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A study on the storage of sterilized soymilk

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ABSTRACT

The study was aimed at the improvement of shelf-life of protein rich soymilk which can be consumed in developing countries such as India to overcome protein energy malnutrition. Sterilized soymilk was prepared by standard procedure and was treated with sterilization method of heat treatment (121°C for 15 min) after bottling. Samples were stored in refrigerated (4°C) as well as at room temperature conditions. Quality attributes like viscosity, TSS, titrable acidity, pH, standard plate count, yeast and mold count were determined at the regular interval of 10 days. On the basis of the quality attributes it was observed that sterilized soymilk samples were acceptable up to 170 days in refrigerated condition while same samples were acceptable up to 90 days in ambient temperature condition from the day of preparation. Thus the study reveals that sterilization treatment successfully increased the shelf-life of soymilk stored at refrigerated condition.

Keywords: Soymilk; sterilization; shelf-life

INTRODUCTION

Soybean (*Glycine max*) with 40 per cent protein and 20 per cent fat assumes the most predominant position in solving the nutritional imbalances prevailing. It not only provides the quality macronutrients but also various other micronutrients which are otherwise required to fight against the hidden hunger. Efforts are being made to popularize various soy-based foods besides the oil which is very popular in India. In India about 9.3 million tons of soybean is produced annually and 80 per cent is utilized for oil extraction. Only 10 per cent is available for direct food uses. Among various foods soymilk is having a great potential as it can be prepared at domestic level using the available resources.

World production of soybean in 2009-2010 at 260838 thousand metric tons (TMT) from an area of 102150 thousand hectares was projected up to 263693 thousand metric tons from an area of 103503 thousand hectares in 2010-

2011. In 2010-2011 India ranked sixth in area and production of soybean producing 9600 thousand metric tons of soybean from an area of 9400 thousand hectares (Anon 2011).

Soymilk is an aqueous extract of soybeans and is inexpensive, highly digestible, nutritious, lactose and cholesterol free and rich in polyunsaturated fatty acids namely linoleic acid. It is quite popular beverage in countries like China, Japan, Taiwan and Thailand (Kanawjia and Singh 2002).

Soymilk has a great potential to supplement the dairy milk and it is nutritionally comparable with the human and cow milk. Soymilk is used as a base in a wide variety of products including tofu, soy yoghurt and cheese. Soymilk yield, quality, flavor, colour, recovery of protein, solids and fat are affected by variety or cultivar (Wang and Murphy 1994), soybean cultivation environment (Schaefer and Love 1992) and soymilk processing methods (Wang and Chang 1995). High grade soybeans generally produce the best soymilk and the large seeded soybeans are considered to be the superior type (Gandhi 2000).

Fresh soymilk has a very short shelf-life which limits consumption to areas close to the production site. Thermal processing is the most common practice used to improve the microbial safety and extend the shelf-life of soymilk because it inactivates vegetative pathogens and many spoilage bacteria (Kwok and Niranjan 1995). The use of ultra-high temperature (UHT) is relatively new for soymilk production. The traditional processing involves temperature of 90-100°C applied up to 30 min (Yuan et al 2008). In some conditions thermal processing however detrimentally affects nutritional and quality attributes of soymilk and produces strong off flavors (Lozano et al 2007). It limits the development of soy foods that are appealing to consumers and negatively impacts the use of heat-treated soymilk as an ingredient (Kwok et al 2000, Achouri et al 2007).

Heating is commonly applied during the soymilk preparation process mostly to ensure food safety and extend the shelf-life of the product. Heat inactivates enzymes such as lipoxygenase and other proteins such as trypsin inhibitors which can negatively impact the quality and nutritional properties of soymilk (Kumar et al 2003).

Plain soymilk is packed in 200/500 ml polythene bags/ glass bottles/tetra packs. It has shelf-life of six months when packed in tetra packs or else for few weeks under refrigerated conditions. It has to be stored and distributed at ambient temperature (Gandhi 2008).

