Development of Chia Seed Oil Supplemented Ice Cream and Assessment of its Efficacy Against Hypercholesterolemia

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ABSTRACT: Chia seeds found to be a rich source of dietary fiber contain oil which is high in omega6 and omega 3 fatty acids and helpful in the control of cardiovascular diseases. Owing to its spectacular significance present research has been designed to explore its effect on cholesterol level of the individuals after consumption of chia seed oil supplemented ice cream. Study was designed in such a manner that fat of ice cream was replaced with chia seed oil in different proportions i.e., 25%, 50%, 75%, 100%. After Physico-chemical and sensory evaluation of ice cream, best treatment was selected and used for efficacy trials. After baseline line study and thorough inclusion criteria 10 individuals were selected and divided into two groups. One group treated as control and the other was given chia seed oil supplemented (50%) ice cream. Significant decrease in cholesterol level was observed in the treated group. 18% decrease in cholesterol level was observed at 40th day followed by 8% at 20th day. Similarly, 20% decrease in LDL cholesterol with 14% increase in HDL cholesterol. It was recommended that further trials be conducted with sophisticated techniques to completely replace saturated fat in ice cream with unsaturated fats and to study its effect in hyperglycemia and oxidative stress.

Key Words: Hypercholesterolemia; Chia seed oil; Low fat ice-cream; omega 3 fatty acids
INTRODUCTION

Chia (Salvia hispanica L.) is an annual herbaceous plant of the mint family (Labiatae). This plant was used by the Aztec tribes in the early history of Mesoamerica. This grain crop was important not only for foods but also for medicines (Peiretti et al. 2009). Its seeds have been investigated and recommended due to its high levels of proteins, antioxidants, dietary fiber, vitamins, and minerals but particularly due to their oil content with the highest proportion of α-linolenic acid (-3) compared to other natural sources known to date (Fuxia et al 2012). Chia seeds contain up to 39% of oil, which has the highest known content of α-linolenic acid, up to 68%. Chia seed is evolving as one of the crucial sources of phytochemicals in the era of functional food. Chia seeds contain high levels of lipids (34.4%) and are rich in Omega-3, Omega-6 and Omega-9, which constituted 62, 17.4 and 10.5% of the total lipids, respectively. Chia seeds also contain fibers (23.7%) and proteins (19.6%) (Alvoraz, 2005; Coelho and Mellado, 2014)

Ice cream being the most delicious and popular food in people of all communities and all ages that’s why its production and consumption is rapidly increasing day by day. The percentage of fat in ice cream vary form 10-20 % and contributes with many functional properties such as hardness, good melting properties and smooth texture (Prindiville et al. 2000). Milk fat also acts as a carrier of many important flavors. (Ohmes et al. 1998). On other end milk fat is a major source of saturated fatty acids and cholesterol. Milk fat contains more than 70% saturated fatty acids, about 26% monounsaturated fatty acids and less than 4% polyunsaturated fatty acids. According to recommendations provided by American Heart Association (AHA) the intake of saturated fats should limited from 7-10 % of total daily calories. Foods having high concentration of saturated fats are known as atherogenic foods (Gonzalez et al. 2003). Due health consciousness consumption of milk and milk products especially ice-cream is declining day by day (Fuxia et al 2012). In contrast 75% increase has been observed in consumption of foods containing high level of polyunsaturated fatty acids (PUFA) (Gonzalez et al. 2003). According to a survey by American Heart Association (AHA) in 1997, cardiovascular diseases were responsible for 41.2% of all the deaths in USA. As milk fat is a big birthplace for cardiovascular diseases, so a mark able decrease in mortality can be achieved by modifying the fatty acid profile of dairy products. It can be achieved by replacing saturated fatty acid content with unsaturated fatty acids. Production of ice cream with vegetable fat instead of animal fat is a way to produce a nutritionally healthier and low cholesterol product (Otero et al. 1999). It yields between 25% to 35% extractable oil, which contain high concentrations of PUFAs. Because of the highly unsaturated nature of the oil, the seeds probably contain potent lipid antioxidants. Chia seed contains the highest known percentage of α-linolenic acid (ALA) of any plant source. Recent studies shows that chia seed possesses the highest amount of PUFA content, more than 80.5% and the best n-6/n-3 ratio compared with flax seed and perilla. ALA leads to small but significant increases in EPA and DPA in humans (Nieman et al. 2009). Nutritionally and potentially therapeutic value of a food plays a key role in the development of new value-added products that are manufactured for health-conscious consumers. As omega-3 and omega -6 fatty acids are present in chia seed
oil abundantly; it can be incorporated in ice cream as a replacer of cream to have its beneficial effects. It may be helpful in lowering the triglycerides and cholesterol level of blood which in turn results in low blood pressure and heart related diseases (Heuer et al. 2002).

