Sunflower Oil in Dairy Substitute

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Abstract

Vegetable oils are preferred over the solid animal fats because of health benefits. Oils contain higher proportion of unsaturated fatty acids, while solid fats contain more saturated fatty acids, which increased the low density lipoprotein (LDL) level of the blood, which is considered harmful for human health. The demand for low fat cheese, a low calorie product, has grown steadily over the last few years and is also gaining popularity. This investigation was carried out to study the effect of using sun flour oil at the rate of (0, 1, 2 and 3%) as a replacer of animal fat on quality of mozzarella cheese. Cheese samples were stored at 4C° to be assayed every 10 days (0, 10, 20, 30 and 40 days) for changes on biophysical and functional properties of cheese. The total volatile fatty acid (TVFA) of cheese increased significantly (P≤ 0.05) with levels of the sun flower oils and storage time. The increased in samples 3% (18.75 ml 0.1 NNaOH) was significantly (P≤ 0.05) more compared to sample 0% (15.0 ml 0.1 NNaOH). The formol ripening index (FRI) were showed increasing trend as the level of the oil increased and storage time progressed significantly (P≤ 0.05). The saturated and unsaturated fatty acids (mg/100g) of Mozzarella cheese were affected significantly (P≤ 0.05) by the level of sun flower oil, the highest values were obtained by samples 3% oils while the lowest were recorded by 0% oils. The meltability and oil separation index of Mozzarella cheese increased while the levels of the stretchability decreased. The microstructure of Mozzarella cheese also showed that, the protein matrix, the little fat globules and small cavities were affected by the level of oil.

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Introduction

Mozzarella cheese is a mild, white fresh cheese made by a special process where the curd is dipped into hot whey then stretched and kneaded to the desired consistency. At one point, Mozzarella was made only from water buffalo milk. Now, it is usually made with cow's milk. There are two forms, regular and fresh. Regular Mozzarella is available in low-fat and nonfat forms and has a semi-soft, elastic texture and is drier than fresh Mozzarella. Fresh Mozzarella is made from whole milk and has a softer texture and sweet, delicate flavour and is typically packed in water or whey. Mozzarella cheese has many health benefits; it is a good source of protein, vitamins and minerals. Consumption of Mozzarella cheese may protect against gout, a painful condition that results in the buildup of uric acid crystals in the joints. The calcium found in Mozzarella cheese also has its contribution in body weight loss and provides protection against breast cancer and metabolic syndrome, which is a group of conditions that increase the main sources of animal fat and dietary cholesterol. Even though cholesterol is essential for membrane structure, hormone and steroid biosynthesis (Mahann and Escott-Stump, 1996).

However, fat contents responsible for many desirable functional, textural, and sensory properties in cheese and its decrease alters physical and flavor properties and lowering cheese quality. Low and reduced fat cheeses have certain disadvantages stipulated by reduction in proportion of moisture in non-fat substances (NFs), level of proteolysis activity, amount of free oil and increased proportion intact casein . In addition, it is possible to produce vegetable oil based milk fat replacement in a more stable and convenient form (Ara *et al*., 2002).

All edible oils consumed in Sudan are vegetable oils which are produced locally. The vegetable oils are produced for both local consumption and exportation.

Sun flower oil has been considered as one of the best vegetable oils, due to high linoleum acid content. Biological value of sun flower oil is estimated on the basis the composition of fatty as well as on the content of lyposoluble vitamins, particularly the vitamin E. The oil should contain large quantities of linoleum and alpha–tocopherol (Bashir, 1986). The objective of this work is to study the effect of using different levels of sun flour oil as fat replacer on biophysical and functional properties of Mozzarella cheese during the storage period.

MATERIALS AND METHODS

Materials

Fresh cow's milk was obtained from a private farm in Shambat Khartoum North Sudan. Salt (sodium chloride) was bought from local market Khartoum North, Rennet powder from Chr-Hansen's Laboratory, Denmark. Sun flower oil was also bought from the local market Khartoum North Sudan.

