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# Effect of Vacuum Packaging on Physico-Chemical Analysis of Paneer Preserved With Formalin at 5-7°C for Analytical Purpose

# Meenatai Kamble, Raman Seth and Harish Kumar\*

ICAR-National Dairy Research Institute, Karnal-132001, Haryana Amity universality Jaipur-303002, Rajasthan \*Corresponding Author E-mail: harishkanwar3@gmail.com Received: 1.03.2018 | Revised: 5.04.2018 | Accepted: 12.04.2018

## **ABSTRACT**

Paneer is heat and acid coagulated milk product. It has great value in diet with respect to its food and nutrition. An attempt was made to evaluate the physico-chemical quality of paneer samples preserved with formalin under vacuum packaging in two type of high barrier packaging pouches. So that it will beneficial to FSO to preserve marketed paneer samples suitable for analytical purpose. In the present study two different packaging pouches i.e. seven layer NYLON/EVOH/70μ polyethylene and 12μPET/70μ polyethylene were used to pack paneer preserved with or without formalin at 5-7°C for 120 and 28 days respectively. The results revealed that on addition of formalin @0.4% in paneer samples showed an increase acidity  $(0.62\pm0.017 \text{ to } 0.67\pm0.011\% \text{ LA})$ , whereas fat decreases  $(24.02\pm0.155 \text{ to } 22.05\pm0.126\%)$ . On storage of paneer in both the pouch, Tyrosine content, acidity (%LA), FFA, peroxide value increased while fat percentage and pH decreased. Paneer sample preserved with formalin in seven layer NYLON/EVOH/70µ polyethylene showed no significant (P>0.05) difference in moisture  $(53.55\pm0.068 \text{ to } 53.52\pm0.042\%)$  and fat  $(22.05\pm0.126 \text{ to } 22.00\pm0.111\%)$  up to 75 days respectively. However  $12\mu PET/70\mu$  polyethylene pouch showed, no significant (P>0.05) difference in moisture content (53.55  $\pm 0.068$  to 53.52 $\pm 0.062\%$ ) and fat percentage (22.05 $\pm 0.127$ to 22.00±0.95%) up to 60 days respectively. Based on results it can be concluded that vacuum packaging technique enhance the shelf life of formalin preserved paneer at 5-7°C packed in seven layer NYLON/EVOH/70µPE pouch up to 75 days.

**Key words:** Paneer, Formalin, Vacuum packaging, Chemical analysis. **Highlights:** 

- Vacuum packaging is an active packaging technique which extend the shelf life of food products 2-3 times.
- Formalin, antimicrobial and generally used as a preservative in milk and milk products for analytical purpose.
- Paneer preserved with 0.4% formalin under vacuum packaging in high barrier packaging materials to remains fit for analytical purpose.

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## INTRODUCTION

India has emerged as the largest milk producer in the world with the success of the Operational Flood Programme. About 7% of milk produced in India is processed into paneer. The Indian soft cheese (paneer) is obtained through heat/acid coagulation of casein component of standardized milk, which entraps complex physico-chemical interactions of all the fat, a part of denatured whey proteins and colloidal salts, as well as a part of the soluble milk solids. An aqueous solution of formaldehyde can be useful as a disinfectant as it kills most bacteria as well as fungi (including their spores). It may be used in food as antimicrobial agent and also used in animal feed to improve handling characteristics. Formaldehyde and its derivatives are also present in a wide variety of consumer products to protect the products from spoilage by microbial contamination. 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<sup>18,13,27,7</sup>. During the collection of market paneer samples, Food safety officers (FSO) may add 37-40% formalin @0.4% (Except in the case where the sample is meant for microbiological analysis) to remains suitable for physico-chemical analysis. The preservation of Paneer is a major problem as its shelf life is hardly one day at room temperature. It provides favourable conditions for the growth of different microorganisms, as a result of which the product undergoes undesirable physicochemical, organoleptic, proteolytic lipolytic changes, which hinder the organized marketing of the product commercially. Chemical spoilage in paneer significantly delayed or prevented by using suitable packaging technique<sup>12</sup>. Vacuum packaging is an active packaging technique which avoids the growth of aerobic spoilage organisms thereby reducing rancidity and moisture evaporation<sup>21</sup>. Scahdeva et al., reported that paneer packaged in laminated

