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Enhancing Soil Fertility through Intercropping, Inoculation and Fertilizer

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Abstract. The present study was conducted to investigate the effects of intercropping grass (*Panicum maximum*) and legumes (*Vicia sativa* and cowpeas) alone or coupled with inoculation or fertilizer on soil fertility. The study comprised of two field experiments conducted under rain fed conditions for two years (June, 2005 to September, 2007) at National Agriculture Research Centre, Islamabad, Pakistan. In one experiment intercropping (33, 50 and 67%) of grass and legumes alone as well as coupled with seed inoculation were studied while, same set of treatments was combined with fertilizer application at the rates of 25, 75 and 50 kg/ha (N, P_2O_5 and K_2O) in the second experiment. Total soil N increased by 0.008% due to symbiotic fixation in addition to plant uptake under best treatment when compared with grass alone while, soil organic matter increased by 0.19%. After crop harvest soil N content was determined to be higher in all the treatments of the experiment compared with growing grass alone. Legumes caused rhizobial N fixation that caused an increase in soil N. Similarly, intercropping and inoculation increased this soil characteristic that was found to be non-significant in the first crop but later on became significant, especially when intercropping of grass with legumes after seed inoculation was investigated or fertilizer was supplemented to the crops. Thus, not only grass used the symbiotically fixed N by companion legumes but also enhanced the soil N content. The effect of fertilizer was not measurable statistically in case of soil organic matter. This parameter, in general, was not affected significantly when assessed after first crop harvest. Nevertheless, legumes alone or intercropped within grass increased this important soil constituent. Inoculation proved further beneficial in this regard but combination of intercropping (especially 67%) either with seed inoculation or application of fertilizer was found as the best technique for increasing soil organic matter.

Keywords: soil fertility, *Panicum maximum,* forage legumes , intercropping, inoculation, fertilizer application

Introduction

Grasses are an important component of Graminae family. Apart from cereals, many grass species provide forage for livestock, protect the soil from erosion, improve soil structure and hence water retention (Ahmad *et al.,* 2001). The grass species *Panicum maximum* var. Tanzani is a tall growing (2-3 m), vigorous, coarse, tufted perennial and shows considerable variation in growth habit. It is a native grass of tropical and sub-tropical Africa but has been introduced in more humid tropics and sub-tropics throughout the world. Vetches (*Vicia* species) are legumes which are well adapted to winter growth in Mediterranean environments throughout the world on a variety of soil types and are used in West Asia and Australia for various purposes as green forage, hay, seed crop or green manure. Cowpeas (the legume species *Vigna unguiculata*) native to South Asia are known for their diverse distribution and range of adoption from the humid sub-tropical to warmed cool temperate climate. It contains higher protein contents, amino acids and vitamins (Bose and Balakarishnan, 2001). Low rhizobial population is the main cause of low legume yield in these areas. The use of inoculation is very low; just below 1-3% of the total area under legumes which is negligible (Aslam *et al.,* 2000). When a legume is introduced in a new locality, it is necessary to inoculate seed with proper rhizobium culture otherwise crop may not thrive and produce nodules. These bacteria although

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present in most of the soil, vary in number, effectiveness in nodulation and N-fixation (Zamaurd *et al.,* 2006). Symbiotic nitrogen fixing bacteria (SNB) are root nodule bacteria and fix nitrogen in association with leguminous plants. Biofertilizers (inoculation material) are apparently environmental friendly, low cost, non bulky agricultural inputs which could play a significant role in plant nutrition as a supplementary and complementary factor to mineral nutrition (Sahai, 1990). It is an attempt to increase nitrogen fixation and the yield at all the sites of harsh climate. Therefore, it is possible to increase nodulation causing improvement in yield from marginal lands by inoculation with rhizobium (Aslam *et al.,* 1990). Thus, keeping in view the limitations and constraints faced by the farmers busy in livestock production, a comprehensive study was conducted to monitor effect of grass-legumes intercropping, inoculation or fertilizer application on soil fertility status.

Materials and Methods

The study comprised of two field experiments conducted under rain fed conditions for two years (2005-2006 & 2006-2007) in the experimental area of Rangeland Research Programme, National Agriculture Research Centre Islamabad, Pakistan (Altitude=518 m, longitude = 73° 08'E & latitude = 33° 42'N). The experimental site is situated in sub-humid, sub-tropical region. There were two separate experiments of the study. Soil samples were prepared and analyzed before sowing having soil pH 8.4; ECe 0.53 dS/m; total N 0.037%; available P 4.7 mg/kg; extractable K 79.6 mg/kg; orgamic matter (OM) 0.53% and textural class was sandy clay loam. *Panicum maximum* grass was planted in 2005 as perennial fodder. After its establishment, winter legume (*Vicia sativa)* commonly known as vetch and summer legume (*Vigna unguiculata*) commonly known as cow peas, were sown as inter crop in the established grass but after its harvesting. Summer legume followed winter legume in the next year. Two lines of legumes with four lines of grass were grown to establish T_3 (33 % legumes) while there were three lines of each in case of T_4 (50 % legumes). In case of T_5, four lines of legumes were grown with two lines of grass to obtain the share of 67 % of the former. Seeds of legumes were inoculated before sowing to obtain T_6 to T_9. The experiment was conducted under rain fed conditions and no irrigation was applied. There was also no fertilizer application either to grass or legumes. Soil samples were obtained from each treatment separately after harvesting of fodder crops and analyzed for different parameters. Methodology for this experiment was almost the same as described under experiment 1. However, fertilizer as a basal dose was applied to the treatments T_6 to T_{10} at the rates of 25, 75 and 50 kg/ha (N, P_2O_5 and K_2O) as urea, single super posphate and sulphate of potash, respectively. Both the experiments were laid out using randomized complete block designs (RCBD) with 4 replications. Soil samples were collected from 0-15 cm after harvesting grass and legumes from all the treatment plots. The samples were air dried, ground and passed, through 2 mm sieve. Soil nitrogen was estimated through sulphuric acid digestion. Distillation was made with micro-Kjeldhal method (AOAC, 1990). Organic matter was determined by applying the following formula:

$$\text{Organic matter in \%} = \text{Organic carbon in \%} \times 1.72$$

Data were analyzed using one-way analysis of variance with the help of software package of MSTAT-C Microcomputer programme, Version 1.3. A least significant difference (LSD) was applied for multiple comparisons (Bicker, 1993).

Results and Discussion

Nitrogen is essential for plant growth as it is a constituent of all proteins and nucleic acids and hence, of all types of protoplasm. The effect of nitrogen in increasing biomass is not only due to its direct effect on the plant growth as structural constituents but also because of rapid synthesis of carbohydrates that are converted to proteins and ultimately to protoplasm when N supply is in ample quantities. The original status of total N was very low because soil analysis indicated its numerical value just as 0.037% (Table1) and response of symbiotic N fixation by rhizobia on legumes was expected because their activity becomes rapid when soil has clear deficit of N. Non-significant performance was observed after the harvest of first crop. However, legume alone had

Table 1. Original soil analysis of experimental site

Determinations	Values
pH_S	8.4
EC_e (dS/m)	0.53
Sand (%)	61
Silt (%)	12
Clay (%)	27
Textural class	Sandy clay
Organic matter (%)	0.53
Total N (%)	0.037
Available P (mg/kg)	4.70
Extractable K (mg/kg)	79.6

slightly more nitrogen than grass alone due to symbiotic nitrogen fixation effect. Intercropping of grass and legumes increased very small N percentage and more N percentage increase was also determined by combination of intercropping and inoculation (Fig. 1). Nevertheless, significant pattern of variation was observed after 2nd, 3rd and 4th crop harvest by all three planting geometries (33, 50 and 67%). The intercropping of 67% increased total N as the highest. Combinations of intercropping and inoculation had more effect on soil nitrogen but it was not found significant. This may be due to consumption of fixed N by the grass under nitrogen deficit conditions. There was a significant difference between legumes and grass alone and inoculation further departed this variation. This revealed clear effect of legumes that were successful to increase soil N as well along with meeting their own requirements. The root nodulation and fixation of atmospheric nitrogen by rhizobia was its only cause. Frame *et al.* (2005) also certified N_2 fixation of forage legumes in terms of rhizobial efficiency, N transference to associated companion grasses. The most widespread and consistent effect of legumes is to improve the N economy of soil through N_2 fixation. The N-balance of legume-cereal sequence in most cases is more positive than that of a cereal-cereal sequence in the same soil. Nitrogen fertility inevitably accompanies intensive agriculture and, at least, reduces the requirements of inputs of fertilizer N (Muhammad *et al.,* 2003). Biological nitrogen fixation occurs mainly through symbiotic association of legumes and some woody species with certain N_2-fixing micro-organisms that convert elemental nitrogen into ammonia (Serraj, 2004). Xia-Yan *et al.* (2006); Reynolds and Frame (2005); Vasilev (2004); Shisany (2003); Zhang and Li (2003); Abbas *et al.* (2001) and Odhiambo and Bombee (2001) also reported the increase in N_2 by intercropping along with inoculation of legume seeds. The overall input of nitrogen into global agriculture for food and feed production is estimated to be approximately 120 million tonnes/year. Biological nitrogen fixation (BNF) accounts for 40 million tonnes/year while 80 million tonnes/year is accounted for by N-fertilizer production from ammonia. In cereal production, fertilizer use dominates because if cereals were able to "fix" their own nitrogen the situation could be very different.

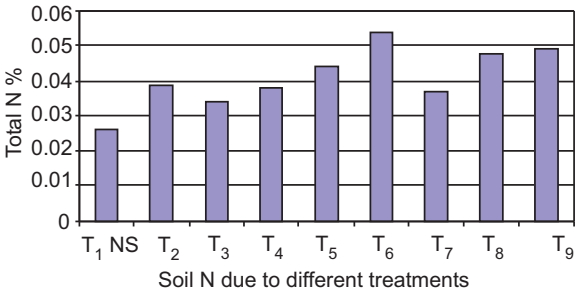

Fig. 1. Changes in soil N due to grass-legumes intercropping and inoculation (n=4, SD = 0.01).

Addition of fertilizer to grass added nothing in soil N during first two crops but significant increase was recorded during last two crops. Maximum values were observed when intercropping was introduced up to the extent of 67% and fertilizer was supplemented (Fig. 2). However, these values of soil N were still found similar with legumes alone. Glacomini *et al.* (2003) concluded that cultivation of oat, common vetch and oil seed radish was more efficient than single crops since it combined the high biomass production capacity of black oat and oil seed radish with the ability of common vetch to fix atmospheric N_2. Geherman and Parol (2004) reported that fertilizer application was one of the main factors affecting the yield of sown pastures by the maximum utilization of readily available nutrients to the crops. Bogomolv and Petrakova (2001) recorded that the use of fodder legumes in the grass helped the reduction of mineral N fertilizer application on energy.

Fertilizer application, intercropping of legumes with grass and combining of intercropping and fertilization showed non-significant performance in all the treatments (Fig. 2).

The original status of organic matter (OM) was very poor (0.53%) (Table1). Non-significant differences were noticed within all the treatments after the harvest of first crop. However, growing of legume or its

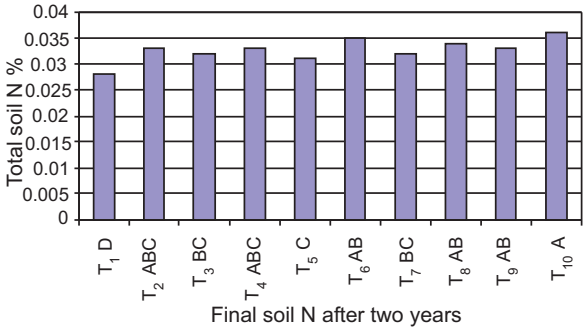

Fig. 2. Effect of intercropping (grass-legumes) and fertilizer after two years on soil nitrogen (n=4, SD = 0.003).

intercropping with grass in subsequent three crops increased soil OM that was significant with 67% intercropping or legume alone. Whereas, inoculation further increased efficiency of intercropping of 33 and 50% in statistical terms, although even OM values were also higher in treatments of legumes alone and 67% intercropping as well. A constant increase in OM content of soil with gradual growing of legumes or intercropping was recorded while the values remained almost the same in case of grass only. The end values were higher in intercropping treatments alone or when these were combined with inoculation (Fig. 3).

Soil organic improves physical condition of soil and increases water holding capacity. Finally, it is the main source of energy for soil microorganisms. Effect of organic matter was noted to be increasing one due to growing of legumes, intercropping of legumes with grass and application of fertilizer but became significant only with combination of intercropping by 67% and fertilizer application when compared with growing of grass alone during first two crops (Fig.1-4). However, during the later two crops legumes alone, intercropping and fertilization became significant except intercropping of 33%. Maximum values were recorded when intercropping of 67% was coupled with fertilizer application (Fig 4). Addition of more crop residues due to increased biomass production in different treatments could only be the reason for enhancement of organic matter status. Goddard *et al.* (2003) concluded that forage legumes played an important role in the production of organic matter in legume alone as well as legume-grass mixed sown pastures that resulted in increase in biomass production.

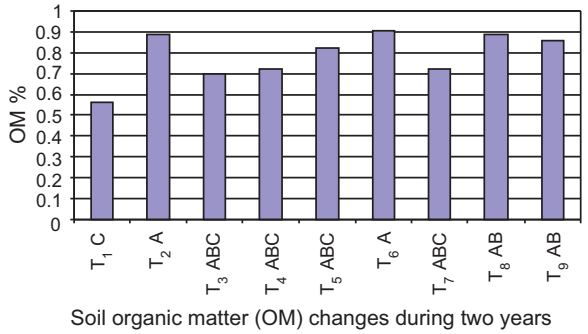

Fig. 3. Variations in soil organic matter due to grass-legumes intercropping and inoculation (n=4 SD = 0.01).

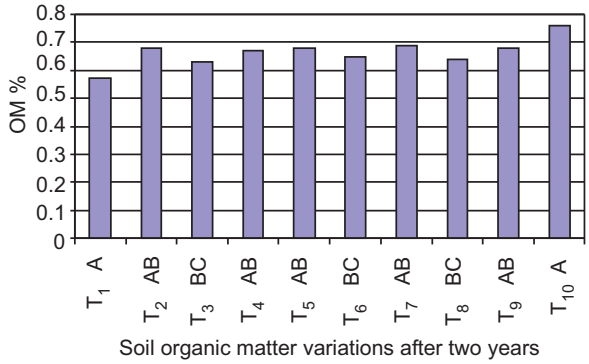

Fig. 4. Effect of intercropping (grass-legumes) and fertilizer after two years on soil organic matter (n = 4, SD = 0.01).

Conclusion

This protocol concluded that intercropping of grass and legumes, inoculation, fertilizer application and their different combinations significantly increase organic matter after second crop. Total soil N was also enhanced, especially where legumes were grown alone or intercropped because of symbiotic atmospheric nitrogen fixation. These techniques were successful to meet plant nutritional requirements because a good biomass production was obtained even in soil highly deficient for supply of nutrients.

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Variation in *Myrtus communis* L. Essential Oil Composition and its Antibacterial Activities Components

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Abstract. The *Myrtus communis* L. leaves samples were collected from five locations of its native grown areas in Lattakia, Syria, during their blooming seasons (June, 2009). Essential oil (EO) extraction was carried out by hydro-distillation in a Clevenger apparatus. The EO was analysed by both gas chromatography -Flame Ionization Detector (GC-FID) and gas chromatography/mass (GC/MS) techniques. The EO yield of the dry samples was found to be around 1.88%. The main identified components of EO were: ∝-pinene 30.40%, 1,8-cineole 17.66%, limonene 8.96%, myrtenol 5.78%, and β-caryophyllene 5.00%. The bulk EO and the separated components were tested for their antibacterial activities against *Escherichia coli* O157, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Yersinia enterocolitica* O9, *Brucella melitensis*, *Proteus* spp., and *Pseudomonas aeruginosa* by using broth micro-dilution method. It was found that citronellal and nerol were the most effective components against all pathogens.

Keywords: essential oil, bacteria, minimum inhibitory concentration, susceptibility, *Myrtus communis*

Introduction

The development of microbial resistance to antibiotics is a global concern. Isolation of microorganisms less susceptible to regular antibiotics and the recovery of increased resistant isolates during antibacterial therapy is rising throughout the world which highlights the need for new principles (Khan and Rauf, 2014).

Several plant species have shown promising microbiostatic and microbiocidal activities against a range of enteric pathogenic microbionta. These have been attributed to the presence of minute doses of bioactive principles referred to as phytochemicals and terpenes which are among the most widespread and chemically diverse groups of natural products (Omojate *et al*., 2014). Since ancient ages, several diseases have been treated with plant extracts (Miyata, 2007). *Myrtus communis* (commonly known as myrtle), is one of the most important aromatic and medicinal species in the Myrtaceae family which includes more than 5650 species that are rich in essential oils (Mulas *et al*., 2002). It is widespread in the Mediterranean regions (Chalcat *et al*., 1998), typically a sub-shrub (height: 1-3 m), with white flowers (blossoming time: June to July), wildly growing in the Syrian coastal region (altitude: 0-300 m). Myrtle is known as ''Al Ass'' or ''Al rihan'' in Arabic. This species is a very aromatic plant due to the high content of essential oils in its leaves, flowers, and fruit glands (Bonjar, 2004). Several studies have focused on the antiviral (Sokmen *et al*., 2004), antifungal (Curini *et al*., 2003), antioxidant (Messaoud and Boussaid, 2011), and antimicrobial (De Laurentis *et al*., 2005) properties of myrtle extracts. The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (Yoshimura *et al*., 2008). The essential oil obtained from the leaves by steam distillation is also important in perfumery.

The first studies on the essential oil from aerial parts of *M. communis* were undertaken by Lawrence (1996; 1993; 1990). The large variability in the chemical composition of its essential oils has attracted many researchers and has been the subject of many studies (Wannes *et al*., 2007; Jamoussi *et al*., 2005). Chemical composition (essential oil, volatile fraction) of *M. communis* aerial parts was studied in this study from five localities in Syria. This is the first report that has been undertaken on the essential oil compositions of Syrian myrtle leaves in order to determine its effect on some locally gram negative bacteria.

Materials and Methods

Study area. The fresh young leaves samples of *M. communis* L. were collected from five locations of the

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plant's native growing areas in Lattakia city located in the coastal regions of Syria. Harvesting period was stretched for 30 days after flowering in June, 2009. The five collection locations differ in the climates and altitude conditions and details of plant collection data are given in Table 1. Young leaves samples of *M. communis* L. were cut into small pieces, shade dried for one week, then ground by special electric mill and stored separately in polyethylene bags until analysis.

Bacterial samples. Local isolates of *Escherichia coli* O157, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Yersinia enterocolitica* O9, *Brucella melitensis*, *Proteus* spp. and *Pseudomonas aeruginosa*, were freshly grown in 2YT broth (peptone, 16 g/L; yeast extract, 10 g/L; NaCl, 5 g/L; [Difco, BD, Spars, MD]) and incubated for 24-48 h. Prior to antimicrobial sensitivity test, 0.2 mL of overnight culture of each organism was dispensed into 20 mL of sterile Mueller Hinton Broth (Hi-media Laboratory Pvt. Ltd., Mumbai, India) and then incubated for about 16-24 h to standardise the cultures to approximately 10^8 CFU/mL. The bacteria were suspended in a sterile phosphate-buffered saline (PBS). Bacteria abundance in PBS was monitored by recording the optical density (OD) at 590 nm. The exact counts were assessed retrospectively by viable counts on 2YT agar plates.

Chemicals. All solvents used in the experiments (acetonitril, tetrahydrofuran, acetone) were purchased from Merck (Germany). Sodium chloride, sulphuric acid, and anhydrous sodium sulphate were obtained from Sigma–Aldrich (Germany).

Standards. Thujone (trans), α-pinene, β-myrcene, terpinen, limonene, 1.8-cineole, linalool, bornyl acetate, citronellal, eugenol, geranyl acetate, nerol, β-caryophyllene were purchased from Aldrich Company, USA.

Essential oil extraction. Essential oil was extracted by hydro-distillation over 180 min using 50 g of dried leaves in 500 mL double distilled water using a Clevenger-type apparatus. After that the essential oil was dried by filtration through anhydrous sodium sulphate, collected in tightened vials and stored at 4 °C until needed. Results were expressed on the basis of dry matter weight.

Gas chromatography (GC-FID) and mass spectrometer. Identification of the essential oil components were done using GC-FID and confirmation of the results was carried out using GC-MS. GC analysis was conducted using a 30 m column HP-5 (0.25 mm i.d 0.25 μm film thickness) with helium as the carrier gas. Fragment energy of 70 eV mass spectra were acquired by using an 70 eV ionisation voltage. Oven temperature was kept at 50 °C for 2 min, programmed to 110 °C at a rate of 2 °C/min and held isothermally for 3 min; then programmed to 175 °C at a rate of 4 °C/min and held isothermally for 2 min; programmed to 250 °C at a rate of 5 °C/min and held isothermally for 5 min. Injection mode was splitless, the injector temperature was 250 °C, and the detector temperature was 275 °C.

GC-MS conditions were (Mass range: 50–500 mass unit, source temperature 260 °C, EI 70-ev). Essential oils have been analysed using GC-FID Agilent technologies 6890 N network GC system, supported with Agilent technologies 5973 inert Mass Selective Detector (Agilent, USA) by the following analysis conditions: the column (HP-5 ms (30 m × 0.25 mm) ID 0.25 μm), carrier gas 0.9 mL/min (constant flow of Helium gas). The GC-MS system was operated by the same conditions like GC-FID. The same conditions of temperature programming were used for oil samples in order to calculate the retention index (RI). Identification of components in the oil was based on RI. Individual components were identified by comparison of both mass spectra and their GC retention data; other identifications were made by comparison of mass spectra with those in the data system libraries. The quantitative analysis of yields were determined according to reference materials and standards obtained from Aldrich. Calculations were made with the use of gas chromatography, Chem Station software, and the injection volume (1 μL). The identity of each essential oil component was determined by comparing retention time values of gas chromatography on polar columns and by comparing mass spectrum and library database (Nist and Willy).

Chromatograms were computed by the normalisation method from the GC peak areas, calculated as mean values of two injections. Essential oil components were identified by comparison of their retention times with those of pure reference standards. Quantitative data were obtained from the electronic integration of the FID peak areas.

Microdilution test. The minimum inhibitory concentrations (MICs) were determined using 96-well microtitre plates in order to determine the antibacterial activity of oils and their components against the human pathogenic bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. Compounds to be investigated were added into Muller-Hinton Agar (MHA, Merck) with bacterial inoculum (1.0×10^4 CFU/100 μL per well). Positive

control was done with the same conditions but without essential oils. Negative control was also done with the same conditions but without adding the bacteria. The microplates were incubated for 24 h at 37 °C. The lowest concentrations without visible growth were defined as the concentrations that completely inhibited bacterial growth (MICs). The optical density of each well was measured at a wavelength of 590 nm by microplate reader (Thermo-lab Systems Reader, Finland) and compared with a blank and the positive control. Three replicates were done for each oil and for each component.

Statistical analysis. All data were reported as means ± standard deviation of three samples. Statistical analysis was performed with ANOVA.

Results and Discussion

The average essential oil in all samples was 1.88% (w/w) of the dry matter (Table 1). The results of the chemical analyses of essential oils investigated are presented in Table 2. The average of yield of *M. communis* L. oil is 1.88% (w/w) for each collection site, the main components are ∝-pinene (30.40%), 1,8-cineole (17.66%) and limonene (8.96%).