Methods

Preparation and Manufacture of Mozzarella Cheese

The manufacture of Mozzarella cheese was done using the traditional method. 133 liters of fresh whole cow's milk, the milk fat was skimmed by centrifuge separation (Appendix 1), the skimmed milk was divided into four equal portions. The first portion was the control, in the next three portions sun flower oil was added at 1%, 2% and 3% respectively. The types of oil were homogenized using Homogenizer (Appendix 2). The rennet powder was added (0.05%) to all four portions.

Appendix 1

Appendix 2

The milk was stirred for 5 minutes and left in room temperature until a smooth thick curd was formed after 30 minutes (coagulation). After coagulation the curd was cut into about 3 centimeters cubes using a stainless steel knife. The curd was squeezed until all the whey was drained. The curds were formed into blocks and left in open areas to drain off the remaining whey. The drained curd was exposed to warm temperature until the required acidity was reached at critical pH (5.2-5.3). The curd was then put in hot water at 70-80C and mixed properly for 5 minutes until a smooth elastic mass was obtained, stretched into proper forms using stainless steel container for 3 hours slightly salted in 5% cold brine solution (Sodium Chloride) for 2 hours. The cheese was removed from the brine and dried lightly, weighed and packaged in polyethylene. The prepared cheese was analyzed for physio-chemical and functional properties during storage periods at 0, 10, 20, 30 and 40 days intervals at 4C.

Biochemical Analysis

Total Volatile Fatty Acids

The total volatile fatty acids (TVFA) contents in the cheese were determined by the direct distillation method of Koiskowski, (1982). Five grams of the cheese were placed in a mortar and ground with successive portion of 10% sulphuric acid until the volume of the acid added to the cheese sample reached 25 ml. About 17.5 gm of magnesium sulphate was added to the flask content followed by few glass beads and exactly 125 ml distilled water. The flask was fitted to the Kjeldahl distillation until the contents were distilled. Distillation was terminated when 140 ml of the distillate were collected. The inside tube of the condenser was rinsed with 5 ml of neutral alcohol to remove the insoluble volatile fatty acids, combined with distillate and then titrated with 0.1N NaOH that neutralize the distillate from it well, filtered and 5 ml 100 gm of cheese.

Formol Ripening Index

The method of Abdel-Tawab and Hofi (1977) was used to measure the formol and ripening index. Five grams of cheese were weighed, emulsified with warm distilled water, made up to 55 ml with distilled water at 60°C, and then filtered 42 filter paper). Ten ml

of the filtrate were titrated with 0.1 NNaOH using 0.5 ml phenol- phethalein indicators (Title A). A 2 ml of a neutralized formaldehyde solution was added to the flask contents and titration was continued with 0.1 NNaOH (Title B).

Formol ripening index (FR1) = (A-B) X 100

Determination of Fatty Acids Content

Fatty acid composition of different samples of Mozzarella cheese was analyzed by Gas Liquid Chromatography (GLC) using GC (Thermo quest) QP2010/Shimad 24 by (Reglament, 1991). Reagent was added 2g of cheese sample were taken in a test tube, 7 mls of alcoholic Sodium hydroxide that prepared by →2g NaOH and complete the volume to 100 ml by methanol, were added, then 7ml of alcoholic sulfuric acid (1 ml H_2SO_4 with 100 ml methanol) were added. The mixture was gently shook and left over night after 24 hrs, Nonanoic acid used internal standard. A standard fatty acids mixture containing 37 fatty acids was used to provide standard retention times. Fatty acids identified by comparing their retention times with those of fatty acids in standard samples. An auto system Thermo quest GC-MS equipped was used to analyze FA of cheese samples.

