Development of Cheese Analogue using Olive Oil and Lactobacillus Bulgaricus

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Abstract
Cheese considered as a well-known dairy product which is manufactured in many varieties according to its texture and flavors. Cheese is formed by coagulation of casein and having high protein contents. Due to increase awareness of modern consumers' fortification of dairy foods including fresh cheese are in demand. Cheese analogues are made for fulfilling the demand of cheese. Cheese analogues are processed cheese-like product and enriched in nutrients. It is healthy and seems to be attractive when it is rearranged and prepared by using ingredients coming from natural source. Cheese analogue produced from olive oil is used as an alternate of cheese. Olive oil improves cardiovascular risk factors, such as endothelial dysfunction, blood pressure, postprandial hyper-lipidemia, lipid profiles, antithrombotic profiles and oxidative stress. The objective of present study is to develop cheese analogue using olive oil and *Lactobacillus bulgaricus*. Single step emulsification was done for fat stabilization. *L. bulgaricus* was isolated from yoghurt. Cheese analogue was subjected to physicochemical, microbiological and sensory analysis. Proximate analysis (moisture, pH, fat contents, ash, total solids and acidity) physicochemical analysis, sensory analysis and rheological analysis were performed. Physiochemical investigation has demonstrated that, with an increase in the olive oil level in cheese, non-significant pH, moisture, fat, total solids, total nitrogen and protein content were considerably influenced by olive oil amounts. Rheological research showed that olive oil quantity has a substantial effect on curd texture. Flavor and overall acceptance were significantly affected by days and concentration. Samples indicate more substantial results and general acceptance compared to other samples treated with minimum olive oil concentrations. The data obtained was analyzed statistically.

Key Words: - Cheese Analogue, Olive Oil, Lactobacillus bulgaricus

Introduction

Cheese is a popular dairy product that has a solid concentrate of milk protein and lipids and is consumed all over the world. Fresh acid cheeses are the most popular among all types of cheeses, and they are processed into a variety of fresh cheese-related goods such as cheese spreads (Repajic *et al*., 2019). Fresh cheese with a moderate acidic flavor that should be taken soon after ripening due to the significant risk of off flavor due to the high moisture content and casein coagulation (Rinaldoni *et al*., 2014). In the case of cottage cheese, smooth acid curd is generated via direct acidification based on many procedures such as draining, washing, cheese integration, and curd structure. Because of the agitation of coagulum in the agitator, various kinds and sizes of curd particles are created after coagulation and cutting of curd in soft type of cheeses like cottage cheese as compared to other acid cheeses. Cottage cheese is available in a variety of shapes, textures, and

sizes due to the lack of an exterior rind. Short set method, medium set method, and long set method cheese setting techniques are utilized in a cheese vat under controlled and hygiene settings before cheese curd cutting, depending on the time and length. In comparison to the long set approach, a high coagulation material or culture is required in the short set method, with temperatures ranging from 22 to 35°C and a period of 5 to 16 hours (Farkye, 2004).

Partially hydrogenated soybean fat or soybean oil contains smaller fat globules which are replaced by butter oil for the development of cheese analogue. Final product showed lower melting, higher hardness and higher values for the elastic viscous moduli and lower spread ability as compared to cheese made from butter oil (Cunha *et al*., 2013). Cheese analogue and other dairy-based perishables are normally produced over time utilizing various cooking procedures rather than invented. Cheese analogues and qurag cheese have been made for decades and are popular among customers. They are also low-fat products made from coagulated skim milk (Bergamaschi and Bittante, 2018). A number of quality issues arise during the production of imitation cheese under controlled conditions, including long setting time and short settling time, milk quality, and starter-related issues such as agglutination, floating of curd, and starter failure. The direct acidification coagulation of skim milk is a technical improvement in the making of cheese by direct acidification, and it helps to lessen and eliminate starter-related difficulties (Makhal *et al.,* 2013). Olive oil is known to be a representative food of the typical Mediterranean diet. Increasing evidence indicates that a decreased risk of cardiovascular disease, obesity, metabolic syndrome and hypertension is due to olive oil. A Mediterranean diet rich in Olive oil improves cardiovascular risk factors, such as endothelial dysfunction, blood pressure, postprandial hyper-lipidemia, lipid profiles, antithrombotic profiles and oxidative stress. Olive oil contains minor components which attributes beneficial effects. Phenolic compounds present in olive oil have shown anti-inflammatory and antioxidant properties which plays vital role in prevention of lipo-peroxidation, disclose antithrombotic properties, improve endothelial function and induce favorable changes of lipid profile. Dietary mono-unsaturated fatty acids in olive oil are protective against Alzheimer's disease and age-related cognitive decline. New reports of the potential protective effect of olive oil on cancer have been provided by experimental and human cellular studies. Furthermore, results of different cohort studies and case-control suggested that mono-unsaturated fatty acid intake including olive oil is associated with lowering the risk of cancer i.e., colorectal, prostate and breast cancers (Lopez *et al*., 2010).

