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CHARACTERIZATION OF CHIA SEEDS OIL AND ITS STORAGE STABILITY IN COOKIES

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Abstract

Chia is an herbaceous plant belongs to Labiatae family which also known as Salvia Hispanica L. Globally, chia seed production is 550-600 kg/ha and it may consume whole or as a part of a product like juices, cakes, breads etc. Generally, chia seeds contain about 35 to 40 % of oil and its oil have a high amount of omega-3 fatty acid. Chia seed comprises of protein, carbohydrates, a good amount of oil, dietary fiber, a few minerals, vitamins, and antioxidants. Chia seed oil extracted through solvent extraction had saponification value, acid value, peroxide value, specific gravity, and iodine value of 194.2 ± 0.6, 1.03 ± 0.01, 1.88 ± 0.02, 0.92 ±0.01 and 190.70 ± 0.6 respectively. Chia seeds are famous for their antioxidant property and the amount of poly unsaturated fatty acids and according to its fatty acid composition, its oil contains α- linolenic acid in the amount of 62.79% followed by Linoleic acid in the amount of 19.89%. The development of new items or advancement in every product is an essential part of the food business. Cookies have distinctive taste and long timeframe of realistic usability, that why cookies are appreciated by all age groups. Chia seed oil added in cookies in different amounts i.e., 25%, 50%, 75%, and 100%. cookies made with chia oil tested for its physicochemical properties (fiber content, moisture content, fatty acid profile, ash contents, crude fat, and crude protein) on different storage days i.e., 1st, 7th, 14th, and 21st days. Cookies with 50% of chia oil shows highest value for protein, fiber, fat, CHO, and ash contents. Sensory analysis

carried out by 9-point hedonic scale. Cookies sample T_2 having 50% chia oil showed good sensory properties as compared to other treatments on all storage days. All the data checked statistically to check the level of significance. It was concluded from the results of the current investigation that chia seed oil significantly influences the physicochemical and organoleptic characteristics of cookies. Furthermore, chia seed oil could be used in different food product as a functional ingredient for the supply of omega-3 fatty acids.

Keywords: *Chia Seed, Chia Seeds Oil , Storage Stability, Chia Cookies, Fatty Acid, Physicochemical analysis, Chia Seed Cookies.*

INTRODUCTION

Chia (Salvia Hispanica L.) has recently gain popularity as one of the good source of polyunsaturated fatty acids. Chia seeds belongs to family Labiatae and considered as staple food in Mexico for since 1500 BC. Chia seeds yield about 35 to 40 % of oil and it has a good concentration of omega-3 fatty acids (Nadeem et al., 2017). It is popular in Mexico, the Southwest US, and America, but it is less well-known in EU. Though, the EU accepted these seeds as a new novel food in 2009, letting them to add a 5% of the total matter in bread products. It is now largely farmed in Bolivia, Australia, Argentina, Mexico, and Guatemala, and its nutritional value has proven. As a crop plant, the species has a huge future potential (Segura-Campos et al., 2014). Their leaves are 4-8 cm long and about 3-5 cm broad and its tree can be up to 1 m tall (Knez Hrnčič et al., 2020). After overblowing it forms round structure fruits, containing numerous small, oval seeds of 2 mm long and 1mm in width. Seed surface is smooth, glossy, going in shading from white to dark brown, with dark spots on it. At first, chia was grown in tropical and subtropical environments(Kulczyński et al., 2019). PUFAS and tend to be lower the amount of cholesterol in meat and meat products (Muhammad et al., 2016).Chia seed oil is a substance that has recently been rediscovered for its use in aesthetics and food. Because chia seed oil is a natural product, its chemical composition varies depending on a variety of circumstances, including the growth, environment, and extraction method (Rosas-Mendoza et al., 2017).They are normally eaten in the form of powder or as entire grain and can be utilized in natural product like juices, milk, soda pops and salads (Fernández-López et al., 2018). Accordingly, this oil might be utilized to make appropriate oil mixes with other oils which contain low degrees of

these mixtures (Fernández-López et al., 2018).Chia is the modern "superfood" because they are abundant in readily digested proteins, essential fatty acids, antioxidants, some vitamins, dietary fiber, minerals(Pareyt et al., 2009). A number of studies on nutritional ingredients have encouraged increased consumer awareness of healthy lifestyles and nutrition (Song et al., 2019). The most significant sugar in cookie making is sucrose. It adds sweetness to cookies, alters their structural and textural features, and is thought to introduce air into the fat during the baking process. Fat helps in spreading cookies and overall look, enhances aeration and cookie volume., which made them easier to break (Pareyt et al., 2009). Fat is the cookie ingredient that contributes to appearance, mouthfeel and has a huge effect on taste and sensation. Particularly soft type cookies contain a huge amount of fat (Zoulias et al., 2002). Baked meals are regarded as less solid, lighter and most prone to melts in the tongue as a result of this phenomenon. (Pareyt and Delcour, 2008).This research aimed at the extraction of chia seed oil using Soxhlet technique, determining nutraceutical benefits of chia seed oil and the development of a functional food i.e., chia seed oil-based cookies which will be prepared by the incorporation of chia seed oil which has excellent antioxidant potential and a rich source of polyunsaturated FAs.

