

# Studies on shelf-life of fresh soymilk

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## SUMMARY :

Fresh soymilk was inspected for quality attributes like viscosity, TSS, titrable acidity, pH, standard plate count, yeast and mould count daily for sample stored in refrigerated condition and at the regular interval of 4 hrs for sample stored in ambient condition. On the basis of the quality attributes, it was observed that fresh soymilk samples were acceptable upto 5 days in refrigerated condition while same samples were acceptable upto 16 hrs in ambient temperature condition from the day of preparation. Thus, the study reveals that, fresh soymilk sample stored at refrigerated condition has better shelf-life.

**KEY WORDS :** Soymilk, Refrigeration, Storage, Shelf life

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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 tonnes of soybeans are 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 assets available.

In 2010-2011 India ranked sixth in area and production of soybean producing 9,600 thousand metric tonnes of soybean from an area of 9,400 thousand hectares (Anonymous, 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 milk and cow milk. Soymilk is used as a base in a wide variety of products including tofu, soy yoghurt and cheese. Soymilk yield, quality, flavour, 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). The soymilk prepared from different varieties exhibited total solids 5.73 - 6.78 per cent, protein 3.01 - 3.67 per cent, fat 1.57 - 1.93 per cent, titratable acidity 0.14 - 0.16 per cent, pH 6.70 - 6.77, viscosity 4.37 - 5.77 cp and specific gravity 1.04 - 1.05 (Harjai and Singh, 2009).

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. In some conditions, thermal processing, however, detrimentally affects nutritional and quality attributes of soymilk, and produces strong off flavours (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 (Achouri *et al.*, 2007).

During packaging the milk can possibly be contaminated with the micro-organisms causing quality deterioration. This can be avoided by hermetic sealing and upright position of the packaging material. There should not be any migration of the low molecular weight substances from the packages. The storage temperature and relative humidity must be below 20°C and 65 per cent, respectively. The place should be air conditioned. Both the temperature and relative humidity must be recorded regularly and corrective actions must be undertaken whenever deviations occur.

Production and consumption of soymilk is rising not only because of the increasing consumer interest for this protein beverage, but also because of its utilization as a base in other food products. Although several studies have been reported on soy however, very little information is available on the changes in physico-chemical properties during storage of fresh soymilk. Therefore, in light of above points, the present investigation was proposed to study the shelf-life of soymilk.

## EXPERIMENTAL METHODS

### Preparation of soymilk :

Soybean seeds were soaked in water for 6-8 hrs. After soaking seeds were cleaned by using clean water. Soaked soybeans were ground with 15 liters water in cooker cum grinder. The slurry was then cooked at 100°C temperature by passing steam through it. After cooking again slurry was ground in order to obtain homogeneity. The slurry was then allowed to pass into deodorizer by opening the butterfly valve and by 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. Now the whole soymilk inside the deodorizer was collected in filter press covered by muslin cloth. After filtration soymilk and okara was collected separately. Soymilk was then again boiled upto 80°C by adding sugar with continuous stirring. The soymilk was allowed to cool then colour and flavour was added. Soymilk was then filled in sterilized glass bottles, each having capacity of 200 ml. Each soymilk bottle was corked firmly. The soymilk samples were then ready to use for subsequent storage study.

### Determination of viscosity :

Viscosity of soymilk was measured by using Brookfield (DV-E) viscometer. Soymilk sample was taken in a 600 ml low

form griffin beaker as recommended. Soymilk level was allowed to reach upto 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 (Liu and Lin, 2008). The refractometer reading adjusted at '0' with the help of rotating small calibration screw. A drop of soymilk was putted on sensor for measurement and the refractometer readings were recorded.

### Determination of titratable acidity :

Acidity of soymilk was determined by titration method (Chinyere and Kenneth, 1997). The known weight of soymilk was titrated against sodium hydroxide using phenolphthalein as an indicator (AOAC, 1995). 10 ml sample was taken in a conical flask and 10 ml distilled water was added. Also 3-4 drops of phenolphthalein indicator was 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.

$$\text{Acidity} = \frac{\text{Titre} \times \text{Normality of alkali (0.1N)} \times \text{Volume make up} \times \text{Eq. wt of acid} \times 100}{\text{Volume of sample taken for estimation of acidity} \times \text{Volume of samle taken for volume make up} \times 1000} \dots\dots(1)$$

### Determination of pH of soymilk :

pH of soymilk was measured by using glass electrode pH (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 are 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 (ISI, 1969 a, 1969 b). The results for exact count were recorded as colony forming units/ml of soymilk *i.e.* CFU/ml. Total plate count was determined by method cited in ISI (1969 a) by using tryptone agar media having following composition. Thus the media 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 in ISI (1969 b) 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 media was also prepared and sterilized in autoclave at 15 PSI for 15-20 minutes.

