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Preservation of Paneer Samples with Formalin under Vacuum Packaging at 27°C for Analytical Purpose

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ABSTRACT

Paneer is an acid cum heat coagulated milk product and is widely consumed in the northern part of India. When the food safety officer collecting the paneer sample for chemical analysis, formalin @0.4% is added to remain fit for analytical analysis. In the present study two different packaging pouches i.e. seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene were used to preserve paneer with or without formalin at 27±1ºC for 120 days. The results revealed that on the addition of formalin @0.4% in paneer samples showed an increase in moisture content (52.88±0.061 to 53.55±0.06%) and acidity (0.62±0.017 to 0.67±0.011% LA), whereas fat percentage decreases (24.02±0.155 to 22.05±0.126%). Tyrosine value, acidity, FFA, peroxide value, of paneer rapidly increased while moisture, fat percentage and pH decreased during storage at 27±1ºC respectively. Paneer sample preserved with formalin in seven layer NYLON/EVOH/70µ polyethylene showed no significant (P>0.05) difference in moisture content (53.55±0.068 to 53.54±0.04%) and fat percentage (22.05±0.126 to 22.04±0.089%) up to 45 days respectively. However, 12µPET/70µ polyethylene pouch showed, no significant (P>0.05) difference in moisture content (53.55±0.068 to 53.53±0.076%) and fat percentage (22.05±0.127 to 21.95±0.080%) upto 30 days respectively. Based on above findings it can be concluded that paneer samples preserved with formalin under vacuum packaging in seven layer NYLON/EVOH/70µ polyethylene pouch at 27±1ºC remained fit for analytical purpose up to 45 days.

Key words: *Food safety officer, Paneer, Formalin, Vacuum packaging, Chemical analysis.*

INTRODUCTION

Paneer is the product obtained from cow or buffalo milk or combination thereof by precipitation with sour milk, lactic acid or citric acid[7]. During the collection of milk and milk product samples, Food safety officer (FSO) may add 37-40% formalin @0.4% to remains fit for chemical analysis. Formalin is antimicrobial. It inactivates the microorganism by alkalytic amino group and sulfhydryl group of proteins and ring of nitrogen atoms of purine bases i.e. adenine, cytosine and guanine in nucleic acid components, denaturing them and resulting in the inhibition of microorganism[9].

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The low shelf life of paneer is generally due to microbial spoilage and physicochemical changes[1]. Vacuum packaging is an advanced technique which can significantly prevent the aerobic spoilage of micro-organism thereby reducing oxidative reactions by eliminating oxygen[12]. It extends the shelf life of paneer by 2-3 times using suitable packaging material[2]. Vacuum packaging has been carried out by using two type of packaging material i.e. seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch.

The objective of this study was to preserve the paneer samples with formalin under vacuum packaging in two types of packaging material i.e. seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch for 120 days. The variation in chemical composition of the paneer samples during storage in two different packaging at 27ºC were monitored to remained fit for analytical purpose.

MATERIALS AND METHODS

Sample preparation of paneer and its vacuum packaging

The paneer samples were obtained from the experimental dairy of National Dairy Research Institute, Karnal. (I) Seven-layer NYLON/EVOH/70µ polyethylene and (II) 12µ PET/70µ polyethylene pouch were used to pack the paneer cubes (1×1 cm size) followed by addition of formalin @0.4% in paneer samples by inverting pouch 8-10 times. Vacuum packaging was done in Indvac vacuum packaging machine at negative pressure (-760 mmHg) within 25 sec. It was stored at 27ºC for 120 days and analysed at a regular interval of 15 days respectively and without formalin were stored at 27ºC for 10 days. Samples were analyzed at a regular interval of 2 days respectively.

Chemicals and Reagents

All the chemicals used in present study were of Analytical Reagent (AR) grade. Diethyl ether, petroleum ether, chloroform, ethanol, ammonia (25%), formalin solution (37-41%), potassium iodide, phenolphthalein indicator, sodium hydroxide pellets, sodium carbonate, copper sulphate pentahydrate, glacial acetic acid, sodium thiosulphate and folin's reagent were purchased from Sigma Aldrich Pvt. Ltd. USA.