The ideal cheese imitation has a meaty texture and is neither too firm nor too tough. Cutting cheese curd at a measured and optimum pH plays a significant role in achieving the best texture, yield, and uniformity of cheese. After cheese coagulation, cheese curd is created, and its cutting at the right pH aids in the production of high-quality cheese. In the case of a cheese analogue, the curd retention capacity required for cream dressing is primarily determined by two factors: the intensity of cheese cooking and the initial pH of the cheese curd. The absorbing property of cream dressing is affected by curd cutting and high temperature cooking at pH 4.8, whereas curd cutting and high temperature cooking at pH 4.5-4.6 makes the curd more delicate and softer, and softer curd retains more moisture (Hickey *et al.,* 2015). Olive oils possess antioxidant properties as well as hypo-lipidemic and anti-inflammatory properties. It also includes enough flavonoids, which have a variety of therapeutic properties and play a role in non-antioxidant activities in the gastro intestinal tract. Consumption of olive oil has been linked to a reduction in blood pressure (Bengana *et al*., 2013).

Objectives

- Development of cheese analogues
- Quality and safety evaluation of cheese analogues

Methodology

Proximate Analysis of milk

pH determination

AOAC (2016) method was used for ph. determination. Electronic digital pH meter was used to check the pH of milk by dipping electrode into the sample. Probe was rinsed through buffered solutions of pH 4.0 and 7.0 before the examination of pH of required sample. Clean the metal rod with tissue paper after determination of pH.

Ash content

Ash content was calculated by using the method of Frau *et al.* (2014). Accurately weigh the empty crucible. Put 5 gram of sample in it and place hydrated samples in muffle furnace at 550°C till persistent weight of sample achieved.

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Fat content

Gerber method was used to calculate fat in milk samples followed by Genis *et al.* (2020). 10.94 ml of skim milk sample was weighted and mixed well. Allow it to stand until the air bubbles were removed. Then place in butyrometer and added 1ml isoamyl alcohol and 10ml of H_2SO_4 in butyrometer. Sample was mixed thoroughly after putting cork on top of tube until the fat was released and curd was dissolved turning content blackish. Butyrometer was placed in centrifuge at 1100-11500rpm for 5 minutes and then in water bath for 2-3 minutes at temperature of 65°C. Make sure that the water level should be adequate to warm fats column. Fat contents were obtained from butyrometer scale.

Solids Not Fat

SNF were calculated by following the method of Gakkhar *et al*. (2015). It was done by subtracting the total solids from total fats. It was examined by evaporating the exact amount of water from accurately weighted sample.

SNF (%) = Total solids- Total fats

Milk acidity

The term of milk souring is also used for milk acidity. Milk acidity was determined by the method of Sengupta *et al.* (2014). A 100ml of flask was taken and 10ml of milk sample was added in it. Phenolphthalein indicator was added and titrate it against solution of 0.1 N NaOH solutions. Initial volume must be noted. Titration stopped when pinkish color was appeared. Milk acidity was calculated using following formula:

Volume of NaOH used = Final volume – Initial volume

$$\text{Acidity}(\%) = \frac{\text{Volume of 0.1 sodium hydroxide(ml)}}{\text{Volume of total sample}} \times 100$$

Protein content

Kjeldahl's method was used for estimation of protein contents in skim milk as used by AOAC (2016). Required regents for protein determination includes: H_2SO_4, sodium hydroxide, solution of boric acid (4%), digestion tablet, methylene indicator. 3 grams weighted milk sample was digested by positioned in distillation tube followed by addition of 30ml of conc. sulphuric acid and distillation tablet. For complete digestion, slow heating for about 45 minutes and then temperature was raised until cleared or greenish color detected. The sample was cooled for at least 30 minutes. Sample was transferred in to volumetric flask of 250ml. Add distilled water to make sample 250ml. In the distillation unit sodium hydroxide of 10ml and digestion sample was added and ammonium was produced then added solution of 4% boric acid. 2-3 drops of methylene indicator were added. If color was changed from red to yellow continued this process for few minutes to get maximum results. Titration was done until solution turns pink.

$$Nitrogen\ (\%) = \frac{\text{Volume of H2SO4 used(ml)} \times 250 \times 0.0014}{\text{Volume of sample} \times \text{Sample used for distillation}} \times 100$$

Total Protein (%) = Nitrogen (%) × 6.38

Emulsification

Single-step emulsification process will be done by following the method of Leong *et al*. (2020). Distilled Mono-glycerides (DMG) was used as emulsifier. Added in olive oil and stirred until mixed properly. After proper mixing olive oil was added to milk samples and homogenized at 40°C for one minute.

Isolation of *Lactobacillus bulgaricus*

Sterilization of glassware

Sterilization of glassware was done by hot air oven by following the method of (Yousef and Caristorm, 2003). The glassware must not remove immediately from hot air oven. Slow cooling period is necessary to avoid the cracking of glassware. Time and temperature for the sterilization of glassware was 171°C for 30min.