The results of antibacterial activity of essential oils are presented in Table 3. Bulk oil and all the components of *M. communis* L. oil were tested in the microdilution method. Bulk oil showed bacteriostatic activity at the concentration of (25-50 µL/mL) against *Y. enterocolitica* O9, *B. melitensis*, *Proteus* spp., *P. aeruginosa* and *S. typhimurium*; but no effect was observed against *E. coli* O157 and *K. Pneumoniae.*Tujene, γ-terpinene, nerol and myrtenol showed antibacterial activity against all bacterial strains tested in the microdilution method, MIC at 0.3-50.0 µL/mL. In contrast, α-pinene, β-myrcene, limonene, bornyl acetate, geranyl acetate, and β-caryophyllene were inactive against *Y. enterocolitica* O9, *B. melitensis*, *Proteus* spp., *P. aeruginosa*, *S. typhimurium*, *E. coli* O157 and *K. pneumoniae*.

The chemical composition of the myrtle leaf essential oils (belonging to different regions and harvested at different periods) has been widely studied (Tuberoso *et al*., 2007; De Laurentis *et al*., 2005). However, this is the first report on the essential oil composition of Syrian myrtle leaves. Thereafter, the leaf and berry essential oil compositions from various mediterranean origins have also been investigated such as in Turkey (Ozek *et al*., 2000), France (Bradesi *et al*., 1997), Italy (Pirisino *et al*., 1996), Portugal (Boelens and Jimenez, 1991), Spain (Boelens and Jimenez, 1992), 1,8-cineole (18.3%), linalool (16.3%) and myrtenyl acetate (14.5%), limonene (5.7-43.4% and 6.2-44.2%), 1, 8-cineole (5.9-26.6% and 8.7-30.40%), respectively. The present results demonstrated that Syrian *M. communis* L. consists of 14 components, which form (90.1-94.5)% of the bulk essential oil. While, the Tunisian *M. communis* L. essential oil combined of 24 components (Jamoussi *et al*., 2005) and other study carried out by Mahboubi and Ghazian Bidgoli (2010) revealed the presence of 70 components, representing 99.23% of the total myrtle essential oil. Tunisian and Algerian *M. communis* L. essential oil combined of 23 identified compounds (Ben Ghnaya *et al.,* 2013) representing 93.73% of total oil, which was found to be rich in monoterpenes hydrocarbons (53.38%) particularly α-pinene (35.30%) and α-limonènes (14.76%). Whereas, Brada *et al*. (2012) demonstrated that Algerian *M. communis* L. leaf oil was composed of 28 compounds representing 95.4% of the total composition of the oil. α-pinene was the major constituent of leaf oil at a concentration of (46.9%), followed by 1,8-cineole (25.2%). The Syrian *M. communis* L. composition characterised by its richness of monoterpene hydrocarbons in leaves, largely due to

Table 1. Collection sites, climatic conditions (year 2009), and yield of essential oils in dry matter of the collected samples

Collection sites	Altitude (m)	Latitude (°)	Longitude (°)	Climatic conditions			EO% (w/w)
				Minimum temperature a year (°C)	Maximum temperature a year (°C)	Rain fall in average/ year (mL)	
Alkirdaha	300	3536	3602	5.5	32.2	1055	1.85±0.13
Shaditi	305	3536	3603	5.3	31.7	1058	1.79±0.11
Salah Aldin	350	3536	3604	6.3	29.3	1089	1.95±0.12
Kismin	120	3541	3559	8.2	29.3	1120	1.89±0.12
Wata Alkhan	120	3541	3559	4	34.6	1135	1.92±0.14

Table 2. The essential oil constituents yields of *M. communis* L. samples for each collection site

RI	Class	Component name	The isolated component yield % (w/w)						Identification*
			Alkirdaha	Shiditi	Salah Aldin	Kismin	Wata Al khan	Average	
933	MH	α-pinene	29.50	28.70	31.40	30.30	32.10	30.40	FID-Std /MS
988	MH	β-myrcene	1.60	1.40	1.70	1.60	2.10	1.68	FID-Std /MS
1034	MH	Limonene	8.70	6.70	9.80	9.20	10.40	8.96	FID-Std /MS
1043	OM	1,8-cineole	19.20	22.20	18.40	16.30	12.20	17.66	FID-Std /MS
1063	MH	γ-Terpinen	2.80	3.30	2.50	2.40	2.30	2.66	FID-Std /MS
973	MH	Sabinene	0.80	1.20	0.70	1.10	0.90	0.94	GC-MS
1225	OM	Geraniol	1.10	0.90	0.70	1.30	0.90	0.98	GC-MS
1342	SH	β-elemene	1.30	1.10	0.90	1.20	0.70	1.04	GC-MS
1375	SH	α-humulene	1.20	0.90	0.70	1.10	0.80	0.94	GC-MS
1479	SH	Germacrene	0.90	0.70	0.60	0.80	0.60	0.72	GC-MS
1097	OM	Linalool	3.40	2.60	5.80	4.70	5.10	4.32	FID-Std /MS
1115	MH	Thujone (*trans*)	2.10	1.90	2.70	2.20	2.90	2.36	FID-Std /MS
1233	OM	Citronellal	1.80	2.10	2.30	2.20	2.70	2.22	FID-Std /MS
1124	OM	Nerol	1.90	2.40	2.60	2.30	3.10	2.46	FID-Std /MS
1289	OM	Bornyl acetate	2.20	2.10	2.70	2.60	2.90	2.50	FID-Std /MS
1273	OM	Geranyl acetate	3.10	2.40	3.40	3.60	3.80	3.26	FID-Std /MS
1143	OM	Eugenol	3.30	3.50	3.70	3.90	4.10	3.70	FID-Std /MS
1119	SH	α- caryophyllene	5.20	4.70	5.40	4.80	4.90	5.00	FID-Std /MS
1324	MH	Myrtenol	5.30	6.40	5.80	5.50	5.90	5.78	GC-MS
Total	**-**	**-**	96.90	97.10	99.70	98.10	98.38	98.53	-

RI = a retention indices; The identification of the compounds (∝-Humulene- Germacrene- β-Elemene- ∝-Phellandrene; Geraniol-Sabinene) are in consistent with library search (Willy-Nist) between 80-85%; Identification* FID-Std /MS =Flame Ionization detector /Mass Spectroscopy; GC-MS = Gas chromatography-mass spectrometry; Monoterpene hydrocarbons (MH)= 53.73%; Oxygenated monoterpenes (OM) = 37.1%; Total Monoterpenes % 90.83; Sesquiterpene hydrocarbons (SH)= 7.7%.

α-pinene (30.40 %), while the Tunisian myrtle essential oil contains 19.20% α-pinene (Messaoud and Boussaid, 2011). Two studies have been published on the antimicrobial activities of plant compounds against different types of microbes, including food-borne pathogens (Gunduz *et al*., 2009; Burt, 2004).

Akalu *et al*. (2007) determined the antimicrobial activity of the essential oils obtained from *M. communis* L. *in vitro* using agar well diffusion method against *S. aureus*, *Bacillus cerus*, *E. coli*, *P. aeruginosa*, *S. pyogens*, *Aspergillus niger* and *Candida albicans*, at different concentrations. The oil showed activity against all tested bacteria and fungi with minimal activity against *P. aeruginosa*. Also Syrian *M. communis* L. essential oil characterised by remarkable yield of γ-terpinene (2.66%) which has a high MIC value (2.3-3.3 μL/mL) as compared to other components' MICs. The yields of all components varied among the populations according to their growing conditions and climate deviation. These variations were not remarkable when compared to the significant deviation between two localities of the Montenegro coastline myrtles. Mimica-Dukic *et al*. (2010) observed that significant differences between the two samples of *M. communis* L. were found in the ranges of α-pinene (14.7%-35.9%) and myrtenyl acetate (5.4%-21.6%). In the present study, it was found that the essential oil of local *M. communis* L. did not contain α-terpineol, β-pinene, p-cymene, linalyl acetate, β-caryophyllene, phellandrene and myrtenyl acetate, while other studies showed that they were among the main components of *M. communis* L. essential oils (Mahboubi and Ghazian Bidgoli, 2010; Mimica-Dukic *et al*., 2010; Wannes *et al*., 2007). The bulk essential oil inhibited 5 of the 7 bacteria in high concentration (25-50 μL/mL), while the essential oil components differed in its influences against the tested bacterial strains. Thujone (*trans*), γ-terpinene, nerol and myrtenol inhibited all tested bacteria in wide range of MICs (1-50 μL/mL). But β-myrcene, α-pinene, limonene, bornyl acetate, geranyl acetate and β-caryophyllene expressed no antibacterial effects. The other components showed inhibition effects against some but not all tested bacterial strains. *E. coli* O157 was the most resistant isolate that was inhibited by 5 of 15 substances (the bulk essential

Table 3. The minimum inhibitory concentrations for the bulk essential oil and for the constituents (MIC µL/mL)

	K. pneumoniae	*S. typhimurium*	*P. aeruginosa*	*Proteus* spp.	*B. melitensis*	*Y. enterocolitica* O9	*E. coli* O157
Bulk oil	NIE	25.00±9.32	50.00±9.62	50.00±20.97	50.00±9.62	25.00±5.78	NIE
α-pinene	NIE	NIE	NIE	NIE	NIE	NIE	NIE
β-myrcene	NIE	NIE	NIE	NIE	NIE	NIE	NIE
Limonene	NIE	NIE	NIE	NIE	NIE	NIE	NIE
1,8-cineole	NIE	50.00±9.62	50.00±20.97	50.00±9.62	50.00±0.00	50.00±9.62	NIE
γ- terpinene	6.25±2.79	12.50±1.33	25.00±1.33	25.00±0.00	12.50±2.88	25.00±1.33	12.50±1.33
Linalool	NIE	50.00±0.00	50.00±0.00	50.00±9.62	NIE	NIE	NIE
Thujone (*trans*)	25±4.8	25.00±5.78	50.00±9.62	50.00±9.62	50.00±9.62	50.00±20.97	50.00±20.97
Citronellal	50±20.97	6.25±2.78	<0.30	3.10±0.00	3.10±0.00	6.25±2.78	NIE
Nerol	3.1±0.86	6.25±0.00	6.25±0.00	6.25±2.79	6.25±0.00	12.50±0.00	6.25±2.78
Bornyl acetate	NIE	NIE	NIE	NIE	NIE	NIE	NIE
Geranyl acetate	NIE	NIE	NIE	NIE	NIE	NIE	NIE
Eugenol	12.5±2.88	50.00±9.62	NIE	6.25±2.79	NIE	NIE	6.25±0.00
β-caryophyllene	NIE	NIE	NIE	NIE	NIE	NIE	NIE
Myrtenol	25±9.32	50.00±9.62	50.00±0.00	25.00±4.80	50.00±20.97	25.00±0.00	25.00±9.32

NIE = No inhibitory effects.

oil and 14 components), while *S. typhimurium* and *Proteus* spp. were the most sensitive isolates that were inhibited by 9 of the 15 of previous substances. Present results confirmed the bioactive properties of *M. communis* L. essential oil and some of its components and reinforced other studies results (Gunduz, 2009; Deriu *et al*., 2007; Takeuchi and Frank, 2000). Previous studies have reported the antimicrobial effects of myrtle leaves oil *in vitro* conditions on *E.coli* and *Y. enterocolitica* (Bouzouita *et al*., 2003; Sagdie *et al*., 2003).

Conclusion

Myrtus communis L. essential oil and its components have exhibited some inhibitory effect against some Syrian gram negative isolates. The EO main components were α-pinene, 1,8-cineole, limonene, myrtenol, and β-caryophyllene. The most effective components were citronellal and nerol against all pathogens used in our study. Synergistic and antagonistic effects and more research are needed for evaluation of these components and it will be tested in a future study.

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Functional Properties and Amino acid Profile of *Spirulina platensis* Protein Isolates

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Abstract. Protein malnutrition and food insecurity represent serious obstructions to sustainable development, poverty reduction and food quality throughout the world. The present study has been designed to evaluate the *Spirulina platensis* (SP) as a protein alternative source for the utilization in food products. A protein isolate was prepared from *S. platensis* powder through extraction with 0.1N NaOH, precipitation at pH 3, neutralization of the dispersed precipitate to pH 6.8-7.0, and subsequent freeze drying. The *S. platensis* isolate amino acids compositions revealed that the total essential amino acids contribution was comparatively higher in SPI (31.16±1.43 g/100 g) as compared with SP (27.75±1.21 g/100 g). Moreover, oil and water absorption capacities, foaming and emulsifying properties, surface hydrophobicity and nitrogen solubility index were found better functional properties under laboratory conditions except emulsion properties. Conclusively, SP and its isolates might be used in various food products to curtail protein energy malnutrition.

Keywords: *Spirulina platensis*, amino acid, protein isolates, functional properties

Introduction

Malnutrition exists in those regions where overall food supply is inadequate due to poor economy; less access to dietary information; political unrest conditions and instability has interrupted food supplies. The tremendous increase in global population and production of insufficient protein has directed to search alternate sources of protein. The greater part of the world, especially developing countries use cereals as the staple foods that are generally deficient in lysine and threonine. Protein quality of the cereals can be improved by algal supplementation as these are good source of essential amino acids (Spolaore *et al*., 2006; Pimentel and Pimentel, 2003).

Spirulina (*Spirulina platensis*) has attained promising position among protein alternative sources due to its amino acids, fatty acids, phytonutrients and vitamin A contents. Both, *Spirulina platensis* and *Spirulina maxima* are commonly used as foods supplement. Spirulina is one of the most prehistoric life forms on earth and has been used as food by humans for centuries (Vigani *et al*., 2015; Habib *et al*., 2008). Marine protein hydrolysates can deliver nutritional benefits and play a vital role as functional ingredients for food industries along with potential use in health issues and as functional ingredients for food processing (Vijaykrishnaraj and Prabhasankar, 2015). Microalgae can be used to enhance the nutritional value of foods due to their abundance in compounds with beneficial attributes (Benelhadj *et al*., 2016).

Spirulina contains about 65-70% protein (dry weight) that is higher than any other natural food, and considered as a good protein source for human consumption. Furthermore, spirulina protein is easily digestible whilst considered more appropriate for malnourished children (Chaiklahan *et al*., 2011; Li *et al*., 2006; Khan *et al*., 2005). Also spirulina has a unique blend of nutrients that no single plant source can provide. It has a high protein concentration (60-70% on dry weight), supplying 18 amino acids, including all essential amino acids in balanced proportion (Kim and Kang, 2011; Fradique *et al*., 2010).

Spirulina has wide food applications ranging from juice smoothies, confectionary, food bars, baked desserts, doughnuts, muffins, pasta, salad dressing, frozen desserts, snack foods, popcorn, corn chips, crackers, breakfast cereals, soups and instant meals. Spirulina protein isolates (SPI) are currently of special interest to processors and consumers due to low fat and high protein content (Widjanarko *et al*., 2011; Fradique *et al*., 2010; Hassan *et al*., 2010). Besides imparting nutritional and therapeutic benefits, spirulina supplementation in the food products improves the texture and colour of the products (Fradique *et al*., 2010).

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Spirulina protein isolates have shown good gelling properties with fairly low minimum critical gelling concentrations. Protein concentrate had a lower water absorption capacity, higher fat absorption and emulsification capacity, similar foaming capacity than its flour. Spirulina protein isolates exhibited lower viscosities at pH 9 due to increase in protein solubility. Subsequent cooling at ambient temperatures caused a further pronounced increase in network elasticity (Devi and Venkataraman, 2006; Chronakis, 2001).

In developing countries of Asia, especially South East, the pressure on food sources is increasing day by day due to escalating population and scarcity of resources. In this context, some unconventional protein sources have been explored for improving nutritional status of population (Voltarelli *et al*., 2011; Simpore *et al*., 2006). There is dire need to explore some algae based high protein sources to formulate nutritious and economical foods. Keeping in view the above facts, present project has been designed to address protein energy malnutrition and micronutrient deficiencies in vulnerable segment of population.

Materials and Methods

Spray-dried spirulina (*S. platensis*) strain Pacifica was purchased from Cyanotech Corporation, Hawaii, USA. The reagents (analytical and HPLC grade) were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich, Tokyo, Japan). The current study was carried out at Institute of Food Science, College of Agriculture and Life Sciences, Cornell University, NY, USA.

Preparation of spirulina protein isolates. *Extraction*. The spirulina protein isolates were extracted by dissolution of spirulina under reductive conditions in 2M NaOH for maximum solubility. The soluble protein was separated by acidic precipitation (2M HCl) and freeze-dried powder was stored at 5 °C for further analysis (Gerde *et al*., 2013; Chronakis, 2001). The nitrogen content was determined by using AACC Method No. 46-10 (AACC, 2000).

***Protein yield*.** Protein yield was calculated using isolate recovery, protein content of isolates and spirulina (Wang *et al*., 1999).

$$\text{Yield (\%)} = \frac{\text{Weight of isolate (g)} \times \text{protein content of isolate (\%)}}{\text{Weight of sample (100 g)} \times \text{protein content of sample (\%)}} \times 100$$

Functional properties. For the measurement of bulk density, spirulina protein isolates were filled in 10 mL graduated cylinder, and gently tapped several times until there was no further attenuation of the sample level. Oil absorption capacity was measured by mixing 0.5 g of spirulina isolate sample in 6 mL of corn oil in centrifuge tube followed by stirring to dissolve the sample in oil. After 30 min, the tube was centrifuged at 3000 × g for 25 min. The separated oil was removed by the pipette and the tubes were inverted for 25 min to remove the oil before reweighing. Water absorption was determined by mixing 3 g of sample in 25 mL of distilled water. The solution was stirred followed by centrifugation for 25 min at 3000 × g. The resultant supernatant was decanted, excess moisture was removed and reweighed. Sample (1 g) was dispersed in 50 mL of distilled water, in a capped test tube, by shaking vigorously for 5 min followed by immediate pouring into a 250 mL graduated cylinder. The volume of the foam formed was then recorded as the foam capacity (mL/100 mL). A final observation was made after 60 min for recording the foam stability. Emulsifying properties were determined following the procedure described by Yasumatsu *et al*. (1972). Sample (0.5 g) of spirulina protein isolates was suspended in 3mL of distilled water contained in a graduated tube followed by the addition of oil (3 mL). The contents were then shaken vigorously for 5 min and the resulting emulsion was centrifuged at 2000 × g for 30 min. The volume of the emulsified layer divided by that of the whole slurry multiplied by 100 was taken as the emulsifying activity (mL/100 mL). To determine the emulsion stability, the homogenised mixture of spirulina isolates, water and oil was heated at 80 °C for 30 min before centrifugation at 2000 × g for 30 min. The emulsifying stability was then calculated as the volume of the emulsifying layer divided by that of the heated slurry multiplied by 100, reported as mL/100 mL. While, surface hydrophobicity of spirulina protein isolates was determined by hydrophobicity florescence probe (1-anilino-8-naphthalene sulphonate ANS) and *cis*-parinaric acid (CPA) probes with some modifications by using SLM 8000 Spectrofluorimeter (Slm Aminco Instruments, Inc. Rochester, NY, USA), with adjusted wavelengths of 390 and 470 nm for excitation and emission, respectively (Alizadeh-Pasdar and Li-Chan, 2000; Kalapathy *et al*., 1997). Nitrogen solubility index was determined following the method described by Shand *et al*. (2007); Betschart (1974) and AACC Method No.46-23 (AACC, 2000) and . The least gelation concentration was determined by the methods of Sathe and Salunkhe (1981) and Siddiq *et al*. (2010).

Amino acid profile. Amino acid profiles of spirulina were determined at the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO), by using AOAC Method No.982.30E (AOAC, 2011) by injecting known volume of the supernatant of the prepared samples using high speed amino acid analyzer (L-8500 A, Hitachi, Tokyo, Japan) by modifying the method described by Adeyeye and Afolabi (2004).

Statistical analysis. All the experiments were run in triplicate. The collected data were statistically analyzed by t-test using Statistical Package (Statistix 8.1) and expressed as mean± SD.

Results and Discussion

Protein contents and yield of spirulina proteins isolates. Mean values for the protein content and yield of spirulina proteins isolates (Table 1) revealed 74.01±1.53% protein content and 52.60±3.46% yield. Isolation of proteins from microalgae further enhances the commercial value by increasing food applications (Schwenzfeier *et al*., 2011).The *Spirulina platensis* strain Pacifica protein isolates were extracted by dissolution of the algae under reductive conditions in NaOH exhibited greater solubility followed by acidic precipitation and freeze-drying (Devi and Venkataraman, 2006). The spirulina extracted protein isolate revealed protein content of 67.9 and 67.8% using method of AOAC (1995), respectively. Minor variations in protein content of fractions (67.9%) were noticed for the sample before fractionation (Chronakis *et al*., 2000). Spirulina protein is generally well balanced, reflecting its potential as human food and as a source of natural products. Spirulina is slightly acidic and carries a negative charge at neutral pH. However, the presence of salts reduces the solubility of spirulina protein, contrasting to other proteins like soybean meal (Chronakis, 2001).

Functional properties of spirulina protein isolates. The functional properties of proteins mainly depend upon interaction with three important components of food systems including oil, water and gas. Protein plays a major role as surface active agent; functionality depends simultaneously on efficiency to absorb into the water-oil or oil-water interfaces, film forming ability, and ability to prevent coalescence. Interaction of protein with water is main factor to establish the solubility behaviour along with viscosity and gelling, though interaction with oil and gas indicates the emulsifying and foaming properties. Processing brings certain structural modifications in proteins altering their functionality. Exposure of proteins to variable temperatures may either dissociate the oligomers into subunits or formation of new units due to coagulation. Sometimes, these irreversible changes may lead to loss of protein quality as well as hydration, viscosity, biological activity and solubility.

Bulk density, absorption capacities and surface hydrophobicity. Bulk density specifies packaging behaviour of manufactured products depends on collective effect of some unified factors like particle size, forces and strength of contact points. The maximum bulk density (Table 2) was observed in spirulina protein isolates (0.64±0.07 g/cm^3) followed by spirulina sample (0.58±0.04 g/cm^3). The lower bulk density of powdered spirulina may have been due to textural porosity leading to lower bulk density whereas in spirulina protein isolates sheerness of particle size facilitated the proper reconciling of isolates thus improving the bulk density value.

Protein has both hydrophobic and hydrophilic properties which influence the interaction with oil and water in various foods. Water and oil binding properties are an evaluation of physicochemical interaction of proteins with other components in food. Spatial rearrangement in protein structure during heat processing may alter its

Table 1. Protein content and yield of spirulina proteins isolates

Parameter	Quantity (%)
Protein content	74.01±1.53
Protein yield	52.60±3.46

Values are means ± SD, (n=3).

Table 2. Functional properties of spirulina and spirulina protein isolates (SPI)

Properties	Spirulina	SPI
Bulk density (g/cm^3)	0.58±0.04	0.64±0.07
Water absorption (mL/g)	2.14±0.18	2.96±0.17
Oil absorption (mL/g)	1.85±0.21	0.59±0.16
Surface hydrophobicity	25.34±0.18	17.13±0.21
Foaming capacity (mL)	10.74±0.27	12.53±0.16
Foaming stability (min)	19.26±3.87	32.40±4.53
Emulsion activity (%)	56.32±1.54	51.54±2.12
Emulsion stability (%)	71.51±1.20	65.20±2.17

Values are means ± SD; SPI = spirulina protein isolates; (n=3).

absorption properties. Higher water absorption capacity of spirulina protein isolates (2.96±0.17 mL/g) may have been due to presence of more polar amino acids at primary cites of protein-water interface (Table 2). Likewise, lower water absorption capacity was noticed in spirulina (2.14±0.18 mL/g). On the contrary, spirulina protein isolates showed lower oil absorption capacity (0.59±0.16 mL/g) than spirulina (1.85±0.21 mL/g).

