PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 11 (9): 823-827, 2012
ISSN 1680-5194

Evaluation of Functional Fat from Interesterified Blends of Butter Oil and *Moringa oleifera* Oil

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Abstract: The main objective of this research work was to develop a functional fat from Butter Oil (BO) and *Moringa oleifera* Oil (MOO) blends by interesterification process. To prevent the separation of liquid and solid phase the blend was chemically interesterified. MOO was incorporated into BO at five different levels i.e. T_1 (90% BO and 10% MOO) T_2 (80% (BO) and 20% MOO) T_3 (70% BO and 30% MOO) T_4 (60% BO and 40% MOO) and T_5 (50% BO and 50% MOO). All these treatments were compared with a control T_0 which did not contain any addition of MOO (100% BO). Melting point of all the treatments along with control increased after interesterification. Melting point of T_5 was 35.5°C with iodine value of 60.44 (g/100 grams). Cholesterol reduction with beta cylodextrin increased as the concentration of MOO increased in the blend. The cholesterol reduction in T_1 and T_5 was 87 and 94% respectively as compared to control 85%. Oxidative stability was significantly increased with increasing augmentation of MOO in the blend. After 5 days in oven at 63°C the peroxide value of T_5 was 2.19 as compared to control 13.14 (M. Eq./kg). It can be concluded that MOO and BO can be used in the formulation of functional and shelf stable fat.

Key words: Butter oil, *Moringa oleifera* oil, interesterification, cholesterol, peroxide value, food security

INTRODUCTION

Butter oil is a popular dairy product in Pakistan and used for frying and cooking purpose. It contains almost 99.5% milk fat (Mc Sweeney and Fox, 2003). It is rich in vitamin A which is necessary for thyroid, adrenal health and good eye vision. The fat contains lecithin, which is important for cholesterol metabolism. Butter oil possess antioxidants which protect the body against free radical damage and weakening of arteries. It contains conjugated linoleic acid that serves as a potent anti-cancer agent, muscle builder and immunity booster. Butter oil protects the calcification of the joints and pineal gland, prevents hardening of arteries and cataract. It is a source of Vitamin K, which is important for treating arthritis, osteoporosis, tooth decay, tuberculosis, emphysema and asthma (Speer, 2005). Pakistan is the 4th largest buyer of palm oil in the world and in 2010 imported palm oil worth 1.31 billion US $ with average price of 94.15$ per metric ton and spending almost 20% of the total budget on the imports of edible oils to feed ever increasing population. 50% of the population sustain their lives on less than one dollar per day and more than 17 millions people suffer from the shortage of edible fats (Anonymous, 2010). Prevailing situation of food shortage demands immediate attentions of all the concerns to explore the indigenous edible oil resources and find out their suitability in the formulation of edible fats. *Moringa oleifera* (MOO) oil is edible and closely resembles to olive oil in fatty acids composition. The oil content of seeds ranges from 30-40%. Vegetable oils rich in monounsaturated fatty acids are generally more stable to oxidative rancidity and are healthier and poise a lower risk of coronary heart disease. It is liquid at room temperature and pale-yellow in colour melting point is 18.9°C (Long *et al*., 1997). Myristic and lauric acids are atherogenic and raise the risk of cardio vascular disease by increasing plasma cholesterol and Low Density Lipo proteins (LDL) although oleic, linoleic and linolenic reduce the increase. The National Health Survey of Pakistan reported that 21.5% of the urban population over 15 years (one in every three persons over the age of 45) suffers from hypertension (Nishtar, 2002). In partial hydrogenation, unsaturated fatty acids are isomerized into *trans* fatty acids from their natural cis configuration. Trans fatty acids are metabolized in the body like saturated fatty acids and increase LDL cholesterol and decrease beneficial HDL cholesterol. Interesterification is a processes used to modify the physico-chemical characteristics of vegetable oils and fats. It is an acyl-rearrangement reaction on the glycerol molecule and may either be random or selective (Osman and Idris, 1999). Cyclodextrins are carbohydrates composed of α-1,4-linked glucopyranose subunits. Several studies on a laboratory scale have

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indicated that cholesterol removal from homogenized milk and cream was most effectively achieved by β-cyclodextrin powder (Kwak and Ahn, 1999). For the reason present study was planned to develop functional fat containing lower contents of saturated fatty acids and cholesterol and evaluate the suitability of MOO in the formulation of healthy fat which can be used to resolve the problem of food security.

MATERIALS AND METHODS

Raw materials: Butter oil was obtained from Haleeb Foods, Phool Nagar distrit Kasur. *Moringa oleifera* seeds were obtained from Muzafar Garh. Oil was obtained by pressing the seeds in laboratory scale locally manufactured expeller (15 kg/hour). The oil from expeller was used for this study. Sodium methylate (Merck) was obtained from a reputed scientific store of Lahore.