## EXPERIMENTAL FINDINGS AND ANALYSIS

The findings of the present study as well as relevant discussion have been presented under following heads :

### Changes during storage in viscosity of soymilk :

It was observed that viscosity of soymilk increased with increase in storage period for 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 was observed from Table 1 and 2 that in sample stored at refrigerated condition, viscosity increased from 4.21 cp to 8.74 cp during 6 days of storage, while in samples stored at ambient temperature condition, it increased from 4.21 cp to 8.86 cp during 20 hrs of storage. The values of viscosity obtained for both soymilk samples at the beginning (0<sup>th</sup> day) of storage were nearly similar to the minimum value of viscosity obtained by Harjai and Singh (2007).

Fig. 1 and 2 illustrates the increased rate of viscosity of both soymilk samples with increasing storage time. The similar trend of increased viscosity 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

**Table 1 : Changes in quality attributes of fresh soymilk stored at refrigerated condition during storage**

Storage days	Viscosity (cp)	TSS ( $^{\circ}$ Brix)	Acidity (%)	pH	SPC (CFU/ml)	Yeast, mould (CFU/ml)
0	4.21	13.0	0.11	6.95	6	0
1	4.70	13.0	0.14	6.81	1029	9
2	5.10	12.2	0.17	6.74	4858	35
3	5.98	11.7	0.23	6.61	9723	57
4	6.50	11.2	0.28	6.41	15217	79
5	7.22	10.9	0.33	6.10	19459	103
6	8.74	10.5	0.38	5.87	22633	128
S.E. $\pm$	0.0124	0.1333	0.0021	0.0088	22.7914	2.1019
C.D. (P=0.01)	0.0537	0.5770	0.0092	0.0382	98.6296	9.0960

**Table 2 : Changes in quality attributes of fresh soymilk stored at ambient condition during storage**

Storage hrs	Viscosity (cp)	TSS ( $^{\circ}$ Brix)	Acidity (%)	pH	SPC (CFU/ml)	Yeast, mould (CFU/ml)
0	4.21	13.0	0.11	6.95	1052	10
4	4.88	13.0	0.14	6.80	3070	28
8	5.70	12.5	0.18	6.51	8320	42
12	6.50	11.8	0.25	6.12	13677	78
16	7.25	11.2	0.30	5.87	18928	101
20	8.86	10.6	0.40	5.60	23636	138
S.E. $\pm$	0.0168	0.0548	0.0042	0.0105	24.7040	1.6228
C.D. (P=0.01)	0.0754	0.2455	0.0189	0.0472	110.7492	7.2749

significant difference with respect to increased number of storage days and change in treatment.

#### Changes during storage in TSS of soymilk :

Initial TSS of fresh soymilk samples stored at refrigerated as well as ambient temperature condition was 13 °Brix. It was observed from Table 1 and 2 that in sample stored at refrigerated condition, TSS decreased from 13 °Brix to 10.5 °Brix during 6 days of storage, while in samples stored at ambient temperature condition it decreased from 13 °Brix to 10.6 °Brix during 20 hrs of storage.

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

Fig. 1 and 2 illustrates 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 its lack

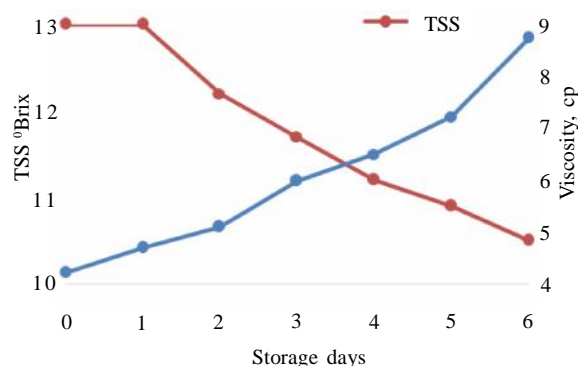


Fig. 1 : Changes in viscosity and TSS of soymilk stored at refrigerated condition during storage