Surface hydrophobicity is an index of protein interaction capacity with other molecules in polar aqueous environment and is strongly correlated with emulsion activity. It is very important factor in determining the emulsifying properties; lower values cannot assist the interaction between proteins and oils. Protein functionality is greatly affected by the presence of hydrophobic patches on the surface of proteins that are available to interact with food system. Means for surface hydrophobicity (Table 2) showed higher surface hydrophobicity (25.34±0.18) for spirulina while spirulina protein isolates had lower values for hydrophobicity (17.13±0.21). Nirmala *et al*. (2006) have reported water absorption (2.92 g/g) and oil absorption (0.56 g/g) capacities of *Spirulina platensis*. In another study, water and oil absorption capacities of spirulina flour (2.2 g/g and 1.9g/g, respectively) were comparable to soybean meal (2.30g/g and 1.20g/g, correspondingly). Protein isolates have lower water absorption capacity and higher oil absorption capacity than its flour samples (Devi and Venkataraman, 2006).

Foaming and emulsion properties of spirulina and spirulina protein isolates. Foaming properties illustrates whipping ability of protein concentrates. Protein's property to form stable foams is vital for diverse food applications. Foam is a two-phase system consisting of air cells separated by a thin continuous liquid layer, lamellar phase. The results regarding foaming and emulsion properties are presented in Table 2. Spirulina protein isolates showed the maximum foaming capacity (12.53±0.16 mL) and stability (32.40±4.53 min), compared with foaming capacity and stability of spirulina (10.74±0.27 mL and 19.26±3.87 min, respectively). Foam obtained from spirulina protein isolates appeared denser and stable due to more interaction at air-water interface. The high values of foaming capacity and stability indicate highly hydrated foams as in case of spirulina protein isolates, while decrease in both parameters was observed in case of spirulina. The foaming stability of spirulina (18%) and spirulina protein concentrate (27%) was poor when compared to soybean meal (55%) (Devi and Venkataraman, 2006). Foaming capacity depends on diffusion of protein at air-water interface by unfolding its structure while foaming stability is dependent on formation of thick cohesive layer around the air bubbles (Damodaran, 1997).

The results pertaining to emulsion activity and stability revealed that spirulina and spirulina protein isolates exhibited 56.32±1.54 and 51.54±2.12% emulsion activity, respectively, while emulsion stability for spirulina and spirulina protein isolates was 71.51±1.20% and 65.20±2.17%, respectively. This was may be due to more surface area and fine particles. Emulsifying capacity and stability of microalgae (*Chlorella vulgaris)* proteins are comparable or superior to the commercial ingredients i.e. sodium caseinate (Ursu *et al*., 2014). Spirulina has similar emulsion and foaming capacity to that of soybean meal. Guil-Guerrero *et al*. (2004) evaluated functional properties of three microalgae species along with soy bean. The emulsifying capacity of three alga (50 g/g of oil at pH 4 and 20 g/g of oil at pH 12) were higher than soybean (50 g/g of oil at pH 4 and 90 g/g at pH 12 of oil).

Least gelation concentration of spirulina and spirulina protein isolates. Least gelation concentration is a qualitative parameter to work out the concentration at which the protein isolates form a gel. Spirulina protein isolates exhibit good gelling properties (Table 3) with least gelation concentration (LGC) of 18% compared with spirulina (14%). Microalgae can be an alternative and novel source of natural ingredient for development of novel food products. Batista *et al*. (2013), findings

Table 3. Least gelation concentration spirulina and spirulina protein isolates

Concentration (%)	Spirulina	SPI
2	(-)	(-)
4	(-)	(-)
6	(-)	(-)
8	(-)	(-)
10	(±)	(-)
12	(±)	(±)
14	(+)	(±)
16	(+)	(±)
18	(+)	(+)
20	(+)	(+)
LGC	14	18

Gelation levels: (-) = liquefied; (±) = gluey; (+) = gel; LGC = least gelation concentration; (n=3); SPI = spirulina protein isolates.

elucidates that, by elevated temperature (upto 90 °C, 5 min) and the effect of time (5-30 min, at 90 °C) was less noticeable to form more structured gels. Spirulina protein isolates have relatively good gelling properties, with a critical gelling concentration of 1.5% w/w in aqueous solution. Hydrophobic interactions were also found to stabilize the structure of spirulina protein gels. Hydrogen bonds are suggested to add the network rigidity. Furthermore, intermolecular disulphide bonds have found to play a trivial role for the network structure of spirulina protein gels (Chronakis *et al*., 2001).

Nitrogen solubility index of spirulina and spirulina protein isolates. Solubility depends on the physico-chemical characteristics of the protein molecules and affects its functional properties like emulsification, foaming and gelling properties. The nitrogen solubility is expressed as the percentage of the total nitrogen that remains in the supernatant of spirulina protein extract that was obtained after acid (0.1N HCl) and alkali (0.1N NaOH) treatment. It is a good index considered while designing any potential applications for flour proteins. Minimum nitrogen solubility (4.55-4.68%) was observed at pH 4.0 might be due to isoelectric region. A marked boost was observed above this point till pH 8.0 (55.04-80.41%), followed by a slight raise up to pH 12.0 where, protein isolates illustrates a solubility index ranging from 77.67% to 90.54% (Fig. 1). Overall, spirulina revealed poor solubility as compared to its isolates. Lower solubility profile of spirulina was due to the strong aggregation during drying and heating conditions.

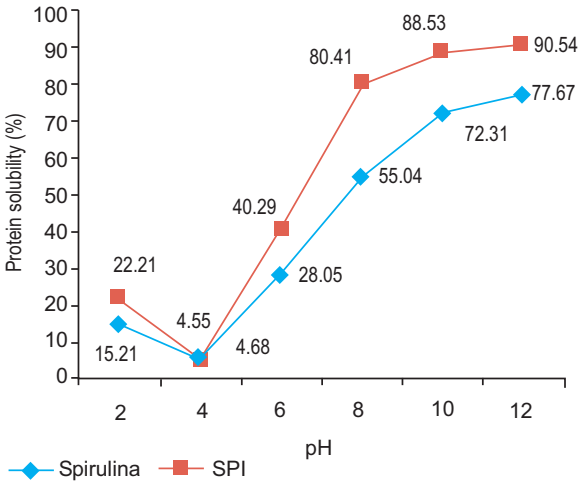

Fig. 1. Nitrogen solubility index of spirulina and spirulina protein isolates (n=3).

There is positive correlation within nitrogen insolubility and protein aggregation index. Protein that shows denaturalization aggregation properties has lower gelation, foaming capacities and emulsification (Guil-Guerrero *et al*., 2004).

The pH has significant impact on nitrogen solubility. In a study, lower nitrogen solubility was noticed at pH 4 in defatted microalgae biomass followed by rapid increase (75%) at pH 12. It might be due to cell wall inhibition in the solubility of the protein at basic pH (Guil-Guerrero *et al*., 2004). Micro-alga biomass consumption without any treatment to break the cell wall e.g., sonication, might affect the maximum body utilization of the algal protein (Oshodi, 1992). Defatted micro-alga biomass can be used in the formulation of acid foods such as milk analog products, and protein-rich carbonated beverages (Kinsella, 1976).

Amino acids profile of spirulina and spirulina protein isolates. Quality of protein depends normally on its essential amino acid content. Results regarding amino acid profile of spirulina and spirulina protein isolates are given in Table 4 and 5. The results for essential amino acids in spirulina and spirulina protein isolates exhibited; leucine in large proportion (6.81±0.19 g/100 g) followed by valine (4.72±0.13 g/100 g) whilst the lowest value (0.98±0.03) was noted for tryptophan in spirulina protein isolates. Likewise, leucine (6.17±0.23 g/100 g) and valine (4.21±0.10 g/100 g) were major essential amino acids in spirulina. The total essential amino acid contribution was comparatively high in spirulina protein isolates (31.16±1.43 g/100 g) than that of spirulina (27.75±1.21 g/100 g).

Table 4. Essential amino acids profile of spirulina and spirulina protein isolates

Amino acids	Spirulina (g/100 g)	SPI (g/100 g)
Phenylalanine	3.33±0.12	3.75±0.14
Methionine	1.71±0.11	1.82±0.05
Threonine	3.31±0.07	3.73±0.10
Valine	4.21±0.10	4.72±0.13
Isoleucine	3.64±0.20	4.24±0.12
Leucine	6.17±0.23	6.81±0.19
Lysine	3.40±0.02	3.85±0.11
Histidine	1.13±0.04	1.26±0.06
Tryptophan	0.85±0.02	0.98±0.03
TEAA	27.75±1.21	31.16±1.43

Values are means ± SD; TEAA = total essential amino acids; SPI = spirulina protein isolates; (n=3).

Table 5. Non-essential amino acids of spirulina and spirulina protein isolates

Amino acids	Spirulina (g/100 g)	SPI (g/100 g)
Alanine	5.02±0.07	5.65±0.16
Arginine	4.47±0.05	5.04±0.14
Aspartic acid	6.31±0.16	7.14±0.20
Glutamic acid	8.47±0.19	9.57±0.27
Glycine	3.43±0.10	3.89±0.11
Proline	2.53±0.04	2.91±0.08
Cysteine	0.64±0.03	0.72±0.02
Tyrosine	3.07±0.05	3.41±0.09

Values are means ± SD; SPI = spirulina protein isolates; (n=3).

The results for non-essential amino acids in spirulina and spirulina protein isolates indicated glutamic acid as major amino acid (9.57±0.27 g/100 g) followed by aspartic acid (7.14±0.20 g/100 g) whereas, the lowest content (20.72±0.02 g/100 g) was noticed for cysteine in spirulina protein isolates. Similarly, glutamic acid (8.47±0.19 g/100 g) and aspartic acid (6.31±0.16 g/100 g) were substantial amounts. It is obvious from the results that both spirulina and spirulina protein isolates are good source of leucine, valine, isoleucine, lysine, glutamic acid, aspartic acid, alanine and arginine. The levels of methionine and alanine are comparatively high in spirulina with most commonly used plant protein source i.e. soybean.

The nutritional quality of a protein is determined mostly by the proportion, content and availability of its amino acids (Becker, 2007; WHO, 2007). The protein contents in algae contain all essential amino acids and some species can be compared with soy and egg protein (Galland-Irmouli *et al*., 1999). In addition, the right combination of plant proteins can ensure the supply of satisfactory amounts of essential amino acids for human health (Day, 2013). The natural dried spirulina of Cuban origin was analyzed for amino acid contents through reverse-phase HPLC. It was observed that, valine and leucine contents were in high proportion (6.1 and 6.0 g/100 g) on dry weight basis. Furthermore, amino acid analysis showed the highest value (101 mg/g) for glutamic acid (Campanella *et al*., 1999). Most of the algae contain lysine and sulphur containing amino acids i.e. methionine and cysteine (Simpore *et al*., 2006). In addition, high concentrations of arginine, aspartic acid and glutamic acid are found in many seaweed species (Dawczynski *et al*., 2007; Fleurence, 1999;). The levels and pattern of amino acids in spirulina are similar as reported by FAO. Cereals are generally low in limiting amino acids like lysine and tryptophan whereas, these amino acids are present in high concentration in spirulina which can be an excellent choice for supplementation in cereals and cereals based diets (WHO, 2007).

Conclusion

Quality of protein depends on its essential amino acid content. It is obvious from the results that both spirulina and spirulina protein isolates are good source of leucine, valine, isoleucine, lysine, glutamic acid, aspartic acid, alanine and arginine. The levels of methionine and alanine are comparatively high in spirulina with most commonly used plant protein source i.e. soybean. Histidine was found to be the first limiting amino acid in both spirulina and spirulina protein isolates. The functional properties of proteins mainly depend upon interaction with three important components of food systems including oil, water and gas. Spirulina protein isolates revealed higher bulk density, water absorption capacity, foaming capacity and foaming stability and good gelling properties.

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Preparation of Sesame Flour Supplemented High Protein and Energy Food Bars

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Abstract. In this study, defatted sesame flour was mixed in different proportions (0, 25, 50, 75 and 100% and given names as T_0, T_1, T_2, T_3, T_4, respectively) with peanut flour and semolina to develop protein enriched sesame bars. These bars were analysed for physicochemical properties. Water activity, texture, calorific value, mineral profile, microbial examination and sensory evaluation were done at ambient temperature for 90 days. Results showed that water activity decreased from T_0-T_4 with mean values 0.6038-0.4308, respectively. Hardness decreased within treatments from T_0-T_4 with mean values ranges from 966.86 to 211.48 g while, factorability increased from 70.41 to 100.33 mm. Calorific value was also increased with maximum energy value found in T_4 (5355.5Kcal/g) and minimum in T_0 (3445.9Kcal/g). During storage, mold growth was increased from 3.2758CFU/g (T_0) to 3.6008CFU/g (T_4). Sensory evaluation results showed that T_2 gave overall best results having moisture content 4.5%, crude protein 35.73%, crude fat 0.61%, crude fibre 2.14%, total ash 2.44% and nitrogen free extract (NFE) 46.04.

Keywords: protein energy, malnutrition, sesame flour, supplemented flour, energy bars

Introduction

Developing countries are facing the challenges of nutritional problems, protein energy malnutrition and micronutrient deficiencies where fast growing population has resulted in limited supply of nutrients and poor sanitation conditions. The specific maladies such as Kwashiorkor and Marasmus are more prevalent in children characterised by odema, restrictions in protein intake, wasting of body tissues, particularly muscles and subcutaneous fat while in adults protein deficiency results in poor health and limited physical and mental stability (Shakeel *et al.,* 2009).

Sesame (*Sesamum indicium* L.) of the family Pedaliaceae also known as gingely, beniseed, sim-sim and till, is an important annual oilseed crop in tropical countries. It is an important potential candidate for protein supplementation in cereal based foods (Alobo, 2001). The world production of sesame is estimated about 3.66 million tonnes mainly from Asia (2.55 MT) and Africa (0.95 MT). Sesame seed is called "queen of the oil seed crops, due to the high production of edible oil. Most of the sesame seeds are used for extraction of oil (Gandhi and Taimini, 2009).

Peanuts (*Arachis hypogaea* L.), also called groundnuts or earthnuts, is known as "The king of oilseeds". It is mainly grown in tropical and the warmer regions of the temperate zone. It is believed to be the nature's blessing to mankind being highly nutritious, tasty and cheapest food as compared to other nuts i.e., walnuts, almonds, pistachio etc. In Pakistan, it is cultivated on an area of about 50,700 hectares, with 85% in Punjab province of Pakistan (Umar, 2006).

Semolina, a product obtained from wheat (*Triticum durum*) as a result of milling process in which bran and germ are removed. In Europe, finely ground semolina is used along with white flour in equal quantities to make quite dense but very flavourful bread (Palumbo *et al*., 2002). In Pakistan, it is usually used to make various types of sweet dishes. Pasta products, mostly consumed all over the world are conventionally manufactured from durum wheat named semolina, known to be the best raw material suitable for pasta production. It is rich source of carbohydrates (74-77%) and its importance is rising due to its nutritional properties, predominantly low glycemic index (GI). It also has proteins 11-15% but deficient in threonine and lysine (the first and second limiting amino acids), common to most cereals (Abdel-Aal *et al*., 2002). This provides an opportunity for the use of non-traditional raw materials to enhance the nutritional quality of pasta (Chillo *et al*., 2008).

Sesame flour is an innovative, economical and traditional ingredient for the preparation of value added products. Sesame bars will not only fulfill the nutritional require-

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ments of school going children, but also act as a healthy product for all age groups. The rising trends of nutritious meals and snacks has promoted food products that combine convenience and nutrition (Izzo and Niness, 2001).

Food products developed through supplementation of defatted sesame and peanut flour provides balanced amount of amino acids required for human body. The present study therefore, has been designed to develop high energy and nutritious bar as an alternative to conventional snacks using sesames indigenous sources. Sesame flour supplemented high protein and energy food bars were prepared and examined to find out their acceptability through physicochemical analysis and sensory evaluation.

Materials and Methods

Procurement and preparation of raw materials. Sesame cultivar (TH-6, White till) were procured from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan while other materials required for the preparation of sesame bar e.g. peanut, suji (semolina), coconut, edible oil and sugar were procured from local market. All reagents (analytical) were procured from Merck (Merck KGa A, Darmstadt, Germany) and Sigma-Aldrich (Sigma Aldrich, Tokyo, Japan). Sesame seeds were partially defatted by using hydraulic press and ground to flour. Coconut were reduced in size by crushing. Peanuts were converted into peanut flour after roasting and grinding. All flours were packed in polyethylene bags and stored at room temperature for further analysis and utilization.

Analysis of raw materials. Sesame flour along with peanut flour and semolina were analysed in triplicate for moisture content, crude protein, crude fat, crude fibre, ash content and nitrogen free extract (NFE) by following respective methods as described in AACC (2000).

Mineral analysis. Raw materials (sesame flour, peanut flour and semolina) were analysed for mineral profile. Each treatment was analysed for Na, K, Ca, and Fe through flame photometer (Sherwood Scientific Ltd., Cambridge) and atomic absorption spectrophotometer (Varian AA240, Australia) by following procedure of AOAC (2006).

Preparation of sesame flour supplemented food bars. Roasted sesame flour, peanut flour, coconut flour, semolina, oil and sugar were mixed on mild heating for 20-30 mins to make a uniform blend. After cooling sheeting were done followed by cutting into bars maintaining a specific size, shape and thickness. Finally bars were baked at 175°C for 20-25 mins followed by packing in aluminium foil. Sesame flour was supplemented in different proportions (Table 1) with remaining ingredients like peanut flour, suji, coconut, sugar and oil kept constant and all compositions were utilized for the preparation of sesame flour supplemented bars.

Table 1: Experimental treatments used for sesame bars

Treatments	Supplementation of sesame flour (%)
T_0	0
T_1	25
T_2	50
T_3	75
T_4	100

Analysis and shelf life study of bars. Sesame flour supplemented bars prepared from all compositions were stored at room temperature (25-30 °C) and analysed for physicochemical, mineral profile, microbial examination, calorific value and sensory evaluation at 0, 30, 60, and 90 days storage interval and the results are summerized in Table 1-9.

Proximate analysis. Sesame flour supplemented food bars were analysed for moisture content, crude protein, crude fat, crude fibre, ash content and NFE according to respective methods given in AACC (2000).

Water activity. An electronic hygropalm water activity meter (Model Aw-Win, Rotronic, equipped with a Karl-Fast probe) was used for estimating the water activity of the sesame flour supplemented food bars at regular storage intervals. Hygropalm water activity meter was caliberated and sesame bars were analysed (AOAC, 1998).

Texture analysis. Texture of bars was determined at different storage intervals according to the method as described by Rehman and Al-Farsi (2005) with some modifications by using a texture analyser (Model TA-XT) plus Stable Microsystems (Surrey, UK) with 5 kg load cell.

Colour measurement. A hand held colorimeter tristimulus colorimeter (colour Test Meter II, Neohaus Neotec) was used to determine the colour of sesame flour supplemented food bars at regular storage intervals

according to the method described by Rocha and Morais (2003).

Mineral profile. Samples of sesame bars were analysed for mineral profile according to the procedures given in AOAC (2006).

Mold growth. Mold growth was done according to the method as described in method 42-50 (AACC, 2000).

Calorific value. Calorific values of the bars were determined by using oxygen bomb calorimeter (IKA-WERKE, C2000 Basic) as described by Krishna and Ranjhan (1981).

Sensory evaluation. Sesame flour supplemented food bars were evaluated for sensory characteristics such as colour, flavour, texture, crispiness, chewability and overall acceptability at room temperature (i.e. 25-30°C) for a storage period of three months in a sensory evaluation laboratory by a panel of five judges on 9-point Hedonic scale (Meilgaard *et al.,* 2006).

Statistical analysis. The results obtained for each parameter were subjected to statistical analysis to determine the level of significance according to the methods described by Steel *et al.* (1997)

Results and Discussion

Raw materials analysis. *Proximate analysis.* Defatted sesame flour subjected to proximate composition (Table 2) indicated that it comprises of moisture 2.19 ± 0.02%, crude protein 51.5 ± 0.4%, crude fibre 3.45±0.03%, ash 6.14 ± 0.09%, crude fat 1.49 ± 0.03% and 45.56 ± 0.19 NFE. Onsaard *et al.* (2010) reported that defatted sesame flour contains moisture 2.19%, crude protein 50.45%, crude fibre 3.46%, ash 6.15%, crude fat 1.49% and NFE 45.56. These results were in conformity with the composition of defatted sesame flour assessed.

Peanuts (*Arachis hypogaea* L.) under proximate analysis showed moisture 4.5 ± 0.03%, crude fat 46.18 ± 0.13%, crude protein 30.59 ± 0.31%, crude fibre 3.87 ± 0.08%, ash 2.72 ± 0.21% and NFE 9.64 ± 0.27%. The earlier findings of Atasie *et al.* (2009) were found in accordance with the composition results who reported that peanut contains crude fat 45%, crude protein 36%, moisture 6.1%, crude fibre 3.25%, ash 2.9% and NFE 6.75%.

Chemical composition of semolina revealed that, it contains moisture 11.22 ± 0.06%, crude protein 13.86 ± 0.17%, crude fat 0.2 ± 0.01%, ash 0.82 ± 0.05%, crude fiber 0.7 ± 0.02% and NFE 73.2 ± 0.15%. The composition of semolina was found in concurrence with the earlier findings of Hussein *et al.* (2011), who reported that semolina contains moisture 12 %, crude protein 14%, crude fat 0.4%, ash 0.9%, crude fibre 0.6% and NFE 73.6%.

Mineral analysis. Defatted sesame flour subjected to mineral composition (Table 2) indicated that, it comprises of K (385 ± 0.3 mg/100g), Na (7.63 ± 0.9 mg/100g), Fe (6.19 ± 0.21 mg/100g) and Ca (20.3 ± 0.9 mg/ 100g). Hahm *et al*. (2009) reported that, defatted sesame flour contains K (382mg/100g), Na (7.60 mg/100g), Fe (6.17 mg/100g) and Ca (420 mg/ 100g). These results were in conformity with the composition of defatted sesame flour assessed.

Peanuts (*Arachis hypogaea* L.) under mineral analysis showed that, they contain K (38.5 ± 0.4 mg/100g), Na (22.6 ± 0.6 mg/100g), Fe (0.8 ± 0.03 mg/100g) and Ca (21.4 ± 0.72 mg/100g). Findings of Vincent *et al*. (2009) were in accordance with the composition results, who

Table 2: Proximate composition and mineral analysis of raw materials

Raw materials	Proximate composition (%)					
	Moisture	Protein	Fat	Fibre	Ash	NFE
Defatted sesame flour	2.19±0.02	51.5±0.4	1.49±0.03	3.46±0.03	6.15±0.09	45.56±0.19
Peanut flour	4.5±0.03	30.59±0.31	46.18±0.13	3.87±0.08	2.72±0.21	9.64±0.27
Semolina	11.22±0.06	13.86±0.17	0.2±0.01	0.7±0.02	0.82±0.05	73.2±0.15
Raw materials	Mineral profile (mg/100g)					
	Potassium (K)	Sodium (Na)	Iron (Fe)	Calcium (Ca)	-	-
Defatted Sesame flour	385±0.3	7.63±0.9	6.19±0.21	20.3±0.9	-	-
Peanut flour	38.5±0.4	22.6±0.6	0.8±0.03	21.4±0.72	-	-
Semolina	3.20±0.6	8.53±0.3	41.5±0.45	278±0.52	-	-

NFE = nitrogen free extract.

reported that peanut contains K (38.1 mg/100g), Na (22.0 mg/100g), Fe (0.78 mg/100g) and Ca (21.1 mg/ 100 g).

Mineral composition of semolina revealed that it contains, K (3.20 ± 0.6 mg/100g), Na (8.53 ± 0.3 mg/100g), Fe (41.5 ± 0.45 mg/100g) and Ca (278 ± 0.52 mg/ 100g). The mineral composition of semolina was found inaccordance with the earlier findings of Cubadda *et al*. (2009), accordance who reported that semolina contains K (3.18 mg/100g), Na (8.51 mg/100g), Fe (41.1 mg/100g) and Ca (276 mg/100g).

