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Effect of Mango (*Mangifera indica* L.) Seed Kernels Pre-extract on the Oxidative Stability of Ghee

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ABSTRACT

*A pre-extract (PE) prepared by heating mango (*Mangifera indica *L.) seed kernel powder (MSKP) and ghee (1:1 w/w) to 120°C contained 430 mg% phospholipids and 224 mg% water-extractable phenolic compounds. The presence of at least eleven phospholipids in a chloroform–methanol extract of MSKP and eight water-soluble phenolic compounds in PE was confirmed. Addition of the PE to ghee at 4, 6, 8 and 10% (v/v) levels increased the phospholipid content of ghee samples over the control to 11·2, 20·9, 26·4 and 34·4 mg% of ghee and those of water-extractable phenolic compounds to 7·4, 11·1, 15·5 and 20·7 mg% of ghee, respectively. The samples of ghee with added BHA contained levels of these compounds similar to those of control samples. The antioxidant potentialities calculated from the induction periods of ghee samples stored at 80°C in comparison to control were in the order: 1·3 (0·02% BHA) < 2·6 (4% PE) < 2·9 (6% PE) < 3·1 (8% PE) < 3·2 (10% PE) suggesting that the phospholipids and the phenolic compounds of MSKP, transferred to ghee, help enhance the shelf-life by protecting against autoxidation.*

INTRODUCTION

Limitations associated with the use of synthetic antioxidants (Hathway, 1966) have stimulated screening of various edible plant materials in a search for harmless, effective and acceptable additives to enhance the shelf-life of ghee (clarified milk fat). Plant materials or their extracts, which have shown

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promising results, include some of the condiments (Sethi & Aggarwal, 1956), soyabean and sunflower seeds (El-Sokkary & Ghoneim, 1951), betel and curry leaves (Patel & Rajorhia, 1979), and tomato seed powder (Guleria *et al.*, 1983). Looking to the edible nature and chemical constituents of mango seed kernels, a study by Parmar and Sharma (1986) revealed that direct addition of kernel powder can help enhance the shelf-life of ghee. This study was, therefore, undertaken to develop a method feasible, under industrial conditions, for transferring the antioxidative principles of the kernels to ghee and also to elucidate the nature of such principles.

MATERIALS AND METHODS

Butter samples prepared from fresh raw cream obtained from fresh raw buffalo milk were boiled down to ghee until the temperature reached to 110°C. The mango seed kernel powder (MSKP) passing through a 100 mesh sieve was obtained from sun-dried kernels collected from raw and ripened mangoes of varieties such as *Rajapuri*, *Langra*, *Kaiser*, *Alphonso* and *Desi*. The MSKP was analysed for moisture, fat, protein, and ash following the methods specified by the Indian Standard Institution (ISI) (1975) and the carbohydrate content was calculated by difference. For the phospholipids of MSKP, appropriate methods were followed for extraction (Folch *et al.*, 1957), estimation (Bartlett, 1959; Rama Murthy & Narayanan, 1966), resolution (Morrison *et al.*, 1980) and detection on thin-layer chromatograms (Stahl, 1969). Total phenolics content of MSKP was determined by the method of Swain and Hillis (1959).

A mixture of MSKP:ghee (1:1 w/w) was allowed to stand in molten condition for about 30 min and then heated to 120°C. The liquid filtered through 4 layers of muslin cloth (pre-extract, PE) was analysed for phospholipids (Bartlett, 1959) and water-extractable phenolics (Swain & Hillis, 1959). The water extractable phenolic compounds were subjected to two dimensional paper chromatography (Roberts & Wood, 1953) and the chromatograms were visualised in an appropriate dip reagent (Barton *et al.*, 1952).

The PE was added to ghee at the rates of 0, 4, 6, 8 and 10% (v/v) and, for comparison in each trial, a further treatment was the addition of butylated hydroxy anisole (BHA) at the rate of 0·02% (w/v). The ghee samples with and without additives were analysed for moisture, free fatty acids and peroxide value (Indian Standard Institution (ISI) 1966), phospholipids (Bartlett, 1959; Rama Murthy & Narayanan, 1966) and water-extractable phenolic compounds (Swain & Hillis, 1959). The ghee samples were stored in an oven at 80° ± 2°C and the peroxide development was monitored at intervals of