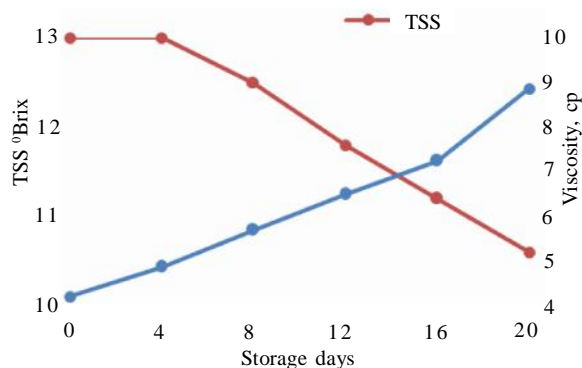


Fig. 2 : Changes in viscosity and TSS of soymilk stored at ambient condition during storage

of stability and sediment precipitation of proteins and other added solid particles such as minerals or flavours which might be the reason for lowered value of TSS of soymilk during storage. Statistically, TSS of soymilk samples showed significant difference with respect to increased number of storage days and change in treatment.

#### Changes during storage in acidity of soymilk :

Initial acidity of fresh soymilk samples stored at refrigerated as well as ambient temperature condition was 0.11. It was observed from Table 1 and 2 that in sample stored at refrigerated condition, acidity increased from 0.11 to 0.38 during 6 days of storage, while in samples stored at ambient temperature condition it increased from 0.11 to 0.4 during 20 hrs 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 illustrates the increased rate of acidity of both soymilk samples with increasing storage time.

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

#### Changes during storage in pH of soymilk :

It was observed that pH of soymilk decreased with increase in storage period. Initial pH of soymilk 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 was observed from Table 1 and 2 that in sample stored at refrigerated condition, pH decreased from 6.95 to 5.87 during 6 days of storage, while in samples stored at ambient temperature condition it decreased from 6.95 to 5.6 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 of soymilk samples was nearly equal to 5.7 and similar value was obtained by Kamaly (1997). He also analysed that coagulation of sterilized soymilk occurred at about pH 5.7.

Fig. 3 and 4 illustrates 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) of soymilk stored at refrigerated and ambient condition :

It was observed that standard plate counts of soymilk increased with increase in storage period for samples stored at both temperature conditions. Standard plate counts increased from 6 CFU/ml to  $2.2633 \times 10^4$  CFU/ml during 6 days of storage,

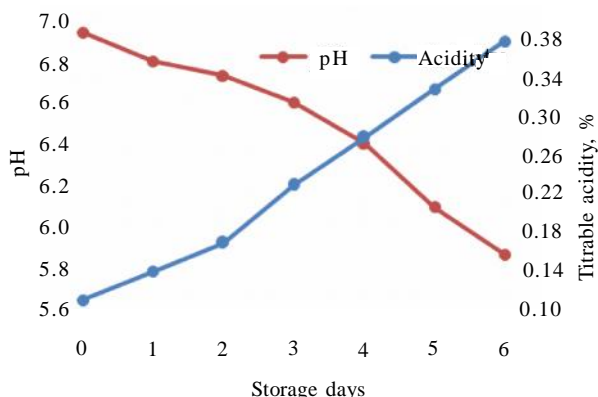


Fig. 3 : Changes in titrable acidity and pH of soymilk stored at refrigerated condition during storage

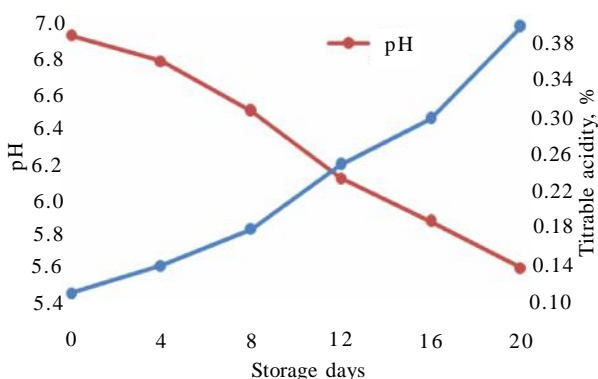


Fig. 4 : Changes in titrable acidity and pH of soymilk stored at ambient condition during storage

while in samples stored at ambient temperature condition it increased from 1052 CFU/ml to  $2.3636 \times 10^4$  CFU/ml during 20 hrs of storage. Similar findings were observed by Onuorah *et al.* (2007) in case of pasteurized soymilk samples.