Moisture content. Moisture of the sesame bars was observed to be highly significant among the treatments. These formulations of sesame bars contained different concentrations of defatted sesame flours at regular storage intervals and ambient temperature were statistically analysed for moisture content ranging from 5.63 ± 0.02 to 3.65 ± 0.05% Table 3. The mean values for moisture content of various sesame bars treatments reveal the peak score for T_0 and the minimum score for T_4. During storage period, the moisture difference was significantly high as well. Having the highest moisture content at 0 day and gradually decreasing to 90 days where it was the lowest, the mean values for storage ranged from 4.73 ± 0.71 to 4.17 ± 0.56% (Table 4) similar to the results in water activity of the sesame bars. There was a steady decrease in moisture content with increasing concentration of defatted sesame flour in treatments. Decrease in the moisture of the sesame bars was vastly significant, possibly due to desertion of water from the products, as a result of two main factors i.e. increased temperature during the hot weather and exposure to the atmosphere at times. Analogous to the case in water activity, moisture content in the last two treatments T_3 and T_4 containing higher concentration of defatted sesame flour showed greater reduction in moisture as compared to treatments containing lower concentration of defatted sesame flour i.e. T_1 and T_2. It could be possible due to the presence of oil in T_3 and T_4 which does not have the tendency to hold water. Also there was highly significant effect of treatment and storage interaction on moisture contents of sesame bars. Outcomes of moisture fluctuations during storage for various treatments is in agreement with the results of Gandhi and Taimini (2009) that moisture content decreased significantly in cereal nut bars from 7.75-6.39% during storage of 30 days at ambient temperature. Results were contradictory to those of Estevez *et al*. (1998), who reported that moisture content remained similar i.e., 7.6-9.6% with no significant storage change in cereal nut bars during a storage period of 90 days at 18-20°C. Low temperature could be the possible reason for constant moisture content during the storage period.

Table 3: Effect of treatments on proximate composition and mineral profile of sesame bars

Treatments	Proximate analysis (%)					
	Moisture	Protein	Fat	Fibre	Ash	NFE
T_0	5.63±0.02 a	33.69± 0.12e	0.64±0.13 c	1.93±0.81 d	2.67±0.03 b	46.04±0.61 c
T_1	4.99±0.07 b	35.57±0.23d	0.61±0.08 d	1.86±0.71 e	2.13±0.08 e	44.85±0.53 e
T_2	4.53±0.04 c	35.73±0.15 c	0.61±0.09 d	2.14±0.07 c	2.44±0.03 d	46.04±0.43 c
T_3	3.73±0.09 d	35.90±0.17 b	0.77±0.11 b	2.34±0.21 b	2.62±0.09 c	46.93±0.27 b
T_4	3.65±0.05 d	36.19±0.23 a	0.97±0.05 a	2.54±0.31 a	3.43±0.11 a	49.17±0.21 a
	Mineral Profile (mg/100g)					
	Potassium	Sodium	Iron	Calcium		
T_0	384.48±9.21 d	46.46±0.52 c	10.22±0.04 a	61.59±2.34 b		
T_1	362.03±8.26 e	44.11±0.91 d	7.49±0.06 b	55.65±1.56 d		
T_2	486.75±5.41 c	38.20±0.74 e	7.90±0.06 b	52.86±3.56 e		
T_3	511.69±8.19 b	48.77±0.75 b	6.48±0.02 c	60.26±2.45 c		
T_4	555.96±6.53 a	51.28±0.48 a	5.35±0.08 d	67.27±1.78 a		

Means carrying same letters in a column for each factor do not differ significantly; NFC= nitrogen free extract; T_0= 0% supplementation of defatted sesame flour; T_1= 25% supplementation of defatted sesame flour; T_2= 50% supplementation of defatted sesame flour; T_3= 75% supplementation of defatted sesame flour; T_4= 100% supplemen-tation of defatted sesame flour.

Crude protein. Protein content differed significantly along the various treatments of the sesame bars according to the statistical results. Table 3 demonstrates the statistical analysis for protein content of different treatments of sesame bars containing assorted concentrations of defatted sesame flours at storage intervals (0, 30, 60 and 90 days). Mean values for protein contents of sesame bars samples ranged from 33.69 ± 0.12 to 36.19 ± 0.23 %, with T_0 having the least protein content and T_4 with the highest protein score. Gradual increase in protein contents with increasing concentrations of defatted sesame flour in treatments was evident from the results. The mean values for treatments also reveal that treatments T_3 and T_4 have significantly higher protein content due to increased quantity of defatted sesame flour used. A non significant effect of storage on protein contents of sesame bars was calculated by the mean values for storage ranged from 35.35 ± 0.65 to 35.48 ± 0.24 % during 90 days storage period as illustrated in Table 4. There were non-significant effect of treatment and storage interaction on protein contents of sesame bars.

Variation in protein content during storage for various treatments is in conformity with the findings of Khalil (1986), who reported that in sesame bars fortified with almonds, skim milk powder, soy protein isolate and single cell proteins, protein content was increased from 4.9-5.3% in the control to 10.7-12.1% in samples containing the high protein ingredients. These supplemented sesame bars not only increased protein content but also possess significantly higher chemical scores and essential amino acids. The protein content changed non-significantly in legume and vegetable based soup powder from 19.35%-19.45% according to Rokhshana *et al*. (2007) during storage of 6 months. Protein results obtained in the present study are compatible with these findings.

Crude fat. Different treatments of sesame bars containing varied concentrations of defatted sesame flour at regular storage intervals at ambient temperature were statistically analysed for fat content and are presented in Table 3. The results in the variation in fat content among different treatments are highly significant. Treatment T_4 had the maximum score where as T_0 having the least score was deducted by the mean values for treatments which ranged from 0.64 ± 0.13-0.97 ± 0.05 %. The mean value for fat contents of sesame bars samples have been presented in Table 3. Evidently from the results, it is affirmed that there was a gradual increase in fat contents with increasing concentration of defatted sesame flours in all the treatments but obviously T_3 and T_4 had more fat content due to the manual inclusion of fat in them used for frying. There was highly significant effect of storage days on fat contents of sesame bars where the mean values ranged from 0.63 ± 0.03 to 0.77 ± 0.03% in Table 4. There was highly significant effect of treatment and storage interaction on fat contents of sesame bars. Similar findings were obtained by Goni and Gamazo (2002) who reported that fat content increased from 1.81-2.60% in wheat pasta after incorporation of chickpea flour in it.

Table 4: Effect of storage on the proximate and mineral profile of sesame bars

Storage intervals	Proximate analysis (%)					
	Moisture	Protein	Fat	Fibre	Ash	NFE
S_0	4.73±0.71 a	35.35±0.65 d	0.63±0.03 d	2.09±0.92 d	2.49±0.72 d	46.01±3.12 d
S_{30}	4.63±0.67 ab	35.40±0.24 c	0.67±0.03 c	2.15±0.78 c	2.65±0.91 c	46.36±2.15 c
S_{60}	4.52±0.24 b	35.44±0.45 b	0.71±0.06 b	2.20±0.82 b	2.73±0.76 b	46.76±4.63 b
S_{90}	4.17±0.56 c	35.48±0.24a a	0.77±0.03 a	2.24±0.56 a	2.79±0.34 a	47.07±2.96 a
	Mineral Profile (mg/100g)					
	Potassium	Sodium	Iron	Calcium		
S_0	459.59±14.24 c	45.69±3.93 d	8.09±0.72 a	59.45±3.67 d		
S_{30}	459.74±18.96 c	45.74±1.46 c	8.01±0.56 a	59.50±1.95 c		
S_{60}	460.47±15.78 b	45.79±2.56 b	6.46±0.73 c	59.56±2.83 b		
S_{90}	460.93±17.93 a	45.85±3.72 a	7.42±0.92 b	59.62±1.57 a		

Means carrying same letters in a column for each factor do not differ significantly; NFE = nitrogen free extract; S_0 = 0 day; S_{30} = 30 days; S_{60} = 60 days; S_{90} = 90 days.

Crude fibre. Fibre content differentiation was highly significant among various treatments of the sesame bars according to the statistical results. Table 3 demonstrates the statistical analysis for fibre content of different treatments of sesame bars containing assorted concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days). The mean values of fibre contents for treatments, ranged from 1.93 ± 0.81 to 2.54 ± 0.31% having the lowest score for T_0 and the highest score in case of T_4. There was a gradual increase in fibre contents with increasing concentrations of defatted sesame flour in treatments according to the results. Treatments T_3 and T_4 (2.34 ± 0.21 and 2.54 ± 0.31%) due to increased quantity of defatted sesame flour, showed higher results of fibre percentage as compared to T_1 and T_2 (1.93 ± 0.81 and 1.86 ± 0.71). Higher dry matter in the sesame bars was seen by the mean values of fibre contents for storage periods ranged from 2.09 ± 0.92 to 2.24 ± 0.56% from 0 to 90 days in Table 4. There was significant effect of treatment and storage interaction on fibre content of sesame bars. The change of fibre during storage for various treatments is in conformity with the findings of Rokhsana *et al*. (2007) who reported that fibre content changed non-significantly in legume and vegetable based soup powder from 0.65-0.70% during storage of 6 months.

Ash content. The analysis of variance for ash contents of different treatments of sesame bars containing varied concentrations of defatted sesame flours at regular intervals showed that the difference in ash contents among different treatments is highly significant (Table 3). The mean values of ash contents for treatments ranged from 2.67 ± 0.03 to 3.43 ± 0.11% having the lowest ash contents for T_0 and the highest ash content in T_4. There was a gradual increase in ash contents with increasing concentrations of defatted sesame flour in treatments and results show that treatments T_3 and T_4, due to increased quantity of defatted sesame flour, showed higher results of ash percentage (2.62±0.03 to 3.43 ± 0.11%) as compared to T_1 and T_2 (2.13±0.08 to 2.44 ± 0.03%) as depicted in Table 3. This could also be due to higher dry matter in the sesame bars of T_3 and T_4. Regarding the dry matter i.e. defatted sesame flour in the sesame bars, ash content is directly related to the fibre content of the sesame bars. There was a highly significant effect of storage days on ash contents of sesame bars. The mean values of ash contents for storage period ranged from 2.49 ± 0.72 to 2.79 ± 0.34% for 0 to 90 days (Table 4). There was a highly significant effect of treatment and storage interaction on ash contents of sesame bars. Results are also in accordance with Gandhi and Taimini (2009) who reported that ash contents were not affected by storage conditions in sesame bars.

Nitrogen free extract (NFE). The data presented in Table 3 showed that NFE among treatments and during storage were highly significant. The interaction of treatment and storage was also highly significant. In Table 3 the mean values of NFE of different treatments showed that minimum NFE was found in T_0 that was 46.04 ± 0.61 and maximum was found in T_4 which was 49.17 ± 0.21. The mean values of NFE contents of supplemented flour showed in Table 4 at 0, 30, 60 and 90 days interval were 46.01 ± 3.12, 46.36 ± 2.15, 46.76 ± 4.63 and 47.07 ± 2.96. The interaction of storage period into treatments showed that the highest NFE value 49.17 ± 0.21 was found in T_4 and the lowest NFE value 46.04 ± 0.61 was observed in T_0 at 90 days of storage period. With the passage of time NFE increased significantly. This is due to increase in fat and protein content. The increasing trend in flours during storage is also observed by Gandhi and Taimini (2009).

Physical characteristics of sesame bars. ***Water activity.*** Water activity fluctuated significant during storage days, ranging the mean values from 0.53 ± 0.08 to 0.49 ± 0.67 for 0 to 90 days, respectively, in Table 7. Water activity among all the treatments naturally decreased due to the decrease in moisture content during storage, as a result of high temperature in the summer. Comparing the defatted sesame flour treatments T_3 and T_4 (0.46 and 0.43) with treatments T_0 and T_1 (0.60 and 0.55), possibly reveal that T_3 and T_4 have lower water activity because of inclusion of vegetable oil in the treatments which could be helpful in binding the water present in the sesame bars. There was a highly significant effect of treatment and storage interaction on water activity of defatted sesame flour bars.

During storage the variation of water activity in various treatments is in compliance with the findings of Estevez *et al*. (1998) in the cereal and nut bars, who informed that water activity significantly reduced in the bars from 0.71-0.52 during storage of 60 days. Similar results were obtained in Amaretti cookies by Piga *et al*. (2005) who reported water activity 0.54 at the start which progressively decreased to 0.40 after storage of 35 days at ambient temperature.

Texture. Two properties of texture were observed i.e., initially hardness of the bars which is described in terms

of the maximum force (g) and fracturability in terms of distance (mm) necessary for the texture analyzer probe to travel through the bars. Reflecting significant differences in the hardness and fracturability of the various sesame bars, the statistical analysis of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) are given in Table 7. The mean values of hardness for treatments ranged from 966.86-211.48 g, having the lowest mark for T_0 and the maximum mark in case of T_4 (Table 7) and fracturability ranged from 70.41-100.33 mm having the least score for T_0 and the highest rank in case of T_4. Increasing concentrations of defatted sesame flour in various treatments apparently resulted in a gradual increase in hardness and fracturability. Hardness of sesame bars increased from 0-30 days progressively. This trend gradually decreased from 60-90 days thereby, decreasing hardness. On the other hand fracturability increased in smooth way from 0-90 days and a relationship with hardness was observed, concluding a highly significant effect of storage days on texture of sesame bars. In general, this might be due to the moisture loss to the atmosphere from the sesame bars but treatments T_3 and T_4 were harder in texture due to cooking treatment which hardened the texture due to excess moisture loss. Thereby, there was a highly significant effect of treatment and storage on hardness and fracturability of sesame bars. The variation of texture during storage for various treatments is in conformity with the hardness that significantly changed rather decreased in Amaretti cookies from 59-383 N during storage of 35 days (Piga *et al*., 2005).

Colour measurement. At regular storage intervals (0, 30, 60 and 90 days) under ambient temperature, the colour values of various treatments of sesame bars containing different concentrations of defatted sesame flour were statistically analysed and are given in Table 7. The observed results obviously reveal that the differences in colour values are highly significant amongst different treatments. The treatment T_0 having the lowest score and the highest score in case of T_4 were increased from the range of mean values of colour from 87.00-180.00 CTn in the treatments. The means for colour values of sesame bars samples have been presented in Table 7. Results apparently show that there was a continual increase in colour values with increased concentrations of defatted sesame flour addition in the bars i.e., greater the amount of defatted sesame flour, higher the colour value. The mean values for storage phase ranged from 125.60 ± 6.21 to 155.60 ± 7.23 CTn for 0-90 days, respectively, showing that there was a highly significant effect of storage on colour of sesame bars as shown in Table 7. The treatments T_3 and T_4 containing greater amounts of defatted sesame flour have significantly higher colour values 141.00-180.00 CTn as compared to T_0 and T_1 containing lesser amount of depatted sesame flour therefore, having lower colour values 87.00-143.00 CTn, which is due to the increase in the quantity of defatted sesame flour used in T_3 and T_4. The results of treatment and storage periods interaction on colour of sesame bars is non-significant. The colour of the bars during storage could be influenced by the Maillard reactions, leading to darken the sesame bars with the passage of time.

Comparing the results with the earlier deductions illustrate that the alteration of colour during storage for various treatments is in conformity with the findings of McMahon *et al*. (2002) who reported that during storage period of 42 days colour changed significantly in high whey protein nutritious bars from 54.73 to 70.30, as the bars became darken in colour during storage depending upon the Maillard reactants.

Mineral profile of sesame bars. ***Potassium content.*** Potassium is an important intracellular cation in the body that plays a vital role in the maintenance of energy metabolism, cell membrane potential and membrane transport of other ions. Because of its role in these processes, optimum potassium intake is vital for the contraction of muscle groups such as the heart.

Potassium content difference was highly significant amongst several treatments on statistical analysis for potassium content of the sesame bars treatments containing different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) as shown in Table 3. The mean values for treatments in Table 3 ranged from 384.48 ± 9.21 mg/100g having the least potassium content for T_0 to 555.96 ± 6.53 mg/100g the highest potassium content in case of T_4. Results reveal that there was a gradual increase in potassium content with increasing concentrations of defatted sesame flour among treatments and T_3 and T_4 have a greater potassium content score (511.69 ± 8.19 and 555.96 ± 6.53 mg/100g) as compared to T_0 and T_1 (384.48 ± 9.21 and 362.03 ± 8.26 mg/100g). A highly significant effect of storage on potassium content of sesame bars was observed by the mean values for storage ranged from 459.59 ± 14.24 to 460.93 ± 17.93 mg/100g from 0 to 90 days, respectively, in Table 4. Effect of

treatment and storage interaction on potassium content of the sesame bars was non-significant, more likely due to its metabolizing disability, which in turn shows no alteration in the potassium content during storage. Change in potassium content during storage for various treatments is in conformity with the findings of (Ismail *et al*., 2008) who reported that potassium content changed non-significantly in cereal and nuts bars during 1 year storage period at room temperature.

Sodium content. Sodium content of different treatments of sesame bars when subjected to statistical analysis reveals a highly significant effect of these treatments on the sodium content of these bars containing different concentrations of defatted sesame flour at ambient temperature at regular storage intervals (0, 30, 60 and 90 days). It is elaborated in Table 3 along with the mean values of sodium content for treatments that the maximum marks 51.28 ± 0.48 mg/100g is in case of T_4 and the least score 46.46 ± 0.52 mg/100g is for T_0. Results reveal that with increasing concentrations of defatted sesame flour among treatments, there was a steady increase in sodium content. The mean values for storage ranging from 45.69 ± 3.93 to 45.84 ± 3.72 mg/100g from 0-90 days, respectively, showed highly significant effect of storage on sodium content of sesame bar in Table 4. Also the effect of treatment and storage interaction on sodium content of sesame bars was significant. The results are in accordance with Ismail *et al.* (2008) that treatments and storage interaction are highly significant.

Iron content. Iron is an essential element and its physiological losses must be compensated regularly (Yip and Dallman, 1996). Iron content difference was highly significant on statistical analysis of various treatments of sesame bars containing different concentrations of defatted sesame flour at storage intervals (0, 30. 60 and 90 days) at ambient temperature. Table 3 elaborates the mean values of iron content for treatments ranging from the highest score 10.22 ± 0.04 mg/100 g in case of T_0 and the lowest score 5.35 ± 0.08 mg/100 g for T_4. A highly significant effect of storage on iron content of sesame bars was observed by the mean values for storage ranging from 8.09 ± 0.72 to7.42 ± 0.92 mg/100 g at 0-90 days, respectively (Table 4). Effect of treatment and storage interaction on iron content was also highly significant. The iron content changed significantly in legume and vegetable based soup powder from 26.75-25.77 mg/100 g during 6 months storage period at room temperature in the findings of Rokhsana *et al*. (2007) which happen to be similar to the iron content variations in the sesame bars during storage.

Calcium content. Calcium content varied highly significant amongst the various treatments in conclusion to the statistical analysis different treatments of sesame bars containing varied concentrations of defatted sesame flour as illustrated in Table 3, at regular storage intervals (0, 30, 60 and 90 days). Table 3 presents the mean values of calcium content for treatments ranging from 61.59 ± 2.34 to 67.27 ± 1.78 mg/100 g, having the lowest calcium content for T_0 and the highest in case of T_4. A gradual increase in calcium content, owing to increasing concentrations of defatted sesame flour among treatments is evident from the results. The mean values for storage ranged from 59.45 ± 3.67 to 59.62 ± 1.57mg/100 g from 0-90 days, respectively, in Table 4. There was highly significant effect of storage and significant effect of treatment-storage interaction on calcium content of sesame bar. Calcium content changed during storage for sesame bars treatments is in conformity with the findings of Rokhsana *et al*. (2007) who reported that calcium content changed non-significantly in legume and vegetable based soup powder from 65.193-69.103mg/100g sample during 6 months storage at room temperature.

Mold growth. With reference to the statistical analysis in Table 5, results obviously demonstrate that highly significant differences were observed in the mold count of various treatments of sesame bars with different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) at room temperature. As presented in Table 5, the highest mean values of mold count was $7.63\times10^2 \pm 3.6$ CFU/g for T_4 and the least was $3.275\times10^2 \pm 2.76$ CFU/g for T_0. The results show that an increase in mold count was not related to increasing concentrations of defatted sesame flour in treatments T_3 and T_4 show higher mold growth (3.160 and 3.600×10^2) as compared to treatments T_0 and T_1 ($3.275\times10^2 \pm 2.76$ and $6.65\times10^2 \pm 2.99$) . This can possibly be due to the cooking treatment in T_0 and T_1 which undoubtedly perishes the mold present up to an extent. Highly significant effect of storage and storage treatment interaction on mold count of sesame bars are deducted from the mean values for storage change periodically from $0.282\text{-}7.065\times10^2 \pm 0.02$ CFU/g at 0 and 90 days, respectively. Thereby increased the mold growth significantly during storage for 3 months at ambient temperature but within tolerable limitations.

Table 5: Effect of treatments and storage on mold count (CFU/g) of sesame bars

Treatments	Mold count (CFU/g) storage intervals				
	S_0	S_{30}	S_{60}	S_{90}	Means
T_0	0.28±0.02	0.95±0.04	4.91±0.34	6.95±0.13	3.27±2.76 d
T_1	0.22±0.04	0.50±0.06	4.61±0.12	6.65±0.42	6.65±2.99 c
T_2	0.41±0.03	1.51±0.02	5.13±0.24	7.14±0.31	3.55±2.71 e
T_3	0.11±0.02	0.65±0.04	4.93±0.31	6.94±0.17	6.94±2.87 b
T_4	0.38±0.01	0.79±0.07	5.59±0.42	7.63±0.24	7.63±3.6 a
Means	0.28±0.11 c	0.88±0.35 c	5.03±0.32 b	7.06±0.32 a	

Means carrying same letters in the column do not differ significantly; Means carrying same letters in the row do not differ significantly; CFU = colony forming unit.

The varied increase in mold growth during storage for different treatments is in consistency with the findings of Al-Hooti *et al*. (1997b), who accounted that mold growth increased significantly in all treatments during 6 months storage ranging from 2.60-3.00×10^2 CFU/g of sesame bar samples.

Calorific value. With reference to the statistical analysis in Table 6, results obviously demonstrate that highly significant differences were observed in the calorific value of various treatments of sesame bars with different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) at room temperature. The highest mean value of gross energy was 5355.5 ± 336.9 Kcal/g for T_4 and the least was 3445.9±312.4 Kcal/g for T_0 (Table 6). The results gives the evidence that an increase in calorific value was not related to increasing concentrations of defatted sesame flour in treatments T_3 and T_4 (4488.2±207.6 Kcal/g and 5355.5±336.9 Kcal/g) show higher gross energy as compared to treatments T_0 and T_1 (3445.9±312.4 Kcal/g and 3602.4±291.3 Kcal/g). Highly significant effect of storage and storage-treatment interaction on gross energy of sesame bars are deducted from the mean values for storage change periodically from 3813.0±691.2 Kcal/g to 4484.7±725.6 Kcal/g at 0 and 90 days, respectively. Increase in gross energy, is thereby highly significant, during storage for 3 months at ambient temperature. Results are in accordance with Gandhi and Taimini (2009) who reported that gross energy increased in storage conditions in sesame bars.

Sensory evaluation of sesame bars. Sensory evaluation happens to be the most essential part of the product development and assessment, which reveals the consumer preferences at the initial stages of developing an innovative product like the defatted sesame flour supplemented food bars. It was carried out to evaluate the response of judges towards the product and their likings were recorded on a hedonic scale. Sesame bars were assessed for colour, flavour, texture, crispiness, chewability and overall acceptability during 90 days of storage periods after every 30 days interval.

