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Short Communication

Analytical Characterization of Butter Oil Enriched with Omega-3 and 6 Fatty Acids Through Chia (*Salvia hispanica* L.) Seed Oil

Muhammad Nadeem, Muhammad Ajmal, Fazal Rahman* and Muhammad Ayaz

Department of Dairy Technology, University of Veterinary and Animal Sciences Lahore

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Abstract

Analytical characterization of blends of butter oil and chia (*Salvia hispanica* L.) seed oil was performed. Chia oil was added in butter oil at four different levels i.e. 6.25%, 12.5%, 18.75% and 25% (T_1, T_2, T_3 and T_4), butter oil without any addition of chia oil served as control. Blends of butter oil and chia oil were packaged in tin containers, stored at ambient temperature (34±2^{o}C) for 90-days. Iodine values of control, T_1, T_2, T_3 and T_4 were 36.85, 45.63, 57.22, 67.45 and 76.37 (cg/g %).Concentration of omega-3 fatty acids in T_1, T_2, T_3 and T_4 was 4.17%, 7.39%, 12.55% and 16.74 %. The extent of omega-6 fatty acids in T_1, T_2, T_3 and T_4 was 2.81%, 2.94%, 3.15% and 3.32%.Concentration of omega-3 and 6 fatty acids in butter oil can be increased by chia oil.

Keywords: Chia oil; Butter Oil; Omega-3 and 6 fatty acids

Introduction

Diet has a great effect on serum lipoprotein and serum lipid profile. Omega-3 fatty acids are comprised of α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid [1, 2]. α-linolenic acid, eicosapentaenoic acids are associated with the synthesis of prostaglandins, leukotrienes, and thromboxanes etc. which are involved in a wide range of physiological activities [3]. The cardiac and neuron protective effects of eicosapentaenoic acid and decosahexaenoic acid are scientifically proven [4]. Omega-3 fatty acids have a positive effect in controlling the harmful cardiac arrhythmias which are caused by the sodium and calcium channel dysfunctions [5]. Chia seed (*Salvia hispanica* L.) possess the highest concentration of omega fatty acids; it contains about 60% omega-3 fatty acids on weight basis [6]. The massive nutritional potential of chia seed has not been utilized to enhance the concentration of beneficial omega fatty acids in food systems. This study was planned to perform the analytical characterization of blends of butter oil and chia oil on the basis of selected chemical characteristics.

Materials and Methods

Materials, experimental plan and analysis

Black Chia seeds were obtained from Market; oil was extracted by mechanical expression and stored in amber glass bottles at -10^{o}C till usage in this experiments. Chia oil was incorporated into butter oil at four different concentrations i.e. 6.25%, 12.5%, 18.75% and 25%. The butter oil without any addition of chia oil served as a control. Butter oils supplemented with chia oil were packaged in tin containers, stored at ambient temperature (34±2^{o}C) for 90 days; peroxide value was measured at 0, 30, 60 and 90 days of storage period. Proximate composition of chia seed was determined as per standard AOAC methods [7]. Free fatty acids, unsaponifiable matter, refractive index and peroxide value were

*Corresponding Author Email: drfazal32@gmail.com

determined according to the standard methods [8]. Fatty acid composition was determined by converting the fatty acid into fatty acid methyl esters, using acid transesterifiation technique through methanolic HCl (14%) on a GC-MS (7890 A GC System Agilent), fitted with MSD detector, ZB-5 fused silica capillary column (Zebron Phenomenex;30m x 0.25 mm). Identification and quantification of individual fatty acids were performed with reference to FAME internal standards (FAME-37 Kit Sigma Aldrich, UK) [9].

Statistical analysis

The experiment was performed in a completely randomized design; all treatments were done in triplicate, to determine the effects of treatments, storage period and their interaction, two way analysis of variance technique was used. Treatments were separated through Duncan Multiple Range Test, the results were reported significant on p-value ($p<0.05$) on SAS 9.1 software [10].

