Antioxidant activity, fatty acids characterization and oxidative stability of Gouda cheese fortified with mango (*Mangifera indica* L.) kernel fat

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Abstract Effect of mango kernel fat (MKF) on antioxidant characteristics and lipolysis of Gouda cheese was investigated. Milk fat (3.5%) was partially replaced with MKF i.e. 5, 10, 15 and 20% concentrations (T₁, T₂, T₃ and T₄). Cheese prepared from 100% milk fat served as control. Cheese samples were ripened for 90 days at 6 ± 1 °C and analysed at 0, 45 and 90 days of ripening. Total phenolic contents of control, T₁, T₂, T₃ and T₄ were 14 ± 0.35, 129 ± 0.75, 188 ± 2.52, 267 ± 10.61 and 391 ± 8.46 mg GAE/g. Total flavonoid content of control, T₁, T₂, T₃ and T₄ were 0.22 ± 0.03, 1.47 ± 0.09, 3.62 ± 0.15, 5.88 ± 0.35, 8.29 ± 0.63 mg quercetin equivalent/ml. DPPH free radical scavenging activity of control and experimental samples increased throughout the ripening period. DPPH free radicals scavenging activity of 90 days old control, T₁, T₂, T₃ and T₄ were 16.38 ± 0.026e, 30.47 ± 0.64d, 68.62 ± 0.91c, 73.29 ± 0.85b, 92.61 ± 1.44a %. HPLC characterization revealed the existence of mangiferin, caffeic acid, catechin, quercetin and chlorogenic acid in MKF fortified Gouda cheese. Fortification of MKF increased the concentration of C18:1, C18:2 and C18:3 in cheese. The concentration of C18:1, C18:2 and C18:3 in control were 24.55 ± 0.95, 1.76 ± 0.09 and 0.31 ± 0.02%. While, the concentration of C18:1, C18:2 and C18:3 in T₄ were 30.11 ± 1.34, 2.79 ± 2.79 and 0.92 ± 0.11%. MKF

fortified Gouda cheese had better oxidative stability and sensory characteristics. These results evidenced that antioxidant capacity, unsaturated fatty acids and oxidative stability of Gouda cheese can be improved with MKF.

Keywords Mango kernel fat · Gouda cheese · Antioxidant characteristics · Lipolysis

Introduction

Foods fortified with bioactive compounds of plant origin are becoming part of regular diet of an individual, food industries have started to manufacture functional foods. Scientific evidences have revealed that consuming antioxidants safeguards the body cells from oxidative damage, that reactive oxygen species can cause singlet oxygen, triplet oxygen, peroxides, superoxides, hydroxyl, perhydroxyl and peroxynitrite radicals are the most important reactive oxygen species (Young and Woodside 2001). Health benefits associated with the intake of phenolic compounds of plant origin are well understood (Mohdaly et al. 2011). Research work related to antioxidant and biological activity of natural antioxidants is enormously increasing in food industries, since numerous synthetic antioxidants are proven to be carcinogenic (Manach et al. 2005). Polyphenols are widely distributed in foods of plant origin, produced as secondary metabolites (Harris et al. 2007). Fortification of foods with polyphenols of plant origin may improve the quality and nutritional value of foods and helps the body to prevent oxidative stresses (Habauzit and Morand 2010). Among the phenolic compounds, phenolic acids and flavonoids are the most important classes, which have been associated with large number of health benefits, such as, cancer, cardiovascular

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diseases, ulcer, atherosclerosis, mutagenic, diarrheal and inflammatory ailments (Jung et al. 2007). During cheese ripening, several biochemical events take place which had a pronounced effect on sensory textural characteristics of cheese; presence of antioxidant substances in cheese may have a great effect on lipolysis and generation of flavouring compounds (Hickey et al. 2007). The increased knowledge of consumers in nutrition and free radicals biology has led to a considerable demand of functional foods. Therefore, it is the need of hour to find out new sources of antioxidants and extend the application of existing sources. Gouda is a typical semi-hard, ripened Dutch cheese prepared from pasteurized and unpasteurized milk, characterized with mild nutty flavour small uniformly distributed eyes, in world cheese competition held in 2002, it was declared as the second best cheese (Ellner 2002). Jabbar et al. (2003) studied the effect of different fat levels on the quality characteristics of Gouda cheese from buffalo milk. However, antioxidant characteristics of Gouda cheese have not been studied before. Mango (*Mangifera indica* L.) is one of the most popular tropical fruit, grown in over a hundred countries of the world, mango kernel contains about 12–15% good quality edible fat (Kittiphoom and Sutasinee, 2013). Studies of Abdalla et al. (2007) and Jafari et al. (2014) disclosed that supplementation of sunflower oil and tallow with mango kernel fat (MKF) significantly inhibited the auto-oxidation and prolonged the shelf life. Soong and Barlow (2006) described that MKF can be used as an alternate of synthetic antioxidants; antioxidant activity of 1% MKF was equivalent 300 ppm BHT. Several studies have been performed regarding the enhancement of antioxidant characteristics of cheese through phenolic compounds of plant origin (Kurcubic et al. 2015). Literature review evidenced that most of the studies were conducted through the supplementation of cheese curd or cheese milk through plant extracts (Branciari et al. 2015). Replacement of milk fat with vegetable fat/oil is not a new strategy to carry unsaturated fatty acids to the cheese matrix, however, little is known regarding the enhancement of antioxidant characteristics of cheese through the partial replacement of milk fat with vegetable fat. This study aimed to partially replace milk fat with MKF, study the antioxidant characteristics and lipolysis of Gouda cheese on the basis of some chemical and sensory techniques.