Currently soymilk in its new domain is becoming a domestic affair (Weingartner 1987). Unfortunately attempts to preserve

soymilk in rural households have remained a problem in that most soymilk if not consumed shortly after production loses its appealing quality. Thus there is a need to systematically assess the influence of production method and storage technique on the quality of soymilk. Although several studies have been reported on soy however very little information is available on the changes in physicochemical properties during storage of soymilk. In light of above the present investigations were proposed to study the shelf-life of soymilk.

MATERIAL and METHODS

Preparation of soymilk

Soybean seeds were soaked in water for 6-8 h, cleaned by using clean water and ground with 15 liters water in cooker cum grinder. The slurry was cooked at 100°C temperature by passing steam through it. After cooking again slurry was ground in order to obtain homogeneity and was allowed to pass into deodorizer by opening the butterfly valve and creating vacuum simultaneously. During the flow of soymilk into the deodorizer there was continuous removal of its beany flavour carried out by using vacuum pump. The whole soymilk inside the deodorizer was collected in filter press covered by muslin cloth. After filtration soymilk and okara were collected separately. Soymilk was again boiled up to 80°C by adding sugar with continuous stirring and allowed to cool. It was then filled in sterilized glass bottles having capacity of 200 ml after adding the colour and flavour. Bottles were corked firmly and finally sterilized in autoclave steam sterilizer at 121°C for 15 min.

Determination of viscosity

Viscosity of soymilk was measured by using viscometer (Brookfield, DV-E). The sample was taken in a 600 ml low form griffin beaker and the level was allowed to reach up to the immersion groove on the spindles shaft. The time required for stabilization was depending on the speed at which the viscometer was running and the characteristics of the sample fluid. Care was taken to maintain constant values of test temperature 30°C (Chinyere and Kenneth 1997), spindle used LV1 and test speed 60 RPM (Harjai and Singh 2007), sample container size (600 ml), sample volume (500 ml), viscometer model (DV-E) and length of time or number of spindle revolutions to record viscosity.

Total Soluble Solids (TSS)

The content of total soluble solids (TSS) in the soymilk was determined with the help of 0-32°Brix hand refractometer (RHB-32) (Liu and Lin 2008). The refractometer reading was adjusted at '0' with the help of rotating small calibration screw. A drop of soymilk was put on sensor for measurement and the refractometer reading was recorded.

Determination of titratable acidity

Acidity of was determined by titration method (Chinyere and Kenneth

1997). The known weight of soymilk was titrated against sodium hydroxide using phenolphthalein as an indicator (Anon 1995).10 ml sample was taken in a conical flask and 10 ml distilled water and 3-4 drops of phenolphthalein indicator were added. The solution was titrated against 0.1 N NaOH solution till the colour of solution was changed to pink. Then burette reading was recorded.

$$\text{Acidity} = \frac{\text{Titre} \times \text{Normality of alkali (0.1 N)} \times \text{Volume make up} \times \text{Equi. wt of acid} \times 100}{\text{Volume of sample taken for estimation of acidity} \times \text{Volume of sample taken for volume make up} \times 1000}$$

Determination of pH

pH was measured by using glass electrode pH meter(Chinyere and Kenneth 1997). The electrode was dipped in the sample up to sufficient depth such that electrode should not touch the bottom of the beaker. The readings were recorded which were displayed constantly on the scale.

Microbial examination of soymilk

The microbial study of sterilized soymilk kept at room temperature as well as refrigerated conditions during storage was carried out as per the method cited in Indian Standard Institute (Anon 1969a, 1969b). The results for exact count were recorded as colony forming units/ml of soymilk ie CFU/ml. Total plate count was determined by method cited by Anon (1969 a) by using tryptone agar medim having following composition. Medium was prepared by adjusting the pH 7.0 to 7.5 and sterilized in autoclave at 15 PSI for 15-20 minutes.