48 h. To test the effectiveness of the additives, antioxygenic indices or protection factors were calculated according to Pruthi *et al.* (1970). Four replications were carried out in a randomised block design and the statistical analysis of data was carried out as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

It is reported that ghee made from buffaloes' milk is comparatively more prone to oxidative deterioration than that made from cow's milk (Rama Murthy *et al.*, 1968) and, irrespective of the source of raw material, ghee made by the creamery-butter method is more susceptible to oxidative deterioration than that made by the other methods (Singh *et al.*, 1979). Therefore, in this study, ghee made from buffaloes' milk by the creamery-butter method was used. The use of MSKP from raw and ripened mangoes of mixed varieties and its addition in the form of PE was preferred because of its practical feasibility.

The MSKP contained, on average, 4·80% moisture, 12·96% fat, 6·74% protein, 2·19% ash and by difference an assumed 73·31% carbohydrates. These results are in general agreement with those reported by Bhatnagar and Subramanyam (1971) and Patel *et al.* (1971). Besides these constituents, the MSKP also contained 6·39% total phenolics and 0·375% phospholipids, thereby reducing the carbohydrates to 66·55%. The phospholipid, on the basis of fat of the MSKP, was found to be 2·88%. This value is close to that reported by Van Pee *et al.* (1981) for the *Gholek* variety. The value of total phenolics is considerably lower than that reported by Das and Banerjee (1952). These differences could be due to the variations in the nature of the material, methods of extraction and estimation.

The ghee samples, used for preparation of PE, contained on average 6·1 mg% phospholipids and 0·28 mg% water-extractable phenolic compounds. The PE obtained by heating MSKP:ghee (1:1 w/w) contained 430 mg% phospholipids and 224 mg% water-extractable phenolic compounds. This suggests that, during preparation of PE, essentially all the phospholipids and (of the total) about 3·5% phenolic compounds (water-extractable) are transferred to the PE. After repeated extraction of the PE with water, the residual material gave a positive test for phenolic compounds, indicating the presence of fat-soluble phenolics.

The data on the phospholipids of ghee samples, with or without additives (Table 1), were in the order: 6·1 mg% (control and 0·02% BHA) < 17·3 mg% (4% PE) < 27·0 mg% (6% PE) < 32·5 mg% (8% PE) < 40·5 mg% (10% PE). The data suggest that with the addition of 4, 6, 8 and 10% PE to ghee there was a gradual increase in the phospholipid content over control or BHA-

TABLE 1

Phospholipids (mg%), Water-Extractable Phenolics (mg%), Induction Period and Antioxygenic Indices of Ghee as Affected by Addition of PE at Various Levels (v/v) and BHA at 0·02% (w/v)

PE-added	*Phospholipids*	*Water-extractable phenolics*	*Induction period*[a]	*Antioxygenic index*[b]
Control	6·1 ± 1·1	0·28 ± 0·04	182 ± 13	
4% PE	17·3 ± 1·2	7·7 ± 0·46	469 ± 12	2·6 ± 0·23
6% PE	27·0 ± 1·8	11·4 ± 0·45	526 ± 25	2·9 ± 0·18
8% PE	32·5 ± 2·6	15·8 ± 0·66	569 ± 13	3·1 ± 0·20
10% PE	40·5 ± 2·0	21·0 ± 1·51	574 ± 30	3·2 ± 0·37
0·02% BHA	6·1 ± 1·1	0·20 ± 0·04	235 ± 11	1·3 ± 0·15

Average of four replications ± standard error of mean.

[a] h to reach a peroxide value of 5 meq. of peroxide oxygen/kg ghee.

[b] Ratio of induction period of treated sample to the induction period of control sample.

added samples and such increases were 11·2, 20·9, 26·4 and 34·4 mg% of ghee, respectively, indicating a significant rise ($P < 0·05$) in the phospholipids content. The non-uniform increase in phospholipids with the addition of relative quantities of PE to ghee could be attributed generally to the presence of sedimentable insoluble material in such extracts. The phospholipids extracted from the MSKP, when subjected to thin-layer chromatography on