***Colour*.** Colour reveals the first impression of a food product before consumed. It is the first score of a likeable and disliked food commodity. Results depict that the

Table 6: Effect of treatments and storage on calorific value of sesame bars

Treatments	Energy value (Kcal/g) storage intervals				
	S_0	S_{30}	S_{60}	S_{90}	Means
T_0	3059.4±54.12	3265.6±38.72	3574.5±27.24	3884.3±30.67	3445.9±312.4 e
T_1	3221.7±36.85	3444.9±32.92	3760.1±29.86	3983.0±52.49	3602.4±291.3 d
T_2	3611.6±48.92	3732.8±29.85	3848.9±31.76	3990.1±42.24	3795.8±140.0 c
T_3	4243.2±62.06	4353.0±31.67	4573.7±41.34	4782.8±30.82	4488.2±207.6 b
T_4	4929.1±49.92	5143.5±29.34	5565.7±43.97	5783.6±28.78	5355.5±336.9 a
Means	3813±691.2 d	3988±685.6 c	4264.6±733.7 b	4484.7±725.6 a	

Means carrying same letters in the column do not differ significantly; Means carrying same letters in the row do not differ significantly.

colour score differed highly significant among different treatments. These treatments of sesame bars containing varied concentrations of defatted sesame flours at regular intervals of storage (0, 30, 60 and 90 days) at ambient temperature were statistically analysed for colour evaluation as shown in Table 8. The results pertaining to mean score for the sesame bars in Table 8 revealed that T_2 (7.85 ± 0.13) was most preferred by judges regarding colour followed by T_1 (6.32 ± 0.14). This could be due to the acceptable range of defatted sesame flour addition in the two treatments. Though remaining treatments got fewer score, yet they were acceptable having a reduced colour score in T_0 (5.63 ± 0.17) and T_4 (5.40 ± 0.12), a highly significant effect of storage on colour score of sesame bars. Communally, it was observed that sesame bars were more readily accepted having the maximum scores which were decreased from 5.92 ± 0.92 to 6.58 ± 0.35 after 90 days storage (Table 9). The decrease in colour score might possibly be due to non enzymatic browning within storage period. There was a highly significant effect of treatment and storage interaction on colour score of sesame bars. A decrease in colour score from 6.8-6.2 during storage of 6 months in sesame bars was recounted by Al-Hooti *et al.* (1997b), whose findings were in concurrence with the changes in colour of sesame bars during their storage period. The colour of sesame bars significantly affected by storage periods, according to Ahmed and Ramswamy (2005). They illustrated that colour score varied from 6.6-6.2 during storage of 6 months at ambient temperature.

Flavour. Flavour is combination of taste and aroma which as a matter of fact compiles the acceptability of food product under a single sensory attribute. Flavour differed highly significant amongst different treatments on their statistical analysis as shown in Table 8, containing different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days). The results concerning to mean score for the sesame bars presented in Table 8, revealed that T_2 (8.40 ± 0.47) was favoured by the panel of judges among the sesame bars treatments, followed by T_0 (6.25 ± 0.82) regarding flavour. This could be due to the satisfactory proportions of defatted sesame flour in the two treatments. Though remaining treatments got fewer score, yet they were acceptable having a reduced flavour score in T_1 (5.37 ± 0.72) and T_4 (5.4 ± 0.12). There was a highly significant effect of storage and significant effect of treatment-storage interaction on flavour of sesame bars. Collectively, the highest scores were observed in fresh sesame bars that gradually increased from 5.82 ± 0.53 to 6.56 ± 0.43 after 90 days storage presented in Table 9. A decrease in flavour from 6.8-6.3 storage of 6 months in sesame bars was observed by Al-Hooti *et al.* (1997b) which was in accordance to the change of flavour during

Table 7: Effect of storage on physical analysis of sesame bars

Storage intervals	Sensory evaluation parameters			
	Water activity (Aw)	Hardness (g)	Fracturability (mm)	Colour (CTn)
S_0	0.5363±0.08 a	645.17±15.58 a	76.707±3.21 d	125.60±6.21 d
S_{30}	0.5315±0.03 b	517.80±20.14 b	85.487±3.53 c	135.60±5.93 c
S_{60}	0.5013±0.75 c	407.74±18.93 c	89.765±6.21 b	145.60±2.56 b
S_{90}	0.4905±0.67 d	388.14±22.12 d	99.223±2.86 a	155.60±7.23 a

Means carrying same letters in a column for each factor do not differ significantly.

Table 8: Effect of treatments on sensory evaluation of sesame bars

Treatment	Sensory evaluation parameters					
	Colour	Flavour	Texture	Crispiness	Chewability	Overall acceptability
T_0	5.63±0.17 d	6.25±0.82 b	5.38±0.09 e	5.91±0.45 d	5.62±0.23 b	5.80±0.24 d
T_1	6.32±0.14 b	5.37±0.72 d	6.40±0.03 b	6.36±0.36 c	5.50 ±0.13cd	6.40±0.23 b
T_2	7.85 ±0.13a	8.40±0.47 a	7.67±0.05 a	7.92±0.25 a	8.42±0.72 a	7.93±0.12 a
T_3	6.07±0.18 c	5.60±0.13 c	5.50±0.07 d	6.55±0.43 b	5.45±0.23 d	5.95±0.82 c
T_4	5.40±0.12 e	5.37±0.09 d	5.65±0.04 c	5.70±0.28 e	5.57±0.23 bc	5.32±0.64 e

Means carrying same letters in a column for each factor do not differ significantly

Table 9: Effect of storage on sensory evaluation of sesame bars

Storage intervals	Sensory evaluation parameters					
	Colour	Flavour	Texture	Crispiness	Chewability	Overall acceptability
S_0	5.92±0.92 d	5.82±0.53 d	5.72±0.21 d	6.10±0.72 d	5.83±0.67 d	5.96±0.27 d
S_{30}	6.14±0.83 c	6.10±0.32 c	6.00±0.92 c	6.35±0.66 c	6.01±0.54 c	6.16±0.51 c
S_{60}	6.38±0.43 b	6.32±0.46 b	6.24±0.25 b	6.63±0.87 b	6.25±0.24 b	6.40±0.72 b
S_{90}	6.58±0.35 a	6.56±0.43 a	6.52±0.92 a	6.88±0.44 a	6.36±0.33 a	6.60±0.45 a

Means carrying same letters in a column for each factor do not differ significantly

storage of various sesame bars samples. (Ahmad and Ramaswany, 2005) reported that storage periods have significant effect on flavour of fruit bars. It was recorded that, flavour acceptability varied from 7.1-6.9 during storage at ambient temperature.

***Texture*.** Texture of sesame bars when statistically analysed, showed highly significant difference amongst different treatments containing different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) at ambient temperature signified in (Table 8). The mean score findings for texture of the sesame bars given in Table 8 revealed that T_2 (7.67 ± 0.05) was favoured by the judges followed by T_1 (6.40 ± 0.03), which could probably be owing to increased amount of defatted sesame flour in a greater quantity in T_2 and T_1 has given a better compact texture to the sesame bars which may be due to lesser moisture content in the treatments as compared to the T_3 and T_4. Significant texture rating in T_3 (5.50 ± 0.07) and T_4 (5.65 ± 0.04) were collected but they were favourably within satisfactory confines. There was a highly significant effect of storage on texture of sesame bars as well. In general, the maximum scores observed have been presented in Table 9, fresh sesame bars but highly significant effect on storage thus gradually increased them from 5.72 ± 0.21 to 6.52 ± 0.92. There was non-significant effect of treatment and storage interaction on texture of sesame bars. A significant decrease in texture from 6.9-5.9 during storage of 6 months was reported by Al-Hooti *et al.,* (1997b) which happened to be in conformity with variations in the texture of the sesame bars.

***Crispiness*.** Crispiness of sesame bars when statistically analysed showed highly significant difference amongst different treatments containing different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) at ambient temperature signified in Table 8. The mean score findings for texture of the sesame bars in Table 8 reveal that T_2 (7.92 ± 0.25) was favored by the judges followed by T_3 (6.55 ± 0.43), which could probably be owing to increased amount of defatted sesame flour in a greater quantity in T_2 and T_3 has given a better compact crispiness (7.92±0.25 and 6.55±0.43) to the sesame bars which may be due to lesser moisture content in the treatments as compared to the T_1 and T_4 (6.36 ± 0.36 and 5.70 ± 0.28). Significant crispiness rating in T_1 (6.36 ± 0.36) and T_4 (5.70 ± 0.28) were collected but they were favourably within satisfactory confines. There was a significant effect of storage on crispiness of sesame bars as well. In general, the maximum scores were observed in fresh sesame bars but highly significant effect on storage thus gradually increased them from 6.10±0.72 to 6.88±0.44 (Table 9). There was non-significant effect of treatment and storage interaction on crispiness of sesame bars. A significant increase in crispiness from 5.5-6.7 during storage of 6 months was reported by Al-Hooti *et al.* (1997b) which happened to be in conformity with variations in the crispiness of the sesame bars.

***Chewability*.** Chewability of sesame bars when statistically analyzed showed highly significant difference amongst different treatments containing different concentrations of defatted sesame flour at storage intervals (0, 30, 60 and 90 days) at ambient temperature signified as in Table 8. The mean score findings for texture of the sesame bar in Table 8 reveal that, T_2 (8.42 ± 0.72) was favuored by the judges followed by T_0 (5.62 ± 0.23), which could probably be owing to increased amount of defatted sesame flour in T_2 and T_0 (8.42 ± 0.72 and 5.62±0.23) has resulted a compact chewability to the sesame bars due to lower moisture content in the treatments as compared to the T_1 and T_4 (5.50±0.13 and 5.57±0.23). Significant chewability rating in T_1 (5.50±0.13) and T_4 (5.57 ± 0.23) were collected but they were favourably within satisfactory confines. There was a highly significant

effect of storage on chewability of sesame bars as well. In general, the maximum scores were observed in Table 9, fresh sesame bars but highly significant effect on storage resulted from 5.83 ± 0.67-6.36 ± 0.33. There was significant effect of treatment and storage interaction on overall acceptability of sesame bars.

The findings of Al-Hooti *et al.* (1997b), showed significant increases in chewability of sesame bars from 6.2-6.9 during storage of 6 month. The results are in accordance with the alteration of chewability during storage for various sesame bar treatments.

Overall acceptability of sesame bars. The quality scores obtained from the evaluation of colour, flavour, texture, crispiness, chewability and overall acceptability, whose statistical analysis at storage intervals (0, 30, 60 and 90 days) is illustrated in Table 8. Results reveal that the overall acceptability differed highly significant among different treatments. The results related to mean score for the sesame bar in Table 8 revealed that T_2 (7.93 ± 0.12) was preferred by the judges followed by T_1 (5.80 ± 0.24) regarding overall acceptability. This could be due to the acceptable range of defatted sesame flour addition in the two treatments. Though having a reduced score in T_3 (5.95 ± 0.82) and T_4 (5.32 ± 0.64) resulted in greater acceptability of the sesame bars. There was a highly significant effect of storage on overall acceptability of sesame bars. Collectively, the maximum scores were observed in Table 9 fresh bars that gradually increased from 5.96 ± 0.27 to 6.60 ± 0.45 after 90 days storage. There was non-significant effect of treatment and storage interaction on overall acceptability of the sesame bars. The findings of Al-Hooti *et al.* (1997a) who reported significant increase in overall acceptability of sesame bars from 6.9 to 7.9 during storage of 6 months are in accordance with the alteration of overall acceptability during storage for various sesame bars treatments.

Conclusion

Protein energy malnutrition can be overcomed by the provision of healthy, tasty, convenient and nutritious sesame snack bars. In the current scenario, development of nutritious bars is a good substitute to other junk foods. Sesame snack bars have great market potential to boost up energy and maintain performance by providing minerals, vitamins, fat, protein and carbohydrates. Sesame supplemented bar can be used for the school nutrition programmes to uplift the nutritional status of the school going children.

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Effect of Different Combinations of Gums and Emulsifiers on the Quality of Bread

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Abstract. A project was designed to evaluate the effect of different combinations of emulsifiers and gums on the quality of bread. Wheat variety AARI-11 was milled to get straight grade flour and mixed with the Emulsifiers (DMG & DATEM) and Gums (G.G & CMC) in a quantity of (0.3- 0.6 %). Both, straight grade flour as well as treated flour (combination with gums and emulsifiers) were subjected to proximate and rheological analysis. Results of the rheological study showed a significant change in water absorption, dough development time, dough stability time and dough viscosity i.e. W/A 61.33-62.93%, D.D.T 3.9-4.8 min, D.S.T 7-9.1 min and 818.33-950.00 BU, respectively. Breads prepared with both flours were also studied for their sensory attributes during storage after the interval of 24 h. The highest score was awarded to T_1 (0.3% DATEM & 0.5% guar gum) on the bases of its excellent external attributes (colour of crust, volume, symmetry of form, evenness of bake and crust character) and internal characteristics (aroma, grain, texture, taste, mastication and colour of crumb). After the sensory and physicochemical analyses, it is concluded that with the addition of DATEM (0.3%) and guar gum (0.5%) resulted in good quality of bread.

Keywords: bakery product**,** straight grade flour, rheological study, water absorption

Introduction

Bread is an important food product and largely consumed all over the world. It is a bakery product having good aroma, texture and taste. It is prepared by baking of dough and having common ingredients like salt, fat, water and yeast. There are also some optional ingredients like egg, sugar, spice, milk, vegetables, nuts and seeds. Bread is leavened by yeast through the fermentation of wheat flour sugars that derives from starches. The leavening property is due to the elastic structure of gluten that has the ability to strengthen and change itself. So, it helps the shaping of bread in bread making process by gas retention. The different characteristics i.e., softness, crust, flavour, sizes, texture, shapes and eating qualities of bread explain that wether it is good quality or bad quality type of bread (Cauvain, 1994).

Bread comes in the categories of perishable food products having very short shelf life and usually consumed around 4-5 days of manufacturing. Ropiness and mouldiness are the major factors responsible for the deterioration and spoilage of the bread. But there are some additives which are added in bread to enhance the storage duration and bread quality. Generally, the fresh baked bread have attractive colour, rubbery and soft crumb texture, a pleasant smell, fine slicing characteristics and the mouth watering feel (Bakke and Vickers, 2007).

According to market demand of bread, different problems related to bread preservation may occur during storage. To overcome these problems, various techniques and different additives are used to enhance the quality of bread and baked products. There are some additives which are mostly being used nowadays in bakery industries for the good quality of baked goods. Hydrocolloids and emulsifiers are major two types of substances which are mostly used in baking units. The emulsifiers consisted of both lipophilic and hydrophilic properties hence, it provides a chance to react at water-oil inter phase. Thus by growing emulsion capabilities, the amphiphilic part takes part to produce the complex structure with protein and starch (Stampfli and Nersten, 1995).

In general, the characteristics of emulsifiers in the bakery industry are to improve the dough strength, crumb structure, water absorption rate, brighter crumb and finer grains. Emulsions reduce the crust thickness, enhance the slicing properties of bread and improve gas retention during fermentation (Selomulyo and Zhou, 2007; Stampfli and Nesstin 1995).

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Practically it was supposed that additives delay in fixing the structure of crumb, which is correlated with staling (Azizi and Rao, 2005). There is another group of additives which is used to enhance the quality of bread and known as hydrocolloids or gums. These are polysaccharides which are extracted from seaweeds, plants, microbial sources and from modified biopolymers through the chemical treatment of cellulose, as well as derived from plants exudates. Commonly used hydrocolloids in the industry are alginates, guar gum, carrageenans, arabic gum, carboxymethyl cellulose and agar (Gomez-Diaz and Navaza, 2003).

Hydrocolloids are also known as water-soluble gums and have been found specifically a wide range of functions i.e., thickening, gelatinization, gel formation, fragmentation, melting, foam stabilization, retro gradation of starch and increasing water-holding capacity. Hydrocolloids may be used alone or in combination to achieve their functional characteristics. When these are used in little amount i.e., <1% (w/w) in flour, then there is chance to increase in water retention and ultimately decrease in the firmness of crumb. Hydrocolloids have hydrophilic nature so prevent ice crystals growth during frozen storage and water migration to coating, so it increases thaw stability. Hydrocolloids have unbiased aroma and taste which allows a free flavour liberate of all recipe components. They provide an oily body to products having reduced fat, in which texturizing properties and water-binding ability compensate for the little fat contents. These compounds have been used in gluten formulation as substitutes of gluten. Additives are used in bakery to help processing, guarantee constant quality and balance for dissimilarity in raw materials and to preserve food properties and freshness (Ribotta *et al*., 2008).

In general, the addition of emulsifiers and hydrocolloids enhance the value as improvers of bakery products. These are broadly used in baked products to increase overall fresh product's quality, dough handling properties and to extend shelf-life of stored goods. Present study was conducted to determine the effect of different combinations of gums and emulsifiers on the quality of bread.

Materials and Methods

The research was carried out to study the effect of different combinations of gums and emulsifiers on the quality of bread. The work was conducted at Institute of Food Science and Nutrition, University of Sargodha, Sargodha and in a local baker in Lahore, Pakistan. Details of raw materials used and the procedures employed are as follow:

Procurement of raw materials. Wheat variety (AARI-11) was procured from Wheat Research Institute, Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan. Hydrocolloids (guar gum, carboxymethyl cellulose) and emulsifiers DATEM (Diacetile tartaric acid of mono glycerides), DMG (dimono glyceride) were purchased from Sakhawat Essence (Lahore, Pakistan).

Physical characteristics of wheat grains. The raw materials were cleaned manually to remove dirt, dust, damaged seeds, seeds of other crops and other foreign matters. Wheat variety (AARI-11) was tested for different physical characteristics like thousand kernel weight by the method No. 84-10 as described in AACC (2000). Three replicates of each measurement were made. Straight grade flour was prepared according to method No.26-95 (AACC, 2000).

Physicochemical analysis. Moisture content, ash, protein, crude fat, crude fibre content in samples was determined according to the procedure described in AACC (2000). Nitrogen free extract (NFE) was calculated by subtracting the percentages of moisture, ash, crude protein, fat and crude fibre from 100 as per the following formula:

NFE (%) = 100 – (moisture % + crude protein % + crude fat % + crude fibre % + ash %)

Rheological studies of acceptable straight grade wheat flour and farinograph. The rheological behaviour of the treated flour samples was evaluated by using Brabender Farinograph (Model: Brabender DUISBURG 380, Germany) according to method described in AACC method no. 54-21 (AACC, 2000). Dough properties such as water absorption, dough development time and dough stability were interpreted by the Farinogram.

α-Amylase activity. Falling numbers were determined using Pertin Falling Number Apparatus 1900 (Pertin Instruments AB, SE 1405, Huddige, Sweden) according to the method No.56-81 as described in AACC (2000).

Experimental design. Various treatments of straight grade flour were made to study the effect of different combinations of gums and emulsifiers on the quality of bread. This treated flour contain gums and emulsifiers in different proportion as shown in Table 1.

Formulation of bread. The breads with each treatment were prepared in a local bakery in Lahore, Pakistan. The basic bread recipe contained flour (5 kg), salt (100 g), yeast (100 g), shortening (50 g), calcium acetate (4 g), calcium propionate (400 g), sugar (400 g) and baking improver (0.5%). Emulsifier (MDG and DATEM) and gums (guar gum and CMC) were added according to the experimental design shown in Table 1. The water added in each formulation was based on farinograph water absorption (Table 1). Dough was mixed in a spiral mixer for 3 min at 90 rpm, then for 7 min at 120 rpm. After resting for 10 min, it was divided into 70 g pieces and rounded. Dough was proofed at 32 °C and 80% relative humidity for 75 min, baked at 230 °C for 2 min with 0.2 L of steam, and finally baked at 170 °C for 10 min. Breads were packaged in polyethylene bags. Physical and sensory analyses were carried out.

Sensory evaluation. All the bread samples were evaluated by the trained panel of twenty five judges from the Institute of Food Science & Nutrition, University of Sargodha, Pakistan for various attributes like colour, flavour, taste, texture and overall acceptability at 0, 1st, and 2nd days according to the method described by Meilgaard and Civile (2007).

Statistics. Results were statistically analysed by using ANOVA. Level of significance within mean was calculated by using complete randomized design Test and two factorial tests (Steel *et al*., 1997).

Table 1. Treatments of flour having gums and emulsifiers prepared during study

Treatments	DATEM	G G	MDG	CMC
	(%)			
T_0	0	0	0	0
T_1	0.3	0.5	0	0
T_2	0.6	1	0	0
T_3	0	0	0.3	0.5
T_4	0	0	0.6	1

Table 2. Proximate composition of emulsifiers-hydrocolloids blended flours and straight grade flour

Treatments/ flour	Moisture	Protein	Fat	Fibre	Ash	NFE
	(%)					
SGF	11.88^{b}	11.32^{c}	1.50^{d}	0.33^{f}	0.46^{e}	74.51^{a}
T_0	11.36^{c}	11.36^{a}	1.47^{a}	0.36^{d}	0.40^{c}	75.05^{a}
T_1	12.20^{d}	11.03^{b}	1.46^{a}	1.03^{b}	0.42^{b}	73.86^{c}
T_2	12.68^{a}	10.73^{b}	1.37^{b}	1.25^{a}	0.47^{a}	73.50^{d}
T_3	12.12^{d}	11.06^{c}	1.37^{b}	0.87^{c}	0.47^{a}	74.11^{b}
T_4	12.53^{b}	10.27^{d}	1.20^{c}	1.26^{a}	0.43^{b}	74.07^{b}

DATEM = diacetylated tartaric acid esters of monoglycerides; MDG = di mono-glycerides; GG = guar-gum; CMC = Carboxymethylcellulose; SGF = straight grade flour; T_0 = 100% white flour; T_1 = 0.3% DATEM+0.5% guar gum+99.2% white flour; T_2 = 0.6% DATEM+1.0% guar gum+98.4% white flour; T_3 = 0.3% MDG+0.5% CMC+99.2% white flour; T_4 = 0.6% MDG+1.0% CMC+98.4% white flour.

Results and Discussion

Proximate composition of straight grade flour. Proximate composition of straight grade wheat flour has been given in Table 2. The straight grade wheat flour contained moisture content 11.88%, crude protein 11.32%, crude fat 1.5%, crude fibre 0.33%, ash content 0.46% and NFE 74.51%. The obtained results are closely related with the findings of Ayaz (1998).

Proximate composition of emulsifiers and hydrocolloids blended flour. Effect of emulsifiers and hydrocolloids on proximate composition of straight grade white flour have been depicted in Table 2. The results showed that the addition of different levels of CMC, DATEM, MDG and guar gum to white flour have non-significant effect on ash, crude fat and crude protein while significant effect on moisture and nitrogen free extracts and highly significant effect on fibre in different treatments of treated flours. The highest significant value for crude fibre 1.25% was observed in T_4 and T_2 and lowest 0.33% in T_0 of white flour. It shows that fibre contents increased by increasing the concentration of additives. These results reveal an increasing trend in moisture content with the increase in additives. Maximum value for moisture content has been found in T_2 (12.69%) while minimum value in T_0 as 11.387%. T_0 (11.367%) showed the maximum value while T_4 (10.26%) showed minimum value for protein content of treatments. Means for nitrogen free extract (NFE) showed (2%) that T_0 contained highest value (75.016%) for NFE followed by T_3 (74.11%), T_4 (74.07%), T_1 (73.86%) while minimum value (73.56%) was observed in T_2. The overall range varied from 73.86-75.06%. Results of present study are in-line with the findings reported by Rodge *et al*. (2012); Guarda *et al*. (2004) and Rehman and Ahmad (2003).

Rheological properties of emulsifiers and hydrocolloids blended flours. Effect of emulsifiers and hydrocolloids on the rheological properties of straight grade flour have been depicted in Table 3. It is obvious from

Table 3 that water absorption capacity ranged from 60.87 to 62.93% in different treatments. The maximum water absorption was observed in T_4 (62.93%) followed by T_3 (62.53%), T_2 (61.83%) and T_1 (61.33%) while the minimum water absorption capacity was observed in T_0 (60.87%). Results showed that water absorption increased as the quantity of additives increased and changed by changing the emulsifiers and hydrocolloids as well. Results of present study are also in-line with the findings of Basak and Ercan (2011) and Sim *et al*. (2009). T_4 contained highest value (4.80 min) for dough development followed by T_3 (4.33 min), T_2 (4.30 min) and T_1 (4.10 min) while minimum value (3.90 min) was observed in T_0. The results showed an increasing trend in dough stability when used the combination of guar gum and DATEM while dough stability time decreased when combination of CMC and MDG emulsifier was used. The results of present study are in accordance with the findings of Shiyong and Yang (2013) and Sim *et al*. (2009).