REVIEW OF LITERATURE

Mohd Ali et al. (2012) stated that interest for useful food sources with different health benefits has expanded as general health awareness has developed across the world. Coelho and Salas-Mellado (2014) stated that chia is a seed that grows in an area that stretches from northern Mexico to some regions of Guatemala and has been studied for addition in food. Bioactive ingredients such as dietary fiber, antioxidants, and other chemicals are found in many of its newly designed functional food. The antioxidant activity of their isolated phenolic components was discovered. Attalla and El-Hussieny (2017) stated that a new nutraceutical dairy product, fortified yogurt mousse with Chia seeds, was investigated. To produce novel dairy products with high nutraceutical and sensory properties, 3 % chia seed fortification is recommended.Ullah et al. (2018)

stated that impact of enhancing cheddar with addition of chia seed oil on unsaturated fats, phenols, and lipid breakdown of cheese was examined. Fat of milk has been replaced by chia oil at 2.5, 5, 7.5 and 10% concentrations. Ullah et al. (2017) stated that chia seed oil has high number of ω-3 FAs. Along these lines, fundamental goal was assurance of impact of different amount of chia oil (olein part) on concentration of ω-3 FAs, oxidation strength, and sensory attributes of ice cream. Derewiaka et al. (2019) stated in this study that chia seed oil was added to yogurt. The point of the investigation remained to assess the impact of adding two percent chia seed oil to characteristic yogurts on the nature of yogurts and to decide chia seed oil could be used as an added substance in matured dairy items. The predominant type of microorganisms found in yogurts was Lactobacillus delbrueckii. . Alwosais et al. (2021) stated that some functional food products have been shown to help control level of blood glucose and maintaining hypertension, as well as improve other risk factors for Cardiovascular diseases (CVD). These include dietary fiber, long chain PUFAs, and moderate protein. Chia seeds are rich in these nutrients, but there have been little studies on the impact of consuming them on physiological and metabolic outcomes. Chia seed gel is a nutritious organic gel that is widely utilized in a wide range of foods as a natural source of fiber, bulking agent, and a fat substitute .Metri-Ojeda et al. (2020) specified chia seeds as a main source of nutritious components such as PUFAS, soluble and insoluble fiber and protein. Oil and soluble fiber were collected in this study to create a vegan mayonnaise. According to Akcicek and Karasu (2018) the major goal of this research was to generate a low-fat salad dressing utilizing waste chilled pressed chia-seed-oil-waste powder. Response surface methodology (RSM) was used to enhance the formulation built on rheological and micro-structural parameters. Rahman et al. (2015) said that in this study the characterization of margarine oil and chia-seed-oil blends carried out. Chia oil added to margarine oil at four dissimilar meditations: 6.25%, 12.5%, 18.75%, and 25% and named in a sequence of 1 to 4. Sample with margarine oil deprived of CO serving as a reference. Arifin et al. (2021) stated chia-seed has a great portion of fiber and polyunsaturated fatty-acids including omega-3-linolenic acid. Chia seed is

high in calcium and phosphorus, as well as other minerals and vitamins. Chia seed has a lot of gel-forming capabilities as well as high water and oil retaining abilities. According to Dinçoğlu and Yeşildemir (2019) stated that this seed has been found to be a decent supply of soluble and insoluble dietary fiber, omega-3 fatty acid, proteins, bioactive and polyphenolic elements. It conjointly possesses omega-3 fatty acid chemical science and practical characteristics that create it a lot of applicable to be used within the food sector. Chia seed are often used as a thickening, chelating agent, foam attention, emulsifier, suspending agent, and rehydration issue. Frozen product, food, drinks, candies, pasta, and sausages could all be supplemented with seeds, and chia oil are often accustomed substitute fat in these foods. Consumption of the seed has been found to be helpful for health problems like dyslipidemia, inflammation, upset, and hypoglycaemic agent resistance in different studies.According to Ayerza (2011). A research was carried out to determine the macromolecule and oil content, as well as the fatty acid composition, of chia seeds matured in several bigger industrial fields, to determine if these seeds are full of elements of that location. Oil saturation cared-for decrease as elevation of seed production inflated, with decreasing levels of palmitic, stearic, oleic, and linoleic fatty acids found. Muñoz-Tébar et al. (2019) expressed that the feasibleness of extracting oil from sage hispanica L. was investigated during this work. Seeds used as a rich source of polyunsaturated fatty acid supply for enriching sheep's cheese. The science behind chemicals, microbiological, and sensory properties of 2 concentrations (3 and 5 g/L) of chia oil shaped as emulsions stabilized with atomic number 20 caseinate were investigated in fortified sheep's milk cheese. Jaddu and Yedida (2018) expressed that Chia seed has a good amount of dietary fiber and polyunsaturated fatty acids. Usually, animal oil has higher levels of PUFAs than the other oil seeds offered. Chia oil, be that as it may, incorporates a higher measure of polyunsaturated unsaturated fat per 100 g than cod liver oil, oil in herring and salmon. Silva et al. (2018) stated that the goal of this study was to describe cheese breads made with varied concentrations of chia seeds in terms of physicochemical and sensory properties during storage. Chia seed meditations of 5g, 7.5g,

and 10g were used to make cheese loaves. Fernandes and de las Mercedes Salas-Mellado (2017) stated that in this study, chocolate cakes and the breads were produced with varying quantities of chia seed mucilage which is dried at about 50°C or as fat, resulted in healthy food products. According to the findings, chia mucilage-based breads and the chocolate cakes can be produced with up to 50% of fat without compromising on physical properties. Razavizadeh and Tabrizi (2021) stated that microcapsules containing chia seed oil were added at varied concentrations of 5, 10, and 15% w/w to make compound milk chocolates that are fortified with chia seed oil. Heck et al. (2019) stated that ultrasonic extraction and standard extraction were both used to include rosemary leaves directly into chia oil. Coming up next is the means by which chia oil was microencapsulated and utilized in burgers: 20% pork back fat kept as control, (10% pork back fat with 7.5% water, and 2.5% chia oil) as T_1, (10% pork back fat, 10% chia oil microparticles) as T_2, (10% pork back fat with 10% chia oil microparticles improved by ultrasound-helped extraction) as T_3, and (10% pork back fat, 10% chia oil microparticles enhanced by regular extraction) kept as T_4.On days 1^{st} and 120^{th} of frozen storage, volatile chemicals and sensory characteristics of burgers were assessed .Mohd Ali et al. (2012) stated that demand of functional foods with many health advantages has increased as public health awareness has grown around the world. Most study has employed high EPA and DHA fish oils, but less emphasis is paid to lineolenic fatty acid, which is a precursor of EPA and DHA.Kaur and Das (2011) stated that a strong relation between meals and healthiness has led to several scientific research attempting to determine the relevance of foods or dietary components on processes in the human body. Functional food refers to food that has certain useful purposes and was first used in Japan.