Ingredient	**Amount**
Peptone	10 g
NaCl	5 g
Beef extract	10 g
Agar	20 g
Distilled water	1000 ml

Yeast and mould count was determined by method cited by Anon (1969b) by using potato dextrose agar media (Momoh et al 2011) having following composition:

Ingredients	**Amount**
Infusion from white potato	200 ml
Dextrose	20 g
Yeast extract	0.1 g
Agar	20 g

The above ingredients were added by adjusting pH to 3.5$\pm$0.1. This medium was also prepared and sterilized in autoclave at 15 PSI for 15-20 minutes.

RESULTS and DISCUSSION

Changes during storage in viscosity of soymilk

Viscosity of soymilk increased with increase in storage period for sterilized samples stored at both temperature conditions. Initial viscosity of sterilized fresh soymilk samples stored at refrigerated as well as ambient temperature condition was 4.21 cp. It can be observed from Table 1 and 2 that in sterilized sample stored at refrigerated condition viscosity increased from 4.21 cp to 7.57 cp during 180 days of storage while in sterilized samples stored at ambient temperature condition it increased from 4.21 cp to 9.26 cp during 100 days of storage. The values of viscosity obtained for both soymilk samples at the beginning (0 day) of storage were nearly similar to the minimum value of viscosity obtained by Harjai and Singh (2007).

Fig 1 and 2 illustrate the increase in viscosity of both soymilk samples with advancement of storage time. The similar trend was studied by Morales-de la Pena et al (2010) in case of fruit juice soymilk beverages throughout the storage. According to Chinyere and Kenneth (1997) under refrigerated and frozen storage the viscosity of soymilk samples was relatively stable over time and then increased. In Fig 1 and 2 viscosity curves also varied in same fashion. As the viscosity is affected by the state and concentration of fats, protein, temperature, pH and milk age the change in viscosity might be due to change in concentration of above factors. Statistically the viscosity of soymilk samples showed a significant difference with respect to increased number of storage days and change in treatment.

Changes during storage in TSS

Initial TSS of sterilized fresh soymilk samples stored at refrigerated as well as ambient temperature conditions was 13°Brix. It can be observed from Table 1 and 2 that in sterilized sample stored at refrigerated condition TSS decreased from 13°Brix to 10°Brix during 180 days of storage while in sterilized samples stored at ambient temperature condition it decreased from 13°Brix to 10.3°Brix during 100 days of storage.

The values of TSS obtained for soymilk samples at the beginning of storage are in line with the findings of Osman and Razig (2010) while values of TSS obtained at the end of storage are supported by Liu et al (2004), Nik et al (2008), Rehman et al (2007) and Smith et al (2009).

Fig 1 and 2 illustrate the decreased rate of TSS of all soymilk samples with increasing storage time. According to Fahmi et al (2011) about half of the solids in soymilk consist of soybean protein. As a common problem with soymilk is lack of stability sediment precipitation of proteins and other added solid particles such as minerals or flavours it might be the reason

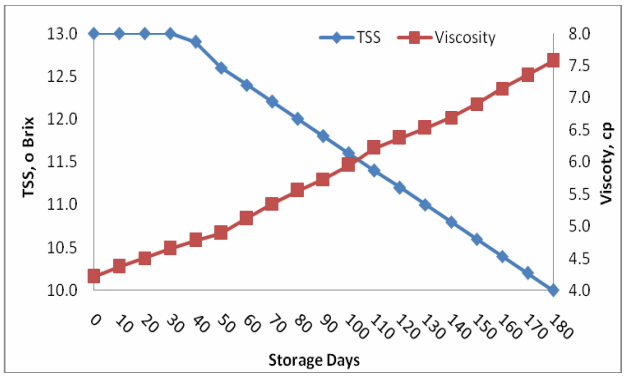

Fig 1. **Changes in viscosity and TSS of sterilized soymilk stored at refrigerated condition during storage**