Materials and methods

Materials

Fat from mango (*Chaunsa* variety) kernel was extracted by *n*-hexane, the solvent was then removed under vacuum at 45 °C in a rotary evaporator (Buchi Rotavapor R-124,

Switzerland). Mono and diglycerides of fatty acids (E471), HPLC grade chemicals used in this experiment were procured from Sigma Aldrich, USA. DVS cultures of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* spp. *cremoris* were purchased from Christian Hansen, Denmark and used in the form of bulk starter culture at 2% concentration. Salt peter (NaNO₃) was used at the rate of 1.5 ml for ten kilogram milk.

Experimental plan

Experiment was planned in a completely randomized design, each treatment was replicated three times. Ten kilogram cow milk was used for the preparation of each replicate of Gouda cheese. Milk fat (3.5%) was partially replaced with MKF at four different concentrations i.e. 5, 10, 15 and 20% (T₁, T₂, T₃ and T₄), cheese prepared from 100% milk fat served as control. Distilled monoglycerides (DIMODAN) of fatty acids were used as emulsifier at 0.25% concentration with no homogenization.

Preparation of Gouda cheese

Milk (10 l) was heated to 60 °C, emulsifier (Distilled monoglycerides, DIMODAN) 0.2% was added, mixed and pasteurized at 65 °C for 30 min, cooled down to 31 °C, starter culture (2%) were mixed followed by the addition of KNO₃ (1 ml), CaCl₂ (6 ml) and Rennet (1.7 ml Christian Hansen; NATUREN®). Fermentation/curd formation time was 30 min, curd was cut into 1 cm³, 40% of the whey was drained from the cheese vat. Temperature of cheese vat increased from 31 to 34 °C by the addition of calculated amount of hot water (50 °C). Cooking of the curd was performed for 45 min, followed by the filling of cheese molds and whey drainage. Whey was allowed to drain by the weight of cheese for 3 h, cheese was pressed at 3.5 bar pressure for 3 h and side of the cheese was turned after 1 h. At pH 5.2, cheese was brined for 8 h, followed by drying and emulsion based coating (CSK Food Specialities, the Netherlands). Experimental and control samples were ripened for 90 days at 6 ± 1 °C, sampled at 0, 45 and 90 days for chemical and sensory analysis.

Proximate composition of mango kernel cake and chemical characteristics of MKF

Mango kernel cake was analysed for moisture, fat, protein, ash content and nitrogen free extract according to the standard methods (AOAC 1997). MKF was analysed for free fatty acids, moisture content, slip melting point, iodine value, refractive index, saponification value, peroxide value and unsaponifiable matter according to the standard methods of the American Oil Chemists Society (AOCS 1995).

Chemical composition of Gouda cheese fortified with MKF

Gouda cheese was analysed for moisture, fat and protein contents by following the standard methods (AOAC 1997).

Antioxidant characteristics of Gouda cheese fortified with mango kernel fat

Total phenolic contents

Total phenolic contents of Gouda cheese fortified with mango kernel fat were determined according to the Folin–Ciocalteu colorimetric method, phenolic contents were determined on the basis of Gallic acid, from the calibration curve using standards of known concentrations ranged from 10 to 500 mg/ml, expressed as Gallic acid equivalent (GAE/g) on a double beam spectrophotometer (Shimadzu, Japan). The value of R^2 for all the series of standards was not less than 0.986 (Rashidinejad et al. 2013).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

DPPH free radical scavenging activity of MKF fortified cheeses was determined according to the method of Bunea et al. (2011). DPPH reagent was prepared by dissolving 0.1 mM in methanol; 2.9 ml of the freshly prepared DPPH solution was reacted with 100 μ l cheese sample, vortex at 500 $\times$ g for 2 min, incubated at 25 °C, in the dark for 30 min, followed by the measurement of absorbance at 517 nm in visible region of spectra (Speccord 200 Plus, Analyticjena).

Total flavonoid content

AlCl₃ solution (dissolved in ethanol, 2%, 0.5 ml) was mixed with 0.5 ml sample, incubation was performed for 60 min at ambient temperature, absorbance (intensity of yellow colour) was measured at 420 nm on a spectrophotometer (Shimadzu, Japan) using series of standards of quercetin ranged from 10 to 410 mg/ml, total flavonoids were determined from the calibration curve, value of R^2 for all the standards used was not less than 0.992 (Ordóñez et al. 2006).

Oxidative stability of Gouda cheese

Free fatty acids and peroxide value value were determined at 0, 45 and 90 days of ripening period according to the standard methods (AOCS 1995).

Determination of cholesterol

Cholesterol was determined at 0, 45 and 90 days of ripening period according to the spectrophotometric method of Rudel and Morris (1973).

Fatty acid profile

Fat was extracted from Gouda cheese using the standard method (AOAC 1997). 50 μ g fat was converted into fatty acid methyl esters using 2 ml methanolic hydrogen chloride (15%, Fluka) at 100 °C for 60 min. Fatty acid methyl esters were extracted with *n*-hexane, washed with 2 ml of deionized water, mixed at 2200 Rpm for 2 min, allowed to settle for 15 min, supernatant was transferred to GC vials, 1 μ l was injected into GC–MS auto sampler (7890 A GC System Agilent) fitted MSD detector, using ZB-5 fused silica capillary column (Zebron Phenomenex). (30 m $\times$ 0.25 mm). FAME 37 kit (Sigma Aldrich, UK) was used to identify and quantify the fatty acids (Qian 2003).