MATERIALS AND METHODS

This research was performed in postgraduate research laboratories of National Institute of Food Science and Technology (NIFSAT, Faculty of Food, Nutrition and Home Sciences (FFNHS), University of Agriculture, Faisalabad (UAF).Oil was extracted by solvent extraction method at postgraduate research laboratories of National Institute of Food Science

and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences (FFNHS), University of Agriculture, Faisalabad (UAF). Raw materials for development of chia oil-based cookies included chia seeds, flour and other ingredients purchased from local market of Faisalabad. Firstly, raw chia seeds were carefully cleaned manually to get rid of foreign materials as in dust particles, stones, damaged seeds, small stalks and may be seeds other than of chia. Then chia seeds grinded to form powder in a lab scale grinder. The powder was pressed through standard sized mesh to obtain uniform particle size. The fine, ground, and sieved chia powder was then sealed in airtight plastic bags for further use. Paper thimbles of ground chia seed powder were made and then added in thistle chamber of Soxhlet. As a solvent n-hexane is used. The chia seed oil was extracted at the temperature of 65^{o}C.CSO was extracted using methods explained by Ixtaina et al. (2011). With slight modifications. Solvent extraction method was utilized to obtain oil from chia seed. Conventional Soxhlet apparatus was utilized to achieve efficient extraction procedure. The fine ground chia seed powder was precisely weighed as 15-20 grams per paper thimble and was placed into the Soxhlet apparatus extraction chamber. Continuous extraction was done with 300ml of (analytical grade, organic solvent) n-hexane at a set temperature of 68°C for 2-3 hours. After completion of extraction, the extract was obtained from round bottom flask. Chia seed oil was separated from organic solvent in a pre-set rotary evaporator. The weight of oil was carefully taken and obtained yield was calculated.Saponification value of CSO was determined using the methodology suggested in AOCS (2006). The samples were carefully taken and filtered through filter papers to get rid of any impurities as well as all traces of remaining moisture. It was ensured that the sample is completely dried. After that, mixed the oil samples thoroughly and then accurately weighed about 2.0 grams and pipetted out 25 ml of alcoholic KOH solution into a 250 ml Erlenmeyer flask. Refluxed the sample for 30 minutes in water bath with occasionally shaking followed by addition of 2-3 drops of indicator ($C_{20}H_{14}O_4$). After that titrated against 0.5N HCL (hydrochloric acid) till to the end point as pink color disappeared to a clear solution. The saponification value of CSO was analyzed by using

the following formula:

$$Saponification\ value = \frac{56.1(B - S) \times N\ of\ HCL}{Weight\ of\ oil}$$

The amount of FFAs present in chia seed oil samples was determined according to the method of AOCS (2006). To determine the free fatty acid (FFA) value accurately, weighed 10 ml of oil into a conical flask and poured 25ml of ethanol (95%). Mixed thoroughly to the point where oil was miscible in ethanol (C_2H_5OH). Subsequently, added 2-3 drops of phenolphthalein (indicator) with vigorous shaking. Titrated this mixture against 0.1N of sodium hydroxide solution (NaOH) with constant stirring till the end point as pink color appeared purple. Computation of Free Fatty Acids percentage as:

$$FFA\% = \frac{Vol.of\ 0.1N\ NaOH\ used \times Normality\ of\ NaOH \times 28.2}{Volume\ of\ oil\ used\ (ml)}$$

The peroxide value of samples was calculated according to the standard analytical procedure suggested in AOCS (2006). To measure peroxide value (PV) weighed five milli-liters of chia seed oil sample into a 250ml of titration/conical flask and added about 30ml solvent mixture of acetic acid-chloroform (3:2) and swiveled for approximately one complete minute till to dissolution. Later, pipetted 1-5ml of standard potassium iodide solution with special Mohr pipette. Let the mixture stand in cool and dark place for one minute and was shaken occasionally. Added about 30ml of water. Afterwards, titrated the liberated iodine against 0.1N $Na_2S_2O_3$ solution with continuous stirring till to the point that mixture turned into yellowish shade/color. Accordingly, starch solution was used as an indicator, constant shaking was done during frequent titration until the formed blue color vanished. The PV was estimated by the following formula:

$$Peroxide\ value\ (meq/kg) = \frac{Titer \times N \times 100}{Weight\ of\ sample}$$

The specific gravity of chia seed oil after extraction was evaluated by using pycnometer method described in standard methods of AOCS (2006). To obtain the specific gravity value, first dried the pycnometer and then filled

with sample (oil) in such a way that there was no entrapment of air bubbles using the cap of pycnometer side arm. Afterwards, inserted stopper and placed into a pre-set water bath for about thirty minutes. Vigilantly, wiped off oil that came out from the capillary opening. Thoroughly cleaned and dried the pycnometer after removing from water bath. Took off the cap from side arm, immediately and accurately weighed and ensured temperature should be 30°C at the time of weighing. Value was figured as follows:

$$\textit{Specific gravity} = \frac{Weight\ of\ oil\ (ml)}{Weight\ of\ water\ (ml)} = \frac{(C-S)}{(B-S)}$$

The iodine number/value of all the designated samples was measured according to the procedures of AOCS (2006). To determine (iodine value) took 5.0 ml of oil into 500 ml Erlenmeyer flask. Pipetted 25 ml of carbon tetrachloride (CCL_4) and was continually stirred, followed by addition of Wij's solution till to the point where all contents were well mixed. Solution was left to stand for about 30 minutes in dark place. Added 20 ml of distilled water and 10% potassium iodide solution. The contents of the flask were titrated against 0.1 normal sodium thiosulphate solution by using starch solution as an indicator.