Analysis

The paneer samples were analyzed for moisture[10], fat %[10], acidity (% LA)[10], pH (pH meter), tyrosine value[13], Free Fatty Acid %[11], Peroxide value[11].

Statistical analysis

Experimental analysis was performed in triplicates. Data was analyzed by Graph pad prism 5.1 software. Significant differences between means were determined by two-way analysis of variance at 5 % level of significance.

RESULT AND DISCUSSION

Effect of vacuum packaging on storage of paneer samples

Paneer samples without formalin were vacuum packed in seven layer NYLON/EVOH/70µ polyethylene and 12µ PET/70µ polyethylene respectively and stored at 27ºC. During storage foul smell and sticky surface appearance was observed in the both the packaging pouches on 2^{th} day respectively. Paneer packed in seven layer NYLON/EVOH/70µ polyethylene pouches was having in foul smell after 45^{th} day and in 12µ PET/70µ polyethylene pouches, foul smell was suspected after 30^{th} day 27ºC respectively.

Effect of formalin under vacuum packaging on chemical analysis of paneer during storage at 27ºC

Moisture content (%)

Moisture content of freshly prepared paneer samples was 52.88±0.061% (Table 3.1). Moisture content of paneer packed in both the packaging pouches showed significant ($P<0.05$) decrease on 4^{th} and 2^{nd} day respectively and continue to decrease upto 10 days respectively (Table 3.1 and Fig. 3.1.a). Moisture content of paneer preserved with formalin (0.4%) showed significantly increase ($P<0.005$) in moisture content from 52.88±0.061 to 53.56±0.068% (Table 3.1 and 3.2). Moisture content in both the packaging pouches showed significant ($P<0.05$) decrease

on 75th and 60th day respectively and continue to decrease upto 120 days respectively (Table 3.2 and Fig. 3.1.b). No significant (P>0.05) difference was observed in moisture content of paneer samples preserved with formalin in both packaging pouches (Fig. 3.1.b). Decreasing trends in moisture level was also reported by Haridas and Narayanan[8] and Singh[14].

Fat (%)

The fat percentage of paneer samples preserved with and without formalin were analyzed by Rose-Gottlieb method and results are presented in tables 3.1- 3.2. and fig. 3.2.a, b). The average fat percentage of fresh paneer samples was 24.02±0.155% (Table 3.1.). Fat percentage of paneer samples packed in both the packaging pouch showed significant (P<0.05) decrease on 2nd day respectively and continue to decrease upto 10 days (Table 3.1 and Fig. 3.2.a). The reduction in fat might be due to lipolytic activity of microorganisms in paneer. No significant (P>0.05) difference was observed in fat percentage of paneer in both packaging pouches (Figure 3.2.a). Initial fat percentage in paneer preserved with formalin was decreased from 24.02±0.155 to 22.05±0.126%. Fat percentage in both the packaging pouches showed significant (P<0.05) decrease on 60th and 45th day respectively. It was continued to decrease upto 120 days respectively (Table 3.1 and Fig. 3.2.b). Lower values of fat percentage in paneer preserved with formalin may be due to aqueous formalin react with milk proteins[5,6]. No significant (P>0.05) difference was observed in fat percentage of paneer preserved with formalin in both packaging pouches (Fig. 3.2.b). Haridas and Narayanan[8], also observed a decrease in the fat percent of paneer when preserved with 0.4% formalin at 37°C after 6th day.

Titratable acidity (% LA)

The average titratable acidity of fresh paneer samples was 0.62±0.017% LA (Table 3.1). Titratable acidity of paneer in both the packaging pouch showed significant (P<0.05) increase on 2nd day respectively and continue to increase upto 10 days (Table 3.1). Increasing trends in % LA were observed, thereby indicating bacterial spoilage by lactic acid fermenting bacteria. Paneer samples preserved with formalin showed increased titratable acidity from 0.62±0.017 to 0.67±0.011% LA (Table 3.1- 3.2). It may be due to the reaction of formalin with primary amino group, amide groups and guanidyl groups of proteins and releases hydrogen ions. Similar results were reported by Bansal and Singhal[3], Boghra and Borkhatriya[4], Singh[14], in milk and paneer on the addition of formalin. Paneer preserved with formalin showed significant (P<0.05) increase in titratable acidity in both the packaging pouch on 30th and 15th days. It was continue to increase upto 120 days respectively (Table 3.2). Significant (P<0.05) differences were observed in titratable acidity (%Lactic acid) of paneer preserved with formalin in both packaging pouches (Fig. 3.3.b). Haridas and Narayanan[8], also reported that an addition of 0.4% formalin in the paneer showed no change in titratable acidity upto 6th day at 37ºC and thereafter an increase in titratable acidity of paneer was observed upto a storage period of 21st day.

