

## Antioxidant Potential of *Moringa oleifera* Leaf Extract for the Stabilisation of Butter at Refrigeration Temperature

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### Abstract

NADEEM M., ABDULLAH M., HUSSAIN I., INAYAT S., JAVID A., ZAHOOR Y. (2013): **Antioxidant potential of *Moringa oleifera* leaf extract for the stabilisation of butter at refrigeration temperature.** Czech J. Food Sci., **31**: 332–339.

The antioxidant potential of a leaf extract of *Moringa oleifera* Lam. (Moringaceae) – LEMO was studied for the stabilisation of butter at refrigeration temperature. LEMO was obtained by extracting the ground and dried leaves with 80% ethanol at room temperature for 48 hours. LEMO was added into butter at three different concentrations, i.e. 400 ppm (T<sub>1</sub>), 600 ppm (T<sub>2</sub>), and 800 ppm (T<sub>3</sub>) and compared with a treatment which was not supplemented with LEMO, i.e. control (T<sub>0</sub>). The addition of LEMO at all three levels did not have any effect on butter composition. Free fatty acids, peroxide value and *p*-anisidine value (AnV) of T<sub>2</sub> after 90 days of storage were 0.10%, 0.71 meq/kg and 14.85 as compared to the control 0.16%, 1.24 meq/kg and 28.85, respectively. Peroxide value of the control and T<sub>2</sub> in Schaal oven test after 5 days in oven was 8.19 and 2.99 meq/kg, respectively. Induction period and overall acceptability score of the control and T<sub>2</sub> were 6.35 h, 8.91 h and 7.6, 7.2, respectively. The results of this study suggest that LEMO at 600 ppm may be used for reasonable storage stability of butter at refrigeration temperature with acceptable sensory characteristics.

**Keywords:** oxidative stability; overall acceptability

Butter is a popular dairy product in the subcontinent and is used for cooking, bakery products, and many kinds of traditional sweets. Most of the butter is made from the mixed milk of cow, buffalo, and goat etc. (Agricultural Statistics of Pakistan 2006). Adulteration is a serious malpractice in milk which leads to the inclusion of many undesirable and health hazardous chemical substances and the partial replacement of milk fat with vegetable oils, which reduces the keeping quality of butter (GARCIA *et al.* 2003). In Pakistan the duration of electric load shedding is prolonged to eighteen hours a day and also the cost of electricity is dramatically increasing every month and the storage of butter at minus temperatures is becoming a costly affair. Therefore, most of the local butter manufacturers use refrigerators instead of freezers for the storage of butter. The storage of butter at relatively higher temperatures leads

to the development of rancid flavour (POTTER & HOTCHKISS 1998) with decreased customer acceptability. The consumption of rancid fats leads to the development of cancer and ailments like the coronary heart disease (FOX & McSWEENEY 2003). Further the partial replacement of milk fat with vegetable oils like canola, sunflower etc. exerts a great set-back in this context. Vegetable oils owing to a higher content of unsaturated fatty acids are more prone to the development of oxidation and oxidation products (ERICKSON 1999). The dairy processing sector of Pakistan is concentrated in the Punjab province especially along the Ravi and Chenab Rivers and dairy products are transported to a distance of thousands of kilometres to the rest of the country and Afghanistan (GARCIA *et al.* 2003). Quality of milk is not so good due to rough, extensive and poor milk handling practices and the use of iron and copper utensils

for milking, storing and transportation causes significant lipolytic activity up till reaching the milk processing plant and churning into butter. To cope with the situation, dairy industries have started to use chemical antioxidants in butter; the use of synthetic antioxidants has many health concerns (GHATAK & BANDYOPADHYAY 2007) and the situation demands for an organic solution of the problem. Extracts of many higher plants have been found to contain appreciable amounts of phenolic antioxidants, tocopherols, flavonoids which possess antiageing, anticarcinogenic, and cardioprotective effects (ANWAR & BHANGER 2003). *Moringa oleifera* Lam (Moringaceae) (also known as drumstick tree, horseradish tree) is cultivated throughout Pakistan, especially in southern Punjab the tree is widely grown near the houses to sit in the shade. Every part of this miraculous tree is full of nutrition and fresh, tender pods are cooked and eaten as a vegetable, leaves are used for animal feeding and manufacturing of many herbal medicines. The leaves of *Moringa oleifera* contain up to 8% antioxidants on dry matter basis. The antioxidant potential of *Moringa oleifera* leaf extract for the stabilisation of sunflower oil was studied by ANWAR *et al.* (2006). No work has been done to investigate the antioxidant potential of *Moringa oleifera* leaves for the stabilisation of butter at refrigeration temperature. For the reason this research work was planned to explore the antioxidant potential of *Moringa oleifera* leaf extract using butter as an oxidation substrate on the basis of certain chemical and sensory parameters.