Conducted a blank reading congruently and subsequent formula was used to figure the iodine value:

$$\textit{Iodine value (g/100)} = \frac{(B-S) \times N \times 12.69}{Weight\ of\ sample\ (ml)}$$

Chromatography technique was utilized to determine the whole composition of FAs (fatty acids) of chia seed oil. Fatty acid methyl esters (FAME) were prepared by trans-esterification of oils. To achieve derivatization, vortexed the 100 µl of oil within 05 ml heptane and added 250 µl sodium methoxide in that mixture subsequently. Vortexed this mixture in a vortex mixer for 5 minutes and a gap was given of 15-20 seconds. Afterwards, added about 5 ml of saturated sodium chloride (NaCl) in the mixture and vortexed the mixture until 3 layers were made. The apical/topmost layer contained the methyl esters. Meanwhile, second and third layers contained foam and impurities in respective order. Collected carefully, the upper layer in a vial with the help of micro syringes and primed

this vial of sample for gas chromatographic analysis. Procedure illustrated by Coates, (2011) were followed with slight modifications.

A gas chromatography (Model G-C- 17.A Shimadzu. Hi-Tech lab) equipped with FID (flame ionization detector), capillary column (30.mL × 0.25. mm.ID) and helium utilized as a gas carrier, was utilized. The column's initial temperature was set at 50°C for 1 minute, moderately raised the temperature as 2°C per minute till to reach maximum 240ºC. All the solutions, standards and solvents used were of analytical grades. Accordingly, unsaturated fats assessed by variance of the peaks and the relative peak regions correlating with standard fats as every unsaturated fat was linked as level of overall fatty acid quantity.The method described by Venturini et al. (2019) for the preparation of chia oil cookies was followed with slight modifications. A formulation without chia seed oil was created as a control. Firstly, flour in the amount of 40.00 g, sugar of amount 17.92 g, salt in amount of 0.38 g, baking soda in amount of 0.90 g, and chia seed oil in partial fat replacement formulations mentioned in Table. 3.1 were combined for 3 minutes. After that, the margarine added in the mixture, and homogenized for two minutes. Then water was added depending on the dough properties, and the amount of water in the other formulas was modified to obtain the same dough consistency, as determined by visual observation. Doughs were spread and standardized to a thickness of 5 mm before being cut into circles of 25-mm diameter and oven-baked for about15 minutes at approx. 200 °C. The cookies were packed in plastic containers and stored at 25 °C after cooling to room temperature.Total Moisture content of the chia cookies was determined using the procedures recommended in AACC (2000). The moisture content analysis was executed as follows, about 10gm of the cookies was carefully weighted and placed in the aluminum moisture dish. The moisture dishes were closed and were placed in hot air oven. Heating was turned on and was set at the temperature of 131-135℃. The samples were left in the oven for 18 hours. After that, to cool the sample, placed in a desiccator at ambient temperature. Then the cooled sample was carefully weighed, and readings were noted. The weight of the sample before and after heating in hot air oven was calculated to conclude the moisture content. The decline in the weights denoted the total

moisture content of chia cookies. Percentage moisture content in chia cookies was computed by using the specified formula:

$$Moisture\ (\%) = \frac{Weight\ of\ the\ original\ sample\ (g) - Weight\ of\ dried\ sample\ (g)}{Weight\ of\ the\ original\ sample\ (g)} \times 100$$

Quantification of total ash contents of chia seed oil base cookies was done by following the procedures illustrated in AACC (2000). Firstly 10 grams of the sample was taken in one dry and clean crucible. The contents of total ash were investigated at high temperatures after complete incineration inside the muffle furnace. The samples were placed inside muffle furnace at the temperature of 585°C, for 24 hours. Afterwards the remaining material was cooled at room temperature. The total ash content was premeditated as % of initial weight of the sample. The ash contents in chia seed oil base cookies were calculated by using the given formula:

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

Protein content of chia seed oil base cookies was analyzed by using Kjeldahl's apparatus following methods of AACC (2000) method No. 46-10.01. 2gm dried sample was taken carefully and then was placed into a Kjeldahl flask. 30 ml of concentrated sulphuric acid was added into it. Then added digestion tablets. In the first phase, the total mixture was heated at 400^{o}C and then at 600^{o}C. The color of mixture was changed to give greenish shade. The digested samples were then diluted with almost 250ml distilled water. 10mL of diluted material was distilled with 40 percent NaOH into 4 percent boric acid from preceding combination. The mixture was subjected to titration with 0.1N H_2SO_4 and the end state was light pink in hue. The nitrogen percentage then was determined using the formula:

$$N\% = \frac{Vol.of\ H2\ SO4\ used\ \times\ Vol.of\ dilution\ \times\ 0.0014}{weight\ of\ sample\ \times vol.of\ sample\ taken} \times 100$$

The crude total fat content in sample was calculated by using hexane as organic solvent using Soxhet apparatus in accordance with the method of

AACC (2000) method No.30-25. Two grams of sample was taken to extract the fat by using hexane as solvent. Extraction of fat from sample was done during seven continuous siphons. Afterwards, solvent was evaporated by rotary evaporator. The amount of the fat was then estimated by using following formula:

Cookies developed by utilizing chia seed oil were assessed for sensorial score by using 9-point hedonic scale which fluctuated from liking extremely towards disliking extremely as 9 scoring implying liked extremely to 1 score implying dislike extremely. It was by a suitable panel of qualified judges from National Institute of Food Science & Technology, Faculty of Food, Nutrition and Home Sciences, University of Agriculture Faisalabad by ensuing the guidelines of Meilgaard et al. (2007). All the product analyses were be performed at different intervals during the storage period of 21 days as mentioned in Brandao et al. (2018).At the end the obtained was statistically analyzed by using Latin square design, the analysis of variance under 2-way factorial design to find the level of significance as the method suggested by Montgomery (2008).