pouches had a shelf life of about 30 days at refrigerated storage (6+1°C). Paneer packaged in high barrier film EVA/EVA/PVDC/EVA) under vacuum and heat treated at 90°C for one min is reported to have a shelf life of 90 days under refrigeration. The objective of this work was carryout to study effect of vacuum packaging on paneer samples preserved with formalin in two different 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 5-7°C were monitored to remained suitable for analytical purpose.

## MATERIAL AND METHODS

# 2.1. Sample preparation of paneer

The paneer samples were obtained from experimental dairy of National Dairy Research Institute, Karnal. Two types of packaging such seven layer NYLON/EVOH/70µ (a) and polyethylene  $12\mu$ PET/70µ (b) polyethylene pouch were used to pack the paneer cubes. Paneer blocks cut into cubes (1×1 cm size) followed by addition of 37-40% formalin solution @0.4% added in paneer cubes and it was mixed by inverting pouch 8-10 times. Vacuum packaging was done in Indvac vacuum packaging machine at negative pressure (-760 mmHg) within 25 sec. Paneer samples preserved with formalin were stored at 5-7°C for 120 days and analyzed at a regular interval of 15 days respectively. Paneer samples without formalin was stored at 5-7°C for 28 days and analyzed at a regular interval of 4 days respectively.

# 2.2. 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 (37-40%), 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.

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# 2.3. Analysis

The paneer samples were analyzed for moisture<sup>16</sup>, fat % <sup>16</sup>, acidity (% LA) <sup>16</sup>, pH (pH meter), tyrosine value<sup>24</sup>, Free Fatty Acid % <sup>17</sup>, Peroxide value <sup>17</sup>.

# 2.4. Statistical analysis

The experiment was carryout in triplicate and the effect of treatment with or without formalin in both the packaging of paneer on physico-chemical attributes were assessed. Interaction between packaging materials were evaluated by analysis of variance (ANOVA) and Tukey's test at a significance level of 5%. Correlation coefficient was used to correlate effect different packaging materials on physico-chemical analysis of paneer preserved with and without formalin after 15 days and 4 days respectively at 5-7°C. Significance was considered at P <0.05 and P<0.01. All the data was analyzed using SAS 9.2 software.

# RESULT AND DISCUSSION

# 3.1. Compare the effect of vacuum packaging on storage of paneer samples

Paneer samples without formalin were vacuum packed in (a) seven layer NYLON/EVOH/70µPE and (b)  $12\mu$ PET/70µPE pouches respectively and stored at 5-7°C. During storage foul smell and sticky surface appearance was observed in the both the packaging pouches on 4<sup>th</sup> day respectively. Paneer preserved with formalin packed in seven layer NYLON/EVOH/70µPE pouches was having in foul smell after 75th day and in 12μ PET/70μ polyethylene pouches, foul smell was felt after  $60^{th}$ day 5-7°C respectively.

# 3.2. Comparing the effect of vacuum packaging on physico-chemical analysis paneer samples preserved with formalin during storage at 5-7°C