HPLC characterization of phenolic compounds of fortified Gouda cheese

HPLC characterization of phenolic compounds of fortified Gouda cheese was performed on HPLC (Agilent 11000). The system was equipped with binary gradient pump (G-1312). 4- μ l sample was injected with an auto sampler (1330-A). Mobile phase was comprised of acetic acid in water (20%) and 0.5% acetic acid water and acetonitrile (50:50). Measurements were performed in 200–600 nm, phenolic compounds were identified and quantified using internal standards (Schiber et al. 2003).

Sensory evaluation

Sensory evaluation of fortified Gouda cheese was performed by a panel of ten trained judges according to the standard method of the American Oil Chemists Society (Rass et al. 2008). 2.5 g cheese samples were tempered to 12 °C for 2 h, offered in individual sensory evaluation booths (23–25 °C). Evaluation of 3 digit randomly coded samples was performed for smell, taste and texture on 9 point scale. Unsalted crackers and distilled water were provided to clean the pallet.

Statistical analysis

Experiment was organized in a completely randomized design; data were reported as Mean $\pm$ SD of triplicate treatment and triplicate analysis of each sample. One way and two way analysis of variance techniques were used to

determine the effect of treatments, storage and their interaction. Means were separated through Duncan Multiple Range Test, using SAS 9.1 software (Steel et al. 1997).

Results and discussion

Proximate composition of mango kernel cake and MKF

Proximate composition of mango kernel cake showed moisture content, fat, crude protein, ash content, crude fibre and nitrogen free extract were 6.57 ± 0.38 , 13.25 ± 0.22 , 7.76 ± 0.27 , 1.46 ± 0.11 , 2.57 ± 0.17 and 68.21 ± 0.61 , respectively. Fat content in mango kernel were in line with earlier investigations. The results of proximate composition of mango kernel recorded in current investigation were similar to the earlier findings (Ashoush and Gadallah 2011). Nadeem et al. (2016a) described that fat content of mango kernel ranged from 12 to 15%. Free fatty acids, moisture content, slip melting point, iodine value, refractive index, saponification value, peroxide value and unsaponifiable of MKF were $0.18 \pm 0.02\%$, 0.14 ± 0.01 , $35.2 \text{ }^{\circ}\text{C} \pm 0.21$, $55.4 \pm 0.74 \text{ cg/100 g}$, 1.4537 ± 0.01 , $191 \pm 0.82 \text{ mg KOH/g}$, 0.25 ± 0.03 and $1.62 \pm 0.06\%$, respectively.

Chemical composition

The results of chemical composition of Gouda cheese fortified with mango kernel are presented in Table 1. Partial replacement of milk fat with MKF did not have any effect on fat, protein and moisture content of cheese ($P > 0.05$). The non-significant variation among various treatments and control can be justified by the identical ingredients and strict adherence to the manufacturing

procedure. Fat content of cheese milk for all the treatments and control was adjusted to 3.5%, only the kind of fat was different, maintained at the same level for all the treatments. Ahmad et al. (2015) prepared Gouda cheese from three different low melting fractions of milk fat; chemical composition of experimental cheeses was similar to the control. Chen et al. (2004) also observed non-significant effect on compositional attributes of dairy products, when original milk fat was replaced with modified version of milk fat. Fatty acid profile of mango kernel indicates that oil is rich source of palmitic, stearic and oleic acid (Kitiphoorn and Sutasinee 2013). Fatty acid profile of milk fat is mainly comprised of palmitic acid, stearic acid and oleic acid (Nadeem et al. 2015). Triglyceride composition of mango kernel fat evidenced that 1,3-distearoyl-2-oleoylglycerol, 1-stearoyl-2,3-dioleoylglycerol, 1-palmitoyl-2-oleoyl-3-stearoylglycerol and 1,3-dipalmitoyl-2-oleoylglycerol are the major triglycerides of MKF (Jahurul et al. 2014). Melting point of MKF ranges from 32 to 35 $^{\circ}\text{C}$, melting point of milk fat also falls in the same range, all these factors may have contributed towards the non-significant differences in treatments and control. Branciarri et al. (2015) also reported a non-significant effect on fat content of pecorino cheese, when rosemary leaves were included as antioxidants in the diet of ewes.

Total phenolic contents

Phenolic compounds are the metabolic products which contain numerous phenol groups, they are very active in the neutralization of free radicals, phenols neutralize the free radicals either by the donation of hydrogen atom or an electron, chelating of metal ions in aqueous phase (Petti and Scully 2009). In addition to the antioxidant activity, phenolic compounds act as antitumor, antimicrobial and antimutagenic agents (Shui and Leong 2002). Fortification of cheese milk with MKF at all concentrations increased the total phenolic contents in fresh cheese ($P < 0.05$). Total phenolic contents of freshly prepared control, T₁, T₂, T₃ and T₄ were 14 ± 0.35 , 129 ± 0.75 , 188 ± 2.52 , 267 ± 10.61 and $391 \pm 8.46 \text{ mg GAE/g}$. The higher phenolic content of cheese can be attributed to occurrence of higher magnitude of phenols in MKF. Total phenolic content of biscuits increased from 3.84 mg GAE/g to 24.37 mg GAE/g, as a function of addition of mango kernel (Ashoush and Gadallah 2011). Blending of tallow with MKF considerably enhanced the total phenolic contents and inhibited the free radical mechanism (Jafari et al. 2014). Abdalla et al. (2007) also reported that total phenolic contents of blends of sunflower oil and MKF were higher than sunflower oil. Total phenolic contents of mango kernel were significantly higher than chia oil (Nadeem et al. 2015). A strong correlation between