Sample collection

For the isolation of *Lactobacillus bulgaricus* yoghurt was used. Yoghurt sample was procured from the Al-Fateh mart of Faisalabad.

Media preparation

Microbial growth was done on De Man, Rogosa and Sharpe (MRS) agar (Yousef and Caristorm, 2003). MRS agar was prepared and used for TPC. It was prepared by sterilizing in the autoclave at 121°C for 20min. MRS was poured into petri dish and allowed to solidify.

Inoculation and Incubation

Inoculation of samples will be done MRS agar. Incubation will be carried out in the incubator (Yousef and Caristorm, 2003). Normally 1% of bacterial culture (*Lactobacillus bulgaricus*) was used for the inoculation. Freeze dried culture of *L. bulgaricus* was obtained by MRS broth. Rehydration was done by using 1ml pipette. After that it was transferred to MRS broth tube and mix well. It is necessary that anaerobic culture should be rehydrated in anaerobic conditions otherwise there is reduction in viability of cells. *L. bulgaricus* is anaerobic bacteria and therefore, rehydration was carried out in anaerobic environment. By following the method of Betoret *et al*. (2017) sample was incubated at 37°C for 48hour by using the incubator.

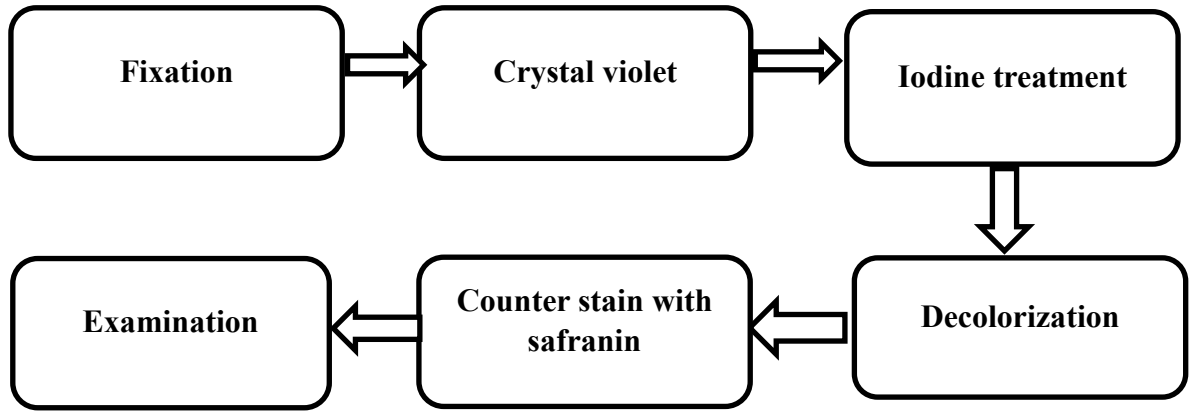
Morphological characterization

To study the morphology of culture, microscope will be used and gram staining will be done (Yousef and Caristorm, 2003). The bacterial cells that isolated were characterized with the application of this technique. Glass slides were rinsed to make them clean and clear. Bacterial sample was smear on the slide with the help of inoculating loop then, air drying of sample was done. Flame was used for the heat fixation of slide. Following steps were followed for gram staining;

a) Staining reagent crystal violet was used. Then, flow it over the slide for 1min and after that slide was rinsed with distilled water.
b) Again, the slide smudge was swamp with Gram's iodine (1min) and rinsed it.

c) Decolorizing agent (Ethyl alcohol) was used which was applied on the smear.
d) Safranin was applied to the smear for 1 minute and washed with distilled water.
e) After drying, slide was examined under the microscope.

Flow chart: Gram's staining



Purification

Recognized colonies of bacteria will be sub-cultured on the MRS agar to obtain pure culture (Yousef and Caristorm, 2003). The purification was done by the following steps at 4°C. The fermentation broth used was centrifuged at 10,000 g for 10 min. The purification was carried out using crude enzyme extract and powdered ammonium sulphate was also added to the crude extract. The activity of *Bulgaricus* was associated with the fraction precipitated at 70 - 100% saturation. After centrifugation at 9,000 rpm for 15 minutes precipitate was collected. Dissolved in sodium borate buffer and dialysed against the same buffer. The dialysed fraction was applied to a DEAE column. An anion exchanger, pre-equilibrated with Tris-HCl buffer had pH 8.6. The enzyme was eluted (1 ml/min) with NaCl gradient (0.1 - 0.5 M), 0.1 M borate buffered at pH 7.0. The active fractions were collected, dialysed and concentrated.