# **3.2.1.** Moisture (%)

Moisture percentage of freshly prepared paneer samples was  $52.88\pm0.061\%$  (Fig. 3.1.a). Moisture percentage of paneer packed in seven layer NYLON/EVOH/70 $\mu$ PE and  $12\mu$  PET/70 $\mu$ PE pouch showed significant (P<0.05) decrease (52.88 $\pm0.061$  to 52.75 $\pm0.030$ ° 52.74 $\pm0.084\%$ ) on  $12^{th}$  day

respectively and continue to decrease  $(52.88\pm0.061 \text{ to } 52.53\pm0.077, 52.49\pm0.080\%)$ up to 28 days (Fig. 3.1.a). Formalin (0.4%) showed significantly increase (P<0.005) in moisture percentage from 52.88±0.061 to 53.56±0.06% (Fig. 3.1.a and 3.1.b). Moisture percentage in both the packaging pouch (P < 0.05)decrease showed significant  $(53.55\pm0.068 \text{ to } 53.50\pm0.063, 53.49\pm0.042\%)$ on 105<sup>th</sup> day of storage respectively and decrease (53.55±0.068 continue to 53.48±0.098, 53.47±0.05%) up to 120 days (Fig. 3.1.b). No significant (P>0.05) difference was observed in moisture percentage 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<sup>14</sup> and Singh<sup>32</sup>. The overall decrease in moisture percentage during storage of paneer may be due to water vapor transmission rates of packaging materials<sup>33,29</sup>. It had a significant (P<0.01) effect on chemical parameters i.e. fat, FFA, tyrosine content, peroxide value. pH and acidity were affected significantly (P<0.05) (Table 3.1).

# 3.2.2. Fat (%)

The fat percentage of paneer samples preserved with and without formalin was analyzed by Rose-Gottlieb method and results are showed in Fig. 3.2.a, and 3.2.b. The average fat percentage of fresh paneer samples was 24.02±0.155% (Fig. 3.2.a). Fat percentage of paneer samples packed in (a) and (b) pouch showed significant (P < 0.05)decrease  $(24.02\pm0.155 \text{ to } 23.99\pm0.097, 23.97\pm0.099\%)$ on 8th day respectively and continue to  $(24.02\pm0.155)$ to  $23.48\pm0.110$ , 23.46±0.112%) up to 28 days (Fig. 3.2.a). The reduction in fat of paneer might be due to lipolytic activity of microorganisms produce on short and medium chain fatty acid which may not be completely recovered by solvent (petroleum ether and diethyl ether) extraction method<sup>25,22</sup>. No significant (P>0.05) difference was observed in fat percentage of paneer in (a) and (b) pouches (Figure 3.2.a). Fat percentage in paneer preserved with formalin was decreased from  $24.02\pm0.155$  to  $22.05\pm0.126\%$ . Initial decrease in fat percentage may be due to

improper dissolution of casein matrix which entraps the fat globules. Fat percentage of paneer preserved with formalin showed significant (P<0.05) decrease in (a) pouch  $(22.05\pm0.126 \text{ to } 21.98\pm0.122\%) \text{ on } 90^{\text{th}} \text{ and in }$ (b) pouch  $(22.05\pm0.126 \text{ to } 21.97\pm0.232\%)$  on 75<sup>th</sup> day respectively. It was continue to decrease in both the pouches (22.05±0.126 to 21.94±0.101, 21.91±0.225%) up to 120 days (Fig. 3.2.b). Lower values of fat percentage in paneer preserved with formalin may be due to reaction between aqueous formalin and milk protein<sup>8,9</sup>. No significant (P>0.05) difference was observed in fat percentage of paneer preserved with formalin packed in both packaging pouches (Fig. 3.2.b). Haridas and Narayanan (1976), also observed a decrease in the fat percent of paneer when preserved with 0.4% formalin at 37°C after 6<sup>th</sup> day. Fat percentage of paneer packed in both the packaging had a significant (P<0.01) effect on moisture, FFA, tyrosine content, peroxide value, pH and acidity (Table 3.2).