RESULTS AND DISCUSSIONS

The present study was mainly designed to consider the "physicochemical prominence of chia seed oil in cookies". After the extraction, the CSO was incorporated for the development of cookies, one of the samples from cookies was kept exempt of the inclusion of chia seed oil and the rest were incorporated with different amount of chia seed oil. All allocated samples of extracted chia seed oil and cookies were additionally investigated, various analyses were performed. The chia seed oil was extracted by the solvent extraction technique using n-hexane as extractant carefully and efficiently to obtain the maximum oil yield. The solvent extracted chia seed oil was further subjected to different analyses before further use.It was determined how long it took to extract chia seed oil with n-hexane as the solvent. The other operation parameters were remained constant as particle size, n-hexane solvent, solid to solvent ratio 1:2, and temperature 65°C. Results indicated that the chia seed had 28.02% maximum oil content with the extraction time 80 minutes. The extraction yields per minute substantially decreased for the rest of the extraction up to 100 to 120 minutes and reached

29.06% approximately.The saponification value of oil can be defined as an indicator that can be used to indicate the usefulness of oil as well as to compare molecular masses. It is the amount of alkali potassium-hydroxide (KOH) mg per gram required to demolish free fatty acids to saponify the eaters show in a certain amount of the substance (1 gram of fat or oil). The degree of saponification values gives a thought about the standard atomic weight of fats and oils. The saponification values of fat lesser that had a higher sub-atomic weight as well as the higher the saponification value predicted that more short-chain fatty acids present in the glycerides of oil or fats and vice versa. Additionally, the saponification value demonstrated the number of the carbon atoms or length of carbon chain of an acid possess in that specific fats and oils. There is the significant impact of temperature on the saponification value of edible vegetable fats and oils. The saponification value decrease with the increase in temperature. This might be due to the transformation of fatty acids to carbonyl compound. Hence, reduces in the FFAs content in the oils and lower the value of saponification. Although, the ratio had least impact on the saponification value. The mean value of saponification of solvent extracted chia seed oil is 194.20 mg KOH/g . Saponification value or SV of 194.20 ± 0.6, mg KOH/g for chia seed oil also talks about the presence of long chain unsaturated fatty acids in chia seed oil. This result was also observed by Uzunova et al. (2019).

The FFA's content measured the degree of extent at which the glycerides compound in the oil have been deteriorated by the lipase activity. The free fatty acid is guide to the purity as well as freshness of oil. The number of FFA's depends upon the several factors like as nature of fats and oils, the action (activity) of lipase enzyme, and method of extraction, humidity, temperature, and storage conditions. It has been ascertained that oil will be susceptible to oxidation phenomenon if higher the free fatty acids value, thus resultantly deterioration of its color and flavor which ultimately leads to rancidity process. These results are also comparable to findings by Uzunova et al. (2019). Normally, the oxidative type of rancidity of oils is assessed by the PV. i.e., peroxide value. The peroxide value as defined is "the amount of peroxide oxygen possess in one liter of oil or fat". Ever since, it is a pointer

of the vital oxidation grade of the oils.The results are close to the literature by Uzunova et al. (2019).The systematic technique of specific gravity is frequently used to evaluate the pureness of the oils. The specific gravity of oils or fats is the ratio of the density of the oil to the density of reference standard substance (i.e., density of water). The specific density of oils or fats is reliant on numerous aspects as number of carbon atoms, fatty acids carbon chain length and double bonds present in oil's structure. The specific gravity value always, lies under 1 and normal values are 0.85 to 0.959 g/cm^3 for the edible oils and fats. The results of specific gravity of chia seed oil are depicted in Table 4.1. The specific gravity of extracted chia seed oil has a mean of 0.92 ±0.01. These results are almost identical to the results of research done by Gazem et al. (2016).There are several analytical methods for assessing unsaturation in fatty acids, among that mythologically the most effective and proficient has be known as iodine number. The IV (iodine value) can be defined as "iodine number is the quantity of iodine used-up by hundred grams of oils or fats". In edible oils and fats, the degree of unsaturation is higher if had more iodine value. Normally, the oil oxidized when in interaction with oxygen and air. As a result of this oxidation the iodine value decay constantly. At the point when oil is repeatedly used for deep-fat frying, there is significant increment in absorption of saturated fat that influence the iodine value. Table. 4.1 exhibit the iodine values of chia seed oil samples. Mean iodine value for extracted chia seed oil is 129.29± 3.56. These results are almost identical to the results of research done by Gazem et al, (2016).

Water content (the quantitative measurement of total water content) is one indicator of a food's reliability and quality. Moisture is an ideal tactile quality in heated items in the meantime, it is unfavorable for most of food products. Nevertheless, too much moisture promotes microbial growth. Findings of the variance analyses are described in Table 4.3.a describing that moisture content in the chia seed oil cookies is highly significantly ($p<0.01$) influenced by treatment while significantly ($p>0.05$) influenced by days and interaction of treatments with storage days.