Identification of pure culture

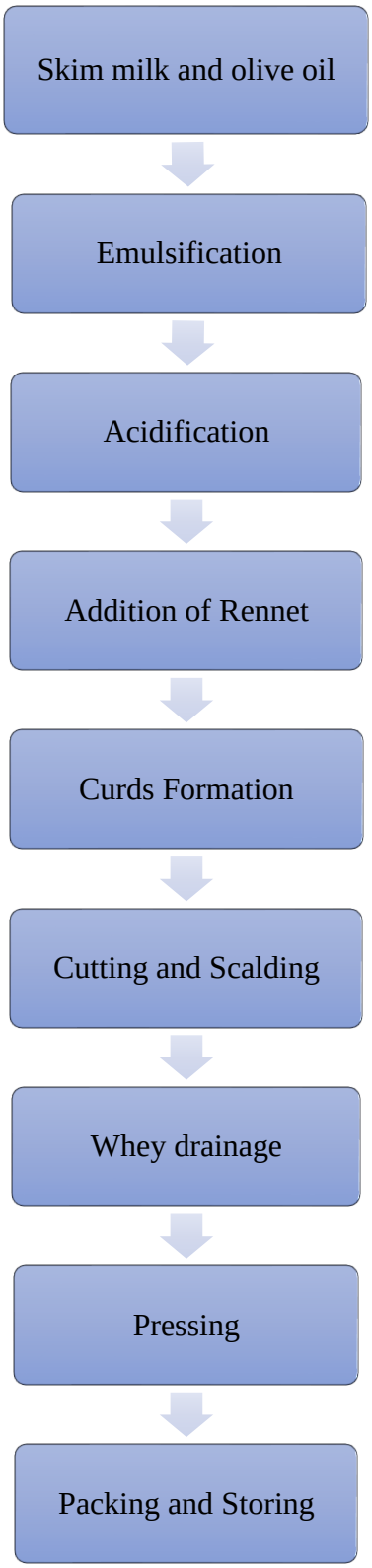
Pure culture will be identified on the basis of morphology (Yousef and Caristorm, 2003). The bacterial cells that isolated were characterized with the application of this technique. Glass slides were rinsed to make them clean and clear. Bacterial sample was smear on the slide with the help of inoculating loop then, air drying of sample was done. Flame was used for the heat fixation of slide. After drying, slide was examined under the microscope.

Preservation of pure culture

Isolated pure culture will be conserved by lyophilization method (Yousef and Caristorm, 2003). After the incubation of 48 hours the bacterial cells were harvested by centrifugation method. The test tube containing bacterial cells and broth was placed into centrifuge machine. Centrifuge at 3000×g for 20min and bacterial cells were settled down. Then, washing was done with the saline water (0.80g/100ml) carefully after removing from broth. Again, centrifugation was done according the above procedure and washed with saline water. The whole procedure was repeated three times and cells were re-suspended to the final concentration of 10^8-10^{10} CFU/ml (colony forming unit/ml). At -20°C, cell pellets were immediately stored.

Preparation of cheese analogue

Cheese analogue was manufactured according to the procedure used by Leong *et al*. (2020) with slight modifications. Milk was Pasteurized at 60-68ºC for half an hour under control conditions. Emulsification was done using emulsifier (DMG) at 40ºC for one minute at 1100-1200rpm. Milk will be poured into stainless steel vat and adjusted to 35ºC. *Lactobacillus bulgaricus* was added at a rate of 0.05 g/kg and mixed well. Acidification was done at pH 4.4 to 4.9. After proper mixing of culture rennet was added at the rate of 1.5 ml/kg. 3 hours was required for gelation at 33°C. Gels were cut into ~1 cm cubes. Curds were cooked and its stirring rate and temperature were increased, respectively. The curds were drained and cheddaring of the cooked curd was performed. Curds were milled and salted chips were packed and pressed overnight at 50 psi. Cheeses was packed and stored at refrigeration temperature.



Flowchart: Flowchart of development of cheese analogue using olive oi

Table: Treatment plan for cheese analogue using olive oil

Treatment	Olive oil (%)
T_o	-
T_1	2
T_2	3
T_3	4

Physicochemical analysis of cheese analogue

pH

AOAC (2016) method was used. Electronic digital pH meter was used to check the pH of milk by dipping electrode into the sample. Probe was rinsed through buffered solutions of pH 4.0 and 7.0 before the examination of pH of required sample. Clean the metal rod with tissue paper after determination of pH.