Amylograph study of emulsifiers and hydrocolloids blended flours. Results showed that peak viscosity derived from amylogram varied highly significantly among different treatments. The mean values of peak viscosity ranged from 818.33 to 950.00 BU among various treatments (Table 3). The highest peak viscosity i.e. 950.00 BU was recorded in T_2, followed by T_1 (908.33 BU), T_0 (860 BU) and T_3 (858.33 BU) while minimum value (818 BU) was observed in T_4. In the present study, differences in the peaks of different treated flour treatments were due to differences in the α-amylase activity. The results of present study are comparable with the findings of Ashwini *et al*. (2009) and Sim *et al*. (2009) who reported similar increasing and decreasing trend of peak viscosity within different treatments of treated flours as the quantity of additives increased in the blends.

Sensory evaluation of emulsifiers-hydrocolloids blended breads. Emulsifiers-hydrocolloids blended breads were prepared and analyzed for sensory attributes like loaf volume, colour of crust, symmetry of form, evenness of bake, break and shred, crust character, grain, crumb colour, aroma, mastication, taste and texture to find out suitable composition of bread showing consumer acceptability. The data about mean scores of different treatments on various parameters has been presented in Table 4. The results showed that, symmetry of form, evenness of bake, break and shred, crust character, grain, crumb colour, mastication, taste were non significantly effected while loaf volume, colour of crust, texture and aroma were significantly affected by the addition of these additives. These results are in line with the observations of earlier researchers (Regine *et al*., 2013; Mohammad *et al*., 2012; Rodge *et al*., 2012; Sara *et al*., 2012; Carr *et al*., 2006; Shahzadi *et al*., 2005; Guarda *et al*., 2004; Qarooni, 2005; 1996).

Table 3. Ferinographic and amylographic study of emulsifiers-hydrocolloids blended flours

Treatments	Water absorption (%)	Dough development DDT (min)	Dough stability (min)	Viscosity (BU)
T_0	60.87^{e}	3.90^{d}	7.80^{c}	860.00^{c}
T_1	61.33^{d}	4.10^{b}	9.00^{b}	908.33^{b}
T_2	61.83^{c}	4.30^{c}	9.10^{a}	950.00^{a}
T_3	62.53^{b}	4.33^{c}	7.30^{d}	858.33^{d}
T_4	62.93^{a}	4.80^{a}	7.00^{e}	818.33^{e}

Table 4. Sensory attributes of emulsifiers-hydrocolloids blended bread

Treatments	T_0	T_1	T_2	T_3	T_4
	External characteristics				
Loaf volume	6.17^{d}	8.28^{ab}	8.72^{a}	7.56^{a}	6.33^{bc}
Colour of crust	6.67^{b}	5.67^{d}	5.89^{cd}	7.56^{a}	6.33^{bc}
Symmetry of form	2.56^{a}	2.50^{b}	2.22^{d}	2.44^{c}	2.50^{b}
Evenness of bake	2.56^{b}	2.11^{d}	2.39^{c}	2.72^{a}	2.39^{c}
Crust character	2.44^{a}	2.33^{c}	2.44^{a}	2.28^{d}	2.39^{b}
Break and shred	2.39^{b}	2.44^{a}	2.33^{c}	2.39^{b}	2.44^{a}
	Internal Characteristics				
Grain	8.22^{b}	8.22^{b}	8.33^{a}	8.00^{d}	8.17^{c}
Colour of crumb	8.44^{b}	8.28^{d}	8.33^{c}	8.67^{a}	8.33^{c}
Aroma	6.67^{c}	7.78^{ab}	8.11^{ab}	8.44^{a}	7.67^{b}
Mastication	8.00^{c}	7.78^{d}	8.22^{b}	8.00^{c}	8.44^{a}
Taste	12.33^{c}	13.33^{b}	13.67^{ab}	14.00^{ab}	14.33^{a}
Texture	11.22^{d}	12.22^{c}	12.56^{bc}	12.89^{ab}	13.22^{a}

T_0 = 100% white flour; T_1 = 0.3% DATEM + 0.5% guar gum + 99.2% white flour; T_2 = 0.6% DATEM + 1.0% guar gum + 98.4% white flour; T_3 = 0.3% MDG + 0.5% CMC + 99.2% white flour; T_4 = 0.6% MDG + 1.0% CMC + 98.4% white flour.

Conclusion

It is concluded from the present investigation that gums and emulsifiers have significant affect on water absorption, dough development time and dough stability. Overall acceptability like colour, volume, taste, aroma, crust colour, crumb texture etc. could be highly improved with the help of gums and emulsifiers according to the

climatic conditions of the specific area with respect to wheat quality. The results acquired from the present study may be very valuable for industries to decide to take best additives/improver for their quality products.

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Improvement of the Physical and Oxidative Stability Characteristics of Ice Cream through Interesterified *Moringa oleifera* Oil

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Abstract. This study aimed to investigate the effect of high melting point interesterified *M. oleifera* oil (35.6°C) with substantial amount of unsaturated fatty acids on physicochemical and oxidative stability characteristics of ice cream. Of the 10% fat in the ice cream, 30% was replaced by interesterified *M. oleifera* oil at three levels i.e. 10, 20 and 30% (T_1, T_2 and T_3, respectively). Oleic acid increased from 26.55% to 31.69%, 36.94% and 42.15% in T_1, T_2 and T_3 with no effect on melting time, compositional attributes and free fatty acid content of ice cream ($P>0.05$). Supplementation of ice cream with interesterified *M. oleifera* oil inhibited the autoxidation process in ice cream during 3 months storage period ($P<0.05$).The loss of oleic and linoleic acid in fresh and 3 months stored control and T_2 was 26.55%, 24.15%, 26.39% and 1.93%, 1.24% and 1.79%, respectively. Peroxide value of three months stored control and T_3 was 1.12 and 0.39 ($meqO_2/kg$). The overall acceptability score of T_2 was 80% of the total score (9).

Keywords: *Moringa oleifera* oil, interesterification, ice cream, oxidative stability

Introduction

Milk fat contains plenty of atherogenic fatty acids which have a great deal of contribution in the enhancement of harmful LDL cholesterol. In addition to the saturated fatty acids, milk fat also contains higher extent of the dietary cholesterol; these factors make it the first choice of criticism for the nutritionists and dieticians (Williams, 2000). About 44% of the US population has shown negative opinion against the milk and dairy products particularly, fat rich dairy products (Hansel *et al*., 2007; Honda *et al*., 2007). The increased rate of mortalities from cardiovascular disease has led to the development of large number of modifications and replacement strategies in milk and dairy products. Partial replacement of milk fat with vegetable oils in ice cream consequences in weak body and lower melting resistance (Nadeem *et al*., 2010). This problem can be ameliorated by using lipids having higher melting points and melting resistance as extent of saturated fatty acids and melting points are usually correlated with each other.

Interesterification is an effective way to increase the slip melting point and solid fat content of vegetable oils without generation of undesirable *trans* isomers (Osman and Idris, 1999).

Moringa oleifera is widely grown in tropics and quality of its oil is almost similar to olive oil (Mohammed *et al*., 2003). Melting point of *M. oleifera* oil can be substantially increased through the random rearrangement of esters (Nadeem *et al*., 2012). Interesterified *M. oleifera* oil can fulfill the above mentioned quality and functionality characteristics simultaneously (Nadeem *et al*., 2012). The oil content of seeds ranges between 30-40%, oil is edible and rich in monounsaturated fatty acids (Tsakinis *et al*., 1998). *Moringa oleifera* oil improved the oxidative stability of vegetable oils and butter oil (Nadeem *et al*., 2013b).

The potential role of *M. oleifera* oil on the physical characteristics and oxidative stability of ice cream has not been studied so far. This study and aimed to evaluate the suitability of interesterified *M. oleifera* oil to partially replace milk fat in the formulation of functional ice cream. Some physicochemical, sensory and oxidative stability characteristics were observed to find out the suitability of chemically modified *M. oleifera* oil in blends with milk fat.

Materials and Methods

Chemical interesterification. *M. oleifera* oil was extracted by laboratory scale expeller, oil was stored in clean and dry pet bottles, sealed and stored at -18 °C till further usage. Melting point of interesterified *M. oleifera* oil and milk fat was 19.8 and 35.2 °C. Chemical interesterification of *M. oleifera* oil was performed to improve the melting resistance of ice cream. Samples were dehydrated in a flask, under reduced pressure, fitted with a vacuum pump in a water bath at 95 °C. The portions were mixed with 0.2% (w/w) of sodium methylate. Interesterification reaction was

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performed under reduced pressure at 70 °C in a 1000 mL stoppered flask in a water bath with constant agitation for 1 h. To stop the interesterification reaction, 5 mL distilled water was added. Treated blend was washed with hot water twice for the complete removal of catalyst (Sreenivasan, 1978). After rearrangement reaction, melting point of *M. oleifera* oil was 35.6 °C.

Experimental plan. Milk fat was partially replaced with interesterified *M. oleifera* oil at 10, 20 and 30% levels (T_1, T_2, T_3, respectively). The formulation of control comprised of 10% milk fat, 11% MSNF, 15% sucrose, 0.5% cremodan with no added colour and flavour.

Analysis. Fat was extracted from ice cream by following the recommended method of AOAC (1997). Fatty acids were analysed as fatty acid methyl esters using sodium methylate as transesterifying agent (Qian, 2003). Fatty acid methyl esters were injected into Gas Chromatograph model Shimadzu, Japan 17-A, fitted with a methyl lignoserate-coated (film thickness 0.25µm), SP-2330 (SUPELCO Inc. Supelco Park Bellefonte, PA 16823-0048, USA) polar capillary column (30 m × 0.32 mm) and a flame ionization detector. Fatty acids were identified and quantified by the FAME-37 internal standards (Sigma Aldrich, UK). Fat, protein, ash, lactose content, total solids, pH and acidity were determined by following the recommended methods of AOAC (2000). The viscosity readings were taken after ageing the mix at 4 °C for 24 h. For the determination of melting time, ice creams were taken out from the storage compartment (-18 °C), exposed to 20 °C, the time required to fall the first drip was recorded as melting time and determined by the method of Abdou *et al*. (1996). Overrun of ice cream was also determined by the method prescribed by Abdou *et al.* (1996). Peroxide and anisidine values were determined as per methods of AOCS (1995). The ice creams were tempered to -18 °C for 2 h before serving. Evaluation was performed by 10-trained judges on a 9-point scale (1 was the worst and 9 the best) as prescribed by Larmond (1987). The statistical analysis of the triplicate experiments was performed by one way and two way analysis of variance techniques as prescribed by Steel *et al*. (1997) in a completely randomized design, the significant difference among the treatments was made by using Duncan's Multiple Range Test.

Results and Discussion

Characterisation of *M. oleifera* oil. Preliminary characterisation of cold pressed *M. oleifera* oil revealed free fatty acids 0.19% (oleic acid), moisture content 0.22%, colour on Lovibond Tintometer scale Red 2.1 and Yellow 3.2, unsaponifiable matter 1.29%, peroxide value 0.22 ($meqO_2/kg$), iodine value 69.4 and slip melting point 19.8 °C. The free radical scavenging activity, flavonoid content and total antioxidant activity of *M. oleifera* oil was 74.19%, 35.14 mg/100 g and 37.68%, respectively. Fatty acid profile of *M. oleifera* oil revealed that concentraction of C16:0, C18:0, C18:1 and C18:2 were 6.55%, 2.72%, 76.04% and 3.16%, respectivey.

Fatty acid profile. Fatty acid composition of milk fat, *M. oleifera* oil and their blends has been presented in Table 1. Marked changes were recorded in fatty acid composition of the blends after the formulation of blends particularly the content of short chain fatty acids declined from 10.65% (T_o) to 8.67, 7.49 and 6.68% in T_1, T_2 and T_3, respectively, which was 19.6, 29.68 and 37.28% less than the control. Oleic acid increased from 26.55% (T_o) to 31.69, 36.94 and 42.15% in T_1, T_2 and T_3, respectively. Oleic acid progressively increased as a function of higher levels of *M. oleifera* oil. Lim *et al*. (2010) studied the effect of partial replacement of milk fat with flaxseed oil and observed progressive increase in the content of unsaturated fatty acids with increasing increments of flaxseed oil. Nadeem *et al*. (2010) incorporated rape seed oil into ice cream and recorded higher concentration of oleic acid in the blends, supplemented sunflower oil by *M. oleifera* oil and observed major difference in fatty acid composition of the blends. Mohammed *et al*. (2003) characterized *M. oleifera* oil with the objective to explore its potential as future's oil and reported that major fatty acid was oleic acid (78.9%). Fatty acid composition of *M.*

Table 1. Fatty acid composition of various treatments of ice cream containing interesterified *Moringa oleifera* oil, milk fat and their blends

Fatty acid	T_0	T_1	T_2	T_3
C4:0	4.47±0.09d	4.10±0.03b	3.57±0.11c	3.12±0.01c
C6:0	2.28±0.12a	2.04±0.05b	1.84±0.02c	1.56±0.04d
C8:0	1.34±0.04a	1.21±0.01a	1.06±0.01b	0.95±0.07b
C10:0	2.56±0.07a	2.32±0.04b	1.85±0.07c	1.75±0.11d
C12:0	2.66±0.15a	2.36±0.06b	2.14±0.02c	1.84±0.04d
C14:0	9.24±0.19a	8.35±0.09b	7.44±0.05c	6.46±0.15d
C16:0	25.84±0.23a	23.95±0.12b	22.05±0.18c	18.15±0.10d
C18:0	14.65±0.11a	13.45±0.02b	12.34±0.07c	11.14±0.06d
C18:1	26.55±0.16d	31.69±0.14c	36.94±0.23b	42.15±0.12a
C18:2	1.93±0.12d	2.14±0.17c	2.34±0.09b	2.58±0.22a
C18:3	1.31±0.05a	1.17±0.05b	1.03±0.03c	0.82±0.09d
C20:0	0.15±0.02d	0.37±0.01c	0.61±0.05b	0.87±0.02a

Means of triplicate experiment; means sharing same letter in same row are statistically non significant; T_0 =100% milk fat; T_1= 90% milk fat and 10% *M. oleifera* oil T_2 = 80% milk fat and 20% *M. oleifera* oil T_3 = 70% milk fat and 30% *M. oleifera* oil.

oleifera oil was almost similar to olive oil. Mariod *et al*. (2005) studied the effect of blending on fatty acid composition of sunflower and *Sclerocarya birrea* oil. Oleic acid increased by 41.3% and linoleic acid decreased by 51% in the blend when blended in 60:40 ratios. Sunflower, palm oil and ground nut oils were blended in various proportions; fatty acid composition of blends was significantly distinguishable from the substrate oils.

Composition. The results of chemical composition of functional ice cream containing interesterified *M. oleifera* oil is given in Table 2. The addition of *M. oleifera* oil at all three levels did not show any effect on pH, acidity, fat, protein, ash content and total solids of ice cream. The reason could be the identical formulation and non-variation in the ingredients. Chemical composition of ice cream formulated from milk fat, palm kernel oil, cotton seed oil, dalda vanaspati, ground nut and rape seed oil was not different from the standard ice cream (Nadeem *et al*., 2010; Abdou *et al*., 1996; Adhikari and Arora, 1994; Miglani *et al*., 1987).

Viscosity. Viscosity of ice cream mix has a direct connection with the whipping process; higher viscosities are associated with better whipping ability (Lim *et al*., 2010). Viscosity of ice cream mixes supplemented with interesterified *M. oleifera* oil was not different from the control (Table 3). Partial replacement of milk fat with vegetable oils had a pronounced effect on the viscosity of ice cream mix (Im and Marshall, 1998; Adhikari and Arora, 1994). The results of the present study are different from the previously conducted research works, although vegetable oil was used yet it was chemically modified with different physical characteristics which definitely contributed in the improvement of the physical characteristics of ice cream.

Overrun. 30% replacement of milk with interesterified *M. oleifera* oil did not reveal any effect on overrun of ice cream (Table 3) however, significant changes were observed beyond this level. Replacement of milk fat with corn and rape seed oils decreased the overrun in mellorine (Nadeem *et al*., 2010). The results of this investigation are in partial confrontation of other researchers. These results can be justified by two; replacement level was not too high and secondly the fat used in this investigation was chemically modified having higher solid fat index and higher melting point.

Melting time. Melting time of all the experimental samples were at par with the control (Table 3). Other studies on milk fat replacement with vegetable oils revealed that, melting time markedly decreased as the milk fat was replaced with liquid vegetable oils. The results of this investigation were different from the literature due to the usage of interesterified *M. oleifera* oil which had greater melting point (35.6 °C) than milk fat (34.6 °C) and higher melting point improved the melting resistance (Adhikari and Arora, 1994) while, studying the replacement of butter fat with unmodified ground nut oil observed that replacement of milk fat significantly decreased the melting time ($P<0.05$). Melting time of the ice cream is directly dependent on the melting point of fats as the fats with lower melting point will melt quickly (Flack, 1988). For ice cream the fats having melting point in the range of 34-36 °C may give the optimum melting time. The fat used in this study was wisely interesterified to increase the melting point from 18 to 34.6 °C which contributed in the melting resistance of ice cream and confer the melting time to be almost similar to milk fat at medium level of fat replacement (T_2).

Changes in fatty acid composition. Determination of fatty acid composition of three months stored ice cream exhibited two different trends; saturated fatty acids increased whereas, unsaturated fatty acid decreased as a function of storage period (Table 4). The increase or decrease in the extents of saturated and unsaturated fatty acids could be attributed to the better oxidative stability of saturated fatty acids and break down of unsaturated fatty acids into the oxidation products. Supplementation of milk fat with *M. oleifera* oil at all three levels tended to inhibit the autoxidation process in the stored ice cream. The strong inhibition of the oxidative breakdown in the supplemented ice creams could be attributed to the presence of higher concentration of polyphenolic compounds in *M. oleifera* oil. The concentration of unsaturated fatty acids decreased

Table 2. Effect of replacing milk fat with interesterified *M. oleifera* oil on composition of ice cream

Treatments	Fat%	Protein%	Ash%	Total solids%	pH	Acidity%
T_0	9.84±0.06	4.05±0.47	0.73±0.17	36.18±0.10	6.70±0.05	0.17±0.04
T_1	9.85±0.09	4.01±0.42	0.70±0.17	36.15±0.10	6.68±0.03	0.18±0.01
T_2	9.94±0.16	3.95±0.21	0.72±0.09	36.09±0.10	6.71±0.01	0.16±0.03
T_3	9.78±0.11	3.81±0.56	0.71±0.09	36.03±0.05	6.75±0.05	0.16±0.08

Refer to Table 1 for the detail of treatment.

Table 3. Effect of replacing milk fat with interesterified *M. oleifera* oil on physical characteristics of ice cream

Treatments	Viscosity (CP)	Overrun (%)	Melting time (min)	MP (°C)
T_o	67.38±1.55	82.2±1.23	6.33±0.90	34.8±0.2
T_1	65.98±1.47	81.9±1.45	6.15±1.71	34.6±0.1
T_2	64.29±1.34	70.3±1.99	5.98±0.95	35.0±0.3
T_3	63.18±1.32	77.5±1.79	5.70±1.10	35.2±0.2

Means of triplicate experiment and means sharing same letter in column are statistically non significant refer to Table 1 for the detail of treatments; MP = Slip Melting Point (°C).

in ice cream supplemented with flaxseed oil during the course of 42-days storage period (Lim *et al*., 2010). Long term storage of butter at refrigeration and freezing conditions increased the extents of oxidation products (Krause *et al*., 2008). In an another study related to the determination of oxidative stability of olein based ice creams it was observed the enhancement of saturated fatty acids and decline of unsaturated fatty acids during 6-months storage (Nadeem *et al*., 2013a).

Peroxide value: Supplementation of milk fat with interesterified *M. oleifera* oil at all concentrations considerably inhibited the formation of primary oxidation products (Table 5). Enhancement of unsaturated fatty acids in ice cream either by using modified milk fat or vegetable oils results in development of oxidized flavour in ice cream during the storage process (Lim *et al*., 2010; Gonzalez *et al*., 2003). In this study, the use of *M. oleifera* oil offered a unique benefit of enhancing the beneficial oleic acid without posing a problem of rancid flavour. The lower extents of primary oxidation products could be attributed to the lower oxidation susceptibility of oleic acid and presence of phenolic compounds. Enhancement of the oxidative stability of soybean and sunflower oils by mixing with *M. oleifera* oil has been reported by strong inhibition of the autoxidation process was also observed when butter oil was blended with *M. oleifera* oil (Nadeem *et al*., 2013b). Flavour score and peroxide value were highly correlated and determination intervals showing higher peroxide value revealed lower flavour score (Fig. 1). Generation of oxidation products decreased the flavour score of olein based ice creams (Nadeem *et al*., 2013a).

Anisidine value. Anisidine value measures the magnitude of secondary oxidation products generated in fats and oils during the course of autoxidation, higher values usually anticipate poor storage stability (Pritchard, 1991). Ice creams supplemented with *M. oleifera* oil exhibited better storage stability over the control due to the strong inhibition of free radical mechanism thus leading to the formation of secondary oxidation products (Table 5). Enhancement of the unsaturated fatty acids in ice cream by the manipulation of the cow's ration with unsaturated fatty acids and flaxseed oil in ice cream rendered it vulnerable to the oxidative breakdown (Lim *et al*., 2010; Gonzalez *et al*., 2003). Olein based ice creams yielded the higher

Table 4. Changes in major fatty acids of fresh and 3 months stored ice cream

Fatty acids	T_0-Fresh	T_0-3M	T_1-3M	T_2-3M	T_3-3M
C16:0	25.84±0.23b	27.42±0.82a	25.36±1.16b	25.51±0.92b	25.72±0.54b
C18:0	14.65±0.11b	15.89±0.73a	15.12±0.38b	15.79±0.61b	15.93±0.71b
C18:1	26.55±0.16b	24.15±0.44c	25.94±0.75b	26.17±0.97b	26.39±0.58b
C18:2	1.93±0.12b	1.24±0.11c	1.65±0.08b	1.74±0.16b	1.79±0.07b

3M = three months stored ice cream at -18 °C; means with different letter in a row are not different; refer Table 1 for the detail of treatments.

Table 5. Effect of interesterified *M. oleifera* oil on storage stability of ice cream

Treatment	Peroxide value		Anisidine value		FFA (%)	
	Fresh	3M	Fresh	3M	Fresh	3M
T_0	0.25±0.03a	1.12±0.08a	3.58±0.12a	13.59±0.43a	0.11±0.1a	0.16±0.02a
T_1	0.28±0.02a	0.72±0.06b	3.62±0.18a	9.72±0.24b	0.10±0.01a	0.16±0.02a
T_2	0.26±0.03a	0.54±0.05c	3.68±0.07a	6.65±0.30c	0.11±0.01a	0.15±0.01a
T_3	0.21±0.01a	0.39±0.02d	3.52±0.04a	5.18±0.22d	0.11±0.02a	0.16±0.01a

Means denoted by different letter in a column are different, 3M = three months stored ice cream at -18 °C, Peroxide value ($MeqO_2$/kg); FFA = free fatty acids.

extents of secondary oxidation products during storage of 6-months (Nadeem *et al*., 2013a).

Free fatty acids. The free fatty acids of the control and all the treatments steadily increased during 3 months storage period and were at par with each other. Supplementation of ice cream with *M. oleifera* oil was ineffective to stop the generation of free fatty acids during the storage period (Table 5). Free fatty acids in food systems are produced due to the hydrolytic activities of lipases, presence of moisture, metal ion contamination etc. and phenolic compounds do not have a mechanism to stop their formation. Free fatty acids are associated with the generation of off flavours in foods; higher values can have a negative influence on the quality of the stuffs (Fox and McSweeney, 2003). Free fatty acid content of three months stored ice creams were within the acceptable limits. Free fatty acids of six months stored olein based ice creams and control were not different from each other (Nadeem *et al*., 2013a).