pH

The pH value of fresh paneer samples was 5.53±0.08. pH value of paneer in both the packaging pouch showed significant (P<0.05) decrease on 2nd day respectively and continue to decrease upto 10 days (Table 3.1). Decrease in pH of paneer samples during storage may be due to utilization of lactose by microorganism and its conversion to lactic acid. No significant (P>0.05) difference was observed in pH value of paneer samples in both the packaging pouches. Average pH value of paneer samples preserved with formalin remains same. No significant change in pH value was observed on addition of formalin (Table 3.1-3.2). pH value of paneer preserved with formalin in both the packaging pouch showed significant (P<0.05) decrease on 15th day respectively and continue to decrease upto 120 days (Table 3.2). No significant (P>0.05) difference was observed in pH of paneer preserved with formalin in both packaging pouches. Upadhyay[15], also reported that pH of

paneer samples preserved with formalin decreased with increase in storage period.

Tyrosine content

The tyrosine content of paneer samples was 6.28±2.732mg/100g. Tyrosine content of paneer packed in both the packaging pouch showed significant (P<0.05) increase on 2nd day respectively and continue to increase upto 10 days (Table 3.1 and Fig. 3.3.a). The increase in tyrosine content of paneer was due to proteolysis of paneer. Significant (P>0.05) difference was observed in tyrosine content of paneer in both packaging pouches (Fig.3.3.a). Tyrosine content of paneer sample preserved with formalin remains same. No significant change in tyrosine content was observed on addition of formalin (Table 3.1-3.2). Tyrosine content in paneer preserved with formalin in both the packaging pouch showed significant (P<0.05) increase on 30th day respectively and continued upto 120 days (Table 3.2 and Fig. 3.3.b). No significant (P>0.05) difference was observed in tyrosine content of paneer preserved with formalin in both packaging pouches (Fig.3.3.b). During storage, increase in tyrosine content revealed that milk proteins present in paneer have been degraded thereby releasing free amino acids. Haridas and Narayanan[8], also reported that the significant increase in tyrosine content of paneer preserved with 0.4% formalin at 30°C upto 21 days. Upadhyay[15], found that the tyrosine content of paneer samples preserved with 0.4% formalin, significantly increased after 1 month at 37°C and further increased upto 6 months of storage.

Free fatty acid (% oleic acid)

The FFA (% oleic acid) of milk fat extracted from fresh paneer were 0.15±0.01% oleic acid (Table 3.1). The FFA of milk fat extracted from paneer samples in both the packaging pouch showed significant (P<0.05) increase on 4th and 2nd day respectively and continue to increase upto 10 days (Table 3.1 and Fig. 3.4.a). Significant (P<0.05) difference were observed in FFA of milk fat extracted from paneer in both packaging pouches (Fig. 3.4.a). FFA of milk fat extracted from fresh paneer sample preserved with formalin remains same. No significant change in FFA of milk fat extracted from paneer was observed on addition of formalin (Table 3.1-3.2). The FFA of milk fat extracted from paneer preserved with formalin in both the packaging pouch showed significant (P<0.05) increase on 30th day respectively and continue to increase upto 120 days (Table 3.2 and Fig. 3.4.b). An increase in FFA of milk fat extracted from paneer with formalin indicated lipolysis of milk fat in paneer during storage. No significant (P>0.05) difference was observed in FFA of milk fat extracted from paneer preserved with formalin in both packaging pouches (Fig. 3.4.b). The increase in FFA during storage may be due to inherent lipases present in the paneer. Singh[14], also reported that FFA of milk fat extracted from paneer with 0.4% formalin were increased significantly upto 6th month at 37°C.