Titratable acidity

The term of milk souring is also used for milk acidity. Milk acidity was determined by the method of Sengupta *et al.* (2014). A 100ml of flask was taken and 10ml of milk sample was added in it. Phenolphthalein indicator was added and titrate it against solution of 0.1 N NaOH solutions. Initial volume must be noted. Titration stopped when pinkish color was appeared. Milk acidity was calculated using following formula:

Volume of NaOH used = Final volume – Initial volume

$$\text{Acidity}(\%) = \frac{\text{Volu254me of 0.1 sodium hydroxide(ml)}}{\text{Volume of total sample}} \times 100$$

Moisture content

Moisture contents of cheese sample were determined by following method of AOAC (2016). Weighted the empty China dish and put 5g of sample in it. Placed sample in hot air oven at 100°C for 3 hours until a constant weight achieved. Then cooled in desiccator for 30 minutes and moisture contents were calculated by using following formula:

$$\text{Moisture}(\%) = \frac{\text{Weight of wet cheese sample} - \text{weight of dry cheese sample}}{\text{Weight of wet cheese sample}} \times 100$$

Fat Content

Gerber method was used to calculate fat in milk samples followed by Genis *et al.* (2020). 10.94 ml of skim milk sample was weighted and mixed well. Allow it to stand until the air bubbles were removed. Then place in butyrometer and added 1ml isoamyl alcohol and 10ml of H_2SO_4 in butyrometer. Sample was mixed thoroughly after putting cork on top of tube until the fat was released and curd was dissolved turning content blackish. Butyrometer was placed in centrifuge at 1100-11500rpm for 5 minutes and then in water bath for 2-3 minutes at temperature of 65°C. Make sure that the water level should be adequate to warm fats column. Fat contents were obtained from butyrometer scale.

Microbiological analysis

Microbiological analysis was determined by following the method of Sengupta *et al.* (2014). It includes total plate count (TPC) and probiotic count.

Total plate count

Total plate count (TPC) was determined by following the method of (Oto *et al*., 2013). MRS agar was prepared and used for TPC. It was prepared by sterilizing in the autoclave at 121°C for 20min. MRS was poured into petri dish and allowed to solidify. 1g Sample was taken and added to 9ml distilled water in test tube. Similarly, again another 1g sample from previous tube was brought to mix with 9ml water in another tube and so on. Similarly bacterial culture was diluted 10 times through serial dilution. Took sample and poured on MRS agar media for growth at incubation temperature. Total number and bacterial colonies were calculated after 48hours through colony counter and was used for the presentation of results were presented in the form of CFU/g.

Probiotic count

Probiotic count from the sample was determined by Zhang *et al*. (2019) using specialized Gly-MRS agar media (glycoprival MRS agar). This media was prepared without adding the sodium acetate. The sample was diluted tenfold by serial dilution. Then, poured onto petri dish having sterilized Gly-MRS agar media. The petri plates were subjected to incubation temperature of 37°C for 48hours. Colony counter was used for detection of probiotic (*Lactobacillus bulgaricus*).

Protein

Kjeldahl's method was used for estimation of protein contents in skim milk as used by AOAC (2016). Required regents for protein determination includes: H_2SO_4, sodium hydroxide, solution of boric acid (4%), digestion tablet, methylene indicator. 3 grams weighted milk sample was digested by positioned in distillation tube followed by addition of 30ml of conc. sulphuric acid and distillation tablet. For complete digestion, slow heating for about 45 minutes and then temperature was raised until cleared or greenish color detected. The sample was cooled for at least 30 minutes. Sample was transferred in to volumetric flask of 250ml. Add distilled water to make sample 250ml. In the distillation unit sodium hydroxide of 10ml and digestion sample was added and ammonium was produced then added solution of 4% boric acid. 2-3 drops of methylene indicator were added. If color was changed from red to yellow continued this process for few minutes to get maximum results. Titration was done until solution turns pink.

$$Nitrogen(\%) = \frac{\text{Volume of H2SO4 used(ml)} \times 250 \times 0.0014}{\text{Volume of sample} \times \text{Sample used for distillation}} \times 100$$

Total Protein (%) = Nitrogen (%) × 6.38

Total nitrogen

After protein determination nitrogen contents were determined by the respective method followed by Margolies *et al*. (2018). Nitrogen from protein converted into ammonium sulphate later ammonium and distilled in solution of boric acid and titration is against standard acid.0.0014 is a content value in formula.

$$\text{Nitrogen}(\%) = \frac{\text{Volume of NaoH used} \times \text{Normality} \times 0.0014}{\text{Weight of origional sample used}} \times 100$$

Phenolic compounds

The phenolic compounds in cheese analogue were calculated by the method of Han *et al*. (2011). Folin Ciocalteu reagent was used. 10ml of cheese sample was taken and placed in cuvettes. 1.0ml of reagent i.e., Folin-Ciocalteu's and Na_2CO_3 (0.8ml) was added. UV-visible spectrophotometer was used with the wavelength of 765 nm for specific time. After that, incubation was done at 30°C. Results were found as milligrams of gallic acid equivalent (GAE) /gram of total sample weight. Gallic acid equivalent value = 0.1008.

TPC was calculated using following equation:

$$\text{TPC}(\text{mgGAE}/100\text{g}) = \text{C XV}/\text{M}$$

Where:

T = Total phenols

C = Gallic acid concentration

V = Volume of extracted solution (ml)

M = Extract weight (grams)

X = phenolic compounds in different extract

Total solids

SNF were calculated by following the method of Gakkhar *et al*. (2015). It was done by subtracting the total solids from total fats. It was examined by evaporating the exact amount of water from accurately weighted sample.