## MATERIAL AND METHODS

**Raw material.** Cream was purchased from Haleeb Foods Ltd. (Phool Nagar, Kasur, Pakistan). *Moringa oleifera* leaves were obtained from Ayub Agricultural Research Institute (Faisalabad, Pakistan). CSK-95 starter culture was procured from CSK Food Specialties (Leeuwarden, the Netherlands). All the reagents used in this study were HPLC grade and obtained from Sigma Chemical Co. (St. Louis, USA).

**Butter manufacturing.** Cream was pasteurised in beakers of 2000 ml in a water bath (Memmert, Hannover, Germany) at 85°C for 2 min, immediately cooled down to 21°C, pooled and inoculated with 2% bulk starter culture of CSK-95 mixed well and incubated at this temperature for 18 h (titratable

acidity 0.75%). Churning was done in a laboratory scale churn (ELBA 80; Elecrem, Vanves, France) and stopped when the butter grains attained the size of peas, washed with cold water (10°C) twice, worked (SPEER 2005) and divided into 12 equal proportions, each containing 500 grams of butter.

**Leaf extract of *Moringa oleifera* (LEMO).** Leaves of *Moringa oleifera* were washed with tap water, dried in the sun under the shade, ground in a grinder (Moulinex, Japan). LEMO was obtained by mixing 500 ml of 80% ethanol with 50 g of dried leaves in a triplicate pyrex beakers of 1000 ml, tightly closed with aluminum foil and mixing was carried out at a low speed magnetic stirrer (100 rpm) for 48 h, ethanol was removed by distillation at a low temperature (45°C) on a soxhlet distillation apparatus. LEMO was stored in light-resistant brown glass bottles at 4°C for subsequent usage as per method of SIDHURAJU and BECKER (2003).

**Experimental plan.** Leaf extract of *Moringa oleifera* was incorporated into butter at three different concentrations, i.e. 400 ppm ( $T_1$ ), 600 ppm ( $T_2$ ), and 800 ppm ( $T_3$ ), and compared with a treatment which was not added any LEMO (control). All the experimental samples along with the control ( $T_0$ ) were packed in air-tight polyethylene bags and put into a refrigerator at  $10 \pm 1^\circ\text{C}$  for 90 days and storage stability was determined at the interval of 30 days. Each treatment was replicated three times.

**Analysis.** Composition of butter, i.e. fat, fat-free dry matter, and moisture content, was determined as per methods of AOAC (2000). Free fatty acids, peroxide value, *p*-anisidine value (AnV) and iodine value were determined by the following methods as given in AOCS (1990).

**Total phenolic content.** Total phenolic content of the leaf extract of *Moringa oleifera* was determined by taking 20  $\mu\text{l}$  of the extract in a screw capped 11-ml test tube, 1.6 ml distilled water and Folin-Ciocalteu reagent (100  $\mu\text{l}$ ), all were mixed with each other, then 300  $\mu\text{l}$  of  $\text{Na}_2\text{CO}_3$  solution (20%) was added and well shaken in a shaking water bath at 40°C for 30 min and total phenolic content was determined from the standard curve plotted by using gallic acid as standard at 760 nm according to the method of ANWAR *et al.* (2006).

**Schaal oven test.** This test was performed by weighing  $20 \pm 0.1$  g butter in 50-ml triplicate pyrex beakers and put into an oven adjusted at  $60 \pm 0.1^\circ\text{C}$  for five days. Oxidative stability was determined by measuring the peroxide value

in all experimental samples and control as per method of AOCS (1990).