Results and Discussion

Chemical characteristics of butter oil and chia oil blends

The results of chemical characteristics of blends of butter oil and chia oil are given in (Table 1). Free fatty acids of blends and control ranged from 0.08% to 0.16%, supplementation of chia oil with butter oil increased the free fatty acids of all the blends in a concentration dependant manner, the increase in free fatty acids of the blends was due to the application of crude chia oil and this situation can be easily encountered by using refined chia oil. The value of 0.16% free fatty acids is usually regarded as acceptable for butter oil. Free fatty acids are considered as an important quality criterion of butter oil; higher proportions of free fatty acids in butter oil are usually responsible for the development of objectionable odours during the storage period [11]. Iodine value of control and chia supplemented butter oils ranged from 36.85 to 76.37 and was in the order of T_4> T_3> T_2> T_1> control. Iodine value of chia oil was 203, which could be the appropriate justification of rise in iodine value as a function of addition of chia oil. The degree of unsaturation in fats and oils is represented by the iodine value; greater values are associated with higher degree of unsaturation [12]. Refractive index of control and T_2 were not different ($p>0.05$); however, in T_3 and T_4 it was significantly influenced from the control ($p<0.05$). Refractive index of fats and oil is connected with degree of unsaturation, higher values exhibit greater unsaturation. Melting point of all the blends decreased when chia oil was blended with butter oil. Melting point dropped from 34.2 to 29.5^{o}C, when 25% chia oil was added in butter oil. Iodine and saponification values of chia seed oil were 207 and 193.3, respectively [13].

Table 1. **Chemical Characteristics of Butter Oil Supplemented with Chia oil.**

Treatments	FFA%	Iodine Value Cg/g	USM%	RI	MP
Control	0.08± 0.02a	36.85± 1.12e	0.73± 0.01a	1.4543± 0.03b	34.2± 0.3a
T_1	0.10± 0.02a	45.63± 0.88d	0.72± 0.02b	1.4561± 0.04b	33.8± 0.2a
T_2	0.11± 0.02a	57.22± 1.55c	0.73± 0.01c	1.4592± 0.02b	33.2± 0.1a
T_3	0.14± 0.01a	67.45± 0.95b	0.75± 0.04d	1.4619± 0.01a	31.6± 0.1b
T_4	0.16± 0.03a	76.37± 1.74a	0.71± 0.03e	1.4643± 0.02a	29.5± 0.1c

Within a column means denoted by a different letter are different

Fatty acid composition

The results of fatty acid composition of chia oil, butter oil and their blends are presented in (Table 2). Fatty acid composition of chia oil revealed that C16:0, C18:0, C18:1, C18:2 and C18:3 were 6.31%, 3.47%, 6.62%, 19.71 and 64.17%, respectively. Omega-3 and 6 fatty acids content of chia oil was 61.45% and 18.96%, as compared to butter oil, which accounted for 0.5% and 1.75%. Addition of chia seed in butter oil had a major effect on the fatty acid composition of blends. The concentration of α-Linolenic fatty acids in T_1, T_2, T_3 and T_4 was 4.17%, 7.39%, 12.55% and 16.74%, respectively. The extent of omega-6 fatty acids in T_1, T_2, T_3 and T_4 was 2.81%, 2.94%, 3.15% and 3.32%. The correlation between the dose of chia oil, omega-3 and 6 fatty acids was 0.9937 and 0.7994 (Fig. 1 and 2).The concentration of eicosanoic acid in T_1, T_2, T_3 and T_4 was 0.15%, 0.22%, 0.29% and 0.35%.Another significant change was observed around the medium chain fatty acids, their concentration

considerably decreased with increasing increments of chia oil. The role of medium chain fatty acids in the promotion of hypercholesterolemia has been well identified, according to the scientific information available; the reduction in concentration of medium chain fatty acids can have health benefits [14]. Blended butter oil and *Moringa oleifera* oil with major changes in the fatty acid composition of blends was reported. The recommendation of American Heart Association had a great deal of switching form saturated fatty acids to the consumption functional foods added with functional ingredients [1]. Chia seeds are regarded as the powerhouse of omega fatty acids. The contain the highest concentration of omega fatty acids [6]. Modification in fatty acid composition of fats and oils as result of blending is extensively reported in literature. However, little is known regarding the blending of chia oil with dietary lipids. Omega-3 fatty acids have the ability to block the dysfunction of calcium and sodium channels, which otherwise can consequences in hypertension [5].