SNF (%) = Total solids- Total fats

Texture analysis

Texture profile was analyzed by the mean of $TAXT_2$ texture analyzer with attach load cell of 25 grams and by using a compression plate probe by the respective method of Leong *et al.* (2014). The cylindrical probe was used to force the depth of about 20.0cm in product at storage temperature of 4°C. The insertion of probe into cheese analogue sample should be smooth.

Sensory analysis

The sensory evaluation of final product was also performed by the panelist at National Institute of Food Science and Technology. Product evaluation was performed by using nine points of Hedonic scale. The product was evaluated for its flavor, taste, aroma, color and overall acceptance (sharif *et al*., 2017).

Statistical analysis

The obtained data after performing different analysis was statistically calculated according to Montgomery (2017) by using two ways ANOVA and factorial design. Data was analyzed by using statistic 8.1software and factorial was applied on each parameter.

Results

Table: Proximate composition of skim milk

Component	Quantity (%)
Moisture	86.3
Ash	0.9
pH	6.8
Fats	0.3
Total solids	10.0
Acidity	0.19

Table: Effect of different olive oil concentration and days on pH of cheese analogues

Treatment	pH					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	4.92	4.75	4.45	4.23	4.06	**4.48**
T_1	4.86	4.75	4.40	4.20	4.02	**4.44**
T_2	4.82	4.65	4.33	4.16	3.90	**4.32**
T_3	4.78	4.57	4.29	4.08	3.64	**4.32**
Mean	**4.84a**	**4.68ab**	**4.37bc**	**4.16c**	**3.90d**	

Means having the same letters within a column or row do not differ significantly ($P<0.05$)
T_0 = Control group (without olive oil)
T_1 = 2% Olive oil cheese analogues
T_2 = 3% Olive oil cheese analogues
T_3 = 4% Olive oil cheese analogues

Table: Effect of different olive oil concentration and days on Titratable acidity (%) of cheese analogues

Treatment	Titratable acidity (%)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	0.30	0.40	0.50	0.60	0.70	**0.50**
T_1	0.35	0.45	0.52	0.60	0.70	**0.53**
T_2	0.40	0.50	0.60	0.70	0.80	**0.60**
T_3	0.44	0.54	0.64	0.74	0.84	**0.64**
Mean	**0.37a**	**0.47d**	**0.57c**	**0.66b**	**0.76a**	

Table: Effect of different olive oil concentration and days on moisture content (%) of cheese analogues

Treatment	Moisture content (%)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	70.20	69.20	68.19	67.19	67.18	**68.19**
T_1	70.13	69.13	68.13	67.14	66.15	**68.14**
T_2	70.11	69.11	68.11	67.12	66.14	**68.12**
T_3	70.05	69.05	68.05	67.06	66.06	**68.05**
Means	**70.12a**	**69.12b**	**68.12c**	**67.13d**	**66.13e**	

Table: Effect of different olive oil concentration and days on fat (%) of cheese analogues

Treatment	Fat (%)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	19.38	19.23	19.15	19.11	19.08	**19.19d**
T_1	21.42	21.33	21.28	21.26	21.22	**21.30c**
T_2	23.16	22.75	22.69	22.65	22.60	**22.77b**
T_3	24.83	24.75	24.71	24.67	24.61	**24.71a**
Mean	**22.19**	**22.01**	**21.96**	**21.92**	**21.88**	

Table: Effect of different olive oil concentration and days on total plate count (log CFU/g) of cheese analogues

Treatment	Total plate count (log CFU/g)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	3.13	3.06	2.96	2.86	2.76	**2.95**
T_1	3.09	3.00	2.90	2.80	2.70	**2.93**
T_2	3.07	2.97	2.87	2.77	2.74	**2.90**
T_3	3.04	3.00	2.94	2.64	2.35	**2.84**
Means	**3.08a**	**2.99ab**	**2.89bc**	**2.79c**	**2.77c**	

Table: Effect of different extra virgin olive oil concentration and days on protein (%) of cheese analogues

Treatment	Protein (%)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	24.29	24.35	24.43	24.24	24.19	**24.30**
T_1	24.30	**24.34**	24.44	24.23	24.17	**24.29**
T_2	24.32	24.33	24.45	24.22	24.16	**24.29**
T_3	24.31	24.32	24.47	24.20	24.12	**24.28**
Mean	**24.30**	**24.33**	**24.44**	**24.22**	**24.16**	

Table: Effect of different olive oil concentration and days on total nitrogen (%) of cheese analogues

Treatment	Total nitrogen (%)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	0.51	0.55	0.58	0.47	0.42	**0.50**
T_1	0.49	0.54	0.59	0.45	0.40	**0.49**
T_2	0.47	0.53	0.60	0.44	0.39	**0.48**
T_3	0.45	0.52	0.61	0.41	0.38	**0.47**
Means	**0.48**	**0.53**	**0.59**	**0.44**	**0.40**	