Table 1 Chemical composition of Gouda cheese fortified with MKF

Treatments	Moisture %	Fat %	Protein %
Control	40.5 ± 0.39	30.5 ± 0.42	27.1 ± 0.15
T ₁	40.8 ± 0.52	29.5 ± 0.21	26.2 ± 0.66
T ₂	41.5 ± 0.61	29.2 ± 0.33	26.6 ± 0.85
T ₃	42.1 ± 0.28	28.5 ± 0.59	25.8 ± 0.23
T ₄	42.6 ± 0.33	28.2 ± 0.45	25.3 ± 0.61

The mean values expressed in all columns of Table 1 are statistically non-significant ($P > 0.05$)

Control: 100% milk fat

T₁: 95% milk fat and 5% mango kernel fat

T₂: 90% milk fat and 10% mango kernel fat

T₃: 85% milk fat and 15% mango kernel fat

T₄: 80% milk fat and 20% mango kernel fat

phenolic contents and antioxidant activity is documented (Song et al. 2010). Milk constituents such as casein, whey proteins, selenium, zinc, catalase, superoxide dismutase, glutathione peroxidase, vitamin C, E and beta-carotene possesses antioxidant capacity (Usta and Yilmaz-Ersan 2013). Rise in total phenolic contents of margarine as a function of addition of chia oil is reported in literature. Total phenolic contents of *trans* free margarine increased when chia (*Salvia hispanica* L.) oil was added from 5 to 20% concentrations. Blending of mango kernel oil improved the antioxidant capacity and oxidative stability of butter oil in ambient and accelerated oxidation (Nadeem et al. 2016b).

Total flavonoid content

Normal metabolic processes produce tremendously active, highly unstable free radicals, which have been recognized as devastating transient chemical species. Cellular redox state is maintained by the enzyme systems, dietary antioxidants and hormones (Martínez-Cayuela 1995). Consumptions of foods with dietary antioxidants can lead to the uncontrolled free radicals in body, oxidative stresses can lead to large number of physiological disorders, such as, cardiovascular diseases, cancers and ageing (Valko et al. 2007). Recent studies have shown that antioxidant activity of natural antioxidants is mainly due to flavonoids, phenolic acids and tannins (Cartea et al. 2010). Total flavonoid content of control, T₁, T₂, T₃ and T₄ were 0.22 ± 0.03 , 1.47 ± 0.09 , 3.62 ± 0.15 , 5.88 ± 0.35 , 8.29 ± 0.63 mg quercetin equivalent/ml. Maisuthisakul (2008) reported that mango kernel is potentially a rich source of flavonoids. In a recent investigation, it was revealed that both cow and buffalo milk has flavonoids, however, their concentration mainly depends upon the type of feed (Khan et al. 2017). Numerous studies have conducted on the characterization of MKF; however, little information is available regarding the flavonoid content of supplemented foods. Muffins prepared from 50:50 blend of MKF and bakery shortening revealed antioxidant characteristics, as compared to bakery shortening based muffins (Abdel-Razik et al. 2012).

DPPH free radical scavenging activity

DPPH free radical scavenging activity of Gouda cheese fortified with MKF increased in a concentration dependant manner and was in the order of $T_4 > T_3 > T_2 > T_1 > \text{control}$. DPPH free radicals scavenging activity of control, T₁, T₂, T₃ and T₄ were 4.61 ± 0.25 , 13.27 ± 0.51 , 33.57 ± 0.73 , 45.18 ± 0.88 and $62.5 \pm 1.14\%$. The strong free radical scavenging activity of MKF can be attributed to the higher magnitudes of phenolic acids,

flavonoids and tannins. DPPH free radical scavenging activity of control and cheese samples increased throughout the ripening period of 90 days and all the testing intervals indicated an increasing trend in antioxidant capacity. However, DPPH free radical scavenging activity of experimental samples was higher than that of the control cheese. DPPH free radicals scavenging activity of 45 days old control, T₁, T₂, T₃ and T₄ were 12.77 ± 0.42 , 25.41 ± 0.66 , 55.22 ± 1.24 , 71.84 ± 1.39 and $84.36 \pm 1.55\%$. DPPH free radicals scavenging activity of 90 days old control, T₁, T₂, T₃ and T₄ were 16.38 ± 0.026 , 30.47 ± 0.64 , 68.62 ± 0.91 , 73.29 ± 0.85 , $92.61 \pm 1.44\%$. Rise in antioxidant capacity of cheese during the ripening period is attributed to the production of water soluble peptides that have antioxidant capacity (Gupta et al. 2009). The interaction between hydroxyl group of phenolic acids, protein and polysaccharides may impact the absorption of natural antioxidants in cheese matrix. With 96% DPPH free radical scavenging activity, the antioxidant activity was greater than potentially recognized strong natural antioxidants. Cheese derived from milk of ewes fed on rosemary leaves had higher antioxidant activity (Branciari et al. 2015). IC₅₀ value for the inhibition of DPPH free radicals in Pirotski Kachkaval cheese supplemented with extracts of *Kitaibelia viti-folia* was 67.45 µg/mL, with higher total antioxidant activity (Kurcubic et al. 2014).