# 3.2.3. Titratable acidity (%LA)

The average percent lactic acid of fresh paneer samples was 0.62±0.017% LA. Percent lactic acid of paneer in (a) and (b) pouch showed significant (P<0.05) increase (0.62±0.017 to  $0.74\pm0.030$ ,  $0.70\pm0.022\%$ ) on 8<sup>th</sup> day respectively and continue to increase  $(0.62\pm0.017 \text{ to } 0.89\pm0.019, 0.88\pm0.038\%) \text{ up}$ to 28 days. Increasing % LA were observed during storage, thereby indicating bacterial spoilage by lactic acid fermenting bacteria<sup>2</sup>. Paneer samples preserved with formalin showed increased titratable acidity from  $0.62\pm0.017$  to  $0.67\pm0.011\%$  LA. 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<sup>3</sup>, Boghra and Borkhatriya<sup>5</sup>, Upadhyay et al.<sup>34</sup>, Singh<sup>32</sup> in milk and paneer on the addition of formalin. Paneer preserved with formalin showed significant (P<0.05) increase in titratable acidity in the (a) and (b) pouch  $(0.67\pm0.011 \text{ to } 0.70\pm0.022, 0.72\pm0.016\%)$  on 30<sup>th</sup> day respectively and continue to increase  $(0.67\pm0.011 \text{ to } 0.87\pm0.01, 0.91\pm0.016\%)$  up to

120 days. Significant (P<0.05) differences were observed in titratable acidity (%LA) of paneer preserved with formalin in both packaging pouches. Addition of 0.4% formalin in the paneer showed no change in titratable acidity upto 6<sup>th</sup> day at 37°C and thereafter an increase in titratable acidity of paneer was observed upto a storage period of 21<sup>st</sup> day<sup>14</sup>.

# 3.2.4. pH

The pH value of fresh paneer samples was 5.53±0.08. pH value of paneer packed in (a) and (b) pouch showed significant (P<0.05) decrease  $(5.53\pm0.08)$ to  $5.46\pm0.006$ , 5.45±0.008) on 4<sup>th</sup> and 8<sup>th</sup> day respectively and continue to decrease (5.53±0.08 to 5.34±0.008, 5.33±0.006) up to 28 days. This decreasing trend of pH in paneer samples during storage may be due to utilization of lactose by microorganism and its conversion to lactic acid<sup>4</sup>. No significant (P>0.05) difference was observed in pH value of paneer samples in both the packaging pouches. The average pH value of paneer samples preserved with formalin was 5.55±0.012. On addition of formalin in paneer cubes, no change in pH value of paneer samples was noticed. pH value of paneer preserved with formalin in both the packaging pouch showed significant (P<0.05) decrease  $(5.55\pm0.012$ to  $5.46 \pm 0.008$ , 5.45±0.01) on 15<sup>th</sup> day respectively and decrease  $(5.55\pm0.012)$ continue to 5.37±0.003, 5.33±0.005) upto 120 days. No significant (P>0.05) difference was observed in pH of paneer preserved with formalin in both packaging pouches. Singh<sup>32</sup>, also reported that pH of paneer samples preserved with formalin decreased with increase in storage period.

# 3.2.5. Tyrosine content

The tyrosine content of paneer samples was 6.28±2.732 mg/100g. Tyrosine content of paneer samples packed in (a) and (b) pouch showed significant (P<0.05) increase (6.28±2.732 to 14.48±2.732, 17.57±0.191 mg/100g) on 4<sup>th</sup> day respectively and continue to increase (6.28±2.732 to 58.50±0.191, 64.75±0.819 mg/100g) up to 28 days (Fig.3.3.a). The increase in tyrosine content of paneer was due to proteolysis of paneer<sup>26,31</sup>.

No significant (P>0.05) difference was observed in tyrosine content of paneer in both packaging pouches. Paneer sample preserved with formalin showed tyrosine value as 6.28±2.732 mg/100g. No effect was observed on addition of formalin in tyrosine content of paneer. Tyrosine content in paneer preserved with formalin in both the packaging pouch significant (P < 0.05)showed increase  $6.83\pm1.791$ ,  $6.83\pm1.521$  $(6.28\pm2.732)$ to mg/100g) on  $30^{th}$ day respectively and continued increased  $(6.28\pm2.732)$ to 28.14±1.661, 30.33±1.706 mg/100g) up to 120 days (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, milk proteins present in paneer have been degraded thereby releasing free amino acids leads to increase in tyrosine content. Haridas and Narayanan<sup>14</sup>, also reported that the significant increase in tyrosine content of paneer preserved with 0.4% formalin at 30°C upto 21 days. Singh, R.32, 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.