Peroxide value

Peroxides are an indicator of oxidative rancidity of milk fat. No peroxide value was observed in fresh paneer samples (Table 3.1). The peroxide value of milk fat extracted from paneer in both the packaging pouch showed significant (P<0.05) increase on 4th day respectively and continue increase upto 10 days (Table 3.1). No significant (P>0.05) difference was observed in peroxide value of milk fat extracted from paneer in both packaging pouches. Peroxide value of milk fat extracted from paneer preserved with formalin remains same. No significant change in peroxide value was observed on addition of formalin (Table 3.1-3.2). Peroxide value of milk fat extracted from paneer preserved with formalin in both the packaging pouch showed significant (P<0.05) increase on 45th and 60th day respectively and continue to increase upto 120 days (Table 3.2). No significant (P>0.05) difference was observed in peroxide value of milk fat extracted from paneer preserved with formalin in both packaging pouches. The increase in peroxide value of milk fat in paneer was directly proportional to the extent of auto oxidation of milk fat. Singh[14], also reported that peroxide value of milk fat extracted from paneer preserve with 0.4% formalin increases continuously during storage upto 6 months at 37°C.

Table 3.1: Chemical analysis of paneer samples without formalin in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C.

Days		0	2	4	6	8	10
Moisture content (%)	(I)	52.88±0.061^{aA}	52.82±0.06^{aA}	52.72±0.128^{bA}	52.62±0.065^{cA}	52.54±0.079^{dA}	52.45±0.043^{eA}
	(II)	52.88±0.061^{aA}	52.78±0.077^{bA}	52.64±0.052^{cA}	52.56±0.067^{dA}	52.47±0.099^{eA}	52.40±0.063^{eA}
Fat (%)	(I)	24.02±0.155^{aA}	23.95±0.169^{bA}	23.87±0.172^{cA}	23.77±0.079^{dA}	23.64±0.175^{eA}	23.52±0.131^{fA}
	(II)	24.02±0.155^{aA}	23.94 ±0.154^{bA}	23.84±0.122^{cA}	23.74±0.116^{dA}	23.61±0.184^{eA}	23.50±0.146^{fA}
Titratable acidity (%LA)	(I)	0.62±0.017^{aA}	0.71±0.019^{bA}	0.83±0.061^{cA}	0.95±0.027^{dA}	1.07±0.019^{eA}	1.27±0.018^{fA}
	(II)	0.62±0.017^{aA}	0.73±0.038^{bA}	0.86±0.018^{cA}	0.98±0.051^{dA}	1.12±0.018^{eA}	1.30±0.024^{fA}
pH	(I)	5.53±0.008^{aA}	5.45±0.017^{bA}	5.40±0.008^{bA}	5.35±0.006^{cA}	5.30±0.008^{cA}	5.24±0.011^{dA}
	(II)	5.53±0.008^{aA}	5.43±0.014^{bA}	5.40±0.008^{bA}	5.34±0.014^{cA}	5.29±0.012^{dA}	5.23±0.008^{eA}
Tyrosine content (mg/100g)	(I)	6.28±2.732^{aA}	15.30±0.985^{bB}	28.14±2.732^{cC}	35.52±2.772^{dD}	49.18±0.473^{eE}	57.38±1.639^{fF}
	(II)	6.28±2.732^{aB}	21.58±1.661^{bC}	31.42±1.791^{cD}	37.16±1.912^{dE}	51.64±2.062^{eF}	63.93±1.706^{fG}
FFA(% oleic acid)	(I)	0.15±0.018^{aA}	0.20±0.016^{aB}	0.24 ±0.018^{bC}	0.30±0.018^{bD}	0.41.±0.018^{cE}	0.48±0.029^{dF}
	(II)	0.15±0.018^{aB}	0.22±0.008^{bC}	0.27±0.010^{bD}	0.33±0.0208^{cE}	0.44±0^{dF}	0.50±0.024^{eG}
Peroxide value (meq/Kg)	(I)	0	0.13±0.06^{aA}	0.33±0.06^{bA}	0.67±0.06^{cA}	1.00±0.06^{dA}	1.20±0.115^{eA}
	(II)	0	0.20±1.96^{aA}	0.39±0.012^{bA}	0.73±0.06^{cA}	1.07±0.066^{dA}	1.27±0.066^{eA}

(I) Seven layer NYLON/EVOH/70µ polyethylene pouch, (II) 12µ PET/70µ polyethylene pouch

Data is represented as means ± S.E (n=3) with different superscript (a, b, c,…) letters are significantly different within rows at (P≤0.05) and mean values with different superscript (A, B, C,…) letters are significantly different within columns at (P≤0.05).