HPLC characterization of phenolic acids of Gouda cheese

Results of HPLC characterization of Gouda cheese fortified with MKF are presented in Figs. 1, 2, 3, 4 and 5. Chlorogenic acid, caffeic acid, catechin, quercetin and mangiferin were detected in MKF fortified cheeses, whereas, quercetin was the only phenolic compound detected in lower concentration in control cheese. The concentrations of mangiferin, caffeic acid, catechin, quercetin and chlorogenic acid in T₁ were 502, 41.6, 174.2, 20.8 and 334 mg/100 g. The amount of mangiferin, caffeic acid, catechin, quercetin and chlorogenic acid in T₂ were 967.4, 76.8, 314.5, 39.2, 39.2 and 692.1 mg/100 g. Extent of mangiferin, caffeic acid, catechin, quercetin and chlorogenic acid in T₃ were 1542, 124.3, 493.6, 64.5 and 1032.6 mg/100 g. Concentration of mangiferin, caffeic acid, catechin, quercetin and chlorogenic acid in T₄ were 2137.4, 166.2, 662.3, 87.6 and 1433.4 mg/100 g. The migration of catechin from cheese milk to low fat cheese has been prescribed (Rashidinejad et al. 2013). Many efforts have been carried out to carry phenolic compounds to cheese, in most of studies, cheese/cheese milk was supplemented with antioxidant extracts, this is the pioneer study in which phenolic compounds were carried to cheese matrix through the addition of vegetable fat. Mostafa (2013) described that chlorogenic

Fig. 1 HPLC characterization of phenolic compounds of control Gouda cheese

Fig. 2 HPLC characterization of phenolic compounds of control Gouda cheese added with 5% MKF

Fig. 3 HPLC characterization of phenolic compounds of control Gouda cheese added with 10% MKF

acid, caffeic acid, catechin, quercetin and mangiferin are the major phenolic acid of MKF. Mangiferin, isomangiferin, mangiferin gallate, isomangiferin gallate, quercetin 3-*O*-diglycoside, quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinopyranoside, quercetin 3-*O*-arabinofuranoside, quercetin 3-*O*-rhamnoside, kaempferol 3-*O*-glucoside,

Fig. 4 HPLC characterization of phenolic compounds of control Gouda cheese added with 15% MKF

Fig. 5 HPLC characterization of phenolic compounds of control Gouda cheese added with 20% MKF

rhamnetin-hexoside, quercetin mangiferin, quercetin 3-*O*-arabino-glucoside, quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, isorhamnetin 3-*O*-glucoside, rhamnetin have been isolated from mango kernel (Schiber et al. 2003). Nadeem et al. (2016a) compared the induction period of MKF with commercial vegetable oils and essential oils, induction period of MKF was highest than all the commercial vegetable oils and essential oils.

Oxidative stability of Gouda cheese

The results of lipolysis of Gouda cheese fortified with MKF are given in Table 2. Primary and secondary biochemical changes in cheese lipids lead to the formation of free fatty, aldehydes, ketones, alcohols and flavouring compounds (Fox et al. 2000). Free fatty acids produced during the lipolysis of cheese vitally contribute in cheese flavour. At zero day, free fatty acids of T₃ and T₄ were greater than those of the control, T₁ and T₂. Free fatty acids content of

Table 2 Oxidative stability of Gouda cheese fortified with MKF

Treatments	Storage Days	Free fatty acids %	Peroxide value (MeqO ₂ /kg Dry Basis)
Control	0	0.11 ± 0.02 h	0.25 ± 0.02i
	45	0.27 ± 0.02 g	0.68 ± 0.07d
	90	0.72 ± 0.05f	2.22 ± 0.10c
T ₁	0	0.12 ± 0.01 h	0.32 ± 0.04 h
	45	0.44 ± 0.03f	0.62 ± 0.07d
	90	0.96 ± 0.04d	1.85 ± 0.15a
T ₂	0	0.11 ± 0.03 h	0.35 ± 0.09 h
	45	0.59 ± 0.04f	0.71 ± 0.13d
	90	1.14 ± 0.08c	1.64 ± 0.06b
T ₃	0	0.14 ± 0.01 g	0.33 ± 0.05 h
	45	0.72 ± 0.02e	0.51 ± 0.04f
	90	1.34 ± 0.04b	1.49 ± 0.08d
T ₄	0	0.15 ± 0.04 g	0.35 ± 0.02 h
	45	0.58 ± 0.06d	0.43 ± 0.04 g
	90	1.65 ± 0.07a	1.27 ± 0.07e

Within a column, means denoted by a different letter are statistically different ($P < 0.05$)

MKF were 0.18%, which may be the reason for higher free fatty acids in T₃ and T₄. The magnitude of free fatty acids in all the treatments and control went on increasing during the whole ripening period of 90 days. Bacterial lipases and moisture content were the major causes for the generation of free fatty acids. The concentration of free fatty acids in all the treatments and control were different at 45 and 90 days of analysis. Cheeses having more MKF underwent high degree of lipolysis and yielded more free fatty acids. Kind of fat had great effect on lipolysis of Gouda cheese. Ahmad et al. (2015) reported that type of fat in cheese matrix had great effect on free fatty acids during the ripening process, cheddar cheese prepared from the low melting fractions of milk fat yielded more free fatty acids after 60 days of ripening period. Antioxidants are usually not linked with degree of hydrolysis, which is different phenomenon from fat oxidation (Nadeem et al. 2014). Peroxide value is used for the determination of oxidation status of fats and oils (Nadeem et al. 2017). Oxidative stability of fats is extremely important during the product development, therefore, in current investigation, peroxide value was used as indicator of oxidative stability of Gouda cheese fortified with MKF. It is usually observed that dairy products containing vegetable fats have lower oxidative stability. Peroxide value of experimental cheeses and control increased throughout the ripening period. Farbod et al. (2013) reported that peroxide value and TBA value of feta cheese fortified with olive oil and fish oil increased during the ripening period of 60 days at 5 °C. Blending of palm oil with mango kernel oil improved the oxidative stability of the blends during long term storage (Arif et al. 2016). Mango kernel oil considerably enhanced the

oxidative stability of watermelon seed oil (Nadeem et al. 2015).