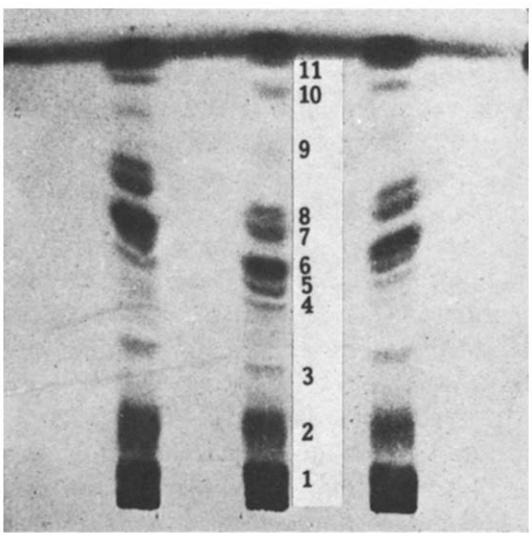

Fig. 1. Thin-layer chromatogram (developed in solvent system—chloroform:methanol: ammonia:water—65:35:5:2·5) of chloroform–methanol (2:1) extract of MSKP representing the total number of phospholipid fractions.

silica gel-G, resolved into at least 11 fractions (Fig. 1) and the pattern was in close resemblance to that identified by Moharram and Moustafa (1982) under similar experimental conditions as followed in this study. The tentative identification of the phospholipid fractions by the above authors were: phosphatidyl serine, lysophosphatidyl choline, phosphatidyl inositol, sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidic acid and glycerophosphatidyl compounds.

The data on water-extractable phenolic compounds (Table 1) indicated that such compounds are transferred from MSKP to PE and, on addition of the latter at the rates of 4, 6, 8 and 10% (v/v) to ghee, the water-extractable phenolics content is found to be in the order: 0·20 mg% (0·02% BHA) < 0·28 mg% (control) < 7·7 mg% (4% PE) < 11·4 mg%

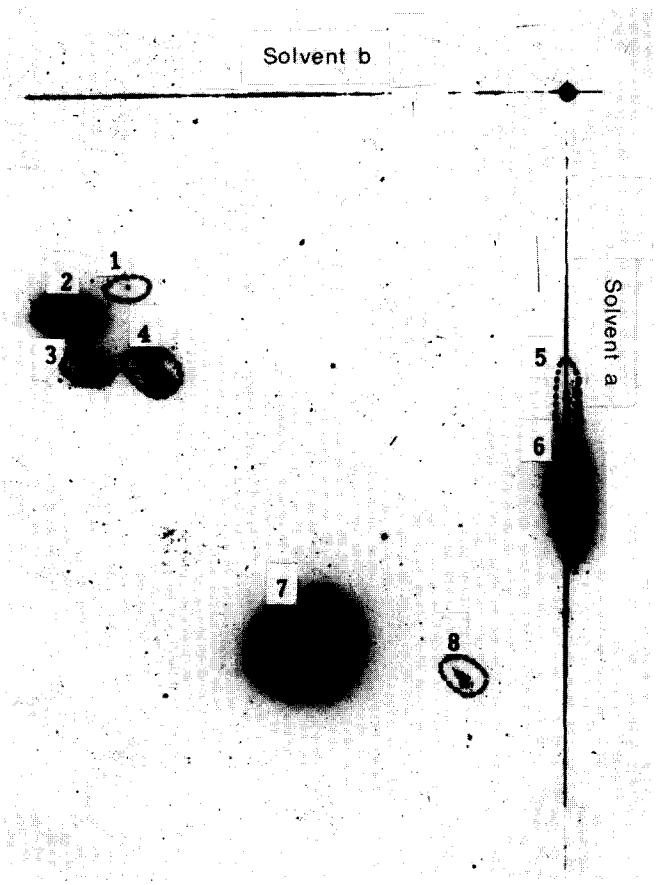

Fig. 2. Paper chromatogram representing the water-soluble phenolic compounds of pre-extract of MSKP:ghee (1:1 w/v). The solvent systems used were (a) *n*-butanol:acetic acid:water—4:1:5 (v/v/v) and (b) 2% acetic acid.

(6% PE) < 15·8 mg% (8% PE) < 21·0 mg% (10% PE). The corresponding increase in water-extractable phenolic compounds over control samples of ghee was about 7·4, 11·1, 15·5 and 20·7 mg% of ghee in the PE-added samples. The aqueous extract, obtained from the PE when subjected to two dimensional paper chromatography, resolved into at least 8 spots (Fig. 2) confirming the transfer of such water-soluble phenolic compounds to ghee.