**Conjugated dienes and trienes.** Samples were diluted in iso-octane and oxidation products in the form of conjugated dienes and trienes were determined by measuring specific extinction at 232 and 268 nm in UV region using the method of IUPAC (PAQUOT 1979).

**Induction period.** Induction period was measured automatically by taking  $2.5 \pm 0.05$  g samples in Rancimat reaction vessels where they were oxidised at  $120 \pm 0.2^\circ\text{C}$  by passing oxygen from the bottom of the samples according to the instructions given in the instruction manual of Metrohm Rancimat 679 (Metrohm 1993).

**Sensory evaluation.** Sensory evaluation of experimental samples and control was carried out at  $25^\circ\text{C}$  in a well illuminated laboratory. Samples of butter were randomly served to a panel of five trained judges (coded with three figure random number) and all orders of servings were fully randomised. Sensory evaluation was done on a 9-point Hedonic scale using 9 the best and 1 the worst, as prescribed by LARMOND (1987).

**Statistical analysis.** The experimental design used in this study was a completely randomized design (CRD) and significant difference ( $P < 0.05$ ) among the treatments and control was determined using Duncan's Multiple Range Test (DMR) as described by STEEL *et al.* (1997).

## RESULTS AND DISCUSSION

Phenolic antioxidants possess the ability to act as scavengers by donating a proton and thus inhibit the autooxidation process. Many higher plants contain useful and potent antioxidants (MIDDLETON *et al.* 2000). Total phenolic content of LEMO was 7.4 g/100 g dry matter basis. SADDIQ *et al.*

(2005) and MOHDALY *et al.* (2011) determined the concentration of phenolic compounds in the cake extract of sesame and found that the seed cake contained  $1.94 \pm 0.02$  g/100 phenolic content in terms of gallic acid. NACZK and SHAHIDI (1998) compared the phenolic content of canola hulls and *Moringa oleifera* leaves and they observed that canola hulls contained appreciably higher levels of phenolic content but the concentration was much lower than the total phenolic content of LEMO (NACZK & SHAHIDI 1998). Although the phenolic compounds are believed to be the major phytochemicals responsible for antioxidant activity of plant materials, *Moringa oleifera* is a rich source of ascorbic acid and flavonoids which also have the antioxidant activity (ANWAR *et al.* 2005).

The results of the chemical composition of butter added different concentrations of LEMO are presented in Table 1, which shows that the addition of LEMO at 400, 600, and 800 ppm did not have any negative effect on compositional attributes of butter. Fat, fat-free dry matter and moisture content of all the treatments were identical to the control ( $P > 0.05$ ). Thus the addition of LEMO to butter from  $T_1$  to  $T_3$  did not pose any problem of standard of identity of butter; Pakistan standards require minimum fat content, maximum concentration of fat-free matter and moisture in the finished butter to be 80, 2, and 16%, respectively. The reason for non-variation in the composition of different treatments and control was due to non-variation in the butter from which the experimental samples were prepared, and also the addition level of LEMO was quite low to affect changes in the composition of butter. KRAUSE *et al.* (2008) reported the fat content of butter ranging from 80.4% to 80.6%. HUSSAIN *et al.* (2011) investigated the composition of butter samples purchased from the local market of Lahore and found that the butter samples contained 84% fat and 13.5% moisture content.

Table 1. Influence of the leaf extract of *Moringa oleifera* on the chemical composition of fresh butter

Parameters	Control	Treatments		
		$T_1$	$T_2$	$T_3$
Fat (%)	$84.20 \pm 0.94$	$84.12 \pm 1.34$	$83.94 \pm 0.65$	$84.17 \pm 1.37$
Fat-free dry matter (%)	$1.92 \pm 0.21$	$1.96 \pm 0.17$	$1.88 \pm 0.11$	$1.94 \pm 0.05$
Moisture content (%)	$13.88 \pm 0.42$	$13.92 \pm 0.19$	$14.18 \pm 0.62$	$13.89 \pm 0.34$