Table: Effect of different olive oil concentration and days on total phenolic compounds (mg GAE/100g) of cheese analogues

Treatment	Total Phenolic compounds (mg GAE/100g)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	2.35	2.34	2.33	2.32	2.31	**2.33d**
T_1	14.44	13.42	12.55	11.55	10.45	**12.48c**
T_2	21.71	20.69	19.95	18.89	17.83	**19.81b**
T_3	30.42	29.58	28.59	26.49	25.04	**27.22a**
Mean	**17.22**	**17.17**	**16.66**	**16.06**	**16.01**	

Table: Effect of different olive oil concentration and days on total solids (%) of cheese analogues

Treatment	Total solids (%)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	19.94	19.91	19.87	19.82	19.78	**19.87c**
T_1	21.93	21.88	21.83	27.83	27.91	**20.50b**
T_2	23.27	20.69	20.37	19.94	19.57	**21.17c**
T_3	26.62	23.23	23.17	23.10	23.05	**21.84a**
Mean	**21.77**	**21.42**	**21.31**	**21.16**	**21.03**	

Table: Effect of olive oil different concentration and days on hardness (mm) of cheese analogues

Treatment	Hardness (mm)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	3.81	3.79	3.75	3.73	3.71	**3.75a**
T_1	3.72	3.69	3.65	3.63	3.61	**3.66b**
T_2	3.61	3.59	3.55	3.53	3.51	**3.55c**
T_3	3.47	3.49	3.45	3.43	3.40	**3.44d**
Mean	**3.65**	**3.64**	**3.60**	**3.58**	**3.55**	

Table: Effect of different olive oil concentration and days on flavor (score) of cheese analogues

Treatment	Flavor (score)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	6.84	6.92	6.95	7.02	7.16	**6.98**
T_1	7.03	7.16	7.37	7.53	7.73	**7.36**
T_2	7.53	7.57	7.72	7.82	7.92	**7.71**
T_3	7.87	7.90	7.94	8.05	8.12	**7.98**
Mean	**7.31d**	**7.38d**	**7.49c**	**7.60b**	**7.73a**	

Table: Effect of different extra virgin olive oil concentration and days on color (score) of cheese analogue

Treatment	Color (score)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	7.85	7.74	7.86	7.95	7.98	**7.14**
T_1	7.36	7.10	6.97	7.10	7.17	**7.47**
T_2	7.23	7.02	7.12	7.17	7.27	**7.37**
T_3	7.44	7.48	7.50	7.46	7.50	**7.16**
Mean	**7.47**	**7.34**	**7.36**	**7.42**	**7.48**	

Table: Effect of olive oil concentration and days on body and texture (score) of cheese analogues

Treatment	Body and texture (score)					Mean
	0 Day	7 Days	14 Days	21 Days	28 Days	
T_0	6.86	6.92	6.97	7.04	7.17	**6.99a**
T_1	7.00	7.06	7.12	7.17	7.25	**7.12b**
T_2	7.85	7.84	7.89	7.95	7.98	**7.90c**
T_3	7.44	7.48	7.53	7.48	7.50	**7.49d**
Mean	**7.29**	**7.32**	**7.35**	**7.37**	**7.38**	

Discussion

This research related to well dairy product was accompanied in order to study the influence of olive oil on various properties of cheese analogues. In first part cheese analogue characterization was done and later on prepared cheese analogues was fortified with olive oil (OO) and subjected to different laboratory analysis. Sensory evaluation was done by trained panelists. The Proximate results are displayed in Table which depicts that fluid skimmed milk consists of 86.3% of total moisture contents and fat traces of 0.3%. Analysis displays that ash matter is 0.9% and total solids was 10.0%. Acidity of the product and pH of milk plays vital role in the texture of end product. pH and acidity readings were 6.8 and 0.19%. There are various factors such as species, season, environment, feed and lactation which cause variations in milk. Milk is a suspension of fat related globules in water based un-solidified material that comprises of crabs and protein mass (Fox *et al*., 2017).

pH of cheese analogues prepared by addition of different concentration of olive oil showed the value range of 3.64 to 4.92. The pH value declined as storage time increase in samples. The pH value among these three concentrations of olive oil did not differ significantly as clear from Table 4.3. Rapid acid activity shows reduction of pH value during storage interval of 28 days in cheese samples. The results were closely related to Abbas *et al*. (2015). Maximum Titratable acidity was detected in case of T_3 followed by T_2, T_1 and T_o. Results are similar to those of Foda *et al.,* (2009), they concluded that there is an increasing trend in the in the value of acidity during the two weeks of storage. The outcomes indicate the store soft cheese. After storage time of 28 days results obtained from analysis of variance shown in Table, indicates that the moisture contents of manufactured cheese analogues were not varied significantly by difference in olive oil concentration and interaction between these variables. While, effect of days is significant. Moisture content of cheese fortify with three various concentrations of olive oil indicates value range of 66.06 to 70.20. The moisture among three concentration of olive oil did not vary significantly which was showed reducing trend with the increase of storage interval up to day 28. T_1 shows maximum value of moisture followed by T_2, T_3. Olive oil had no effect on moisture but loss in moisture occurs as time increase. It was due to relocation of moisture content and release of whey protein from cheese analogues. These results are relatable with (Shekhar *et al.*, 2015.