Sensory evaluation. The results of sensory evaluation of fresh ice cream depicted that the addition of interesterified *M. oleifera* oil at 20% level did not impart any effect on colour score (Table 6). Milk fat is unique among the edible fats for having appreciable amount of short chain fatty acids which are responsible for characteristics flavour of milk and milk products (Marshall *et al*., 2003). Replacement of milk fat up to 20% level created non distinguishable difference in flavour of ice cream. As the replacement level increased to 30% flavour score decreased from 7.1 (T_0) to 6.5 (T_3). The decline in flavour score could be attributed to the lower concentration of short chain fatty acids in T_3. The texture score of the control and T_3 were at par with each other (P>0.05). The results of the present investigation are not in line with the findings of Nadeem *et al*. (2010) who reported lower texture score when milk fat was replaced with rape seed oil. The deviation in texture score was due to the difference in the type of edible oil. Interesterified *M. oleifera* oil was used which had higher melting point than the milk fat, which definitely contributed in the texture of the ice creams.

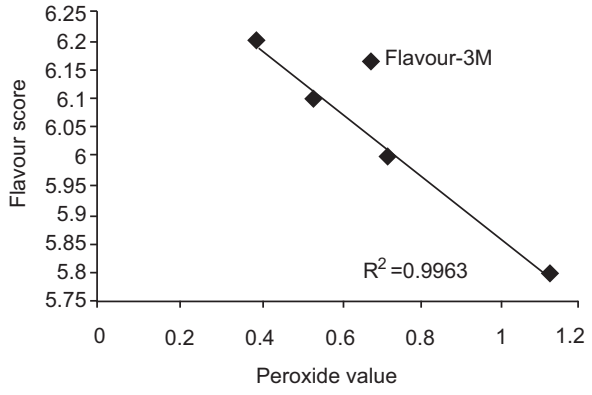

Fig. 1. Correlation between PV and flavour score.

Conclusion

Addition of interesterified *M. oleifera* oil did not have any effect on compositional attributes, overrun, viscosity and melting time, rather it improved the texture and storage stability of ice cream.

Table 6. Effect of replacing milk fat with interesterified *M. oleifera* oil on sensory characteristics of fresh ice cream

Treatment	Colour	Flavour	Texture	Overall acceptability
T_o	7.4±0.25a	7.1±0.41a	7.7±0.15a	7.4±0.24a
T_1	7.2±0.31a	7.0.±0.05a	7.6±0.19a	7.2±0.08a
T_2	7.2±0.45a	6.9±0.43a	7.4±0.24a	7.2±0.31a
T_3	6.9±0.34a	6.5±0.17b	7.5±0.14a	6.7±0.28b

Means of triplicate experiment and means sharing same letter in a column are non significant, refer Table 1 for the detail of treatments.

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Short Communication

The Resistance of Exotic Wheat Germplasm to Stripe Rust (*Puccinia striiformis* f. sp. *tritici*) under Nature Infection at Dera Ismail Khan, Pakistan

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Abstract. An experiment comprising of 49 exotic wheat germ plasm accessions was conducted at Arid Zone Research Institute, Dera Ismail Khan, Pakistan under rainfed condition during 2013-14 for their resistance against stripe rust disease. The trial was laid out in randomised complete block design with three replications. All entries were planted in a four-row plot with 3 m for row length and 25 cm for space among rows. A local susceptible check was repeatedly sown after every 10 test entries. The crop was maintained under rainfed conditions. Results revealed that all the exotic genotypes were genetically divergent in response to stripe rust disease. The disease score ranged from very highly susceptible to very highly resistant. Among 49 exotic lines, 27 exhibited from very highly resistant while 10 were susceptible to very highly susceptible.

Keywords: wheat germ plasm, stripe rust, *Puccinia striiformis* f. sp. *tritici*, rainfed condition

Wheat is cultivated on an area of about 21.465 million acres with production of 24.303 million tonnes annually (PBS, 2014). Stripe rust (*Puccinia striiformis* f. sp. *tritici*) is world-wide threat to wheat production, causing 10 to 70% grain losses depending on susceptibility of the cultivar, earliness of the initial infection, rate of disease development and duration of disease (Chen, 2005). Afzal *et al*. (2009) and Rattu *et al*. (2009) determined variability for yield based-partial resistance against stripe rust among wheat breeding lines. Mirza *et al*. (2003) concluded that up to 50% improvement in wheat yield has been achieved by introducing new high yielding cultivar in Pakistan. It is the genetic makeup of a variety that is expressed in a favourable environment and produces different yields in different environments (Khan *et al*., 2011). They had also screened high yielding and rust resistant wheat lines out of CIMMYT germ plasm. Rahman *et al*. (2012) released a high yielding rust resistance wheat variety BARS-09 selected from CIMMYT nursery in Pakistan. Therefore, using host plant resistance is the most economical, effective and ecologically sustainable method for controlling the disease. To diversify the resistance in wheat breeding programmes, introducing wheat grem plasm from other countries and identifying new resistance source are widely used in breeding practices. In the present study, some exotic wheat lines were evaluated under rainfed condition to identify the best source of resistance against stripe rust for further utilization in wheat breeding programme.

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Forty nine exotic wheat genotypes received from CIMMYT International Wheat Improvement Network (Table 1) were planted in a triplicated randomised complete block design at Arid Zone Research Institute, Dera Ismail Khan, Pakistan in November, 2013. A local susceptible check (CBN-47) was also involved for properly assessment of disease austerity in field conditions. Each line was planted in four rows of 3 meters long plots having row to row distance of 25 cm. The crop had received enough rain during heading and grain formation stage which ultimately helped the intensification of disease pressure in field conditions. The temperature remained low from December (6 °C) to February (7 °C) while, total rain fall (118 mm) occurred in March (50 mm) and April (68 mm) during the entire cropping season.

The modified Cobb's scale (Peterson *et al*., 1948) was used to assess the wheat genotypes for adult plant stem rust resistance based on the percentage of the leaf area covered with stem rust pustules (Table 2). Disease data was recorded when susceptible check showed about 30% infection during the evaluation seasons.

Table 1. CIMMYT wheat germ plasm accessions used in experiment at Arid Zone Research Institute, Dera Ismail Khan, Pakistan

Entry*	Cross name
1	FRANCOLIN #1/WBLL1
2	BAJ #1/TECUE #1
3	MUTUS/AKURI
4	BECARD/KACHU
5	WBLL1*2/4/SNI/TRAP#1/3/KAUZ*2/TRA P//KAUZ/5/BAJ #1
6	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU
7	WBLL1*2/BRAMBLING/5/BABAX/LR42//BABAX*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
8	MUU/5/WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/6/WBLL1*2/4/SNI/TRAP #1/3/KAUZ*2/TRAP//KAUZ
9	FRANCOLIN #1//WBLL1*2/KURUKU
10	BAJ #1/AKURI
11	FRANCOLIN #1//WBLL1*2/BRAMBLING
12	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP //KAUZ*2/5/DEMAI 4
13	WBLL1/4/BOW/NKT//CBRD/3/CBRD/5/WBLL1*2/TUKURU
14	SUP152/BLOUK #1
15	MUTUS/ROLF07
16	WBLL1/FRET2//PASTOR*2/3/MURGA
17	KA/NAC//TRCH/3/DANPHE #1
18	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU//FRET2
19	WBLL1*2/4/BABAX/LR42//BABAX/3/BABAX/LR42//BABAX
20	KA/NAC//TRCH/3/DANPHE #1
21	EMB16/CBRD//CBRD/4/BETTY/3/CHEN/AE.S Q//2*OPATA
22	FRET2/TUKURU//FRET2/3/MUNIA/CHTO//AMSEL/4/FRET2/TUKURU//FRET2
23	ROLF07*2/5/REH/HARE//2*BCN/3/CROC/AE.SQUARROSA (213)//PGO/4/HUITES
24	TILILA/JUCHI/4/SERI.1B//KAUZ/HEVO/3/AMAD
25	BAV92//IRENA/KAUZ/3/HUITES/4/2*ROLF07
26	KA/NAC//TRCH/4/MILAN/KAUZ//DHARWAR DRY/3/BAV92
27	KA/NAC//TRCH/3/VORB
28	OB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAU /FRET2/7/PASTOR//MILAN/KAUZ/3/BAV92
29	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KAU6/FRET2/7/PASTOR//
30	TOB/ERA//TOB/CNO67/3/PLO/4/VEE#5/5/KA UZ/6/FRET2/7/MINO
31	METSO/ER2000//MUU
32	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/4/WEAVER/5/2*JANZ/6/SKAUZ/BAV92
33	SNLG/3/EMB16/CBRD//CBRD/4/KA/NAC//TRCH
34	SNLG/3/EMB16/CBRD//CBRD/4/KA/NAC//TRCH
35	SLVS/3/CROC_1/AE.SQUARROSA(224)//OPATA/5/VEE/LIRA//BOW/3/BCN/4/KAUZ/6/2*KA/NAC//TRCH
36	C80.1/3*BATAVIA//2*WBLL1/3/EMB16/ CBRD//CBRD/4/CHEWINK #1
37	1447/PASTOR//KRICHAUFF/5/2*SERI*3 /RL6010/4*YR/3/PASTOR/4/BAV92
38	METSO/ER2000//MONARCA F2007/3/WBLL1*2/KKTS
39	BERKUT/MUU//DANPHE #1
40	QING HAIBEI/WBLL1//BRBT2/3/PAURAQ
41	KA/NAC//TRCH/3/DANPHE #1
42	WORRAKATTA/2*PASTOR//DANPHE #1
43	METSO/ER2000/5/2*SERI*3//RL6010/4 *YR/3/PASTOR/4/BAV92
44	MILAN/KAUZ//DHARWAR DRY/3/BAV92/4/PAURAQ
45	1447/PASTOR//KRICHAUFF/3/PAURAQ
46	DHARWAR DRY
47	HIDDAB
48	SUNCO.6/FRAME//PASTOR/3/PAURAQ
49	CHAM 6
50	Local Check " CBN-47"

*wheat genotypes have been arranged according to their merit of resistance against stripe rust disease.

Table 2. A modified disease rating scale for recording stripe rust in wheat genotypes

Symptoms	Infection (%)	Rating
No visible signs or symptom	0	VHR
Necrotic and/or chlorotic flecks; no sporulation	5	HR
Necrotic and/or chlorotic blotches or stripes; no sporulation	6-10	R
Necrotic and/or chlorotic blotches or stripes; trace sporulation	11-15	MR
Necrotic and/or chlorotic blotches or stripes; light sporulation	16-25	MS
Necrotic and/or chlorotic blotches or stripes; intermediate sporulation	26-40	MS to S
Necrotic and /or chlorotic blotches or stripes; moderate sporulation	41-70	S
Necrotic and/or chlorotic blotches or stripes; abundant sporulation	71-90	HS
Chlorosis behind sporulating areas; abundant sporulation	91-100	VHS

VHR = very highly resistance; HR = highly resistant; R = resistant; MR = moderately resistant; MS = moderately susceptible; MS to S = moderately susceptible to susceptible; S = susceptible; HS = highly susceptible; VHS = very highly susceptible.

A set of 49 wheat genotypes was studied to evaluate their response against stripe rust disease. A local cultivar (CBN-47) was also involved as susceptible check for properly assessment of disease severity in the field conditions. The crop had experienced severe drought stress up to month of February as it was maintained under rainfed conditions. The frequent rain (50 to 68 mm in March and April, respectively), high relative humidity (67 to 83%) coupled with cool temperature (12 - 24 °C in March, and 18 - 23 °C in April) during heading and grain formation stage had created quite favourite conditions for stripe rust infection and spread on wheat. Imported germ plasms were screened under high disease pressure in field conditions. Therefore, a narrow disease rating scale (0-100) was used for most opposite evaluation and rating of genotypes. Recorded data revealed highly significant variability among the genotypes regarding their response to stripe rust disease. The reaction of various entries ranged from very highly resistant to very highly susceptible depending upon the genetic makeup of genotypes. Out of 49 test entries, 11 remained very highly resistant, 4 highly resistant, 12 resistant, 3 moderately resistant, 2 moderately susceptible, 8 moderately susceptible to susceptible, 1 susceptible, 2 highly susceptible and 6 were very highly susceptible to yellow rust disease (Table 3). Similar results have been reported by Afzal *et al*. (2009), who determine significant variability for field based-partial resistance against stripe rust among 188 wheat breeding lines. Rattu *et al*. (2009) have also found three resistant lines against leaf and yellow rust among 29 wheat candidate lines with desirable Relative Resistance Index (RRI). The expression of resistance and production of different yields in different environments depends upon the genetic makeup of a variety (Khan *et al*., 2011).

Mirza *et al*. (2003) reported that, up to 50% yield improvement in wheat has been attained through introducing new high yielding disease resistance cultivars in Pakistan. Kolmer (2003) and Oelke and Kolmer (2004), suggested that prior to exercising any gene pyramiding approach, it is necessary to identify the effective and genetically diversified sources of resistance. Host resistance is the most economical, effective and ecologically sustainable method of controlling the disease (Vanzetti *et al*., 2011). Rajaram *et al*. (1996) suggested simultaneous evaluation of germ plasm for drought tolerant and disease resistance genotypes. In present studies, all the entries have shown quite inimitable and incomparable response to disease. The entries No. 1 to 27 possessing resistant genes against stripe rust disease may be conceivably exploited; either through direct selection and/or involving them in wheat hybridization programme for development of stripe rust resistant varieties.

Table 3. Disease scoring of CIMMYT wheat accession at AZRI, Dera Ismail Khan, Pakistan

Entry	Disease infection	Genotypic reaction
1	0 J	VHR
2	0 J	VHR
3	0 J	VHR
4	0 J	VHR
5	0 J	VHR
6	0 J	VHR
7	0 J	VHR
8	0 J	VHR
9	0 J	VHR
10	0 J	VHR
11	0 J	VHR
12	3.3 IJ	HR
13	3.3 IJ	HR
14	3.3 IJ	HR
15	3.3 IJ	HR
16	6.7 HIJ	R
17	6.7 HIJ	R
18	6.7 HIJ	R
19	6.7 HIJ	R
20	6.7 HIJ	R
21	6.7 HIJ	R
22	6.7 HIJ	R
23	6.7 HIJ	R
24	6.7 HIJ	R
25	6.7 HIJ	R
26	10 HIJ	R
27	10 HIJ	R
28	13 HI	MR
29	13 HI	MR
30	15 H	MR
31	17 GH	MS
32	17 GH	MS
33	27 FG	MS to S
34	28 F	MS to S
35	30 EF	MS to S
36	33 EF	MS to S
37	33 EF	MS to S
38	33 EF	MS to S
39	37 EF	MS to S
40	40 E	MS to S
41	70 D	S
42	83 C	HS
43	87 BC	HS
44	93 ABC	VHS
45	97 AB	VHS
46	100 A	VHS
47	100 A	VHS
48	100 A	VHS
49	100 A	VHS
50	100 A	VHS

Genotypes sharing common letter(s) are statistically similar at 5% probability level.

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Short Communication

Control of Cabbage Aphid *Brevicoryne brassicae* (Homoptera: Aphididae) through Allelopathic Water Extracts

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Abstract. Laboratory experiments were conducted to evaluate the effect of sorghum, sunflower, brassica and mulberry water extracts on mortality of cabbage aphid *Brevicoryne brassicae* (L.) (Homoptera: Aphididae) which damages the canola crop. The aphids were collected from canola field and applied with different concentrations of allelopathic water extracts or their combinations under laboratory conditions. Allelopathic water extracts of crops such as sorghum, brassica, sorghum + mulberry, sorghum + sunflower and sunflower alone were effective in controlling the aphid. The higher concentrations of these extracts (8 or 16%) were most effective in controlling aphid (>50%) at 24 h after application.

Keywords: allelopathy, cabbage aphid, control, water extracts

Canola (*Brassica napus* L.) crop is among the important sources of edible oil in Pakistan. (Naeem *et al.,* 2013; Khan *et al.,* 2012). Canola crop is damaged by different kinds of pests including weeds, insect pests and disease pathogens (McNairn *et al.,* 2014; Saeed and Razaq, 2014; Jabran *et al.,* 2010a; 2008), and cabbage aphid (*Brevicoryne brassicae*) is one of these pests (Saeed and Razaq, 2014; Razaq *et al.,* 2012). This insect feeds on upper parts of stem, leaves and reproductive structures to disturb seed set and pod filling in conola. Insect pest infestation can reduce canola yield by more than 30% (Brown *et al.,* 1999).

Application of insecticides is one way to control cabbage aphid in canola (Amer *et al.,* 2010; Lashkari *et al.,* 2007). However, aphid has evolved resistance against several insecticides in Pakistan and other parts of the world (Edwards *et al.,* 2008; Ahmad and Aslam, 2005). Also, sometimes the insecticides fail to fully control cabbage aphid in canola (Razaq *et al.,* 2014) and residues of applied insecticide may be included in food chain and badly affect the human health (Schecter *et al.,* 2010). The allelopathic potential of different plants as sorghum, sunflower, brassica, and mulberry has been reported in various studies (Jabran *et al.,* 2015; Jabran and Farooq, 2013) that can be sprayed just like insecticides (Farooq *et al.,* 2011).

The present studies were conducted with the objective to find out the effect of allelopathic water extracts from sorghum, sunflower, brassica and mulberry on mortality of cabbage aphid which severely damages canola crop in Pakistan.

Five laboratory experiments were conducted at the toxicology laboratory of Entomological Research Institute, Faisalabad, Pakistan. All the experiments were done with randomized complete block design using eight replications.

Crop herbage (sorghum, sunflower, and brassica) was harvested at maturity; mulberry leaves were collected from the mulberry tree, dried under shade and then chopped into 2 cm pieces with the help of fodder cutter. These chopped materials were used to prepare allelopathic water extracts according to methods suggested by Jabran *et al.* (2010a) and Farooq *et al.* (2008). This chopped material was soaked in the distilled water in a tub with a ratio of 1:10 (w/v) for 24 h. Water extracts were collected by passing through sieves (10 and 80 mesh). The filtrate was boiled at 100 °C for reducing the volume by 20 times. The concentrated extract was stored at room temperature and then used. The percentage of different allelopathic water extracts or their combinations used in these studies have been summarized in Table 1.

Aphids, *Brevicoryne brassicae* L. (Homoptera: Aphididae) and canola leaves were collected from canola crop grown at the Oilseed Research Area of Ayub Agricultural Research Institute, Faisalabad, Pakistan. Leaves were cut with the help of iron cutter and fitted

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Table 1: Concentrations of different water extracts used in these studies

Study-I Sorghum	Study-II Brassica	Study-III Sorghum +mulberry	Study-IV Sorghum +sunflower	Study-V Sunflower
Control	Control	Control	Control	Control
SWE-0.25%	BWE-0.25%	SOR+MUL-1%	SOR+SUN-1%	SNFWE-1%
SWE-0.5%	BWE-0.5%	SOR+MUL-2%	SOR+SUN-2%	SNFWE-2%
SWE-1%	BWE-1%	SOR+MUL-4%	SOR+SUN-4%	SNFWE-4%
SWE-2%	BWE-2%	SOR+MUL-8%	SOR+SUN-8%	SNFWE-8%
SWE-4%	BWE-4%	SOR+MUL-16%	SOR+SUN-S 16%	N F W E - 16%
SWE-6%	BWE-6%	-	-	-
SWE-8%	BWE-8%	-	-	-

SWE = Sorghum water extract; BWE = Brassica water extract; SOR+MUL = Sorghum + mulberry water extracts; SOR + SUN = Sorghum + sunflower water extracts; SNFWE = Sunflower water extract.

in plastic petri plates having a diameter of 8 cm. Leaf dip method was used to treat the plant leaves with allelopathic water extracts. Canola leaves were dipped in the respective solutions for a period of 10 sec, allowed to dry under air pressured drier, and fitted in the petri plates. All petri plates were labelled for its replication number and treatment. The leaves in control were dipped in distilled water for 10 sec. Forty aphids were dropped in eight petri plates (five in each petri plate) and allowed to feed on canola leaves for 24 h. The dead and alive aphids were counted after treatment of 24 h .

Percent mortalities in each treatment were calculated after adjusting control mortality according to Abbott (1925). Mean mortality (%) and standard errors were calculated for each treatment using Microsoft Excel Programme. The aphid mortality caused by allelopathic water extracts was presented in the form of bar graphs fitted with vertical standard error bars. Further, the data was subjected to analysis of variance test (ANOVA) using IBM SPSS Statistics 20.0 (Field, 2013). The difference among the means was calculated using Duncan's Multiple Range Test. The bar graphs were fitted with the lettering of respective treatments.

All allelopathic water extracts investigated in our studies had a significant effect on aphid mortality (Table 2). Sorghum water extract (8% concentration) caused the highest aphid mortality (63%) followed by 6% concentration of this allelopathic extract (Fig. 1). The sorghum water extracts with lower concentrations (0.25-0.5%) had the statistically same aphid mortality as noted for control while, sorghum water extracts (1-4% concentrations) caused an aphid mortality at par with 6% concentration as well as control (Fig. 1). Brassica water extracts (8, 6 and 2% concentrations) had caused highest aphid mortality (25-30%) (Fig. 1). Brassica

Table 2: Analysis of variance (p values) for indicating significance of various allelopathic water extracts on aphid mortality

	Study-I Sorghum	Study-II Brassica	Study-III Sorghum +mulberry	Study-IV Sorghum+ sunflower	Study-V Sunflower
Treat-ment	0.0001	0.002	0.0001	0.0001	0.0001

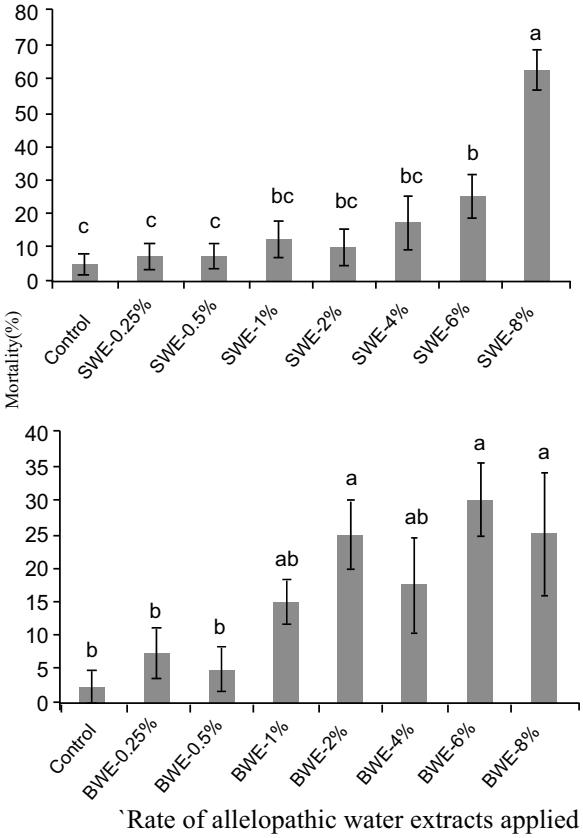

Fig. 1. Effect of sorghum and brassica alleopathic water extracts aphid mortality. SWE = Sorghum water extracts; BWE = Brassica water extracts; Bars not haring a letter in common differ significantly at $p \leq 0.01$.

water extracts (0.25 and 0.5% concentrations) had aphid mortality statistically similar with control (Fig. 1). A combination of sorghum + mulberry water extracts with 16% concentration showed highest aphid mortality compared with control and other water extract concentrations (Fig. 2). A combination of sorghum + sunflower water extract with 16% concentration resulted the highest aphid mortality followed by 8% and 4% concentrations of the same combinations (Fig. 2). For sunflower water extract, the concentrations of 16% caused significantly higher mortality over control and other treatments (Fig. 3).