$T_1$  – 400 ppm LEMO;  $T_2$  – 600 ppm LEMO;  $T_3$  – 800 ppm LEMO; control – without any addition of LEMO; LEMO – leaf extract of *Moringa oleifera*; all the results shown in Table 1 are statistically non-significant ( $P > 0.05$ ); non-significant ( $P > 0.05$ ); significant ( $P < 0.05$ )

Table 2. Influence of the leaf extract of *Moringa oleifera* on storage stability of butter stored at refrigeration temperature

Parameters	Storage period (days)	Control	Treatments		
			T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Free fatty acids (%)	0	0.08 ± 0.02 <sup>a</sup>			
	45	0.11 ± 0.01 <sup>a</sup>	0.10 ± 0.02 <sup>a</sup>	0.08 ± 0.03 <sup>b</sup>	0.08 ± 0.01 <sup>b</sup>
	90	0.16 ± 0.03 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>	0.11 ± 0.02 <sup>b</sup>	0.10 ± 0.02 <sup>b</sup>
Peroxide value (meq/kg)	0	0.15 ± 0.03 <sup>a</sup>	0.15 ± 0.02 <sup>a</sup>	0.15 ± 0.02 <sup>a</sup>	0.15 ± 0.02 <sup>a</sup>
	45	0.55 ± 0.05 <sup>a</sup>	0.52 ± 0.03 <sup>a</sup>	0.38 ± 0.01 <sup>b</sup>	0.42 ± 0.03 <sup>b</sup>
	90	1.24 ± 0.12 <sup>a</sup>	0.98 ± 0.09 <sup>b</sup>	0.75 ± 0.16 <sup>c</sup>	0.71 ± 0.09 <sup>c</sup>
Iodine value (Wijs)	0	35.68 ± 0.95 <sup>a</sup>	35.86 ± 1.13 <sup>a</sup>	35.91 ± 0.53 <sup>a</sup>	35.96 ± 0.62 <sup>a</sup>
	45	35.63 ± 0.62 <sup>a</sup>	35.78 ± 0.75 <sup>a</sup>	35.87 ± 0.24 <sup>a</sup>	35.92 ± 0.15 <sup>a</sup>
	90	35.43 ± 0.11 <sup>a</sup>	35.54 ± 0.35 <sup>a</sup>	35.80 ± 0.08 <sup>a</sup>	35.85 ± 0.04 <sup>a</sup>
AnV-value	0	4.56 ± 0.23 <sup>a</sup>	4.75 ± 0.34 <sup>a</sup>	4.48 ± 0.13 <sup>a</sup>	4.51 ± 0.07 <sup>a</sup>
	45	16.69 ± 0.35 <sup>a</sup>	14.98 ± 0.76 <sup>a</sup>	9.58 ± 0.14 <sup>b</sup>	8.99 ± 0.27 <sup>b</sup>
	90	28.85 ± 1.13 <sup>a</sup>	25.75 ± 0.81 <sup>b</sup>	15.63 ± 0.22 <sup>c</sup>	14.85 ± 0.41 <sup>c</sup>

$n = 3$  where  $n$  is the number of replicates; within the rows of a parameter means sharing the same letters are statistically non-significant; AnV –  $p$ -anisidine value; for the details of treatments see Table 1

It is evident from the results in Table 2 that the addition of LEMO at all levels inhibited the formation of free fatty acids in all the treatments of butter stored at refrigeration temperature. After 90 days of storage at refrigeration temperature the lowest free fatty acid content of 0.10% was recorded in T<sub>3</sub> (containing 800 ppm LEMO) as compared to the control 0.16% (without any addition of LEMO). Free fatty acid contents of T<sub>2</sub> and T<sub>3</sub> were identical ( $P > 0.05$ ). The increase in free fatty acid contents of all the treatments and control during the storage of 90 days was due to lipolytic enzymes which hydrolysed the triglycerides and contributed to this phenomenon. The

lower content of free fatty acids at T<sub>2</sub> and T<sub>3</sub> levels (addition of LEMO at 600 and 800 ppm) was due to the presence of natural antioxidants in the leaves of *Moringa oleifera*. The relatively higher content of free fatty acids in control butter was probably due to the improper/long cold storage and contamination of milk with iron and copper utensils, which resulted in the formation of lipolytic enzymes by the psychrotrophic bacteria and prooxidant behaviour of metals in fat oxidation. With an increase in one ppm of iron concentration, the rate of catalytic oxidation in fats and oils is increased ten times (FEREIDON 2005). Higher levels of free fatty acids result in the development

