementation of multigrain bread enriched with sunflower seed flour posed no effect on the body fat percent of the subjects. The body fat percent during both pre and post intervention period ( $29.67 \pm 1.48\%$ ) was found to be more than the standard value of 18–24% as suggested by American Council on Exercise (ACE 2009).

### Impact of supplementation on biochemical profile of the subjects

The data pertaining to biochemical profile of the selected subjects is given in Table 5. The parameters like total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), triglycerides and fasting blood glucose (FBG) levels of the subjects were analyzed before and after the intervention. At the end of 4 months of supplementation, the mean level of total cholesterol significantly decreased ( $p < 0.01$ ) by 4% from  $237.73 \pm 31.52$  mg/dl to  $228.78 \pm 31.74$  mg/dl. This may be attributed to the decrease in daily intake of saturated fats and increase in the intake of MUFA and PUFA after supplementation. Farid et al. (2015) claimed hypolipidemic, hypoglycemic and hepar protection properties of sunflower seeds on alloxan induced diabetic rats. The seeds had high amount of linoleic acid, phytosterols and phenolic compounds in them responsible for reducing the total cholesterol.

The mean value of LDL cholesterol before the study period was analyzed to be  $163.19 \pm 36.94$  mg/dl which reduced to  $159.78 \pm 36.56$  mg/dl after four months of intervention. This reduction by 2% was significant at 5% level of significance but the corresponding value was higher than the standards during both the periods. LDL reducing properties of sunflower seed oil had been reported by Bester et al. (2010). The levels of some lipid profile

parameters improved when male and female subjects aging 45–55 years were supplemented with 2 g of roasted sunflower seeds for a period of 6 months. The total cholesterol and triglycerides in the experimental group reduced from 154.90 mg/dl to 146.03 mg/dl, 128.93 mg/dl to 120.33 mg/dl (Cheenam and Leena 2016). In the present study, the mean values of HDL and VLDL cholesterol non-significantly reduced from  $38.67 \pm 4.85$  mg/dl and  $35.56 \pm 3.95$  mg/dl to  $37.93 \pm 5.55$  mg/dl and  $34.89 \pm 3.54$  mg/dl after the subsequent time period of four months. Luka et al. (2013) observed a significant decrease ( $p < 0.05$ ) in the levels of HDL cholesterol after sunflower seed extract was supplemented in the diet of diabetic rats for 14 days. Additionally, supplementation of the developed multigrain product resulted in a significant decrease ( $p < 0.01$ ) of serum triglyceride level from  $188.28 \pm 13.25$  mg/dl to  $174.43 \pm 17.74$  mg/dl by 7%. Guo et al. (2017) stated that the predominant monounsaturated fatty acid in sunflower seeds i.e. oleic acid has the ability to lower the levels of triglycerides and LDL cholesterol and therefore reduces the risk of heart disease. Also, a slight non-significant reduction in fasting blood glucose level by 2% (from  $111.27 \pm 22.15$  mg/dl to  $109.13 \pm 16.74$  mg/dl) was detected after the intervention period. Saini and Sharma (2013) reported that the alcoholic extract of sunflower seeds at a dosage of 250 mg per Kg and 500 mg per Kg significantly reduced the blood glucose levels of non-diabetic rats by 17.78% and 24.83% and of diabetic rats by 22.03% and 27.31% respectively after 30 min of oral administration of glucose during oral glucose tolerance test. Luka et al. (2013) studied that the hypoglycemic effect of aqueous extracts of *Helianthus annuus* seeds in diabetic rats may be due to alkaloids, plant sterols and high phytochemicals in the sunflower seeds. It was found that the extract reduced plasma blood glucose level from 500 mg/dl to 160 mg/dl in 14 days and competed with the drug metformin. Wang et al. (2017) reported that intake of  $\beta$ -glucan resulted in the reduction of total cholesterol. Lowering blood cholesterol levels were attributed to increased bile acid synthesis. Consumption of sufficient quantities of oat products reduce host cholesterol and modulate cardiovascular disease risk. The effects are due to gel-forming properties of oat  $\beta$ -glucan which modulates host bile acid and cholesterol metabolism and potentially removes intestinal cholesterol for excretion (Joyce et al. 2019).

### Conclusion

The investigation of the present study revealed that roasting of sunflower seeds resulted in significant increase in carbohydrate, in vitro protein digestibility, phosphorus,

**Table 5** Biochemical profile of the subjects pre and post intervention (n = 60)

Biochemical measurements	Pre intervention	Post intervention	% change	Paired t value	Standard
Total cholesterol (mg/dl)	237.73 ± 31.52	228.78 ± 31.74	− 3.76	4.46**	< 200 <sup>a</sup>
LDL cholesterol (mg/dl)	163.19 ± 36.94	159.78 ± 36.56	− 2.09	2.00*	< 100 <sup>a</sup>
HDL cholesterol (mg/dl)	38.67 ± 4.85	37.93 ± 5.55	− 1.91	1.52 <sup>NS</sup>	40–60 <sup>a</sup>
VLDL cholesterol (mg/dl)	35.56 ± 3.95	34.89 ± 3.54	− 1.88	1.79 <sup>NS</sup>	2–30 <sup>a</sup>
Triglycerides (mg/dl)	188.28 ± 13.25	174.43 ± 17.74	− 7.36	3.94**	< 150 <sup>a</sup>
Fasting blood glucose (mg/dl)	111.27 ± 22.15	109.13 ± 16.74	− 1.92	0.90 <sup>NS</sup>	≤126 <sup>b</sup>

Values are Mean ± S.D.

\* Values are significant at 5% level

\*\* Values are significant at 1% level

NS – Non significant

a –NCEP (2001)

b - American Diabetes Association (2019)

magnesium and copper contents. The most acceptable product (with 7.5% sunflower seed flour supplementation) had higher amounts of protein, fat, fibre, ash, energy, total antioxidant activity, total phenols, flavonoids, omega 3 and omega 6 fatty acids as well as minerals as compared to the control sample. Supplementation of acceptable product in hyperlipidemic subjects for a period of four months increased the daily intake of nutrients i.e. energy, protein, fat, MUFA, PUFA, linoleic acid, alpha linolenic acid, fibre, ascorbic acid, alpha tocopherols, calcium, copper and phosphorus and decreased the intake of carbohydrates, saturated fats, trans fatty acids, β carotene, magnesium and sodium. A significant improvement in the lipid, anthropometric and blood glucose profile of the subjects (as indicated from the decrease in serum levels of total cholesterol, LDL cholesterol, triglycerides, body weight, BMI and fasting blood glucose) was reported. The amelioration in the above stated parameters was due to the therapeutic benefits of sunflower seed flour supplemented multigrain bread in lowering the cholesterol levels, consequently lowering the risk of cardiovascular disease. Hence, sunflower seeds can be an economical food based approach to improve the lipid profile of hyperlipidemics. Their daily usage should be encouraged to reduce their risk of cardiovascular disease.

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