# 3.2.6. Free Fatty Acid (% oleic acid)

Free fatty acids (FFA) are often used as indicators of lipolysis<sup>19</sup>. The FFA (% oleic acid) of milk fat extracted from fresh paneer was 0.15±0.01% oleic acid. The FFA of milk fat extracted from paneer samples in both the packaging pouch showed significant (P<0.05)  $(0.15\pm0.01$  $0.21\pm0.015$ , increase to 0.21±0.018% oleic acid) on 8<sup>th</sup> and 4<sup>th</sup> day respectively. It was continue to increase  $(0.15\pm0.01 \text{ to } 0.47\pm0.0188, 0.48\pm0.015\% \text{ oleic}$ acid) up to 28 days (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). The FFA of milk fat extracted from fresh paneer preserved with formalin was 0.15±0.01% oleic acid. Addition of formalin in fresh paneer samples has no effect on free fatty acids. The FFA of milk fat extracted from paneer preserved with formalin in (a) and (b) pouch showed significant (P<0.05) increase (0.15±0.01 to

0.21±0.016, 0.21±0.018% oleic acid) on 90<sup>th</sup> and 75<sup>th</sup> day respectively and continue to  $(0.15\pm0.01$ increase to  $0.22\pm0.018$ , 0.24±0.018% oleic acid) up to 120 days (Fig. 3.4.b). An increase in FFA of milk fat extracted from paneer with formalin indicated lipolysis of 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<sup>32</sup>, also reported that FFA of milk fat extracted from paneer 0.4% formalin were increased significantly upto 6<sup>th</sup> month at 37°C.

# 3.2.7. Peroxide value

Peroxides are an indicator of oxidative rancidity of milk fat. No peroxide value was observed in fresh paneer samples. The peroxide value of milk fat extracted from paneer in (a) and (b) pouch showed significant (P<0.05)increase (0 to  $0.23\pm0.028$ 0.27±0.066 meq/Kg) on 12<sup>th</sup> day respectively. It was continued increase (0 to 0.47±0.066, 0.53±0.066 meg/Kg) upto 28 days. No significant (P>0.05) difference was observed in peroxide value of milk fat extracted from paneer 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. Peroxide value of milk fat extracted from paneer preserved with formalin in (a) and (b) pouch showed significant (P<0.05) increase (0 to  $0.23\pm0.066$ ,  $0.27\pm0.066$  meg/Kg) on  $75^{th}$  and 60<sup>th</sup> day respectively. It was continued to increase (0 to 0.40±3.93, 0.47±0.066meq/Kg) upto 120 days. 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<sup>32</sup>, 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 Correlation of moisture content on different chemical parameters of paneer packed in seven layer NYLON/EVOH/70μ polyethylene and 12μ PET/70μ polyethylene pouch

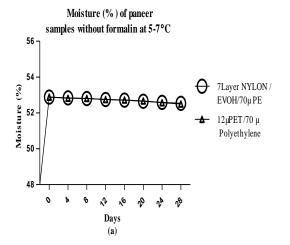
correlation coefficient between	Seven layer NYLON/EVOH/70 μ polyethylene	12µPET/70µ polyethylene
Moisture Vs. Fat	0.3832**	0.4083**
Moisture Vs. pH	0.1145*	0.1139*
Moisture Vs. FFA(% oleic acid)	-0.4454**	-0.4834**
Moisture Vs. Tyrosine content	-0.4971**	-0.4965**
Moisture Vs. Titratable acidity( % LA)	-0.1223*	-0.1328*
Moisture Vs. Peroxide value	-0.4606**	-0.3546**