The results of our studies indicated that allelopathic water extracts caused a considerable mortality (>50%) in cabbage aphid populations at 24 h after application.

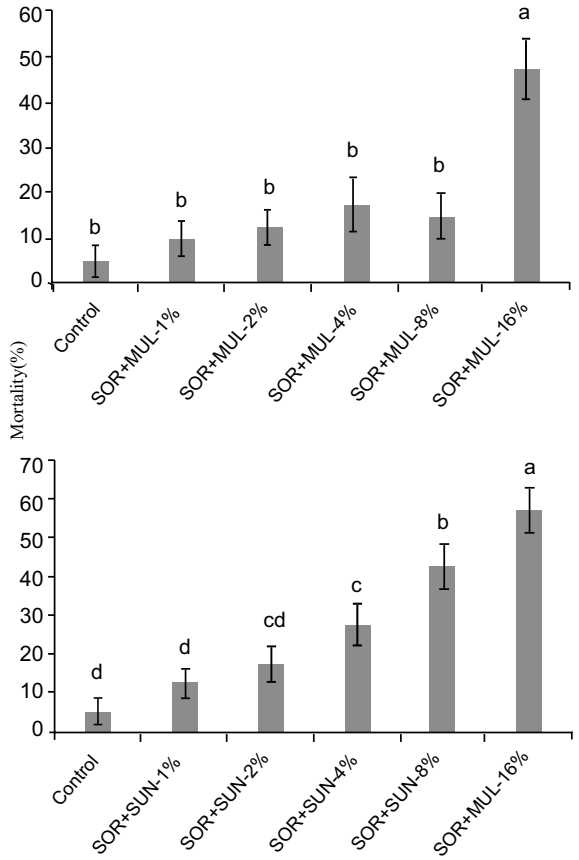

Fig. 2. Effect of sorghum + mulberry, and sorghum + sunflower allelopathic water extracts on mortality of aphid. SOR+MUL = Sorghum + mulberry water extracts; SOR+SUN = Sorghum + sunflower water extracts; Bars not sharing a letter in common differ significantly at $p \leq 0.01$.

This implies that chemicals from natural source can be applied to control cabbage aphid. This will help to avoid the addition of hazardous insecticide residues in canola oil.

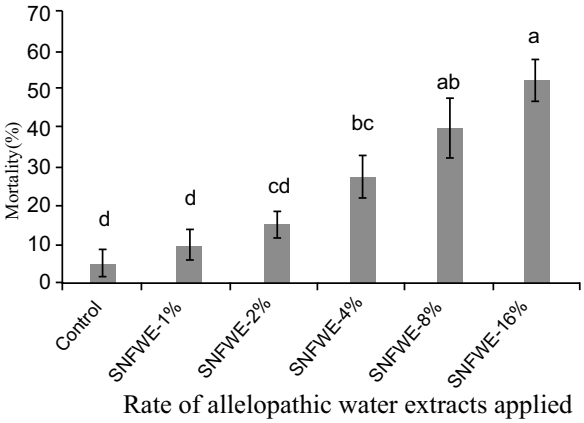

Fig. 3. Effect of sunflower allelopathic water extracts on mortality of aphid. SNFWE = Sunflower water extracts; Bars not sharing a letter in common differ significantly at $p \leq 0.01$.

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Review

Role of Alphasatellite in Begomoviral Disease Complex

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Abstract. A circular single stranded satellite, called as alphasatellite (initially identified as DNA-1), was characterised and confirmed to be associated with the Geminivirus begomovirus-betasatellite complexes. Alphasatellites are single stranded DNA (ssDNA) components, frequently accompanying with monopartite begomovirus or some time with bipartite begomovirus and/or betasatellite complex. The genome of alphasatellite DNAs are nearly half size of its helper virus genome and have no similarity with it. Furthermore, their function in begomovirus-betasatellite complex is still unclear. Recent advances in application of molecular tools helped in finding new viruses and allied satellite components that further help in advancing our understanding of this satellite DNA and this evolution.

Keywords. Geminivirus, begomovirus, alphasatellite

Introduction

Geminiviruses are an emerging group of plant viruses infecting most of economically important crops and ornamental plants throughout the world (Mansoor *et al.*, 2003). Based on the host range, genome organisation and the vector, the Geminiviruses are classified into seven genera: *Becurtovirus, Eragrovirus, Turncutovirus Topocuvirus, Curtovirus, Mastrevirus* and *Begomovirus* (Adam *et al.*, 2013; Brown *et al.*, 2012). However, majority of the members of this family belongs to the genus *Begomovirus* and are spread by the whitefly, *Bemisia tabaci* (Briddon and Stanley, 2006). Viruses of this genus are distributed into two sub-groups; bipartite begomoviruses with DNA-A+DNA-B genomes and monopartite begomoviruses that have a single DNA chain homologous to the DNA-A of bipartite begomovirus. DNA-A component of bipartite and the single component of monopartite begomoviruses (homologues to the DNA-A) encodes all viral functions required for virus replication, control of gene expression and insect transmission. All begomoviruses have a potential stem-loop structure containing the nono-nucleotide sequence TAA/TATT/AC, necessary for replication.

In the last few years alphasatellite, the member of mono-partite betasatellite/begomoviruses complexes, with a monomer of approximately 1375 nucleotide sequences, has attracted much attention and has become, probably, the most attentive scientific topic in the study of single stranded DNA (ssDNA) viruses. After the discovery of this satellite in 1999, more than 150 alphasatellite sequences have been deposited in database to date, however, very little is known about their function(s) during begomovirus-satellites infections. Examples of stability and maintaining of the alphasatellite component in natural infection with several begomoviruses complex have been shown several times since its first discovery, but without gaining further insights on their function (Shahid *et al.*, 2014; Amrao *et al.*, 2010; Mubin *et al.*, 2010). Certainly, alphasatellites are non-essential for virus infection and appear to play no major role in the etiology of the infections with which they are associated (Mansoor *et al.*, 1999). However, recent reports showed that some alphasatellites can attenuate disease symptoms caused by begomovirus-betasatellite complexes in the early stages of infection (Idris *et al.*, 2011; Nawaz-ul-Rehman *et al.*, 2010). An overview of the origin and evolution of alphasatellites including the recent advances in understanding their molecular structure and their applications for reverse genetics are discussed.

General characteristics of alphasatellites. Despite that alphasatellites were discovered virtually 15 years ago, very little information is available up til now about its function(s). Alphasatellite molecules are mostly associated with monopartite begomovirus-betasatellite complex and also monopartite begomovirus can contain this component frequently (Shahid *et al.*, 2014; Harimalala *et al.*, 2013; Zhou, 2013; Zia-Ur-Rehman *et al.*, 2013; Mubin *et al.*, 2010; Dry *et al.*, 1997). On the contrary, a few bipartite begomoviruses have been reported to be associated with alphasatellite (Satya *et al.*, 2014; Paprotka *et al.*, 2010).

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Initially, alphasatellites were found in association with the begomovirus-betasatellite complex from the old world (OW). Nevertheless, some distinctive alphasatellites were recently discovered to be associated with the new world (NW) begomovirus complex (Fiallo-Olive *et al.*, 2012). Alphasatellites are believed to have evolved from satellite-like, Rep-encoding components associated with nanoviruses (Wyant *et al.*, 2012; Briddon and Stanley 2006; Saunders and Stanley, 1999), another family of plant ssDNA viruses. Alphasatellite was also found in association with a yellow vein disease in *Ageratum conyzoides* (weeds) (Saunders and Stanley, 1999).

Genome, genomic organization and replication mechanism. The size of alphasatellite is between 1,300 bp to 1,400 bp nucleotides in length and has three conserved domains: a hairpin structure, a rolling circle replication initiator protein (Rep) and a rich region (A-rich) (Fig. 1). The hairpin structure has a loop that includes a unique nono-nucleotide sequence, which usually varies from rest of the begomovirus components, TAG/TAT/TAC and differs from the TAA/TAT/TAC sequence of geminiviruses by one nucleotide (G instead of A on a third nucleotide). In both begomoviruses and nanoviruses this sequence contains the origin of replication (ori) and is nicked by the rolling circle replication initiator protein to initiate viral DNA replication. The Rep of alphasatellite is the only single large open reading frame in the virion-sense which is predicted to encode a 315 amino acid product similar to the replication associated protein of nanoviruses. An adenine-rich region (approximately 200 bp with 45-52% adenine content) is also present, which is hypothesised to be a stuffer sequence that serves to fulfill the size constrain imposed by helper virus-mediated movement or encapsidation (Shahid *et al.*, 2014; Zhou, 2013). Alphasatellite can replicate autonomously and its replication is specifically mediated by its Rep (Tao *et al.*, 2004), while the replication of other components including betasatellite, is specifically mediated by the begomovirus Rep. This would suggest a difference in the begomovirus and alphasatellite replication origins. Recently, an alphasatellite associated with Okra leaf curl disease from West Africa (Kon *et al.*, 2009) is highly divergent molecule from previously characterized alphasatellites (Fiallo-Olive *et al.*, 2012; Saunders *et al.*, 2002) indicating geographically isolated evolution of a West African lineage of these satellites. The geographical distribution and the genetic diversity of these satellites are consistent with a long term association with monopartite begomoviruses (Briddon and Stanley, 2006).

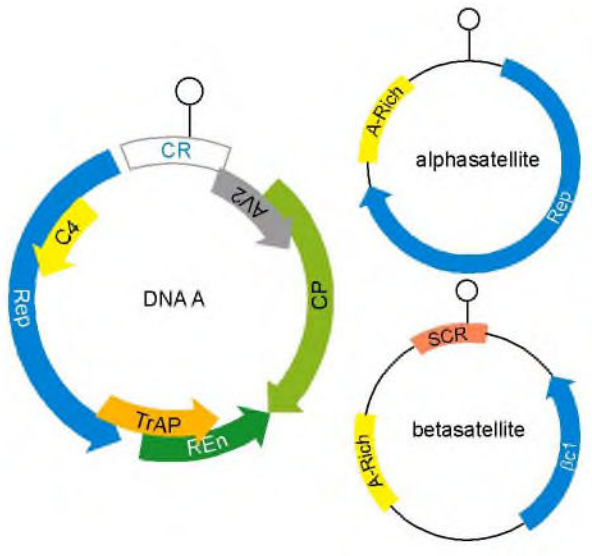

Fig. 1. Typical genome organisation of a monopartite-begomovirus satellites complex.

Genetic variability. Most of the monopartite begomovirus-betasatellite complex associated with alphasatellites have been characterised in the OW. Previous studies have shown that cotton leaf curl Multan virus (CLCuMV), cotton leaf curl Burewala virus (CLCuBV), tobacco leaf curl Pusa virus (TbLCuPuV), Ageratum yellow vein virus (AYVV), tobacco curly shoot virus (TbCSV), tomato yellow leaf curl virus (TYLCV), East African cassava mosaic Kenya virus (EACMKnV) and mungbean yellow mosaic virus (MYMV) are usually associated with alphasatellite (Satya *et al.*, 2014; Shahid *et al.*, 2014; Harimalala *et al.*, 2013; Kumar *et al.*, 2011; Singh *et al.*, 2011; Mubin *et al.*, 2010; Xie *et al.*, 2010; Mansoor *et al.*, 1999) (Fig. 2). Recently, different alphasatellites such as cassava mosaic (virus) alphasatellite, *Gossypium darwinii* symptomless alpha-satellite, Vernonia yellow vein Fijian alphasatellite associated with EACMKnV, and CLCuBV, MYMV were isolated from different hosts i.e., cassava, cucurbits and legumes (Satya *et al.*, 2014; Harimalala *et al.*, 2013; Zia-Ur-Rehman *et al.*, 2013). Interestingly, a strain of TYLCV originating from Oman has been shown to be associated with an unusual alphasatellite (Ageratum yellow vein Singapore alphasatellite), the only alphasatellite that was previously reported from Singapore back in 1999 (Idris *et al.*, 2011; Saunders and Stanley, 1999). Recently, Sida yellow vein China alphasatellite (SiYVCNA) has been identified in association with TYLCVV from main land Japan. However, the low levels of sequence divergence between all isolates of SiYVCNA suggests that this has only recently spread into Japan (Shahid *et al.*, 2014).

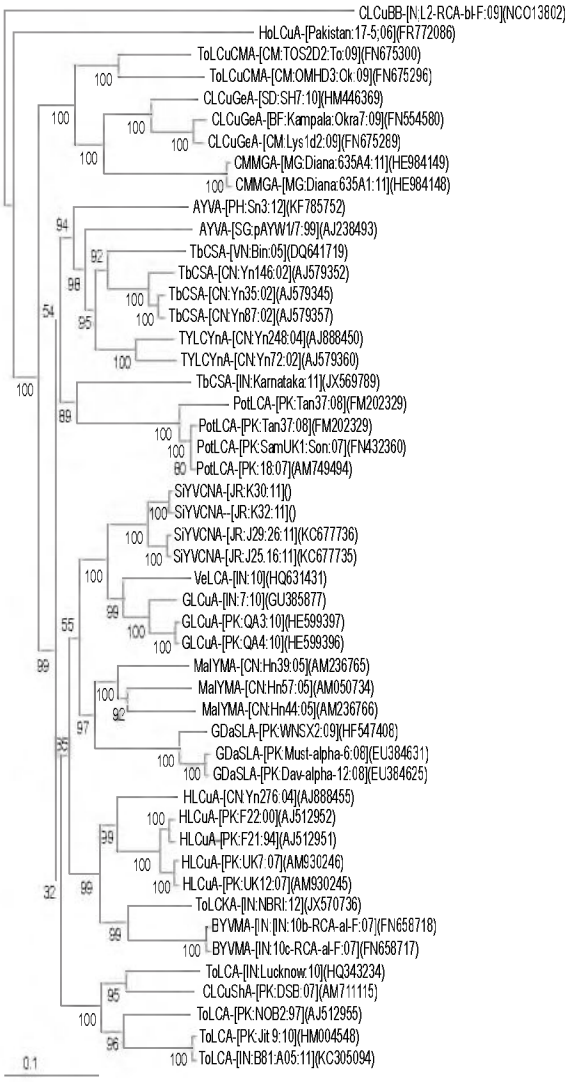

Fig. 2. Phylogenetic tree of commonly associated alphasatellites species.

Potential alphasatellite functions. Alphasatellites have no obvious contribution to symptoms induced by begomovirus-betasatellite disease complexes and appear to affect betasatellite replication but do not affect helper virus replication. However, some alphasatellites can attenuate disease symptoms caused by begomovirus-betasatellite complex in the early stages of infection. For example, Nawaz-ul-Rehman *et al.* (2010) have shown the alphasatellite Rep proteins encoded by two non-pathogenic alphasatellites, *Gossypium darwinii* symptomless alphasatellite (GDarSLA) and *Gossypium mustelinium* symptomless alphasatellite (GMusSLA). They can interact with Cotton leaf curl Rajasthan virus (CLCuRaV) Rep proteins (Table 1). Betasatellites depend solely for replication on the Rep proteins encoded by their helper begomoviruses: binding between alphasatellite-Rep and helper virus Rep proteins may inhibit betasatellite replication and results in down regulated expression of βC1 and correspondent symptom amelioration. Also GDarSLA and GMusSLA alphasatellite-Reps have strong gene silencing suppressor activities (Nawaz-ul-Rehman *et al.*, 2010). Although further investigations are required to prove whether alphasatellite-Reps encoded by other alphasatellites also have silencing suppressor activities. Recently, alphasatellites have been found in association with bipartite begomoviruses in Venezuela and Brazil (Zia-Ur-Rehman *et al.*, 2013; Romay *et al.*, 2010), respectively. The DNA-2 type alphasatellite, a different alphasatellite (only two members) of this alphasatellite are found until now, one from *Ageratum* in Singapore and the other from tomato from Oman (Idris *et al.*, 2011; Saunders *et al.*, 2002). Although all these members contain conserved alphasatellite genome features, the DNA-2 type molecules are less homogeneous and have less than 50% nucleotide sequence identity with each other. The DNA-2 type alphasatellite identified in Oman can attenuate begomovirus symptoms and reduce accumulations of betasatellites (Idris *et al.*, 2011). Further studies are needed to confirm whether these satellite molecules are replicated by their helper virus (es) and whether they have role in pathogenesis similar to those of betasatellites and some alphasatellites. New technologies like vector-enabled metagenomics and the recent circular DNA genomics (Ng *et al.*, 2011) are anticipated to soon provide additional information about the field distributions of these novel satellites and their associated begomoviruses. The promising study about the function of this satellite indicate that the alphasatellite is most likely a molecular parasite of the helper begomovirus (Kon *et al.*, 2009).

Viral vectors based on alphasatellites. Many plant viruses have been adapted into expression and VIGS vectors for external protein expression (Gleba *et al.*, 2007) and silencing (Purkayastha and Dasgupta, 2009) of target genes in main crop plants. Recently, tobacco curly shoot alphasatellite (TbCSA) was successfully used to silence β-glucuronidase and the sulphur desaturase genes in different *Nictoiana tabacum* cultivars (Purkayastha and Dasgupta, 2009). Among that it can be used to investigate gene expression (or as an expression vector) on the entire host range of the begomoviruses/curtoviruses. Alphasatellite has some unique properties that make this component distinctive among other molecules. For example, it has Rep gene which makes the alphasatellite autonomous in replication, secondly it has a-rich region if deleted cannot effect on

Table 1. Alphasatellite associated with monopartite-betasatellite complex

Alphasatellite	Acc. no.	Associated Virus	Source
Ageratum yellow vein alphasatellite	AJ238493	*Ageratum yellow vein virus* (AYVV)	Saunders *et al.*, 1997
Ageratum yellow vein India alphasatellite	JX570736	*Tomato leaf curl Karnataka virus*	Chatchawankanphanich and Maxwell, 2002
Ageratum yellow vein Kenya alphasatellite	AJ512960	Diversity of alphasatellite	Briddon *et al.*, 2004
Ageratum yellow vein Pakistan alphasatellite	FR772085	*Cotton leaf curl Burewala virus*	Iqbal *et al.*, 2013
Ageratum yellow vein Singapore alphasatellite	AJ416153	AYVV	Saunders, 1999
Cassava mosaic Madagascar alphasatellite	HE984148	*East African cassava mosaic Kenya virus*	Harimalala *et al.*, 2013
Cleome leaf crumple alphasatellite	FN436007	*Cleome leaf crumple virus*	Paprotka *et al*, 2010
Cotton leaf curl Dabwali alphasatellite	AJ512957	Diversity of alphasatellite	Briddon *et al.*, 2004
Cotton leaf curl Gezira alphasatellite	FM164740	AYVV	Leke *et al.*, 2013
Croton yellow vein mosaic alphasatellite	FN658711	*Croton yellow vein mosaic virus*	Zaffalon *et al.*, 2011
Euphorbia yellow mosaic alphasatellite	FN436008	*Euphorbia yellow mosaic virus*	Fernanda *et al.*, 2011
Gossypium darwinii symptomless alphasatellite	EU384606	*Cototn leaf curl Rajasthan virus*	Nawazul-Rehman *et al.*, 2010
Hibiscus leaf curl alphasatellite	AJ512950	Diversity of alphasatellite	Briddon *et al.*, 2004
Hollyhock yellow vein symptomless alphasatellite	FR772086	*Hollyhock yellow vein virus*	Saunders *et al.*, 2000
Lantana yellow vein alphasatellite	KC206075	*Lantana yellow vein virus*	Marwal *et al.*, 2013a
Malvastrum yellow mosaic alphasatellite	AM050734	*Malvastrum yellow mosaic virus*	Guo *et al.*, 2006
Malvastrum yellow mosaic Cameroon alphasatellite	FN675297	*Tomato yellow leaf curl China virus* (ToLCCNV)	Leke *et al.*, 2011
Melon chlorotic mosaic virus alphasatellite	FM163578	*Melonchlorotic leaf curl virus*	Romay *et al*, 2010
Mesta yellow vein mosaic alphasatellite	JX183090	*Mesta yellow vein mosaic virus*	Chatterjee *et al.*, 2005
Okra leaf curl alphasatellite	AJ512954	Diversity of alphasatellite	Briddon *et al.*, 2004
Sida yellow vein Vietnam alphasatellite	DQ641718	*Sida yellow vein Vietnam virus*	Ha *et al.*, 2006
Tobacco curly shoot alphasatellite	AJ579361	*Tomato yellow leaf curl China virus* (ToLCCNV)	Xie *et al.*, 2010
Tomato yellow leaf curl China alphasatellite	AJ579358	ToLCCNV	Xie *et al.*, 2010
Verbesina encelioides leaf curl alphasatellite	HQ631431	*Hollyhock yellow vein virus*	Prajapat *et al.*, 2011
Vernonia yellow vein Fujian alphasatellite	JF733780	*Vernonia yellow vein Fujian virus*	Zulfiqar *et al.*, 2012
Vinca yellow vein alphasatellite	KC206076	*Vinca yellow vein virus*	Marwal *et al.*, 2013b

its replication, lastly this molecule is quiet small and easy to manipulate. Shahid *et al.* (2009) have shown by agro-inoculation studies with a-rich deleted cotton leaf curl Multan alphasatellite (CLCuMA) that this sequence is not required for the infectivity or maintenance of CLCuMA. Also CLCuMA has a wider host range and can successfully be maintained by a large number of diverse *Begomovirus* species. The ability to amplify itself is useful in a vector since it will increase the copy number (and thus also expression) of inserted sequences, deletion of a-rich region to increase the insert size and wider host range makes it a potentially useful vector (Tao and Zhou, 2004). The a-rich deleted CLCuMA was maintained in plants in the presence of a begomovirus. Although little is yet known about the maintenance of alphasatellites by begomoviruses, it is likely that high-level replication of these molecules is required for their maintenance, which depends upon its own Rep. There is no evidence for a (strong) selection mechanism for maintenance of alphasatellites. Maintenance of alphasatellites can simply be a selection of numbers; plants containing high levels of the satellite allow cell-to-cell movement by the virus encoded movement proteins or infection to the next plant by the vector of the helper begomovirus. Tao and Zhou (2004) used modified CLCuMA for virus induced gene silencing vector in plants. The same vector was used to successfully silence the chelates gene (ChlI). One of the advantages of an alphasatellite vector, over many of the other vectors, is that it can, at least in theory, be used with different *Begomovirus* or even *Curtovirus* (Saunders *et al.* 2002).

Recent research advances. Recent progresses in research comprises of the construction of the alphasatellite-based vectors, the development of the first VIGS system for different agricultural crops, the description of new alphasatellites, improvement in diagnostics, and new information on the begomovirus-satellite complex.

Conclusion

The role that alphasatellites play in begomovirus-satellite disease complex is still generally unidentified. The recent advancement and emerging potential of Next Generation Sequencing approaches will undoubtedly contribute considerably to the elucidation of the aetiology of many of these alphasatellite associated diseases. The fairly recent discovery of alphasatellite in Japan (Shahid *et al.*, 2014)

and its presence in papaya gardens in Nepal (Shahid *et al.*, 2013) suggest that its occurrence and possible role in disease in other agricultural-producing regions need to be investigated. What effect the presence of an alphasatellite and the defective allied component may have on future begomovirus-betasatellite complex is not clear.

Whereas outdated research focused on the detection and characterisation of prevailing and new begomovirus-satellite complexes, we believe research on (i) the elucidation of the etiology of these disease complexes (ii) the development of resistance using non-transgenic approaches, and (iii) studies on the molecular interaction of alphasatellites and their helper viruses with their original host. As efficient tools are being developed now, future research with begomoviruses, as well as with all other whitefly-vectoring viruses, has to move from typical (model) plants like *Nicotiana benthamiana* towards other host plants to allow the study of symptomology, pathogenicity, host-plant response and viral determinants of vector transmission in their natural host.

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