Table 3. Influence of the leaf extract of *Moringa oleifera* on conjugated dienes and trienes of butter at refrigeration temperature

Parameters	Storage period (days)	Control	Treatments		
			T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Conjugated dienes	0	0.42 ± 0.03 <sup>a</sup>	0.42 ± 0.03 <sup>a</sup>	0.42 ± 0.03 <sup>a</sup>	0.42 ± 0.03 <sup>a</sup>
	45	2.58 ± 0.09 <sup>a</sup>	2.45 ± 0.12 <sup>a</sup>	1.21 ± 0.03 <sup>b</sup>	1.15 ± 0.12 <sup>b</sup>
	90	5.19 ± 0.27 <sup>a</sup>	4.97 ± 0.45 <sup>b</sup>	1.86 ± 0.14 <sup>c</sup>	2.05 ± 0.21 <sup>c</sup>
Conjugated trienes	0	0.18 ± 0.04 <sup>a</sup>	0.18 ± 0.04 <sup>a</sup>	0.18 ± 0.04 <sup>a</sup>	0.18 ± 0.04 <sup>a</sup>
	45	0.57 ± 0.12 <sup>a</sup>	0.49 ± 0.08 <sup>a</sup>	0.31 ± 0.04 <sup>b</sup>	0.29 ± 0.10 <sup>b</sup>
	90	1.36 ± 0.19 <sup>a</sup>	1.29 ± 0.21 <sup>a</sup>	0.51 ± 0.06 <sup>b</sup>	0.54 ± 0.02 <sup>b</sup>
Conjugated dienes	( <sup>1%</sup> ε1cm [λ232])		Conjugated trienes	( <sup>1%</sup> ε1cm [λ268])	

$n = 3$  where  $n$  is the number of replicates; within the rows of a parameter means sharing the same letters are statistically non-significant ( $P > 0.05$ ); for the details of treatments see Table 1

Table 4. Influence of the leaf extract of *Moringa oleifera* on oxidative stability of butter at refrigeration temperature

Parameters	Control	Treatments		
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Induction period (h)	6.35 ± 0.21 <sup>a</sup>	5.95 ± 0.05 <sup>a</sup>	8.75 ± 0.29 <sup>b</sup>	8.91 ± 0.14 <sup>b</sup>
PV (after five days)	8.19 ± 0.35 <sup>a</sup>	7.93 ± 0.53 <sup>a</sup>	3.15 ± 0.18 <sup>b</sup>	2.99 ± 0.08 <sup>b</sup>

$n = 3$  where  $n$  is the number of replicates; within the rows of a parameter means sharing the same letters are statistically non-significant ( $P > 0.05$ ); PV – peroxide value (meq/kg); for the details of treatments see Table 1

of undesirable flavours in fats. Free fatty acid determination is a good parameter to determine the quality of butter fat. Free fatty acids are associated with keeping quality of fats and fat-based foods; higher levels are associated with poor keeping quality (GHATAK & BANDYOPADHYAY 2007). ANWAR *et al.* (2006) investigated the effect of methanolic and acetic leaf extract of *Moringa oleifera* on the stabilisation of sunflower oil and concluded that the methanolic extract at 600 ppm concentration was more efficient in the retardation of oxidative breakdown and formation of oxidation products at ambient storage temperature.

The peroxide value of all treatments and control linearly increased throughout the storage period of 90 days. The highest value of peroxide after 90 days of storage was recorded in the control (without any addition of LEMO) and the lowest peroxide value was observed in T<sub>3</sub> (containing 800 ppm LEMO). Peroxide values of T<sub>2</sub> and T<sub>3</sub> ( $P > 0.05$ ) were identical and significantly ( $P < 0.05$ ) different from the control. The oxidation process was successfully and efficiently inhibited by LEMO at 600 and 800 ppm concentrations. KRAUSE *et al.* (2008) studied the impact of refrigeration and freezing storage on the keeping quality of butter for one year and found that butter stored at refrigeration temperature had a higher peroxide value than butter stored at freezing temperature. In this study the reason for the low peroxide value

of experimental samples stored at refrigeration temperature could be attributed to the antioxidant potential of the leaf extract of *Moringa oleifera*. JEBSON *et al.* (1974) reported an increase in the peroxide value of butter samples stored at 4°C after 120 and 240 days of storage.