For treatments, with increasing the concentration of chia oil, moisture content of the cookies was increased. T_3 showed maximum moisture content

(5.58%) which reduced non-significantly for T_2 (5.55%), T_4 (5.55%), T_1 (5.35%) and finally minimum value was obtained at T_o (4.97%). Cookies with varying concentration of chia oil were stored at cool place for 21 days. Moisture contents were determined at different regular intervals of 1st, 7th, 14th and 21st days. During storage, a slight reduction in mean values of the moisture content was observed. Cookies T_3, attained maximum moisture content (5.67%) at the 21st day and minimum moisture content was obtained by T_o which ended at (4.84%) at 0 day. These results revealed that adding chia seed oil increased the moisture content.The analysis of variance for moisture content of different treatments of chia seed oil base cookies has been presenting mean values for moisture content of different chia seed oil cookies treatments have been presented in Table 4.3.b. Results are closely related to findings of Venturini et al. (2019). The ash content by and large addresses the centralization of mineral substance present in the given item. The presence of elevated amount of ash content by implication mirrors the accessibility of more measures of minerals. The ash content is for the most part impacted by the ecological conditions and properties of the seed. It has been accounted for that the ash content of these crude materials is affected by hereditary just as non-hereditary components like soil, climatic conditions, and utilization of fertilizers. Findings of the variance analyses are described in that ash content in the chia seed oil cookies is highly significant ($p<0.01$) influenced by treatment as well as by days and significantly influenced by interaction of treatments with storage days. For treatments, ash content of the cookies was increased. T_2 showed maximum ash content (1.84%) which gradually reduced for T_3 (1.69%), T_0 (1.48%) and finally minimum value was obtained at T_1 (1.14%) and for T_4 (1.07%). Cookies with varying concentration of chia oil were stored at cool place for 21 days. Ash contents were determined at different regular intervals of 1, 7, 14 and 21 days. During storage, a slight reduction in mean values of the ash content was observed. Cookies T_2 attained maximum ash content (1.70%) at the 21st day and minimum ash content was obtained by T_4 which ended at (1.04%) at the 21st day. The results of the current investigation are in accordance to the findings of Venturini et al. (2019) who found that chia oil

had a good amount of minerals indicating a increase in ash fraction of cookies and there was significant association between ash content and storage periods. Table 4.4.a shows the results of the analysis of variance for ash content of different treatments. and mean values for ash content of different chia seed oil cookies treatments have been presented in Table 4.4.b.Crude protein is vital for repairing and manufacturing new muscles fiber. It plays key role in development and growth of muscles cell and control the function of organ and tissues and all enzyme activity by protein. It gives strength to muscles and facilitates sport and movement to body. Protein in muscles provides energy to body in the absence of fats and carbohydrates. It is typically possible to estimate the crude protein content of a sample by measuring the nitrogen content. Findings of the variance analyses are described in Table 4.5.a describing that protein fraction in the chia seed oil cookies is significantly ($p<0.01$) influenced by treatment as well as by days while significantly ($p<0.05$) by interaction of treatments with storage days. For treatments, protein content of the cookies was increased. T_2 showed maximum protein content (5.90%) which gradually reduced for T_0 (5.60%), T_3 (5.52%) and finally minimum value was obtained at T_1 (5.37%) and for T_4 (5.17%). Cookies with varying concentration of chia oil were stored at cool place for 21 days. Protein contents were determined at different regular intervals of 1^{st}, 7^{th}, 14^{th} and 21^{st} days. During storage, a slight reduction in mean values of the protein content was observed. Cookies T_2 showed maximum protein content (5.69%) at the 21^{st} day and minimum protein content was obtained by T_4 which ended at (5.09%) at the 21^{st} day.

The results of the current investigation are in accordance with the findings of Venturini et al. (2019) who found that oils had reduced protein contents indicating a decrease in protein fraction of cookies and there was significant association between protein content and storage periods. For each treatment, analysis of variance for protein content of cookies, have shown in Table 4.5a. and mean values for protein content of different chia seed oil cookies treatments have been presented in Table 4.5.b.Food products' quality and shelf life are affected by fats, which are an excellent source of energy. Fatty acids may also impact the quality of processed foods through surfactant effects, mouth feel when react with protein. Findings of

the variance analyses are described in Table 4.6.a describing that fat fraction in the chia seed oil cookies is significantly ($p<0.01$) influenced by treatment while significantly ($p>0.05$) influenced by days and interaction of treatments with storage days. For treatments, with increasing the concentration of chia oil, fat content of the cookies was increased. T_2 showed maximum fat content (35.57%) which gradually reduced for T_3 (32.67%), T_0 (30.36%) and finally minimum value was obtained at T_1 (28.70%) and for T_4 (26.52%). Cookies with varying concentration of chia oil were stored at cool place for 21 days. Fat contents were determined at different regular intervals of 1^{st}, 7^{th}, 14^{th} and 21^{st} days. During storage, a slight reduction in mean values of the fat content was observed. Cookies T_2 attained maximum fat content (33.80%) at the 21^{st} day and minimum fat content was obtained by T_4 which ended at (25.23%) at the 21^{st} day.

The results of the current investigation are in accordance to the findings of Venturini et al. (2019) who found that oils are fats so a significant increase in the amount of fat of cookies was seen in this case. and there was significant association between fat content and storage periods. Fat contents decrease with increase in storage study that may be due to a number of reasons e.g.,storage conditions or packaging material. As shown in Table 4.6.a, the analysis of variance for chia seed oil cookies fat content is provided, and the mean fat content values for the different treatments depicted in Table 4.6.b.Dietary fibers are essential for human health. They are linked to the prevention, management, and treatment of a variety of illnesses, including biventricular and coronary heart disease. Although crude fiber has minimal nutritional value, it adds weight to meals and helps to control various physiological activities. Findings of the variance analyses are described in Table 4.7.a describing that fiber fraction in the chia seed oil cookies is significantly ($p<0.01$) influenced by treatment and days and significantly influenced by interaction of treatments with storage days. For treatments, with increasing the concentration of chia oil, fiber content of the cookies was increased. T_2 showed maximum protein content (12.90%) which gradually reduced for T_3 (12.27%), T_0 (11.51%) and finally minimum value was obtained at T_1(10.29%) and T_4 (9.50%). Cookies with varying

concentration of chia oil were stored at cool place for 21 days. Fiber contents were determined at different regular intervals of 1st, 7th, 14th and 21st days. During storage, a slight reduction in mean values of the fiber content was observed. Cookies T_2 attained maximum fiber content (12.36%) at the 21st day and minimum fiber content was obtained by T_4 which ended at (9.09%) at the 21st day.