Effect of MKF on cholesterol content

Supplementation of Gouda cheese with MKF decreased the concentration of cholesterol in cheese samples. Cholesterol content of freshly prepared control, T₁, T₂, T₃ and T₄ was 156 ± 2.64, 145 ± 3.17, 132 ± 5.17, 124 ± 4.28 and 117 ± 1.15 mg/100 g. Concentration of cholesterol decreased during the ripening the whole ripening period and both the determination frequencies revealed a decreasing trend. After 90 days of ripening period, concentration of cholesterol in control, T₁, T₂, T₃ and T₄ was 113 ± 1.25, 102 ± 1.75, 81 ± 3.65, 65 ± 6.87 and 47 ± 0.92 mg/100 g. The decline in cholesterol content of fermented dairy products is reported in literature. The decline in cholesterol content of fermented dairy products may be due to the utilization of cholesterol in microbial activities of starter culture. The decline of cholesterol in ripening cheese may be due to the lipolysis. Ahmad et al. (2015) monitored the changes in cholesterol at different stages of processing, cholesterol decrease with the progression of ripening period. Fermented dairy products have lower cholesterol as compared to parent milk (Fujishiro et al. 2002). Arif et al. (2016) formulated blends of mango kernel fat and palm oil for probable use as vanaspati, all the blends were virtually *trans* free. Blending of butter oil with mango kernel reduced the concentration of cholesterol in a dose dependant manner (Nadeem et al. 2016b). Mango kernel oil is potentially a strong source of natural antioxidants, Gouda cheese supplemented with mango kernel oil

Table 3 Changes in concentration of cholesterol (mg/100 g) of Gouda cheese

Treatments	0 day	45 days	90 days
Control	156 ± 2.64a	142 ± 1.88b	113 ± 1.25e
T ₁	145 ± 3.17b	128 ± 2.54c	102 ± 1.75f
T ₂	132 ± 5.17c	112 ± 1.14e	81 ± 3.65 h
T ₃	124 ± 4.28d	90 ± 2.37 g	65 ± 6.87i
T ₄	117 ± 1.15e	68 ± 2.25i	47 ± 0.92j

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

has higher oxidative stability, wide range of phenolic compounds and lower content of dietary cholesterol (Table 3).

Fatty acid profile of Gouda cheese fortified with MKF

The results of fatty acid composition of various treatments of Gouda cheese fortified with MKF are given in Table 4. Fortification of MKF induced major changes in fatty acid profile of cheese, main variations were recorded around short-chain, medium-chain and long-chain fatty acids. Concentration of short-chain fatty acids decreased in cheese samples as a function of addition of MKF. Concentrations of C4:0, C6:0, C8:0 and C10:0 in T₄ were 2.84, 1.78, 0.95 and 2.59%. Concentration of C4:0, C6:0, C8:0 and C10:0 in control were 3.55, 2.27, 1.24 and 3.12%, respectively. The lower concentration of short-chain fatty acids in Gouda cheese samples was due to the absence of short-chain fatty acids in MKF and in cheese, their concentration decreased on percentage basis. Magnitude of medium-chain fatty acids also decreased in Gouda cheese

when MKF was added. Concentrations of C12:0, C14:0, C16:0 in T₄ were 3.52, 9.72 and 26.45%. The concentration of C12:0, C14:0, C16:0 in control were 4.35, 12.16 and 31.27%. The role of unsaturated fatty acids in the reduction of harmful LDL cholesterol is scientifically established. Nutritionists recommend to take 50% of oil derived calories from C18:1 to minimize the risks of cardiovascular diseases. In current investigation, it was noted that MKF considerably improved the concentration of C18:1, C18:2 and C18:3. The concentration of C18:1, C18:2 and C18:3 in control were 24.55, 1.76 and 0.31%. While, the concentration of C18:1, C18:2 and C18:3 in T₄ were 30.11, 2.79 and 0.92%. *Trans* fatty acids were not detected in any treatment. Fatty acid profile of mango kernel fat is ideal for application in large number of food and dairy products without partial hydrogenation. Partial hydrogenation of fats and oils is necessary to improve functional properties and oxidative stability. MKF possesses excellent functional and oxidative stability perspectives (Nadeem et al. 2016a). Nadeem et al. (2016b) blended mango kernel oil and butter oil, fatty acid profile of the blends were different from the substrate oils. Mango kernel oil considerably altered the fatty acid profile of watermelon seed oil (Azeem et al. 2015).