Experiment*:* (MOO) was incorporated into (BO) at five different levels i.e. T_1 (90% BO and 10% MOO) T_2 (80% BO and 20% MOO) T_3 (70% BO and 30% MOO) T_4 (60% BO and 40% MOO) and T_5 (50% BO and 50% MOO). All these treatments were compared with a control T_0 which did not contain any addition of MOO (100% butter oil). All these treatments were performed in triplicate.

Chemical interesterification: Chemical inter-esterification of different blends of BO and MOO were carried out according to the method of (Sreenivasan, 1978). 1000 mL of the samples were dried in a flask, under reduced pressure, fitted with a vacuum pump in a waterbath at 95°C. The portions were mixed with 0.2% (w/w) of sodium methylate. Interesterification reaction was performed under reduced pressure at 70°C in a 1000 mL stoppered flask in a waterbath with constant agitation for one hour. To terminate the reaction, 5 mL of distilled water was added.

Cholesterol reduction: Cholesterol was removed from all the treatments T_1 to T_5 along with control by encapsulating cholesterol with beta cyclo dextrin according to the method of (Kwak *et al*., 2002). Beta cyclo dextrin was added at 0.5%. Cholesterol removal was carried out at 60°C. Beta cyclo dextrin was separated by filtration.

Determination of cholesterol: Cholesterol content of all the samples was determined by the method of Rudel and Morris (1973).

Oxidative stability: Schaal oven test was performed by following the method of (AOCS, 1990) with 20±0.1 gram samples, in triplicate 50 mL beakers, were kept in an oven at 63±1°C, for 5 days. Peroxide values were analyzed in the experimental samples.

Statistical analysis: The data was obtained by applying Completely Randomized Design (CRD) and the outcome of the data was analyzed through analysis of variance technique (Steel *et al*., 1997). The separation of means or significant difference comparisons was made using Tukey's HSD test.

RESULTS AND DISCUSSION

Addition of MOO at all levels increased the free fatty acid content of the blend. Free fatty acids of all the treatments from T_1 to T_5 before interesterification were at par with each other ($p>0.05$). Highest value of free fatty acids was recorded in T_0 followed by T_3 and T_2. Moisture content in all the treatments from T_1 to T_5 increased as the concentration of MOO was increased in the blend. This non significant increase in moisture content was due to comparatively higher moisture content of MOO. Moisture in crude oils is inherited from seeds and removed during subsequent stages of processing. Fereidoon (2005) reported the moisture content of palm oil, palm olein and cotton seed oils in the range of 0.20-0.30%. The higher levels of moisture are regarded unacceptable may result in low yields. The oils having higher levels of moisture has poor keeping quality and low market value. Addition of *morinag olifera* oil at all levels increased the iodine value of blend. The highest iodine value (59.5) was observed in T_5 (50% BO and 50% MOO). Melting point of all the treatments decreased as the concentration of *Morinag olifera* oil was increased in the blend. The decline in melting point of blend at all levels was due to lower melting point of *Morinag olifera* oil (19.2°C) which contributed in this phenomenon. Iodine value and melting point are inter related parameters, fats having higher degree of saturation have low melting point as compared to those having more unsaturated fatty acids. 65-70% of the fatty acids in milk fat are recognized as saturated; therefore the melting point of milk fat is more than *Moringa oleifera* which has higher concentration of unsaturated fatty acids. Abdulkarim *et al*. (2005) reported that melting point of *Moringa oleifera* was 18.9°C. The milk fat of (BO) was reported to be in the range of 32-34°C by Speer (2005); Potter and Hotchkiss (1990). Free fatty acids of all the treatments along with control were significantly ($p>0.05$) decreased after interesterification reaction. The difference in free fatty acids content of all the treatments and control was non significant ($p>0.05$). The decline in free fatty acids was due to the alkaline nature of catalyst (sodium methylate) used as catalyst for rearrangements of fatty acids on glycerol molecules. Sodium methylate is regarded as strong alkali and almost 70% of the catalyst is used in neutralization of free fatty acids, only 30% initiates and maintain the rearrangement reaction (Ecrickson, 1999). Fermani *et al*. (2006) while studying the effects of chemical interesterification on chemical composition of palm oil and palm olein reported that free fatty acids of all the blends were significantly less than

Table 1: Effect of interesterification on chemical parameters of interesterified blend of BO and MOO