Fat contents of prepared product fortify by means of different application of olive oil fallen in the range of 19.38 to 24.61. Samples of cheese represents alternative increasing trend from day 1 to 28 as displayed in Table. The fat content decreased as storage time increased. The results were harmony with the study Felfoul *et al*. (2015) and the fat contents of full fat, and reduced fat cheese were inconstant in the course of the storage period of 2 months. The results obtained from variance of analysis for total plate count obtained after the storage period of 28 days are displayed in Table. The total plate count of cheese analogues does not vary significantly by difference in olive oil concentration and interaction between these two variables. While, effect of days is significant. Total plate count of prepared product fortify by means of different application of olive oil fallen in the range of 2.35 to 3.13. Sample of cheese represents alternative decreasing trend from day 1 to 28 as displayed in Table. Protein content increased for the first seven days of storage then began to decline. This drop in protein levels after storage could be attributed to protein decomposition and whey loss from the sample. Other, investigation on protein content of yoghurt cheese enhanced with highly extracted EVOO showed an increasing trend as the storage interval and EVOO concentration increased (Abbas *et al*. 2015).

The total nitrogen matter of cream cottage cheese did not vary significantly by difference in olive oil concentration and interaction between these two variables. While, effect of days is significant. Total nitrogen content in olive oil cheese analogues ranged from 0.38 to 0.61 as clear from the results of Table that the three concentration of olive oil do not varies significantly T_3 had less quantity of total nitrogen as storage period increased than that of T_o, T_1 and T_2. The results were relatable with Abbas *et al*. (2015) who described that total nitrogen matter increased till degradation of protein not occurs.

It was cleared from the outcomes as represented Table that total phenolic compounds of prepared cheese analogues fortified by means of three altered concentrations of olive oil ranged from 2.31 to 30.42 and all the three concentrations varied significantly. Olive oil comprises an adequate number of phenolic compounds as its application increased amount TPC also raised. T_o has lowest amount of TPC followed by T_1 T_2 and T_3. Moreover, with the increase in olive oil concentration the amount of TPC significantly improved. The results were agreed with Kostadinovik and Mitrev (2013).

It was cleared from the results that total solid contents in following product fortify by means of different concentration of extra virgin olive oil falls in the range of 19.78 to 26.62 as shown in Table. All the three concentrations of olive oil differ significantly. Total solids were affected by the increasing application of oil but with days it shows non-significantly decreasing trend in cheese. Results were similar to the conclusions of Abbas *et al*. (2015) olive oil in cheese yoghurt showed that the sample which was fresh had high solid matter and as the storage time passed solid contents were decreased.

Rheological analysis of cheese analogues curd fluctuates non-significantly by difference in olive oil concentration. While, the effect of treatment is significant. It was cleared from the outcomes as shown in Table that texture of product ranged from 3.40 to 3.81. All the three concentration of olive oil differed significantly which were found after statistical analysis. The fortification of olive oil expressed a noteworthy change in texture profile of cheese curd. Up to 28 days of storage control sample shows a significant decrease in rheological activities. This may be due to the presence of high melting fats. Presence of olive oil in cheese directly influences the firmness of product.

It is cleared from the data which is represented in Table, showed that body and texture of olive oil cheese analogues falls in the range of 6.86 to 7.98. It represented that T_2 scored more by the evaluators as its texture and body appearance seems to be better than others. Owing to the greasy and thin curd look and T_3 scored least. As product was kept for long duration it becomes thin and viscosity also affected. It is cleared from results that the flavor and taste in case of sensory analysis is in the range of 6.84 to 8.12 as shown in Table. It showed an alternative increasing trend. T_o has less flavor followed by other experimental samples. T_1 with the storage time increase seems to owe more flavors and taste. T_3 due to high oil concentration was less enjoyed by consumer as upon taste it gives bitter flavor. Olive oil in product had a significant outcome on the physical look of final product as storage duration increase but small change in oil quantity did not affect color. T_1 be more liked by consumers as it looks fresher followed by T_0 and T_2. T_3 shows more changes in color with increase storage time. Olive oil concentration and storage time are indirectly related to color as well as appearance of product. It was cleared from the statistical analysis of overall acceptability ranged from 6.86 to 7.98 as showed in Table. All three olive oil concentrations differed significantly. T_2 was preferred by panelists above other fortified samples; however, T3 with the highest amount of olive oil had a bitter flavor and received lower scores.

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