Table 3.2: Chemical analysis of paneer samples preserved with formalin in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C

Days		0	15	30	45	60	75	90	105	120
Moisture content (%)	(I)	53.56±0.068^{aA}	53.53±0.06^{aA}	53.54±0.06^{aA}	53.54±0.04^{aA}	53.53±0.081^{aA}	53.50±0.023^{bA}	53.46±0.079^{bA}	53.44±0.055^{bA}	53.41±0.066^{bA}
	(II)	53.56±0.068^{aA}	53.53±0.052^{aA}	53.53±0.027^{aA}	53.53±0.076^{aA}	53.49±0.08^{bA}	53.45±0.059^{bA}	53.42±0.098^{bA}	53.40±0.063^{bA}	53.38±0.032^{cA}
Fat (%)	(I)	22.05±0.126^{aA}	22.05±0.101^{aA}	22.04±0.091^{aA}	22.04±0.089^{aA}	21.94±0.111^{bA}	21.82±0.099^{cA}	21.77±0.125^{dA}	21.72±0.102^{dA}	21.68±0.103^{eA}
	(II)	22.05±0.127^{aA}	22.05±0.105^{aA}	22.04±0.093^{aA}	21.95±0.080^{bA}	21.83±0.085^{cA}	21.79±0.215^{dA}	21.77±0.101^{dA}	21.70±0.146^{dA}	21.66±0.089^{eA}
Titratable acidity (% LA)	(I)	0.67±0.011^{aA}	0.70±0.015^{aB}	0.72±0.03^{bC}	0.79±0.015^{bD}	0.87±0.016^{cE}	0.96±0.03^{dF}	1.05±0.011^{eG}	1.16±0.029^{fH}	1.28±0.021^{gI}
	(II)	0.67±0.011^{aB}	0.70±0.016^{bC}	0.71±0.01^{bD}	0.77±0.018^{bE}	0.82±0.013^{cF}	0.93±0.036^{dG}	1.05±0.03^{eH}	1.22±0.013^{fI}	1.33±0.030^{gJ}
pH	(I)	5.55±0.012^{aA}	5.44±0.008^{bA}	5.42±0.008^{bA}	5.41±0.006^{bA}	5.38±0.008^{cA}	5.36±0.005^{cA}	5.31±0.008^{cA}	5.28±0.008^{dA}	5.23±0.008^{dA}
	(II)	5.55±0.012^{aA}	5.42±0.014^{bA}	5.40±0.01^{bA}	5.38±0.011^{bA}	5.36±0.01^{bA}	5.34±0.015^{bA}	5.30±0.01^{bA}	5.26±0.005^{cA}	5.23±0.003^{cA}
Tyrosine content (mg/100g)	(I)	6.28±2.732^{aA}	6.28±2.732^{aA}	8.20±0.718^{bA}	14.48±0.722^{cA}	20.22±0.985^{dA}	29.78±1.791^{eA}	39.62±1.190^{fA}	44.53±2.732^{gA}	53.00±2.236^{hA}
	(II)	6.28±2.732^{aA}	6.28±2.732^{aA}	9.016±0^{bA}	17.49±1.521^{cA}	23.50±0.546^{dA}	32.51±1.092^{eA}	43.17±2.6063^{fA}	46.99±1.4457^{gA}	57.10±0.722^{hA}
FFA(% oleic acid)	(I)	0.15±0.018^{aA}	0.19±0.018^{aA}	0.21±0.018^{bA}	0.24±0.018^{bA}	0.30±0.018^{bA}	0.38±0.011^{cA}	0.43±0.017^{dA}	0.52±0.015^{eA}	0.60±0.025^{fA}
	(II)	0.15±0.018^{aA}	0.19±0.018^{aA}	0.21±0.015^{bA}	0.26±0.018^{bA}	0.32±0.011^{cA}	0.41±0.018^{dA}	0.45±0.009^{dA}	0.52±0.014^{eA}	0.62±0.014^{fA}
Peroxide value (meq/Kg)	(I)	0	0	0.067±0.066^{aA}	0.13±0.057^{aA}	0.27±0.033^{bA}	0.47±0.022^{cA}	0.67±0.066^{dA}	0.87±0.066^{eA}	1.07±0.066^{fA}
	(II)	0	0.07±0.066^{aA}	0.13±0.066^{aA}	0.27±0.066^{bA}	0.40±0.115^{cA}	0.53±0.066^{dA}	0.73±0.066^{eA}	0.93±0.066^{fA}	1.20±0.115^{gA}