	Before interesterification				After interesterification			
Trt.	FFA%	MC%	IV	MP °C	FFA%	MC%	IV	MP °C
T_0	0.080±0.03[a]	0.19±0.04[a]	53.46±0.45[d]	38.60±0.45[a]	0.02±0.01[a]	0.01±0.01[a]	53.87±0.76[d]	46.40±0.54[a]
T_1	0.090±0.02[a]	0.14±0.05[a]	54.30±0.66[c]	36.50±0.23[b]	0.02±0.01[a]	0.02±0.01[a]	54.67±0.97[d]	42.80±0.38[b]
T_2	0.092±0.03[a]	0.16±0.07[a]	55.60±0.81[b]	34.80±0.15[c]	0.02±0.02[a]	0.01±0.01[a]	55.99±1.14[c]	40.50±0.26[c]
T_3	0.097±0.01[a]	0.18±0.08[a]	56.90±0.91[b]	33.20±0.11[d]	0.03±0.01[a]	0.01±0.01[a]	56.58±1.43[c]	39.30±0.22[d]
T_4	0.104±0.04[a]	0.21±0.09[a]	58.24±0.95[a]	31.30±0.10[e]	0.02±0.01[a]	0.01±0.01[a]	58.67±1.53[b]	37.20±0.14[e]
T_5	0.11±0.05[a]	0.23±0.11[a]	59.50±0.99[a]	29.10±0.08[f]	0.02±0.01[a]	0.01±0.01[a]	60.44±1.63[a]	35.50±0.09[f]

Means of triplicate experiment; means with same superscripts in columns and rows are statistically non significant by Tuckey's t-test at 0.05 level of significance. T_0 (Control; 100% (BO); T_1 (90% (BO) and 10% (MOO); T_2 (80% BO and 20% MOO); T_3 (70% (BO) and 30% (MOO); T_4 (60% (BO) and 40% (MOO); T_5 (50% (BO) and 50% (MOO*)*; Trt. = Treatments

the original free fatty acids of the blend before rearrangement reaction; they attributed it to the alkaline nature of catalyst. Moisture content of all the treatments were almost zero after interesterification, the reason for very low moisture content in interesterified blends was the drying of samples before the addition of interesterification catalyst. Fereidoon (2005) reported that moisture content of feed stock for chemical interesterificaion should be less than 0.1%. During analytical work, samples absorbed some moisture from the atmosphere. Iodine value of all the treatments was not significantly affected by ineresterification reaction. Iodine value of all the blends from T_1 to T_5 and control (T_0) was not influenced by chemical intereserification before and after rearrangement of esters. Interesterification is a randomization reaction which results in rearrangement of fatty acid esters on glycerol molecule without causing any saturation or unsaturation of fatty acids so iodine value is not changed by interesterification. Erickson (1999) reported that chemical interesterification of soybean oil with sodium methylate (0.2%) did not have any significant effect on iodine value. Melting point of all the treatments and control increased after interesetrification, the increase in melting point of interesterified blends may probably be the rearrangements of esters and change in position of the double bonds in fatty acids molecule as a result of positional isomerization. The melting point of ineresteified blends may be increased, decreased or it might not suffer any change. The change in melting point depends upon the formulation of feed stock for interesterification. Farmani *et al.* (2006) reported that as the percentage of liquid oil in a blend increases, causes the dilution and solubilization of the triacylglycerols with higher melting points.

Removal of cholesterol from interesterified blend of (BO) and *Morinag olifera* oil: T_0 (100% (BO)) yielded the highest concentration of cholesterol 230 mg/100 gram, which decreased as the concentration of MOO was increased in the blend (Table 2). The lowest concentration of cholesterol was recorded in T_5 (115.89/100 gram). The reason for decline in cholesterol content of all the treatments may be the very low level of

Table 2: Cholesterol content of interesterified BO and MOO blends

Treatments	BD	AD
	(mg/100 gram)	
T_0 (Control; 100% (BO))	230.11±2.34[a]	226.89±2.33[a]
T_1 (90% (BO and 10% MOO)	207.42±2.11[b]	26.76±1.45[b]
T_2 (80% (BO and 20% MOO)	184.34±1.89[c]	15.64±0.89[c]
T_3 (70% (BO and 30% MOO)	161.23±1.66[d]	13.45±0.67[d]
T_4 (60% (BO and 40% MOO)	138.67±1.56[e]	10.58±0.45[e]
T_5 (50% (BO and 50% MOO)	115.89±1.34[f]	6.69±0.33[f]