Table 2. **Fatty acid composition (%) of Butter Oil and Chia oil Blends.**

Fatty Acid	Milk Fat	Chia oil	T_1	T_2	T_3	T_4
C4:0	1.81± 0.02a	----	1.69± 0.09b	1.55± 0.07c	1.47± 0.04d	1.35± 0.11e
C6:0	2.13± 0.05a	----	2.04± 0.06b	1.95± 0.12c	1.82± 0.03d	1.67± 0.05e
C8:0	2.32± 0.06a	----	2.21± 0.08b	2.12± 0.13c	2.01± 0.07d	1.88± 0.04e
C10:0	2.41± 0.10a	----	2.27± 0.02b	2.11± 0.06c	1.95± 0.03d	1.81± 0.12e
C12:0	2.65± 0.13a	----	2.46± 0.14b	2.27± 0.05c	2.08± 0.09d	1.85± 0.16e
C14:0	10.19± 0.19a	----	9.51± 0.26b	8.84± 0.19c	8.13± 0.17d	7.32± 0.29e
C16:0	31.11± 0.52a	6.31± 0.04f	29.47± 0.35b	28.72± 0.26c	27.19± 0.55d	26.11± 0.14e
C18:0	9.81± 0.17a	3.47± 0.09f	9.36± 0.13b	8.83± 0.17c	8.22± 0.13d	7.46± 0.18e
C18:1	24.57± 0.35a	6.62± 0.07f	23.55± 0.48b	22.47± 0.36c	21.84± 0.38d	20.52± 0.64e
C18:2	4.21± 0.09f	19.71± 0.24a	5.17± 0.08e	6.34± 0.14d	7.51± 0.16c	8.79± 0.21b
C18:3	1.32± 0.05f	64.17± 0.43a	5.19± 0.07e	9.28± 0.13d	13.72± 0.22c	18.12± 0.09b
α-Linolenic ω 6	0.50± 0.02f 1.75± 0.06f	61.45± 0.29a 18.96± 0.19a	4.17± 0.11e 2.81± 0.03e	7.39± 0.41d 2.94± 0.04d	12.55± 0.18c 3.15± 0.07c	16.74± 0.32b 3.32± 0.13b
Eicosanoic	ND	0.95± 0.02e	0.15± 0.02d	0.22± 0.03c	0.29± 0.05b	0.35± 0.04a

Means of triplicate experiments; within a row means with a different letter are statistically different (P<0.05)
ND: Not Detected

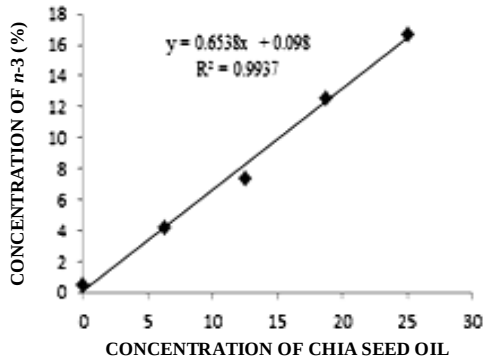

Figure 1. **Corelation Between Doses of Chia Seed Oil and Omega-3 Fatty Acids**

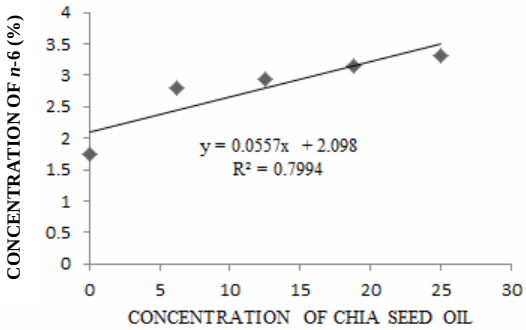

Figure 1. **Corelation Between Doses of Chia Seed Oil and Omega-3 Fatty Acids**

Peroxide value

Peroxide value of all the treatments and control increased during the storage period of 90-days, increase in peroxide value of experimental samples and control was non-significant (P>0.05) up to 30 days of storage, rest of the determination frequencies revealed an increasing trend (Table 3). The rise in peroxide value was dependent upon the concentration of chia oil, treatments containing higher dosage of chia oil revealed greater peroxide value. The peroxide value of control and treatments after 90 days of storage was in the order of control < T_1< T_2< T_3< T_4. The rise in peroxide values of the experimental samples was due to the higher concentration of unsaturated fatty acids [15]. The non-significant changes in peroxide value of control and experimental samples during the first 30-days of storage period could be connected to the presence of phenolic compounds in chia oil. Caffeic acid, chlorogenic acid and quercetin has also been found in chia oil, these compounds are known to have antioxidant properties [16].

Table 3. **Peroxide Value (mev/kg) of Butter Oil Supplemented with Chia oil.**

Treatments	0-Day	30-Days	60-Days	90-Days
Control	0.42± 0.03h	0.45± 0.02h	1.12± 0.05g	2.41± 0.08b
T_1	0.45± 0.02h	0.49± 0.02h	1.35± 0.05f	1.89± 0.08d
T_2	0.0.48± 0.05h	0.51± 0.06h	1.62± 0.04e	2.17± 0.10c
T_3	0.44± 0.03h	0.55± 0.04h	1.93± 0.11d	2.49± 0.13b
T_4	0.47± 0.02h	0.58± 0.03h	2.54± 0.05b	3.07± 0.15a

Within the rows and columns means denoted by different a different letter are different (P<0.05)

Conclusion

Supplementation of chia seed in butter oil at all levels improved the nutritional value, Peroxide value of one month stored chia oil supplemented butter oils was not different from the control. The concentration of omega-3 and 6 fatty acids can be enhanced in butter oil through supplementation of chia seed oil, with reasonable storage stability.

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