(I) Seven layer NYLON/EVOH/70µ polyethylene pouch, (II) 12µ PET/70µ polyethylene pouch

Data is represented as means ± S.E (n=3) with different superscript (a, b, c,…) letters are significantly different within rows at (P≤0.05) and mean values with different superscript (A, B, C,…) letters are significantly different within columns at (P≤0.05)

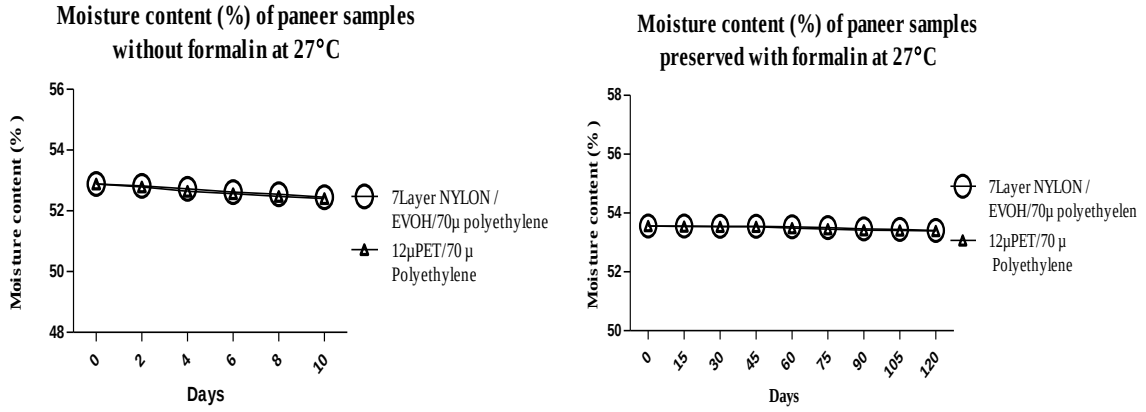

Fig. 3.1 (a) Moisture content (%) of paneer samples without formalin in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C., (b) Moisture content (%) of paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C

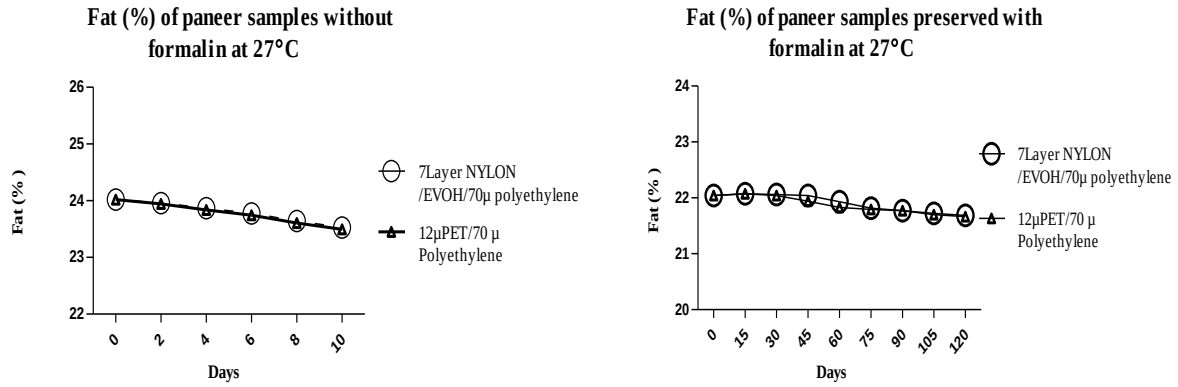

Fig. 3.2 (a) Fat (%) of paneer samples without formalin in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C., (b) Fat (%) of paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C