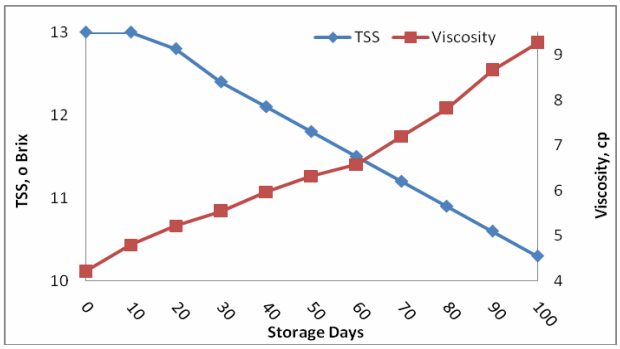

Fig 2. **Changes in viscosity and TSS of sterilized soymilk stored at ambient condition during storage**

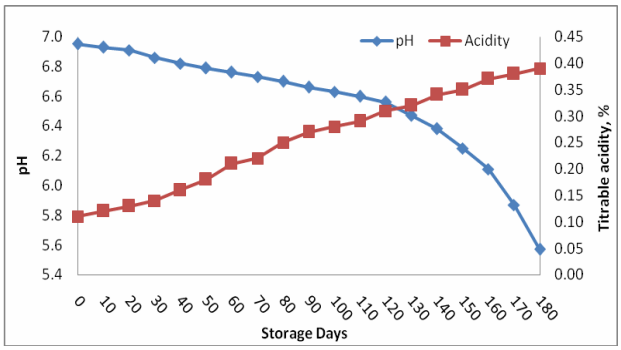

Fig 3. **Changes in titrable acidity and pH of sterilized soymilk stored at refrigerated condition during storage**

for lowered value of TSS of during storage. Statistically TSS of samples showed significant difference with respect to increased number of storage days and change in treatment.

Changes during storage in acidity

Initial acidity of sterilized fresh soymilk samples stored at refrigerated as well as ambient temperature condition was 0.11. It was can be observed from Table 1 and 2 that in sterilized sample stored at refrigerated condition acidity increased from 0.11 to 0.37 during 180 days of storage while in sterilized samples stored at ambient temperature condition it increased from 0.11 to 0.38 during 100 days of storage.

Initial values of acidity obtained for all soymilk samples were close to the values quoted by Harjai and Singh (2007) while final values of acidity were slightly greater than values evaluated by Mnkeni and Nyaruhucha (1994). Fig 3 and 4 illustrate the increased rate of acidity of both the samples with increasing storage time.

Above trend of increase in acidity of soymilk is also comparable with those reported before (Hepburn et al 1930, Chinyere and Kenneth 1997). Increase in acidity of soymilk might be due to decrease in pH and better survival of *Lactobacillus acidophilus* and activity of psychrotrophic bacteria during storage.

Changes during storage in pH

It was observed that pH of soymilk decreased with increase in storage period. Initial pH samples stored at refrigerated as well as ambient temperature condition was 6.95. The obtained pH was close agreement with the value reported by Onuorah et al (2007). It can be observed from Table 1 and 2 that in sterilized sample stored at refrigerated condition pH decreased from 6.95 to 5.57 during 180 days of storage while in sterilized samples stored at ambient temperature condition it decreased from 6.95 to 5.70 during 100 days of storage.

A similar trend of changes in pH was also noted by Mnkeni and Nyaruhucha (1994). The pH value obtained at the end of storage samples was nearly equal to 5.7 and similar value was obtained by Kamaly (1997) who also analyzed that coagulation of sterilized soymilk occurred at about pH 5.7.

Fig 3 and 4 illustrate the decreased rate of pH of all soymilk samples with increasing storage time. Decrease in pH value of samples may be due to an increase in titrable acidity.