Rheological Analyses

Determination of Meltability

Meltability of cheese was measured in duplicate as outlined by Olson and Price (1958) and as modified by Savello *et al.,* (1989), where a Pyrex glass tube 30 mm in diameter and 250 mm long was used to hold the cheese during the melting test. One end of the tube closed with a rubber stopper perforated by 1 mm glass tube to act as a vent. A reference line was marked on the opposite end of the melting tube. This end of the tube was also closed with a rubber stopper. A solid 15g cheese cylinder is placed in the tube with its front edge aligned with the marked reference line. Melting tubes were placed in a vertical position on a rack for 40 min at approx. 40˚C then in a horizontal position in an oven at 110˚C for 30 min Flow of the hot cheese mass was stopped instantly for measurement with a control rack. The distance of flow from the reference line to the leading edge of the melted cheese was quickly measured and recorded in millimeters as "cheese meltability".

Determination of Oil Separation

Oil separation was determined according to the method outlined by Thomas *et al.,* (1980) as follows: a cork borer was used to obtain cylindrical sample of processed cheese, approximately 17.0X 17.0mm. . The sample was pressed gently between two sheets of Whatman No. 41 filter paper and incubated at 45C for two hours. The diameter of the spread oil zone was measured in mm and was used as index of oil separation according to following equation:

OSI = (D2-D1) D1x 100

Where:

OS1 = Oil Separation Index

D1 = Diameter of cheese fat zone before heating.

D2 = Diameter of cheese fat zone after heating

Stretchability

An apparatus was designed and constructed measuring the Stretchability of cheese (Davis, 1966). It is based on heating a specified amount of cheese under specified conditions on a thermostatically controlled hot plate, followed by measuring the distance between the hot plate and mobile stretching plate before the cheese strands are torn off. Measuring procedure: A desalted 15g cheese sample is manually crumbled using a spatula and spread evenly on the filter over the hot plate surface and preheated to 8C (40 sec).The stretching plate is lowered by the pulley handle until settling on the heated sample and held for 5 sec. The stretching plate is then consciously and steadily lifted by the pulley handle until the stretched cheese strands are torn off. The distance between the two plates in cm is considered as a measure of Stretchability.

Microscopic Analyses

Scanning electron microscopy is a valuable technique in dairy research because it provides information on microstructure of dairy products which can be related to physical properties. Small cubes of the cheese analogues were fixed with 2.5% (v/v) glutaraldehyde in water for 1 hour and rinsed three times with phosphate buffer. After that, the samples were then put in 0.2% (w/v) left overnight. The samples were critical point dried through CO_2. They were then fractured and coated by diode sputter coating (Graviver *et al.,* 2004). Micrographs were made with a Quanta- 200 at an acceleration voltage of 10.0 kv.

Statistical Analyses

Statistical analyses were done using the statistical analyses system SAS, (1997) using 5% level of significance. Mean were separated using Duncan’s Multiple Range Test.

Results and Discussion

Total Volatile Fatty Acid

Table 1 shows the total volatile fatty acid (TVFA) of Mozzarella cheese. The levels of sunflower oil significantly (P≤0.05) affected TVFA of Mozzarella cheese. Sample 0% sunflower oil gave the lowest TVFA (15.0mls0.INNaOH) while sample 3% sunflower oil was the highest (18.75mls 0.INNaOH) with sample 1% and 2% sunflower oil being in an intermediate position (16.50 and 17.60mls0.INNaOH) respectively. The total volatile fatty acid content increased with increasing levels of sunflower oil. Sulieman, (2011) found that, the TVFA of cheese using the different levels of fat milk increased with increasing levels of fat milk. Abdel-Razig *et al.,* (2013) mentioned that the total volatile fatty acid increased with increasing levels of sunflower oil.