Means of triplicate experiment; means with same superscripts in columns are statistically non significant by Tuckey's t-test at 0.05 level of significance. BD = Before Decholesterolization (mg/100 gram); AD = After Decholesterolization (mg/100 gram)

cholesterol in MOO. The highest level of cholesterol removal (94%) was observed in T_5 (50% BO and 50% MOO). While control sample (100% butter oil) exhibited the lowest cholesterol reduction (85%) with a dose of 0.5% beta cyclo dextrine. This may be due to the presence of natural cholesterol in high concentrations (230 mg/100 gram). Cholesterol reduction with beta cyclo dextrin increased with increasing augmentation of MOO in BO. T_1, T_2, T_3 and T_4 exhibited 87, 89 and 92 and 93% cholesterol reduction with 0.5% dose of beta cyclo dextrin (Table 2). Several studies on a laboratory scale have indicated that cholesterol removal from homogenized milk and cream was most effectively achieved by β-cyclodextrin powder (Lee *et al.*, 1999). Kim *et al.* (2010) conducted study to examine changes in the chemical and sensory properties of butter in which the cholesterol was reduced and to which evening primrose oil and phytosterols were added. Crosslinked β-cyclodextrin was used and approximately 90% of the cholesterol was removed. Differences in sensory characteristics did not result from the β-cyclo dextrin treatment. In microscopic examinations, no noticeable differences were found among the treatments and a smooth texture and a fine, uniform crystalline structure were observed. Results indicated β-cyclo dextrin treatment itself did not adversely influence the chemical and sensory properties of the butter. Dias *et al.* (2009) studied the removal of cholesterol from butter through three complexation methods with beta-cyclodextrin: co-precipitation, kneading and physical mixture.

Table 3: Peroxide value of interesterified blend of (BO) and (MOO) in the schaal oven test for 5 days at 63°C

Treatments	Peroxide value (meq. O_2/kg)
T_0 (100% BO)	13.14±0.65[a]
T_1 (90% BO and 10% *MOO*)	11.29±0.49[b]
T_2 (50% BO and 50% *sesamum indicum* oil)	7.45±0.31[c]
T_3 (25% (BO) and 75% *sesamum indicum* oil)	5.30±0.18[d]
T_4 (60% (BO) and 40% (MOO)	4.40±0.21[d]
T_5 (50% (BO) and 50% (MOO)	2.19±0.15[d]

Means of triplicate experiment; means with same superscripts in column are statistically non significant by Tuckey's t-test at 0.05 level of significance

Photoacoustic Spectroscopy (PAS) was used to evaluate the inclusion complex cholesterol in beta-cyclo dextrin. The co-precipitation method was the most appropriate for removal of butter cholesterol. The composition in fatty acids was not affected by the adopted process. The standardization of the extraction technique of butter cholesterol for its quantification was efficient. The comparison between three quantification methods, enzymatic, high-performance liquid chromatography and gas chromatography, showed no significant differences.

Oxidative stability: As can be seen in Table 3, after 5-days in an oven at 63°C control sample (100% BO) presented the highest peroxide value of (16.14 meq O_2/kg). Peroxide value decreased as the concentration of (MOO) increased in the blend. The lowest peroxide value (2.19 meq O_2/kg) was observed in T_5 (100% (MOO)). Peroxide value of T_3, T_4 and T_5 was significantly different from each other ($p<0.05$). Peroxide value indicates the primary stages of auto oxidation and important parameter for storage stability of fats and oils. Higher levels of peroxide value are associated with poor keeping quality, this may be due to the presence of natural antioxidants in (MOO). Quality of milk in Pakistan is poor due to the lack of cold chain and lack of knowledge of milk handling which could be the probable reason for higher peroxide value of the control sample. Tsaknis *et al.* (2002) reported that *Moringa oleifera* has excellent oxidative stability during frying. So, great potential exists for blending of *Moringa oleifera* with other oils and fats. Anwar *et al.* (2007) studied the effect of *Moringa oleifera* addition on oxidative stability of some vegetable oils and found that induction time of blend of soybean and sunflower containing 80% (MOO) has induction time from 1.12-5.99 hrs and 1.47-6.22 hrs and recorded the increase in oxidative stability 435% and 323% respectively. It was noted that each 20% addition of *Moringa oleifera* in pure sunflower oil and soybean oil enhanced their induction time by (on average) 56.5% and 43.4%, factors of 1.58 and 1.47, respectively.

Conclusion: It can be seen from the results of Table 1 that melting point of T_4 (37.20°C) is almost similar to body temperature with comparatively high iodine value 58.67. Results of oxidative stability indicated that addition of MOO 50% level significantly improved the stability of blend. Cholesterol reduction in T_5 was 94% as compared to control 87% reduction. From the results of this study it may be concluded that *Moringa oleifera* oil can be added by interesterification process up to 50% in butter oil to formulate a healthier and stable fat with acceptable physico-chemical properties.

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