Changes during storage in standard plate counts (SPC) stored at refrigerated and ambient condition

It was observed that standard plate counts of soymilk increased with increase in storage period for sterilized samples stored at both temperature conditions. Initial

Table 1. Changes in quality attributes of sterilized soymilk stored at refrigerated condition during storage

Storage days	Viscosity (cp)	TSS (°Brix)	Acidity (%)	pH	SPC (CFU/ml)	Yeast, mould (CFU/ml)
0	4.21	13.0	0.11	6.95	0	0
10	4.37	13.0	0.12	6.93	0	0
20	4.50	13.0	0.13	6.91	2	0
30	4.65	13.0	0.14	6.86	6	1
40	4.78	12.9	0.16	6.82	10	3
50	4.89	12.6	0.18	6.79	24	8
60	5.12	12.4	0.21	6.76	55	11
70	5.35	12.2	0.22	6.73	105	14
80	5.55	12.0	0.25	6.70	429	19
90	5.73	11.8	0.27	6.66	785	28
100	5.95	11.6	0.28	6.63	1052	37
110	6.22	11.4	0.29	6.60	3666	43
120	6.37	11.2	0.31	6.56	6280	47
130	6.53	11.0	0.32	6.47	9399	56
140	6.69	10.8	0.34	6.38	12702	61
150	6.90	10.6	0.35	6.25	15370	72
160	7.14	10.4	0.37	6.11	18026	85
170	7.35	10.2	0.38	5.87	19200	100
180	7.57	10.0	0.39	5.57	20721	111
SE±	0.0084	0.0622	0.0025	0.0055	56.9774	1.6863
$CD_{0.01}$	0.0320	0.2358	0.0095	0.0208	215.9497	6.3914

standard plate counts on sterilized samples stored at refrigerated as well as ambient temperature conditions was 0 CFU/ml which increased to 2.0721 x 10^4 CFU/ml during 180 days of storage while in sterilized samples stored at ambient temperature condition it increased to 2.1989 x 10^4 CFU/ml during 100 days of storage. The initial 0 CFU/ml in case of both sterilized samples showed that the sterilization temperature (121°C) and time (15 min) were adequate. Similar findings were observed by Onuorah et al (2007) in case of pasteurized soymilk samples.

Changes during storage in yeast and mould counts of sterilized soymilk stored at refrigerated and ambient condition

Yeast and mould counts of soymilk increased with increase in storage period for sterilized samples stored at both

Table 2. Changes in quality attributes of sterilized soymilk stored at ambient condition during storage

Storage days	Viscosity (cp)	TSS (°Brix)	Acidity (%)	pH	SPC (CFU/ml)	Yeast, mould (CFU/ml)
0	4.21	13.0	0.11	6.95	0	0
10	4.80	13.0	0.14	6.91	353	5
20	5.22	12.8	0.16	6.85	1045	17
30	5.54	12.4	0.19	6.79	3174	27
40	5.97	12.1	0.21	6.71	5634	34
50	6.32	11.8	0.24	6.63	8279	41
60	6.58	11.5	0.28	6.58	10822	50
70	7.20	11.2	0.31	6.44	14205	65
80	7.81	10.9	0.33	6.15	17003	80
90	8.66	10.6	0.36	5.87	19296	101
100	9.26	10.3	0.37	5.70	21989	120
SE±	0.0167	0.0000	0.0012	0.0100	63.9441	2.4079
$CD_{0.01}$	0.0672	0.0000	0.0049	0.0402	257.7274	9.7051

temperature conditions. Initial yeast and mould counts on sterilized fresh soymilk samples stored at refrigerated as well as ambient temperature conditions were 0 CFU/ml. It can be observed from Table 1 and 2 that in sterilized sample stored at refrigerated condition yeast and mould counts increased from 0 CFU/ml to 111 CFU/ml during 180 days of storage while in sterilized samples stored at ambient temperature condition it increased to 120 CFU/ml during 100 days of storage. The initial 0 CFU/ml in case of both sterilized samples showed that the sterilization temperature (121°C) and time (15 min) were adequate. Above trend of yeast and mould count was comparable with the results quoted by Momoh et al (2011) in which total inhibition of yeasts and moulds were achieved when soymilk preserved with a combination of 700-800 ppm of sodium benzoate, pasteurization and refrigeration.