Table 1. Effect of levels of sunflower oil (%) on total volatile fatty acid (mls 0.INNaOH)* of Mozzarella cheese during storage period

Storage period (days)	Levels of sunflower oil (%)			
	0	1	2	3
0.0	15.00±0.20^{o}	16.50±0.19^{n}	17.60±0.23^{m}	18.75±0.18^{l}
10	19.60±0.17^{k}	20.80±0.16^{j}	21.70±0.15^{h}	22.75±0.25^{g}
20	20.50±0.13^{j}	22.05±0.14^{g}	23.15±0.16^{f}	24.50±0.20^{de}
30	20.80±0.19^{i}	23.00±0.11^{f}	24.60±0.12^{d}	25.85±0.22^{c}
40	21.25±0.16^{h}	24.15±0.19^{cd}	26.75±0.17^{b}	27.50±0.14^{a}

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

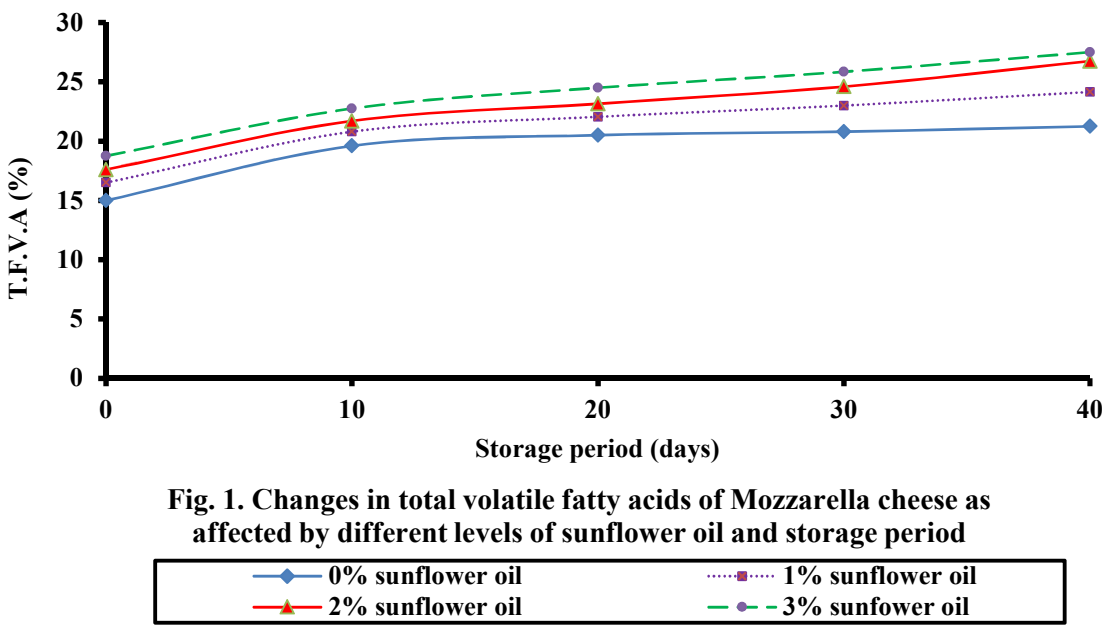

Fig. 1. Changes in total volatile fatty acids of Mozzarella cheese as affected by different levels of sunflower oil and storage period

Formol Ripening Index

Table2 shows the formol ripening index (FRI) of Mozzarella cheese as affected by the levels of sunflower oil. The levels of sunflower oil significantly (P≤0.05) affected the formol ripening index of Mozzarella cheese. Sample 0% sunflower gave the lowest FRI (20.50%), while sample 3% sunflower oil was the highest (27.50%) with sample 1% and sample 2% sunflower oil being in an intermediate position (23.30% and 25.60%, respectively) the formol ripening index increased with increasing levels of sunflower oil. Badwai, *et al.,* (2006) found that, FRI of Mozzarella cheese ranged from 12.08- 19.98%. Abdel-Razig *et al.,* (2013) found that, the formol ripening index increased with increasing levels of sunflower oil.