The results of the current investigation are almost like the results of Venturini et al. (2019) who found that oils had some amount of CHO contents as well as flour use for the production of cookies has also CHO contents indicating a increase in CHO fraction of cookies and there was significant association between CHO content and storage periods.

ANALYSIS OF VARIANCE FOR MOISTURE CONTENT (%) OF CHIA SEED OIL COOKIES

Source	***Degrees of freedom(df)***	***Sum of squares***	***Mean squares***	***F-value***
Days (D)	*3*	*0.26980*	*0.08992*	*3.94**
Treatment (T)	*4*	*16.74780*	*4.18695*	*183.29***
D x T	*12*	*0.56160*	*0.04680*	*2.05**
Error	*40*	*0.91370*	*0.02284*	
Total	*59*	*18.49290*		

NS = Nonsignificant (P>0.05)

**= Significant p<0.05*

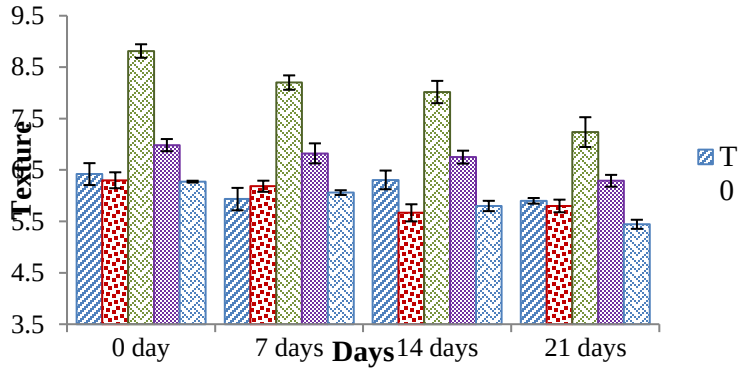
*** = highly significant*

Mean values of moisture (%) for different treatments of chia seed oil cookies

Treatment	***Days***				***Mean***
	0 day	***7 days***	***14 days***	***21 days***	
T_0	*4.84±0.03*	*4.98±0.16*	*5.00±0.06*	*5.03±0.09*	4.97^d
T_1	*5.25±0.14*	*5.31±0.05*	*5.35±0.10*	*5.48±0.07*	5.35^c
T_2	*5.42±0.0*	*5.52±0.12*	*5.62±0.04*	*5.65±0.03*	5.55^b

	6				
T₃	*5.51±0.09*	*5.52±0.04*	*5.62±0.08*	*5.67±0.05*	*5.58[a]*
T₄	*5.59±0.04*	*5.62±0.09*	*5.69±0.01*	*5.30±0.15*	*5.55[b]*
Mean	*4.80±0.12[d]*	*4.89±0.09[c]*	*4.96±0.05[b]*	*4.97±0.08[a]*	

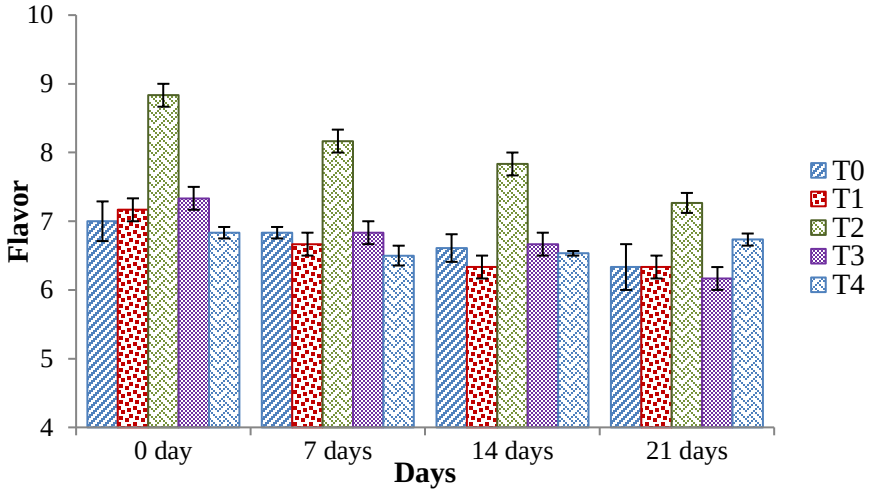
T_0 = control



EFFECT OF TREATMENTS AND STORAGE ON THE TEXTURE OF COOKIES

Flavor is an essential feature in an aesthetic evaluation of the product; it attracts customers with a collective understanding of taste, smell, and mouthfeel. Table 4.13.b. showed the mean values of cookies for flavor. In 21 days of evaluation, the overall flavor score for cookies was significantly reduced ($p<0.05$). In T_2 (7.70), the highest score was recorded with T_3 (6.56), in T_1 (6.40), while T_0 (6.35) and T_4 (6.25) recorded the minimum mean for flavor at 0 day.

The maximum reduction after storage has been reported. The statistical application shows that the impact of treatment and storage intervals on the flavor of cookies is significantly ($p<0.05$). The data observed for the flavor of cookies is like those of Almeida et al. (2018) who reported a reduced acceptability trend in chia oil cookies and their flavor. The above debate suggests that the flavor of cookies varies with oil concentration and storage of cookies.