Ripening effect on fatty acid profile

Results of transition in fatty acid profile of Gouda cheese fortified with MKF are presented in Table 5. Noticeable changes were observed in the fatty acid profile of all the treatments and control, after the end of ripening period. Short-chain fatty acids increased, whereas, long chain unsaturated fatty acids decreased, all the determination intervals revealed the same trend. Ahmad et al. (2015)

Table 4 Effect of MKF on fatty acid profile of Gouda cheese (mg/kg)

Fatty acid	MKF	Control	T ₁	T ₂	T ₃	T ₄
C4:0%	–	3.55 ± 0.02a	3.34 ± 0.05b	3.18 ± 0.10c	3.04 ± 0.19d	2.84 ± 0.21e
C6:0%	–	2.27 ± 0.03a	2.13 ± 0.02b	2.05 ± 0.18c	1.92 ± 0.06d	1.78 ± 0.09e
C8:0%	–	1.24 ± 0.07a	1.19 ± 0.09b	1.11 ± 0.06c	1.04 ± 0.04d	0.95 ± 0.05e
C10:0%	–	3.12 ± 0.01a	2.95 ± 0.07b	2.82 ± 0.15c	2.77 ± 0.14d	2.59 ± 0.16e
C12:0%	–	4.35 ± 0.11a	4.13 ± 0.06b	3.98 ± 0.13c	3.71 ± 0.08d	3.52 ± 0.23e
C14:0%	–	12.16 ± 0.15a	11.61 ± 0.48b	10.93 ± 0.35c	10.38 ± 0.79d	9.72 ± 0.44e
C16:0%	7.55 ± 0.19f	31.27 ± 0.76a	30.11 ± 0.95b	28.94 ± 0.76c	27.64 ± 1.15d	26.45 ± 0.84e
C18:0%	38.22 ± 1.62a	10.88 ± 0.53f	12.37 ± 0.28e	13.81 ± 0.43d	15.73 ± 0.24c	16.64 ± 0.37b
C18:1%	46.61 ± 0.94a	24.55 ± 0.95f	25.97 ± 0.41e	27.19 ± 1.03d	28.91 ± 0.78c	30.11 ± 1.34b
C18:2%	5.74 ± 0.36a	1.76 ± 0.09f	1.98 ± 0.05e	2.29 ± 0.07d	2.51 ± 0.06c	2.79 ± 0.12b
C18:3%	0.45 ± 0.03d	0.31 ± 0.02e	0.42 ± 0.02d	0.55 ± 0.06c	0.71 ± 0.04b	0.92 ± 0.11a

MKF mango kernel fat

Within a row, means carrying a different letter are statistically significant ($P < 0.05$)

Table 5 Effect of MKF on fatty acid profile during ripening of Gouda cheese (mg/Kg)

Fatty acid	Control		T ₁		T ₂		T ₃		T ₄	
	0-day	90-days	0-day	90-days	0-day	90-days	0-day	90-days	0-day	90-days
C4:0%	3.55 ± 0.02c	5.12 ± 0.12a	3.34 ± 0.05d	4.98 ± 0.15a	3.18 ± 0.10d	4.27 ± 0.12b	3.04 ± 0.19e	3.25 ± 0.08d	2.84 ± 0.21f	3.12 ± 0.10d
C6:0%	2.27 ± 0.03d	3.82 ± 0.11a	2.13 ± 0.02e	3.48 ± 0.06b	2.05 ± 0.18e	3.17 ± 0.08c	1.92 ± 0.06f	1.98 ± 0.14f	1.78 ± 0.09 g	1.81 ± 0.13 g
C8:0%	1.24 ± 0.07d	2.16 ± 0.08a	1.19 ± 0.09	1.95 ± 0.08b	1.11 ± 0.06	1.76 ± 0.04c	1.04 ± 0.04e	1.22 ± 0.11d	0.95 ± 0.05f	1.05 ± 0.03e
C10:0%	3.12 ± 0.01d	4.29 ± 0.16a	2.95 ± 0.07e	4.11 ± 0.19b	2.82 ± 0.15	3.95 ± 0.25c	2.77 ± 0.14d	2.55 ± 0.16e	2.59 ± 0.16e	2.71 ± 0.15d
C12:0%	4.35 ± 0.11a	3.74 ± 0.11	4.13 ± 0.06b	3.27 ± 0.09 g	3.98 ± 0.13c	3.21 ± 0.31	3.71 ± 0.08d	3.62 ± 0.29e	3.52 ± 0.23f	3.36 ± 0.19 h
C14:0%	12.16 ± 0.15a	10.47 ± 0.76c	11.61 ± 0.48b	10.33 ± 0.15c	10.93 ± 0.35c	10.19 ± 0.17c	10.38 ± 0.79c	10.13 ± 0.56c	9.72 ± 0.44d	8.67 ± 0.75e
C16:0%	31.27 ± 0.76a	29.53 ± 0.69c	30.11 ± 0.95b	29.17 ± 0.97c	28.94 ± 0.76d	28.57 ± 0.66d	27.64 ± 1.15e	27.39 ± 1.39e	26.45 ± 0.84f	25.97 ± 1.27f
C18:0%	10.88 ± 0.53f	10.26 ± 0.26f	12.37 ± 0.28e	10.55 ± 0.73f	13.81 ± 0.43d	12.42 ± 0.73e	15.73 ± 0.24b	14.55 ± 0.73c	16.64 ± 0.37a	15.89 ± 0.42a
C18:1%	24.55 ± 0.95e	20.72 ± 0.42 g	25.97 ± 0.41d	19.83 ± 0.34 h	27.19 ± 1.03c	21.48 ± 0.85f	28.91 ± 0.78b	27.34 ± 1.55c	30.11 ± 1.34a	29.71 ± 1.62a
C18:2%	1.76 ± 0.09e	0.87 ± 0.05f	1.98 ± 0.05d	0.72 ± 0.05 g	2.29 ± 0.07c	0.92 ± 0.03f	2.51 ± 0.06b	2.21 ± 0.16c	2.79 ± 0.12a	2.68 ± 0.14a
C18:3%	0.31 ± 0.02f	0.14 ± 0.03 g	0.42 ± 0.02e	0.16 ± 0.04 g	0.55 ± 0.06d	0.19 ± 0.05 g	0.71 ± 0.04c	0.59 ± 0.12d	0.92 ± 0.11a	0.64 ± 0.09b