**MATERIALS AND METHODS**

Raw material for ice cream preparation as well as chia seeds purchased from market. Extraction and Purification of chia seed oil: In order to extract oil from chia seeds, after size reduction oil was extracted and then passed to a steamer where temperature (more than 100°C) was applied in combination with vacuum in order to deodorize crude chia seed oil. After deodorization crude oil was passed through different steel filters to make it refine. Finally oil was passed through a cooling unit to lower down its temperature for further processing. Analysis of chia seeds oil. Refined and deodorized chia seed oil was analyzed for free fatty acids, iodine value, per-oxide value, color, melting point and moisture according to the method of (AOAC, 2006).

**Preparation of Ice Cream**

Vanilla ice cream was prepared by using formulation of (Potter and Hotchkiss 1995). As mentioned in Table 1, Fig. 1. Five different batches of ice cream were prepared according to formulation given by Potter and Hotchkiss. In formulation of all treatments the only difference was the type and percentage of fat added. Control group was prepared according to standard formulation. While 25%, 50%, 75% and 100 % milk fat was replaced with chia seed oil in T1, T2 and T3 and T4 respectively. All the ingredients were mixed. Pasteurization was done by heating the mixture at 80°C for few minutes; to kill the pathogenic microorganisms in order to maintain the fat globules size. After that mixture was homogenized in a clean and sanitized double stage homogenizer with speed of 100 kg/cm2 and 35 kg/cm2 in the first and second stage respectively. The pasteurized mix was transferred into refrigerator/freezer to drop temperature <10 °C. Then ageing was done for 4-6 hours at 4 °C. After ageing vanilla flavor was added in ice cream mix. Analysis of ice-cream: pH, acidity, fat, protein, total solids, ash, whipping ability, melting test, overrun and sensory evaluation was carried out according to prescribed standard procedures by AOAC(2006). Chia seed oil supplemented ice cream was subjected to sensory evaluation for color, flavor and overall acceptability by a trained panel of 10 judges as described by (Meilgard et al., 2007). The testing was also carried out at 10, 20 and 30 days of storage interval. All evaluations were carried out at room temperature on the same day in well illuminated laboratory in Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore.

**Efficacy Studies**

To determine the effect of chia seed oil supplemented ice cream on blood lipid profile of hypercholesterolemia people, human trails were performed under the supervision of medical practitioner.

**Inclusion Criteria**

Inclusion criteria included adult patient of age 30-60 years diagnosed with hyperlipidemia (serum cholesterol level 200-300 mg/dl) with no complication of CVD or...
any other underlying disease and patients who were not taking any type of medicine. It was also made compulsory that patients should free from any milk allergy.