Gandhi (2009) has given the nutritional standards for soymilk and quoted the critical limit of yeast and mould counts as 100 CFU/ml. Considering this standard critical limit of yeast and mould counts the spoilage of soymilk and thereby shelf- life of soymilk samples was decided. It was observed that all the samples with sterilization treatment were within safe limit up to 170 days and 90 days in case of refrigerated and ambient storage condition respectively.

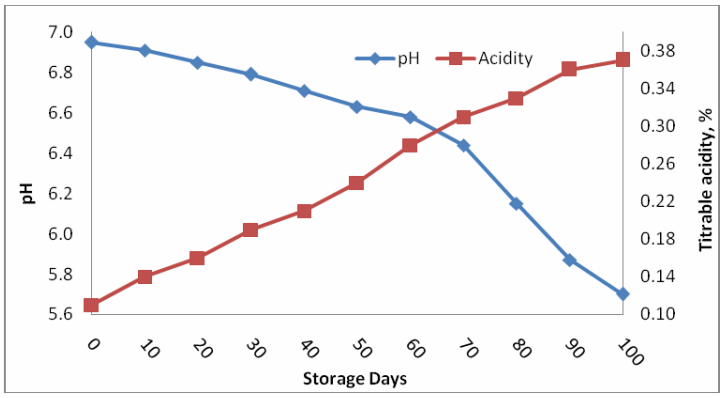

Fig 4. Changes in titrable acidity and pH of sterilized soymilk stored at ambient condition during storage

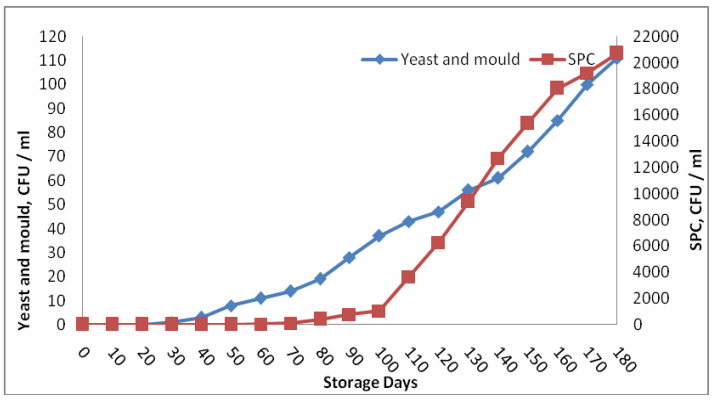

Fig 5. Changes in SPC and yeast and mould count of sterilized soymilk stored at refrigerated condition during storage

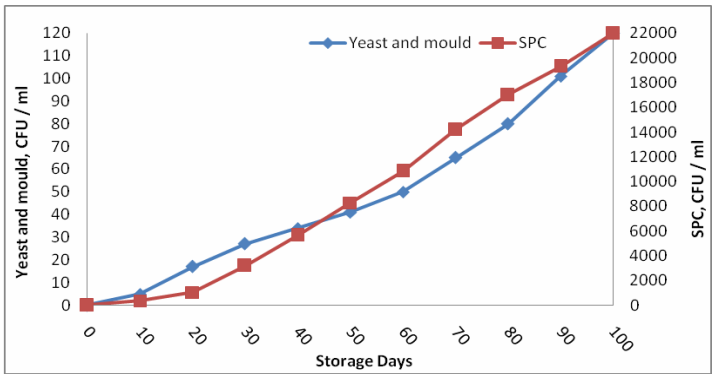

Fig 6. Changes in SPC and yeast and mould count of sterilized soymilk stored at ambient condition during storage

Increase in yeast and mould counts load in soymilk might be due to its susceptibility as the availability of carbohydrates, proteins and fat together with the neutral pH that makes milk a perfect medium for yeast and mould counts growth. Further yeast and mould counts growth caused rancid taste of soymilk (Hayes and Boor 2001).