Within a row means, denoted by a different letter are statistically different ($P < 0.05$)

described that concentration of short-chain fatty acids increased in cheddar cheese during the ripening period. Antioxidant of MKF inhibited the oxidation of unsaturated fatty acids. After 90 days of ripening, concentration of C18:1 in control decreased from 24.55 to 20.72%. After 90 days of ripening period, concentration of C18:1 in T₄ decreased from 30.11 to 29.71%. After 90 days of ripening, C18:2 in control and T₄ decreased by 50 and 4%. After 90 days of ripening period of 90 days, C18:3 in control and T₄ decreased by 55 and 31%. Nadeem et al. (2017) described that addition of vegetable oil considerably inhibited the auto-oxidation in *trans* free margarine. Kwak et al. (2002) monitored the changes in fatty acid composition of low cholesterol Gouda cheese, after ripening period, fatty acid composition of cheese was considerably different from the fresh cheese, short-chain fatty acids increased and long chain fatty acid decreased during the ripening. The association of short-chain fatty acids with typical dairy flavour of milk and milk products is established. T₁ and T₂ had lower concentration of short-chain fatty acids than control, yet their contribution in cheese flavour was similar to control. Kurcubic et al. (2015) also described an increase in the concentration of short-chain fatty acids as a function of cheese ripening. During 90 days of storage, predominant changes took place in fatty acid profile of cheddar cheese (Ahmad et al. 2015). Fatty acid profile of 3 months stored ice cream was different from the fresh ice cream (Nadeem et al. 2015). Nadeem et al. (2014) also described a variation in fatty acid profile of fresh and 90 days stored olein based butter. Supplementation of butter oil with mango kernel fat significantly inhibited the autooxidation and breakdown of fatty acids into oxidation products, in ambient and accelerated oxidation (Nadeem et al. 2015).

Sensory evaluation

The results of sensory characteristics of Gouda cheese fortified with MKF are presented in Table 6. Fortification of cheddar cheese with MKF considerably improved the sensory characteristics of cheddar cheese. After 90 days of ripening period, smell score of control, T₁, T₂, T₃ and T₄ was 8.1, 7.6, 8.0, 8.1 and 8.4. After 90 days of ripening, taste score of control, T₁, T₂, T₃ and T₄ was 8.4, 8.1, 8.1, 8.0 and 8.3. After 90 days of ripening, texture score of control, T₁, T₂, T₃ and T₄ was 8.2, 7.9, 8.1, 8.2 and 8.3. Kittiphoom and Sutasinee (2013) described that oil from MKF light yellow in colour, bland in flavour. MKF is successfully used in wide array of foods, with no negative effect reported, sunflower oil, tallow, butter oil and muffins are among the few examples in which MKF has been successfully used (Abdalla et al. 2007; Jafari et al. 2014; Youssef, 1999; Abdel-Razik et al. 2012).

Table 6 Sensory evaluation Gouda cheese fortified with MKF

Treatments	Ripening days	Smell	Taste	Texture
Control	0	7.0 ± 0.11c	6.5 ± 0.31c	7.3 ± 0.14b
	45	7.4 ± 0.15b	7.7 ± 0.08b	7.6 ± 0.18b
	90	8.1 ± 0.13b	8.4 ± 0.05a	8.2 ± 0.21b
T ₁	0	7.1 ± 0.12c	6.4 ± 0.09c	7.4 ± 0.08b
	45	7.5 ± 0.17b	7.8 ± 0.12b	7.7 ± 0.10b
	90	7.6 ± 0.22b	8.1 ± 0.17a	7.9 ± 0.26b
T ₂	0	7.1 ± 0.16c	6.6 ± 0.22c	7.3 ± 0.05b
	45	7.0 ± 0.23c	7.8 ± 0.34b	7.7 ± 0.02b
	90	8.0 ± 0.25a	8.1 ± 0.27a	8.1 ± 0.07a
T ₃	0	7.2 ± 0.18b	7.0 ± 0.11c	7.4 ± 0.13b
	45	8.1 ± 0.07a	7.7 ± 0.04b	7.9 ± 0.18b
	90	8.1 ± 0.14a	8.0 ± 0.07a	8.2 ± 0.31a
T ₄	0	7.1 ± 0.15c	6.5 ± 0.16c	7.5 ± 0.09b
	45	8.2 ± 0.26a	8.1 ± 0.24b	7.8 ± 0.05b
	90	8.4 ± 0.07a	8.3 ± 0.35a	8.3 ± 0.29a

Within a column means represent by a different letter are statistically different ($P < 0.05$)

Conclusion

Fortification of Gouda cheese with mango kernel fat significantly improved the total phenolic contents, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity, increased total flavonoids. Fortification of mango kernel fat significantly improved the concentration of C18:1, C18:2 and C18:3 in Gouda Cheese. Fortified cheese revealed the existence of mangiferin, caffeic acid, catechin, quercetin and chlorogenic acid. Sensory characteristics of cheddar cheese fortified with 20% mango kernel fat was better than control. These results suggest that, antioxidant characteristics of cheese can be improved through mango kernel fat.

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