**Study Design**

In these trails, 10 volunteers were recruited through flyers and personal contacts. Associated risks of study were explained to each of study participant in detail. The patients were provided with bilingual (English & Urdu) consent form. Trial was initiated after taking written informed consent from the subjects. The subjects were randomized to experimental and control group under single blinded procedures. The control group was not given any treatment as saturated fatty acids present in ice cream may be harmful for their health and the experimental group was given chia seed oil replaced ice cream. Each group was divided in 5 individuals having the same gender. Since the study participants were having cholesterol level >200 mg/dl, it was ethically unacceptable to not to console them regarding their dietary practices. Therefore all the study participants were given verbal dietary counseling. Subjects who met the entry criteria were randomly assigned to the treatment or control group. Subjects in the treatment group received 120 ml ice cream daily and subjects in the control group received counseled diet for 40 days. Blood samples were collected after an overnight fasting of 12 hours. They were collected at day 1, 20 and 40 respectively for lipid profile to evaluate the efficacy of chia seed oil supplemented ice cream consumption against hyperlipidemia. Baseline data also include patient’s socio-demo-graphic factors, dietary and life habits, and anthropometric measurements.

**Baseline Data**

Baseline data included anthropometric measurements including height, weight and body mass index (BMI). The parameters also included age, gender, dietary habits, and life style habits respectively.

**Serum biochemical Analyses**

Cholesterol in serum samples was measured by liquid cholesterol CHOD-PAP as described by (Stockbridge et al. 1989). Serum HDL was determined by HDL cholesterol precipitant method as described by (Assmann 1979). LDL in all the serum samples was estimated by using the procedure outlined by (Mc Namara et al. 1990).

**Statistical Analysis**

The design of study was completely randomized (CRD). Comparability of the treatment and control groups at baseline was determined by using analysis of variance with repeated measures. Level of significance was defined as \( P \leq 0.05 \) (Steel et al. 1997). The means were compared for significant difference using Duncan’s Multiple Ranges Test (Duncan 1955). The collected data of physico-chemical analysis and sensory evaluation was analyzed by using t-test and (ANOVA) analysis of variance technique under CRD for product development. Costat-2003 version 6.303 software was used for all statistical analyses.
Table 1: Standard Formulation of ice cream

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>10%</td>
</tr>
<tr>
<td>MSNF</td>
<td>12%</td>
</tr>
<tr>
<td>Sugar</td>
<td>15%</td>
</tr>
<tr>
<td>Stabilizer</td>
<td>0.25%</td>
</tr>
<tr>
<td>Emulsifier</td>
<td>0.25%</td>
</tr>
<tr>
<td>Water</td>
<td>makes up the volume 100 ml</td>
</tr>
</tbody>
</table>

Mixing of ingredients  
Butter, sugar, skim milk powder,  
Stabilizer, emulsifiers

Heating 70°C – few Mins

Homogenization at 70°C  
100kg/cm² & 35kg/cm²

Pasteurization 1-3 Mins at 85°C

Cooling – 6°C

Ageing 6°C for 12 hours

Packaging and storage at -25°C

Freezing & Over run (-10°C)

Addition of aroma and colorant

Fig 1: Flow chart for ice-cream preparation
RESULTS AND DISCUSSION

Physicochemical analyses of chia seed oil were performed to assess the suitability of oil’s supplementation in ice cream. Preliminary characterization of chia seed oil revealed that the oil yield was 30-35%, lower than data published by (Alvoraz, 2005). The oil was 99.9 percent pure, having pale yellow color and typical fatty oily odor. Iodine value (IV) indicates the degree of unsaturation of the oil. It is defined as the number of grams of iodine absorbed by 100 grams of oil. Iodine value of chia seed oil was 192.73 similar to data presented by (Ixtaina et al. 2011), which was much more higher than all other edible oils; sunflower oil (177.06), Corn oil (206.49) Rapeseed oil (172.29) and peanut oil 179.83. Peroxide value gives the initial evidence of rancidity in unsaturated fats and oils. Observed Peroxide value was 4.39 meq/kg which was very lesser than other edible oils; sunflower oil (9.99), corn oil (22.4), rapeseed oil (9.99) and peanut oil 9.99 (Nayga et al., 1999). Lower peroxide value may be due to presence of higher amount of anti-oxidants in chia seed oil, which increase the acceptability of oil.