From Fig. 5 and 6 it was observed that the growth rate of standard plate counts at refrigerated condition was less as

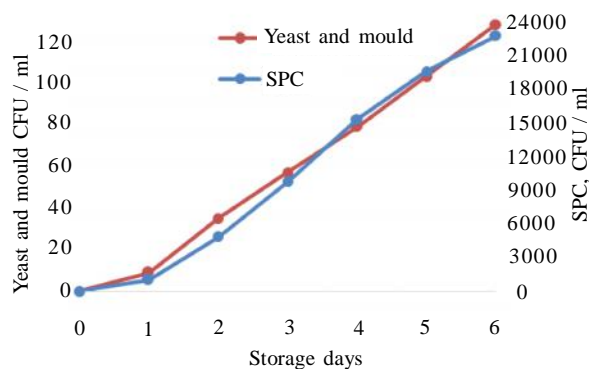


Fig. 5 : Changes in SPC and yeast and mould count of soymilk stored at refrigerated condition during storage

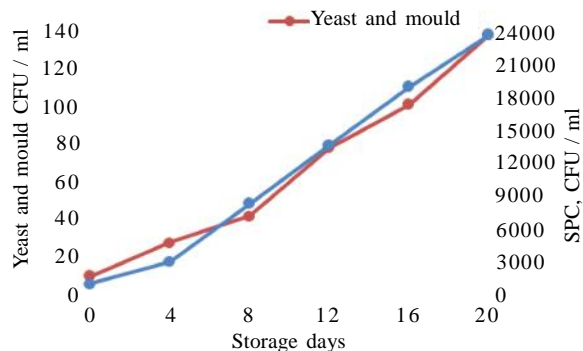


Fig. 6 : Changes in SPC and yeast and mould count of soymilk stored at ambient condition during storage

compared to room temperature. It means that freezing drastically reduced the microbial load on soymilk samples during storage. Similar trends were noted by Farinde *et al.* (2010) in case of yoghurt samples.

Gandhi (2009b) had given the nutritional standards for soymilk and quoted the critical limit of SPC as 20000 CFU/ml. Considering this standard critical limit of SPC, the spoilage of soymilk and thereby shelf-life of soymilk samples was decided. It was observed that all the samples were within safe limit upto 5 days and 16 hrs in case of refrigerated and ambient storage condition, respectively. Further the microbial attack was 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 soymilk samples showed significant difference with respect to increased number of storage days and change in treatment.

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

It was observed that yeast and mould counts of soymilk increased with increase in storage period for samples stored at both temperature conditions. Initial yeast and mould counts on fresh soymilk samples stored at refrigerated as well as ambient temperature condition were 0 CFU/ml and 10 CFU/ml. It was observed from Table 1 and 2 that in sample stored at refrigerated condition, yeast and mould counts increased from 0 CFU/ml to 128 CFU/ml during 6 days of storage, while in samples stored at ambient temperature condition it increased from 10 CFU/ml to 138 CFU/ml during 20 hrs of storage. 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 molds were achieved when soymilk preserved with

a combination of 700-800 ppm of sodium benzoate, pasteurization and refrigeration.

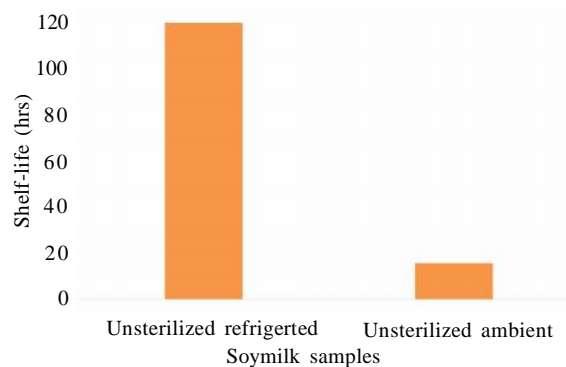
Gandhi (2009b) had given the nutritional standards for soymilk and quoted the critical limit of yeast and mould counts as 100 CFU/ml. Considering this standard critical limit of yeast and mould counts, the spoilage of soymilk and thereby shelf-life of soymilk samples was decided. It was observed that all the samples were within safe limit upto 5 days and 16 hrs in case of refrigerated and ambient storage condition, respectively.

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 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).

#### Evaluation of best treatment and shelf-life of fresh 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 both treatments, treatment with refrigeration storage condition was most suitable for soymilk storage.

Fig. 7 represents the shelf-life of soymilk samples stored at refrigerated and ambient temperature condition. Fig. revealed



**Fig. 7 : Shelf-life of fresh soymilk sample stored at refrigerated and ambient temperature condition**

that shelf-life of soymilk samples at refrigerated condition was 5 days and that of soymilk samples stored at ambient condition had shelf-life of 16 hrs.

#### Conclusion :

One hundred four hours increase in shelf-life of soymilk was observed in Fresh soymilk samples stored in refrigerated condition over fresh soymilk samples stored in ambient condition. Hence, among both the treatments refrigerated storage condition was best suitable for storage and better shelf-life of soymilk.

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