From Fig 5 and 6 it can be observed that the growth rate of standard plate counts at refrigerated condition was less as compared to room temperature meaning thereby that freezing drastically reduced the microbial load on samples during storage. Similar trends were noted by Farinde et al (2010) in case of yoghurt samples.

Gandhi (2009) gave the nutritional standards for soymilk and quoted the critical limit of SPC as 20,000 CFU/ml. Considering this standard critical limit of SPC the spoilage of soymilk and thereby shelf-life was decided. It was observed that all the samples with sterilization treatment were within safe limit up to 170 days and 90 days in case of refrigerated and ambient storage condition respectively. Further the microbial attack increased drastically and beyond the standard limit making product unsafe for consumption.

Increase in microbial load in soymilk might be due to its susceptibility as the availability of carbohydrates, proteins and fat together with the neutral pH makes milk a perfect medium for microbial growth. These may include staphylococcus, coliform and other gram-negative bacteria (Hayes and Boor 2001). Statistically the microbial counts on samples showed significant difference with respect to increased number of storage days and change in treatment.

Evaluation of best treatment and shelf-life of sterilized soymilk stored at refrigerated and ambient condition.

Evaluation of best treatment and shelf-life of soymilk was done as per the results obtained for changes viscosity, TSS, titratable acidity, pH, standard plate counts, yeast and mould counts during storage. Among the all treatments sterilization treatment with refrigeration storage condition was most suitable for soymilk storage.

Fig 7 represents the shelf-life of sterilized soymilk samples stored at refrigerated and ambient temperature condition. It reveals that shelf-life of sterilized soymilk samples at refrigerated condition was 170 days and that of soymilk samples stored at ambient condition had shelf-life of 90 days.

CONCLUSION

Eighty eight per cent increase in shelf-life of soymilk was observed in sterilized soymilk samples stored in refrigerated condition over sterilized soymilk samples stored in ambient

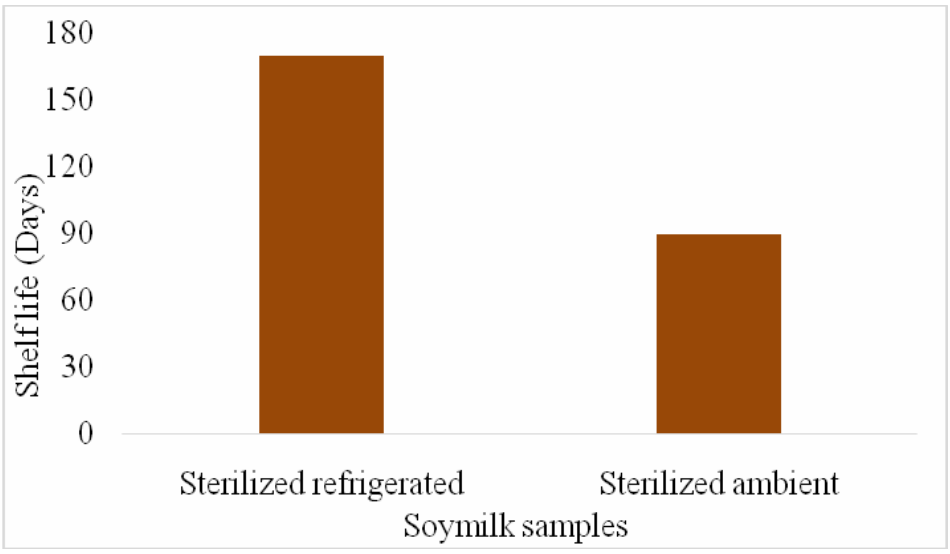

Fig 7. Shelf life of sterilized soymilk sample stored at refrigerated and ambient temperature condition

condition. Hence between both the treatments sterilization treatment with refrigerated storage condition was best suitable for storage and better shelf-life of soymilk.

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