Fat give rich flavor and improve food perception including look, texture, body and mouth feel. Fat is a concentrated source of calories and contribute to higher energy values of the final consumer product (Potter et al 1995). The data concerning mean value of fat content for different treatments of fat shows that treatments had no significant effect on fat content. The results regarding analysis of variance for different treatments of ice cream indicated that fat content of all treatments vary non-significant among each other. Fat content varied from 9.70 to 9.71% between different treatments of chia seed oil supplemented ice cream.

The mean square values for protein content of different treatments indicated that the protein contents were not significantly affected as addition of chia seed oil in ice cream. As skim milk with 33.4 % protein content was used in preparation of different formulation of ice cream, so expected protein contents were 4.08 %. Statistical data revealed that protein content was not significantly varied among different treatments. The lowest protein content was observed in T4 (3.95) while maximum protein content was observed in T1 (9.96). A variable trend of protein content was observed with respect of treatment from T0 to T4.

The results regarding analysis of variance of total solids of different treatments of ice cream indicated that addition of chia seed oil has a non-significant effect on percentage of total solids present in ice cream. Minimum value of total solids was observed in T0 (36.53%), maximum value of total solids was observed in T2 (36.56 %) while a random trend of total solids was observed with respect of treatment from T0 to T4. Similar results were reported by (Abdou et al.,2000) and (Kirk2005)when he studied the effect of replacing butter oil by hydrogenated palm kernel oil reported that all the blends had nearly the same composition and reported non-significant effect on total solids of ice cream. Minimum value of ash content was observed in T0 (0.753%), maximum value of total solids was observed in T1 (0.754%) while a random trend of total solids was observed with respect of treatment from T0 to T4. The result of this study are inline agreement with (Nadeem et al., 2009), they prepared ice cream by replacing milk fat with palm oil and reported non-significant effect of palm oil on ash content of ice cream. The mean values of pH of various treatments are showed in Table 2, pH ranges from 6.62 to 6.58 in different
Efficacy of Chia Seed Oil Supplemented Ice Cream Against Hypercholesterolemia

Minimum mean value for pH was observed in T4 (6.58) and maximum mean value for pH was observed in T0 (6.62). A decreasing trend of pH was observed with respect of treatment from T0 to T4. As p value is equal 0.05 which means that null hypothesis is accepted and a non-significant difference in pH value of at least two of five treatments of ice cream is existed. (Arbuckle, 1986) mentioned that the normal pH value of ice cream is about 6.6. The decrease of pH value could be due to the increased percentage of CSO concentration in ice cream formulation because the pH of cream is higher as compared to the pH of CSO. Acidity has an inverse relation with pH, if value of pH increase acidity tends to decrease and vice versa. As CSO has low pH value as compared to cream so acidity of CSO supplemented ice cream will defiantly be high. The results were within the level reported by (Coates 2011) who mentioned that the ice cream mix containing 11.7% MSNF would have a normal acidity range of 0.190 %. The mean values of acidity of various treatments are showed in Table 2. Acidity ranges from 0.193% to 0.197 % in different treatments. Minimum mean value for acidity was observed in T0 (0.193) while an increasing trend of acidity was observed with respect of treatment from T0 to T4. As p value is lesser than 0.05 which means that null hypothesis is rejected and a significant difference in acidity level of at least two of five treatments of ice cream existed. After sensory evaluation (color, taste, flavor, overall acceptability) and physical characteristics of chia seed oil supplemented ice-cream like overrun, whipping ability and melting resistance one best treatment was selected for efficacy studies.