The addition of LEMO at all the three levels, i.e. T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub>, did not have any negative impact on iodine value. The iodine value of all experimental samples and control ( $P > 0.05$ ) decreased insignificantly during the storage of 90 days. The decline in iodine value was due to the saturation of some double and triple bonds with oxygen, which resulted in lower iodine absorption sites on the fatty acid moiety. HUSSAIN *et al.* (2011) characterised the samples of butter collected from the market of Lahore and found iodine value in the range of 34–42. The iodine value of rancid fats is lower than that of fresh fats (ERICKSON 1999).

*Para*-anisidine value is a good parameter to determine the antioxidant potential of antioxidants. *p*-Anisidine value measures the secondary and tertiary stages of autooxidation of fats and oils (FEREIDON 2005). *p*-Anisidine value linearly increased during storage of 90 days in all the treatments and control. After 90 days of storage the highest *p*-anisidine value (28.85) was determined in the control while the lowest value was found in T<sub>3</sub> (14.85). The reason for the higher peroxide value of control might be due to the presence of prooxi-

Table 5. Sensory characteristics of fresh butter added various concentrations of the leaf extract of *Moringa oleifera*

Parameters	Control	Treatments		
		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
Smell	8.4 ± 0.13 <sup>a</sup>	8.50 ± 0.39 <sup>a</sup>	8.3 ± 0.24 <sup>a</sup>	7.5 ± 0.19 <sup>b</sup>
Taste	8.3 ± 0.46 <sup>a</sup>	8.3 ± 0.11 <sup>a</sup>	8.0 ± 0.52 <sup>a</sup>	7.3 ± 0.23 <sup>b</sup>
Colour	8.1 ± 0.29 <sup>a</sup>	7.8 ± 0.64 <sup>a</sup>	7.7 ± 0.16 <sup>a</sup>	6.3 ± 0.36 <sup>b</sup>
Overall acceptability	7.6 ± 0.45 <sup>a</sup>	7.50 ± 0.89 <sup>a</sup>	7.2 ± 0.55 <sup>b</sup>	6.6 ± 0.22 <sup>c</sup>

$n = 3$  where  $n$  is the number of replicates; within the rows of a parameter means sharing the same letters are statistically non-significant ( $P > 0.05$ ); for the details of treatments see Table 1

dants which speeded the autooxidation process and yielded higher concentrations of oxidation products. ANWAR *et al.* (2005) characterised different antioxidants of *Moringa oleifera* leaves and found them effective in the inhibition of autooxidation. ANWAR *et al.* (2006) investigated the effect of the methanolic leaf extract of *Moringa oleifera* on the enhancement of oxidative stability of sunflower oil for 90 days at ambient storage temperature and reported lower values of the sample added 600 ppm concentration of the *Moringa oleifera* leaf extract.

Measurement of conjugated dienes and trienes is a good indication of oxidative damage occurring in fats and oils. The values of conjugated dienes and trienes increased throughout the storage period of 90 days, the butter added 600-ppm LEMO yielded the lowest conjugated dienes and trienes ( $^{1\%}\epsilon_{1\text{CM}}[\lambda 232]$ ) and ( $^{1\%}\epsilon_{1\text{cm}}[\lambda 268]$ ), while the control butter exhibited a higher content of conjugated dienes. The antioxidant potential of LEMO T<sub>2</sub> and T<sub>3</sub> was identical ( $P > 0.05$ ). While studying the antioxidant potential of the *Moringa oleifera* leaf extract using sunflower oil as oxidation substrate, ANWAR *et al.* (2006) found that the treatment containing 600-ppm methanolic extract was superior to other treatments for the better stabilisation and lower yields of oxidation products. ECONOMU *et al.* (1991) studied the influence of a plant extract of the family Labiatae for the stabilisation of oils rich in polyunsaturated fatty acids and concluded that plant extracts were highly efficient in the retardation of oxidative breakdown. LALAS *et al.* (2002) investigated the antioxidant potential of seed extracts of *Moringa oleifera* grown in Malawi and reported that seeds are rich in phenolic antioxidants (7–8% on dry mass basis) and they recommend the stabilisation of vegetable oils by the seed extract of *Moringa oleifera*. NADEEM *et al.* (2011) reported a higher content of oxidation products in the samples of butter collected from various parts of Lahore and attributed this to improper handling of milk, cream, metal contamination and refrigeration storage of butter in the shops.