Table 2. Effect of levels of sunflower oil (%) on formol ripening index* of Mozzarella cheese during storage period

Storage period (days)	Levels of sunflower oil (%)			
	0	1	2	3
0.0	20.50±0.15^{m}	23.30±0.13^{l}	25.60±0.16^{k}	27.50±0.12^{i}
10	23.80±0.11^{l}	26.50±0.14^{j}	28.75±0.09^{h}	30.05±0.18^{g}
20	25.40±0.19^{k}	29.30±0.15^{gh}	30.85±0.11^{ef}	32.25±0.17^{d}
30	27.85±0.13^{i}	31.65±0.14^{e}	33.50±0.16^{cd}	35.75±0.18^{c}
40	30.15±0.12^{f}	34.30±0.15^{cd}	37.50±0.17^{b}	39.85±0.14^{a}

*Mean±SD having different superscript letters in columns and rows are significantly different (P≤0.05).

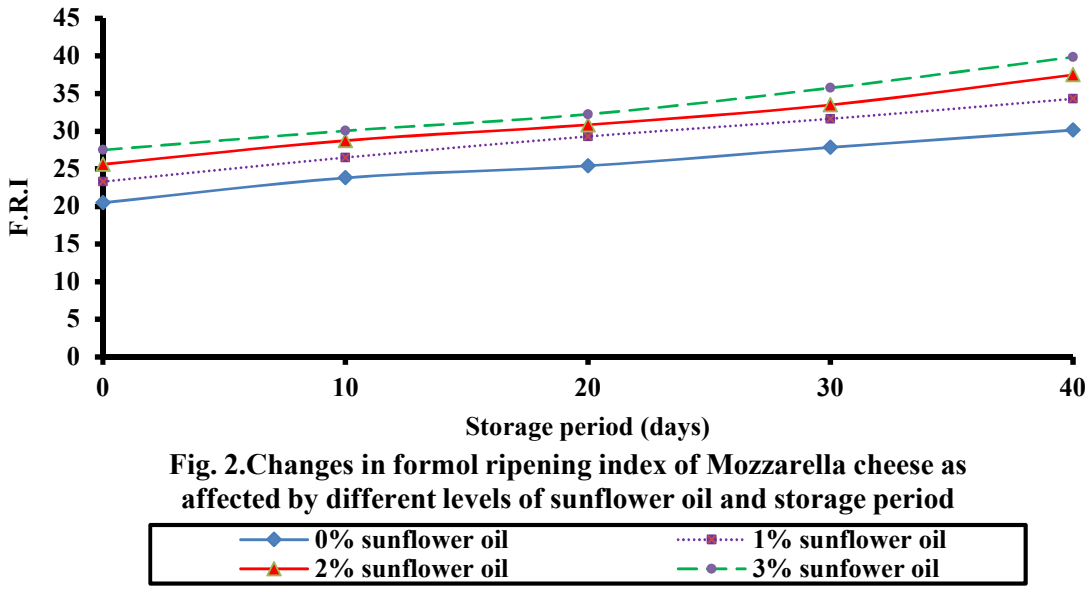

Fig. 2.Changes in formol ripening index of Mozzarella cheese as affected by different levels of sunflower oil and storage period

Fatty acids composition

Saturated fatty acids

Table 3. shows the effect of levels of sunflower on saturated fatty acids (mg/100g). The saturated fatty acids affected significantly (P≤0.05) by levels of sunflower oil. The highest values of (Carprylic, carpic, Tridecnoate, Myristic,Palmitic, Stearic and Arachidic acid (0.42,1.52,3.60,360,18.60,0.86 and0.68mg/100g), respectively were obtained by samples 3% sunflower oil while the lowest values (0.20,0.50,0.04,0.17,0.02,0.15 and 0.50 mg/100g, respectively)were recorded by 0%. Mustafa *et al.,* (2013) found that, the concentration of long chain were higher than those recorded by Kinik *et al.,* (2005) who found a ranged of 0.45 to 0.71 (mg/100g) in the Turkish cheese.