EFFECT OF TREATMENTS AND STORAGE ON THE FLAVOR OF COOKIES

Overall acceptance is the main factor in the acceptance of the products by customers (Dhankhar, 2013). Table 4.14.b. provided the mean results of judges for overall acceptability. In 21 days of storage study, the overall cookie acceptability score ($p < 0.05$) decreased significantly. The high score at 0 day was in T_2 (8.60), while T_3 (8.00), T_0 (7.40), T_1 (7.20) and T_4 (7.20) showed below values than T_2. The maximum decrease in all samples was recorded after 21 days of storage study. Almeida et al. (2018) also found results matched with present study.The statistical implementation shows that the impact and storage intervals on overall acceptance of cookies on the treatment and storage are significant ($p<0.05$).

TREATMENTS MEAN FOR OVERALL ACCEPTABILITY OF COOKIES DURING 21 DAYS OF STORAGE

Treatment	***Days***				***Mean***
	0 day	***7 days***	***14 days***	***21 days***	
T_0	*7.40±0.55*	*6.80±0.44*	*6.00±0.71*	*5.40±0.54*	*6.40^d*
T_1	*7.20±0.84*	*6.80±0.83*	*6.20±0.45*	*5.80±0.44*	*6.50^c*
T_2	*8.60±0.54*	*8.00±0.71*	*7.60±0.55*	*7.00±0.70*	*7.80^a*
T_3	*8.00±0.71*	*7.80±0.42*	*7.20±0.45*	*6.60±0.55*	*7.40^b*
T_4	*7.20±0.83*	*6.60±0.89*	*6.00±0.70*	*5.60±0.54*	*6.35^e*

Mean	*7.68±0.69^{a}*	*7.20±0.65^{b}*	*6.60±0.57^{c}*	*6.08±0.55^{d}*	

T_0 = control

T_1 = cookies containing 25% chia oil

T_2 = cookies containing 50% chia oil

T_3 = cookies containing 75% chia oil

T_4 = cookies containing 100% chia oil

SUMMARY

Chia seeds have recently gained attention as a major source of polyunsaturated fatty acids. Chia seeds, also known as Salvia Hispanica L., member of the Labiatae family and have been used as a staple food in Mexico since 1500 BC. Chia seeds have a high amount of omega-3 fatty acids and generate around 35 to 40% oil. Chia seed is well-known in Mexico, the Southwest United States, and South America, but not so much in Europe. Chia seeds, on the other hand, were approved as a new food by the European Union in 2009, enabling them to constitute up to 5% of the total matter in bread products. It was the second most significant crop after beans for several pre-Columbian cultures. Chia seeds were employed by Aztec tribes for food, cosmetics, and spiritual ceremonies. Chia seed oil is a substance that has recently been rediscovered for use in aesthetics and food. The chia seed consists of protein 22 to 24 g/100 g, CHO 26 to 41 g/100 g, food fiber 18 to 30 g/100 g, ash 4 to 6/100 g, some minerals, vitamins, and antioxidants. Chia seeds are high in Ca, Mg, Fe, Zn, Br, and niacin. All necessary amino acids are present along vitamin B, E and D. Its dry matter and oil contents consist of 91 to 93 g/100 g and 32 and 39 g/100, respectively.The growth of new products or innovation in each commodity is a strategic domain of the food industry. Generally, consumer demands two things in a food product; the first thing is traditional nutritional value of food and the second is its daily ingestion that gives additional health benefits. Cookies are baked goods that are low in moisture, delicious and crispy, consisting of three main ingredients: flour, sugar, and butter. Due to their distinctive taste and long shelf life, cookies are enjoyed by all age groups. Several studies on nutritional ingredients have encouraged increased consumer awareness of healthy lifestyles and nutritionPresent study designed to achieve a few

objectives i.e., to observe the physicochemical properties of chia seed oil extracted through solvent extraction as well as to observe the effect of chia seed oil on cookies in comparison to regular shortening. And to observe the sensory attributes of cookies on different storage days. Chia seed oil cookies prepared with different amount of oil i.e., 25%, 50%, 75% and 100% and treatments represents as T_o (control), T_1 (with 25% chia oil), T_2 (with 50% chia oil), T_3 (with 75% chia oil) and T_4 (with 100% chia oil).

First of all oil from chia seeds extract through solvent extraction using n-hexane as a solvent.Then chia seed oil assessed for its physicochemical propeties such as saponification value, acid value, peroxide value, specific gravity of oil ,iodine value and fatty acid profile of chia seed oil. Results showed the saponifcation number of chia seed is 194.2, free fatty acid value of oil is 1.03,peroxide value of oil is 1.88,specific gravity is 0.92 and iodine value is 190.69. Then using this oil with different concentrations, cookies were made. And proximate analysis of cookies done. Chia oil has more protein and fiber so its ability to absorbs moisture is also greater so cookies with higher concentration of chia oil exhibits greater moisture contents. Ash content is the indication of mineral contents in a product, so cookies with 50% amount of chia oil exibits higher ash contents that is 1.77%. Chia seeds have a reasonable amount of protein so cookies with 50% replacement of shortening exhibits 5.79% of protein contents. Crude fat is also high in T_2 and the amount of fiber and carbohydrates also greater in cookies with 50% amount of chia oil that shows replacement of shortening with chia oil in cookies significantly affect the properties of cookies.In 21 days of storage moisture content increase because cookies absorbs moisture from the enviornment,ash,protein,fiber,fat and carbohydreate, decrease to a minor content,that may be due to storage conditions that fluctuate during storage.T_2 shows the best values in storage days.9-point hedonic scale ranging from extreme liking to disliking will be used for the purpose of sensory analysis. Sensory analysis of cookies showed the best results with T_2 (50% of chia oil). Storage studies showed a slight reduction in the amount of proetin, fat , fiber and other nutrients. That may be affected due to enviormental condition, packaging material and storage conditions.It was concluded from the results of the current investigation that chia seed oil

significantly influences the physicochemical and organoleptic characteristics of cookies. Furthermore, chia seed oil could be used in different food product as a functional ingredient for the supply of omega-3 fatty acids.

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