Cholesterol

In present study participants had serum cholesterol level >200 mg/dl with mean serum cholesterol (229.80± 12.30 mg/dl). There was no substantial difference observed within the different treatment groups (p=.058ns). A remarkable but non-significant difference in serum cholesterol was observed with passage of time (0.116ns). Changes were observed in serum cholesterol level in the study group after 20 days of intervention, however a substantial decrease was observed after 40 days intervention. The decrease in serum cholesterol levels of treatment group was 16.6 %after 40 days which explained that decrease cholesterol level of patients who consumed chia seed oil supplemented ice cream was better than control group. These results were similar to (Ebrahimi et al 2009) where there was reduction in blood cholesterol level after consumption of omega 3 supplements was observed.

LDL

LDL cholesterol is considered as bad cholesterol because it contributes to plaque, a thick, hard deposit that can clog arteries and make them less flexible. The effect of different treatments on serum LDL level of both groups is illustrated in Table 3. A remarkable but non-significant difference was observed between both groups with the passage of time (p=.0.339 NS). The decrease in LDL level in treatment group was 19% when compared with control which was 6%, which explains that chia seed oil supplemented ice cream contribute to a remarkable decrease in LDL level. The results were in agreement with (Fuxia, 2012), who supplemented ten menopausal women with 20 grams daily milled chia seed oil for four
weeks and 20% reduction in LDL levels was observed.

**HDL**

HDL cholesterol is considered as good cholesterol because it helps to remove LDL cholesterol from the arteries. A healthy level of HDL cholesterol may also protect against heart attack and strokes. The effect of different treatment groups on serum HDL level was observed as illustrated in table 4. HDL level of both groups was significantly different from each other (p=0.044*). But no significant difference was observed between control and treatments groups with the passage of time (p=0.119 ns). Similar to (Eftakhari et al 2014), (Ayerza and Coats, 2007) as no significant difference was observed in this study. The increase in HDL level in treatment group was 13.6 % when compared with control which was 2.47 %, (Figure 2) which explains that chia seed oil supplemented ice cream contribute to an increase in HDL level.

**CHOL: HDL**

The effect of different treatments revealed a non-significant effect on cholesterol to HDL ratio (p=0.162ns) as mentioned in Table 3. This ratio its calculated by physician to determine the risk of heart disease. According to AHA (American Heart Association) cholesterol to HDL ratio should be less than 5:1. The results of a study (Lemieux et al. 2011) revealed that increased cholesterol to HDL ratio may have stronger link to heart disease. Although the decrease in cholesterol to HDL ratio was insignificant but with reference to Figure the maximum decrease was observed in treatment group which was 26.38% as compared to control group which was 6.04%. Study results demonstrated that patients were overweight with BMI >25 g/m2 at the time of study. No significant difference for cholesterol, LDL and HDL was observed after 20 days of treatment. Although a minor positive change was observed in all patients regarding all parameters. It may be due to lesser time of treatment or lesser amount of fat present in ice cream. Amazing results were obtained after 20 days of treatment. The effect of different treatments on serum cholesterol levels demonstrate overall non-significant difference in both groups. But decrease in serum cholesterol levels was observed in the group who consumed CSO supplemented ice cream but this was evident after 40 days of intervention. A slight but no significant effect of CSO supplemented ice cream on HDL level was observed. The decline in serum LDL levels of treatment group was also evident. So it was concluded that chia seed oil addition has defiantly positive effect on blood profile of Hypercholesterolemic persons. Data from the present research suggest human trail with larger sample size and more time duration be conducted. Moreover benefits of chia seed oil can be studied on other diseases like diabetes.
Table 2: Proximate Composition of ice-cream

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Total Solids (%)</th>
<th>Ash (%)</th>
<th>pH</th>
<th>Acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>9.71</td>
<td>3.95</td>
<td>36.53</td>
<td>.753</td>
<td>6.63</td>
<td>.193</td>
</tr>
<tr>
<td>T₁</td>
<td>9.71</td>
<td>3.96</td>
<td>36.54</td>
<td>.754</td>
<td>6.61</td>
<td>.195</td>
</tr>
<tr>
<td>T₂</td>
<td>9.70</td>
<td>3.95</td>
<td>36.56</td>
<td>.753</td>
<td>6.60</td>
<td>.196</td>
</tr>
<tr>
<td>T₃</td>
<td>9.71</td>
<td>3.96</td>
<td>36.53</td>
<td>.754</td>
<td>6.59</td>
<td>.197</td>
</tr>
<tr>
<td>T₄</td>
<td>9.71</td>
<td>3.95</td>
<td>36.55</td>
<td>.754</td>
<td>6.58</td>
<td>.197</td>
</tr>
</tbody>
</table>