Table 3. Effect of levels of sunflower oil (%) on saturated fatty acids (mg/100g)* of Mozzarella cheese

Scientific name	Common name	No. of carbon atom	Levels of sunflower oil (%)			
			0	1	2	3
Octanoic	Carprylic	8	0.20^{d} ±0.06	0.22^{c} ±0.07	0.24^{b} ±0.08	0.42^{a} ±0.04
Decanoic	Capric	10	0.05^{d} ±0.02	0.81^{c} ±0.03	0.95^{b} ±0.09	1.52^{a} ±0.01
Tridecanoic	Tridecanoate	13	0.04^{d} ±0.01	0.06^{c} ±0.04	1.82^{b} ±0.09	3.60^{a} ±0.04
Tetradecanoic	Myristic	14	0.17^{d} ±0.04	0.20^{c} ±0.06	1.37^{b} ±0.08	3.60^{a} ±0.02
Pentadecanoic	Pentadecanoate	15	1.18^{a} ±0.08	0.80^{b} ±0.06	0.11^{d} ±0.07	0.18^{c} ±0.05
Hexadecanoic	Palmitic	16	0.02^{d} ±0.01	2.55^{c} ±0.12	16.35^{b} ±0.02	18.60^{a} ±0.04
Heptadecauoic	Heptadecanoate	17	0.55^{b} ±0.12	0.55^{b} ±0.09	0.46^{c} ±0.07	0.73^{a} ±0.06
Octadecanoic	Stearic	18	0.15^{d} ±0.05	0.47^{c} ±0.06	0.54^{b} ±0.08	0.86^{a} ±0.09
Eicesanoic	Arachidic	20	0.50^{d} ±0.03	0.47^{c} ±0.05	0.58^{b} ±0.08	0.68^{a} ±0.07

*Mean±SD having different superscript letters in rows are significantly different (P≤0.05).

Unsaturated fatty acids

Table 4. shows the unsaturated fatty acid (mg/100g) of Mozzarella cheese as affected by levels of sunflower oil significantly (P≤0.05). The highest values of Lauric (0.72mg/100g), Palmitoleic (1.22mg/100g), Oleic (28.80mg/100g) and Linoleic (34.22mg/100g) were obtained by sample 3% sunflower, while lowest (0.05, 0.45, 0.32 and 2.20mg/100g, respectively) recorded by 0% sunflower oil. Mustafa *et al.,* (2013) mentioned that, the oleic acid was found in a higher concentration (21.95-36.89mg/100g) in all cheese samples.

Table 4. Effect of levels of sunflower oil (%) on unsaturated fatty acids (mg/100g)* of Mozzarella cheese

Scientific name	Common name	No. of carbon atom	Levels of sunflower oil (%)			
			0	1	2	3
Dodecanoic	Lauric	12	0.05^{d} ±0.12	0.24^{c} ±0.15	0.52^{b} ±0.14	0.72^{a} ±0.13
9-Hexadecanoic	Palmitoleic	16	0.45^{d} ±0.07	0.76^{c} ±0.06	0.94^{b} ±0.04	1.22^{a} ±0.03
9-Octadecanoic	Oleic	18	0.32^{c} ±0.06	16.20^{b} ±0.03	28.30^{a} ±0.02	28.80^{a} ±0.04
9.12-Octadecadienoic	Linoleic	18:2	2.20^{d} ±0.13	20.40^{c} ±0.16	31.68^{b} ±0.12	34.22^{a} ±0.14
9.12.15-Octadecatrienoic	Alphlinoleic	18:3	0.30^{b} ±0.15	0.18^{d} ±0.07	0.22^{c} ±0.16	0.32^{a} ±0.13
9-Eicosenoic	Cadoleic	20	0.50^{d} ±0.11	0.64^{c} ±0.09	0.68^{b} ±0.08	0.93^{a} ±0.07

*Mean±SD having different superscript letters in rows are significantly different (P≤0.05)