The peroxide value (Fig. 3) of ghee samples, with and without additives, indicated that the development of peroxides was faster in control samples than those with the additives. The induction periods (time taken in hours to reach a peroxide value of 5 as meq. of peroxide oxygen per kg of ghee) were in the order (Table 1): 182 h (control) < 235 h (0·02% BHA) < 469 h

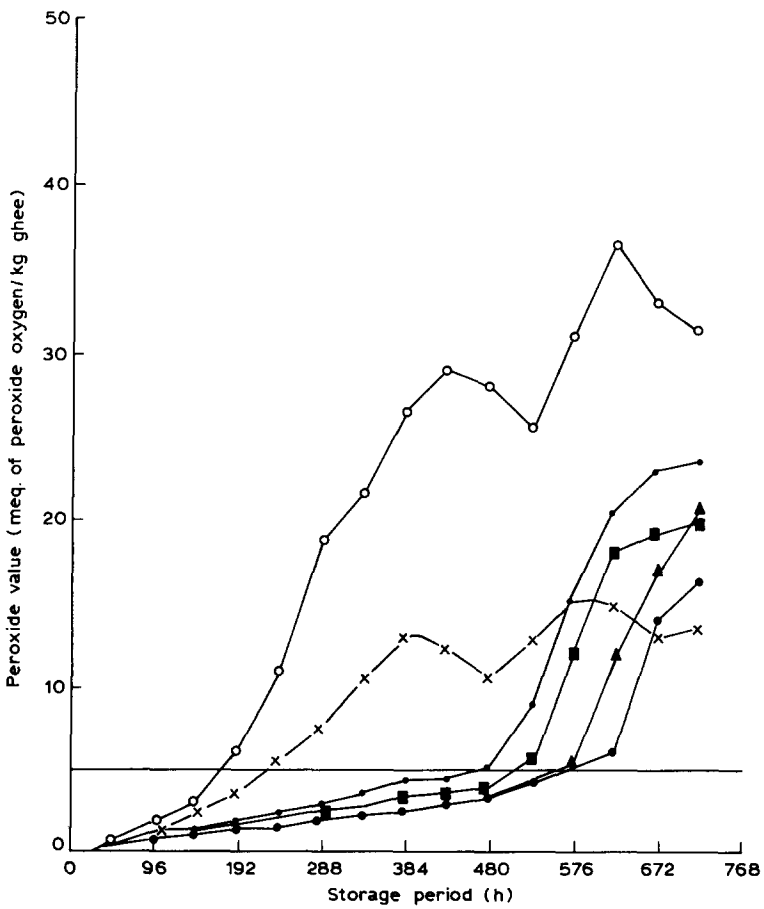

Fig. 3. Effect of addition of pre-extract of mango seed kernel powder (v/v) on the development of peroxide in ghee stored at 80° ± 2°C. ○, Control; ×, 0·02% BHA; ●, 4% pre-extract; ■, 6% pre-extract; ▲, 8% pre-extract; ●, 10% pre-extract.

(4% PE) < 526 h (6% PE) > 569 h (8% PE) < 574 h (10% PE). These results suggested that addition of PE offers resistance against autoxidation to ghee. This effectiveness is clearly elucidated further when the antioxygenic indices (ratio of induction period of treated sample to induction period of control sample) are calculated (Table 1). The antioxygenic indices for treatments were in the order: 1·3 (0·02% BHA) < 2·6 (4% PE) < 2·9 (6% PE) < 3·1 (8% PE) < 3·2 (10% PE) over the control.

The results on peroxide value (Fig. 3), induction periods and the antioxygenic indices (Table 1) suggest that addition of the PE helps enhance the stability of ghee against autoxidation. The results also show that the addition of the PE, at a level of 4% (v/v) and above, doubled the oxidative stability of ghee. This could be attributed to the increase in the phospholipids and the phenolic compounds (Table 1) as these are transferred in significant quantities from MSKP to ghee. It is known that both the phospholipids (Bhatia *et al.*, 1978) and the phenolic compounds (Stuckey, 1962) act as potential agents in enhancing the induction periods through various mechanisms. Besides these two major compounds, the other factors such as tocopherols and carotenoids present in minor quantities (Bhatnagar & Subramanyam, 1971) may also be involved in the effectiveness of MSKP against autoxidation. The other possibility could be the sugar-amino acids browning reaction products (Evans *et al.*, 1958) formed during the course of heating the MSKP–ghee mixture.

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