Significant at \*(P<0.05), \*\* (P<0.01)

Table 3.2. Correlation of fat percentage on different chemical parameters of paneer packed in seven layer NYLON/EVOH/70u polvethylene and 12uPET/70u polvethylene pouch

correlation coefficient between	Seven layer NYLON/EVOH/70µ polyethylene	12µPET/70µ polyethylene
Fat Vs. Moisture	0.3832**	0.4083**
Fat Vs. pH	0.4207**	0.4642**
Fat Vs. FFA(% oleic acid)	-0.6345**	-0.6748**
Fat Vs. Tyrosine content	-0.6342**	-0.6642**
Fat Vs. Titratable Acidity (%LA)	-0.4591**	-0.4270**
Fat Vs. Peroxide value	-0.5255**	-0.5956**

Significant at \*(P<0.05), \*\* (P<0.01)



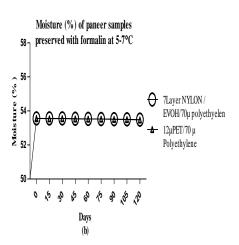


Fig. 3.1 (a) Moisture (%) of paneer samples without formalin in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C., (b) Moisture (%) of paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C

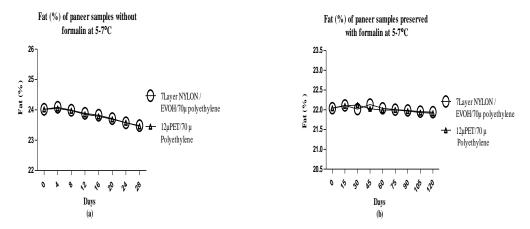


Fig. 3.2 (a) Fat (%) of paneer samples without formalin in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C., (b) Fat (%) of paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C

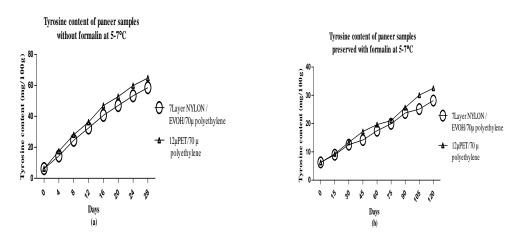


Fig. 3.3 (a) Tyrosine content (mg/100g) of paneer samples without formalin in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C., (b) Tyrosine content (mg/100g) of paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C

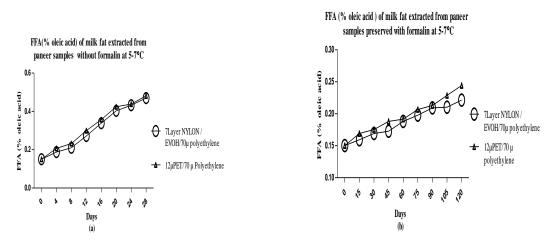


Fig. 3.4 (a) FFA (% oleic acid) of milk fat extracted from paneer samples without formalin in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C., (b) FFA (% oleic acid) of milk fat extracted from paneer samples preserved with formalin packed in seven layer NYLON/EVOH/70 $\mu$  polyethylene and 12 $\mu$  PET/70  $\mu$  polyethylene pouch at 5-7°C

# **CONCLUSIONS**

From present investigation it can be concluded that Paneer samples preserved with 37-40% formalin solution @0.4% under vacuum packaging layer in (a) seven pouch NYLON/EVOH/70µPE 5-7°C showed no change in major physico-chemical parameters up to 75<sup>th</sup> day i.e. moisture and fat percentage. However in 12µ PET/ 70µ polyethylene pouch, no significant difference was observed in physico-chemical parameters up to 60<sup>th</sup> day. Therefore, it can be concluded vacuum packaging use technique increases the shelf life of paneer samples preserved with formalin at 5-7°C.

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