Rheological properties

Table 5 shows the effect of levels of sunflower oil on meltability, stretchability and oil separation index of mozzarella cheese.The meltability (mm), stretchability (cm) and oil separation index significantly (P≤0.05) affected by levels of sunflower oil. The maltability(Fig. 3) increased as levels of sunflower oil increased. Sample 3% sunflower oil gave the highest meltability (170.0 mm), while sample 0% reordered the lowest (10.0 mm). The stretchability (Fig. 4) decreased as the levels of sunflower oil increased. Sample 0% sunflower oil gave the highest stretchability (400.0cm) while the lowest value (220.0cm) reordered by sample 3% sunflower oil. The oil separation index (Fig. 5) significantly (P≤0.05) affected by the levels of sunflower oil. Sample 3% sunflower gave the highest value (40.00), while sample 0% (0.00) recorded lowest value.

Sameen *et al.,* (2008) found that, the meltabihty of cheese containing 2% fat was higher than the other sample followed by 1.5% fat and 0.75% fat, while the cheeses containing 0.75% fat was found to have more stretchability which is the characteristic of mozzarella cheese..El-Batawy *et al.,* (2004) found that, there were increasing in oil separation index when using high levels of vegetable oil in mozzarella cheese. The increase in melt is attributed to increase proteolysis, fat coalescence and water binding capacity of casein matrix, which promotes heat induced displacement of adjoining layers of the casein matrix on heating (Guinee,2003).

Table 5. Effect of levels of sunflower oil (%) on rheological properties* of Mozzarella cheese

Parameter	Levels of sunflower oil (%)			
	0	1	2	3
Meltability (mm)	10.00 ± 0.08^{d}	60.00 ± 0.11^{c}	125.00 ± 0.09^{b}	170.00 ± 0.07^{a}
Oil separation index	0.0 ± 0.02^{d}	24.00 ± 0.07^{c}	30.00 ± 0.06^{b}	40.00 ± 0.05^{a}
Stretchability (cm)	400.00 ± 0.15^{d}	345.00 ± 0.13^{a}	320.00 ± 0.16^{b}	220.00 ± 0.14^{c}

*Mean±SD having different superscript letters in rows is significantly different (P≤0.05).

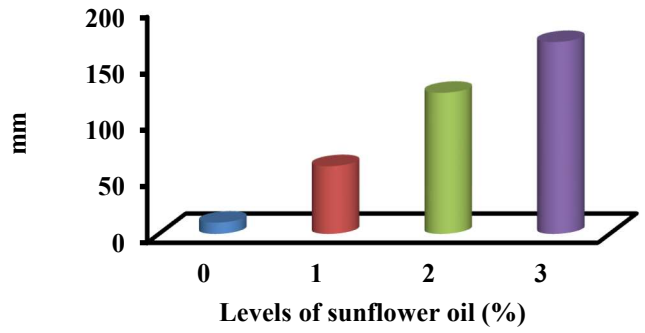

Fig. 3. Effect of levels of sunflower oil on metability of Mozzarella cheese

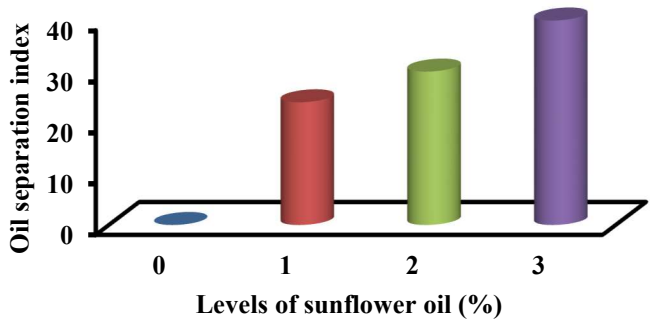

Fig. 4. Effect of levels of sunflower oil on oil separation index of Mozzarella cheese