After 5 days in an oven at 63°C the control sample showed the highest peroxide value (Table 1) followed by T<sub>1</sub>. It is evident from results of the Schaal oven test that LEMO up to T<sub>1</sub> level was almost inefficient to stop the autooxidation process; when the concentration of LEMO was increased from 400 ppm to 600 ppm, the oxidation process and oxidation products were significantly ( $P < 0.05$ ) inhibited. There was no discernible difference ( $P > 0.05$ ) between the antioxidant potentials of T<sub>2</sub>

and T<sub>3</sub> (600 and 800 ppm). ZIA-UR-REHMAN *et al.* (2003) investigated the impact of ginger extract on the oxidative stability of sunflower oil and reported that the vegetable extract was quite effective for the stabilisation of sunflower oil. MOHDALY *et al.* (2011) studied the antioxidant potential of sesame (*Sesamum indicum*) cake extract to prolong the shelf life of sunflower and soybean oils and found that the incorporation of sesame cake extract significantly ( $P < 0.05$ ) improved the keeping quality of sunflower and soybean oils without having a drastic negative effect on sensory characteristics.

Determination of induction period may serve as a parameter to forecast the expected shelf life/keeping quality of fats and oils; a higher induction period is associated with prolonged keeping quality and vice versa (RAMADAN & MORSEL 2004; MAHUYA *et al.* 2008). Addition of LEMO at all levels (400–800 ppm) increased the induction period as measured on Rancimat. The induction periods of T<sub>2</sub> and T<sub>3</sub> were identical ( $P > 0.05$ ) and significantly ( $P < 0.05$ ) different from the control (Table 4). The higher induction period of T<sub>2</sub> and T<sub>3</sub> may be attributed to the presence of a higher concentration of phenolic antioxidants in LEMO. Similar results were reported by ANWAR *et al.* (2005).

The results of the sensory evaluation of treatments of butter added different concentrations of LEMO are the values adjudged by a panel of five trained judges. Addition of LEMO up to T<sub>2</sub> level did not have any negative effect on taste, smell and colour scores; when the addition level was increased to T<sub>3</sub> level (800 ppm), the sensory scores of these parameters significantly ( $P > 0.05$ ) decreased. Some of the panellists criticised T<sub>3</sub> for having phenolic smell and taste, which resulted in lower smell and taste scores. The reason for the low colour score of T<sub>3</sub> was due to the dark green colour of LEMO which has a slight but definite effect on this parameter.

## CONCLUSIONS

It is evident from the results in Table 1 that the addition of LEMO at all levels did not have any negative effect on butter composition. At T<sub>2</sub> level (600 ppm) LEMO significantly inhibited the formation of free fatty acids, oxidation products. After 5 days in an oven the peroxide value of T<sub>2</sub> was 2.99 as compared to 8.35 (meq/kg) in the control. Induction period of T<sub>2</sub> was 8.91 h as compared to 6.35 h in the con-

trol. The colour, smell and taste score of T<sub>2</sub> was not significantly different from the control. The overall acceptability score of T<sub>2</sub> was 7.2 out of 9, which was 80% of the total score. Hence the *Moringa oleifera* leaf extract at the rate of 600 ppm may be used for the enhancement of storage stability of butter stored at refrigeration temperature for three months with acceptable sensory characteristics.

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Received for publication September 18, 2012  
Accepted after corrections November 13, 2012

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