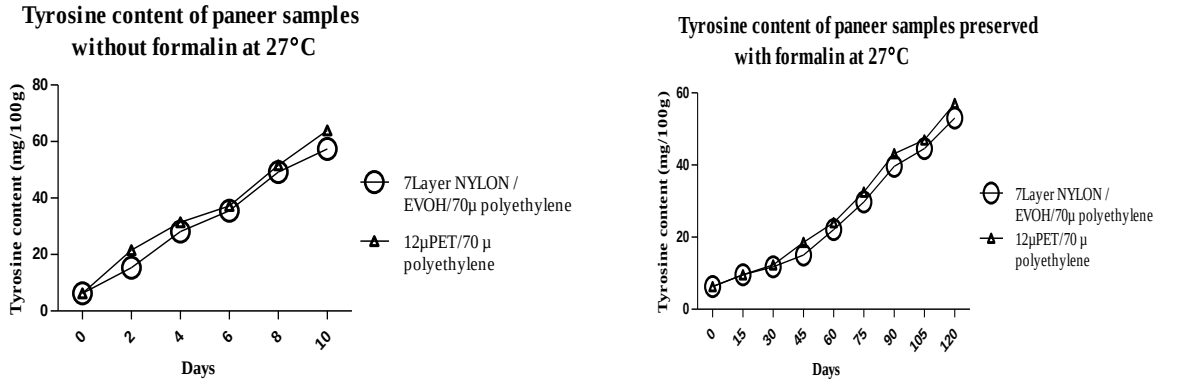

Fig. 3.3 (a) Tyrosine content (mg/100g) of paneer samples without formalin in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C., (b) Tyrosine content (mg/100g) of paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70µ polyethylene and 12µ PET/70 µ polyethylene pouch at 27°C

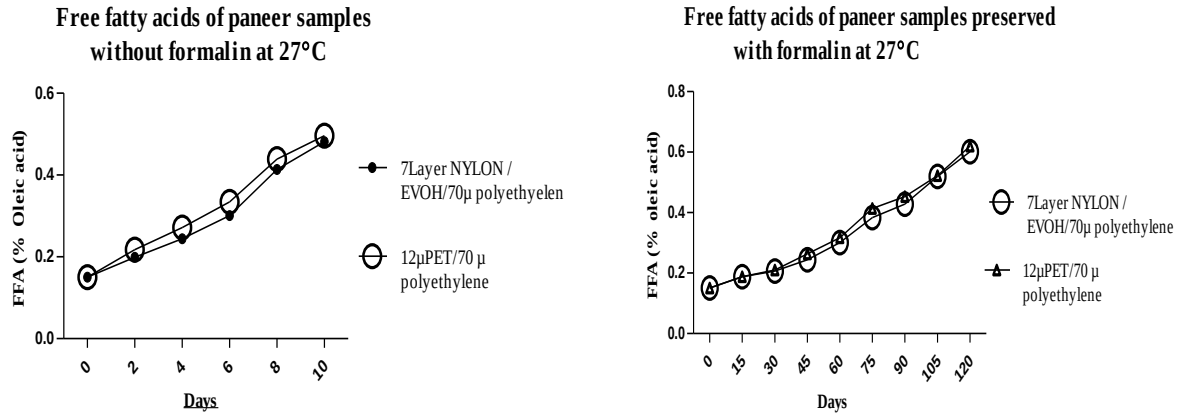

Fig. 3.4 (a) FFA (%oleic acid) of milk fat extracted from paneer samples without formalin in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C., (b) FFA (%oleic acid) of milk fat extracted from paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70µ polyethylene and 12µPET/70µ polyethylene pouch at 27°C

CONCLUSION

From present investigation it can be concluded that Paneer samples preserved with (0.4%) formalin under vacuum packaging in seven layer NYLON/EVOH/70µ polyethylene pouch at 27°C showed no change in major chemical parameters upto 45th day i.e. moisture (%) and fat percentage. However in 12µ PET/70µ polyethylene pouch, no significant difference was observed in chemical parameters upto 30th day. Therefore, it can be concluded that use of seven layer NYLON/EVOH/70µ polyethylene pouch for packaging of paneer samples preserved with formalin at 27°C increases the stability of paneer to remains fit for analytical purpose as compaired to 12µ PET/70µ polyethylene pouch.

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