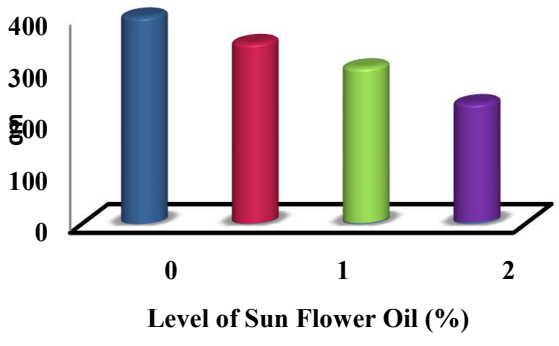

Fig:5. Effect of levels of sun flower oil on…

Microstructure of Mozzarella cheese

Plate1. shows the structural features using scanning electron micrographs of mozzarella cheese with different levels of sunflower oil. In Plate A. the protein matrix appeared as smooth linear area containing little fat globules and small cavities, the complete substitution of milk fat by sunflower vegetable oil (0, 1, 2, and 3%) confirmed noticeably different structural characteristic of fat globules embedded in the cheese samples, the smoothness protein matrix decreased as the sunflower oil level increased, at the same time cavities with different size and fats globules increased. The samples made with full-fat contained higher concentrations of fat globules compared with low-fat cheese analogues (Liu *et al.,* 2008). The quantities of cavities in low-fat samples cheese were less than those in full-fat samples (Willatsa *et al.,* 2006). Microstructure of Mozzarella cheese showed that low fat cheese tends to be harder, more crumbly and less smooth than normal. Flavour and appearance score increased and storage period progress while body and texture score decreased (Abdel- Rafee *et al.,*2004).

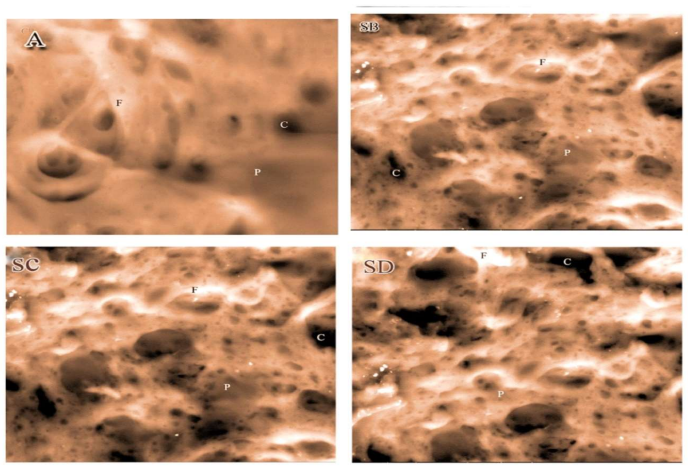

Plate 1. Scanning electron micrographs of Mozzarella cheese with different levels of sunflower oil

Legend:

A : 0% sunflower oil
SB : 1% sunflower oil
SC : 2% sunflower oil
SD : 3% sunflower oil
C: cavity formed after water or air in the cheese
P: protein aggregate
F: fat globule

Conclusions

- Fat content of cheese has undergone obvious lipolysis as shown from significant liberation of total volatile fatty acids.
- The formol ripening index increased significantly during the storage period.
- The fatty acids significantly ($P \leq 0.05$) increased with levels of the oils during storage period.
- The meltability and the oil separation index of the cheese increased, while the streatchability decreased.
- In microstructure the protein matrix, fat globules and cavities showed changes within the level of the vegetable oils.
- Sample 2%of the sun flower oil significantly ($P \leq 0.05$) secured the best followed by 1% and 3% while the worst recorded by 0% oil.

Recommendations

- Cheese producer should be give special consideration to the level of oils substances of vegetable oils.
- Since Mozzarella cheese in still prepared traditionally an improvement of quality through introduction of modern technology is needed.
- Evaluation of microbial quality of this cheese during manufacturing and storage to cater for HACCP system for the end product.
- Special Mozzarella cheese should be produced for those suffering from heart disease.
- Production of Mozzarella cheese by using sunflower oil is considered useful for the individuals having health problems.

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