Table 3: Means of Different Treatments for Lipid Profile at Different Time Intervals

<table>
<thead>
<tr>
<th>Parameter (mg/dl)</th>
<th>Treatment</th>
<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 Days</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>-8.13±0.03</td>
<td>-17.66±0.03</td>
</tr>
<tr>
<td>Control</td>
<td>-4.65±0.03</td>
<td>-4.92±0.03</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>8.47±0.02</td>
<td>12.32±0.04</td>
</tr>
<tr>
<td>Control</td>
<td>4.79±0.02</td>
<td>0.304±0.02</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>-13.52±0.05</td>
<td>-19.91±0.04</td>
</tr>
<tr>
<td>Control</td>
<td>-12.38±0.04</td>
<td>-7.50±0.02</td>
</tr>
<tr>
<td>CHOL: HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>-15.26±0.03</td>
<td>-26.47±0.05</td>
</tr>
<tr>
<td>Control</td>
<td>-9.0±0.02</td>
<td>-5.15±0.08</td>
</tr>
</tbody>
</table>

* Negative sign (-) indicates percent decrease
  Positive indicates percent increase
**Table 4: Comparison of Means for lipid profile of the groups under study**

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>CHOL: HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group</td>
<td>-4.79</td>
<td>2.57</td>
<td>-9.94</td>
<td>-7.07</td>
</tr>
<tr>
<td>Treated Group</td>
<td>-12.90</td>
<td>10.40</td>
<td>-16.72</td>
<td>-20.86</td>
</tr>
</tbody>
</table>

**Fig 2: Graphical representation for the percent decrease/increase in cholesterol level in control vs treated group**

**CONCLUSION**

Chia seed is known to be one of the crucial sources of phytochemicals in the era of functional foods. Chia seeds contain high levels of lipids that is rich in Omega-3, Omega-6. They are found to be a rich source of dietary fiber and helpful in the control of cardiovascular diseases. Due to its magnificent implication current research had been formulated to investigate its effect on cholesterol level of the individuals after consumption of chia seed oil supplemented ice cream. The study was designed in such a manner that fat of ice cream was replaced with chia seed oil in different proportions i.e., 25%, 50%, 75%, 100%.

After Physico-chemical and sensory evaluation of ice cream, best treatment was selected and used for efficacy trials. Following baseline line study and thorough inclusion criteria 10 individuals were selected.
and divided into two groups. One group treated as control and the other was given chia seed oil supplemented (50%) ice cream. Subjects in the treatment group received 120 ml ice cream daily and subjects in the control group received counseled diet for 40 days. Blood samples were collected after an overnight fasting of 12 hours. They were collected at day 1, 20 and 40 respectively for lipid profile to evaluate the efficacy of chia seed oil supplemented ice cream consumption against hyperlipidemia. Baseline data also include patient’s socio-demo-graphic factors, dietary and life habits, and anthropometric measurements. The decrease in LDL level in treatment group was 19% when compared with control which was 6%, which depicted that chia seed oil supplemented ice cream contribute to a remarkable decrease in LDL level.

Significant decrease in cholesterol level was observed in the treated group. 18% decrease in cholesterol level was observed at 40th day followed by 8% at 20th day. Similarly 20% decrease in LDL cholesterol with 14% increase in HDL cholesterol.

It was suggested that auxiliary trials be conducted with latest techniques to completely replace saturated fat in ice cream with unsaturated fats and to study its effect in hyperglycemia and oxidative stress.

REFERENCES


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