





# EFFECT OF TREATMENTS, CMC AND STORAGE CONDITIONS ON SENSORIAL QUALITY OF MANGO FLAVOURED SOYMILK

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doi: 10.15414/jmbfs.2014.4.1.06-09

#### ARTICLE INFO

Received 3. 12. 2013 Revised 1. 3. 2014 Accepted 21. 5. 2014 Published 1. 8. 2014

Regular article



#### ABSTRACT

In the present research work effect of different treatments on physico-chemical and sensory properties of soymilk was studied. Also, effect of Sodium carboxymethyl cellulose (CMC), sucrose and mango flavor on quality of soymilk was determined. Soymilk samples were prepared by three different methods. The control sample (sample A) was prepared as per the standard procedure reported in literature. The other two samples were prepared by slight modification in the process use for control sample preparation. Microwave treatment at 2450 MHz for 5 min prior to grinding was given in sample B. Sample C was prepared by giving autoclave treatment to the soaked soybeans and grinding was performed with the use of hot water. Among all the treatments autoclave treatment gave best quality soymilk (Sample C) with respect to flavor, taste and overall acceptability. The quality of soymilk (Sample C) was further improved by using 0.2% CMC with respect to viscosity and mouth feel. Also, mango flavored soymilk containing 0.2% CMC, 15% sucrose and 0.1% mango essence scored maximum sensory score among all formulations with respect to color, flavor, taste, mouth feel and overall acceptability and was stable for consumption for about 25 days at room temperature storage and 60 days at refrigeration temperature storage.

Keywords: Soymilk, off flavor, treatments, oxidative stability, sensory parameters, flavored soymilk, storage condition

#### INTRODUCTION

Soybean is a highly nutritious, which contains about 30% protein and 20% fat (Salunkhe and Kadam, 1989). In addition amino acid pattern of soy bean compares well with those of animal proteins (Mathur, 2004 and Christina and Patience, 2003). Recently, many functions of soybeans have been in the spotlight, for example, reducing the risk of heart diseases, cancer, osteoporosis and kidney diseases (Wang et al., 2001). Soymilk, a beverage made from whole soybeans has emerged in the market in recent years as a nutritious food product. It is also an intermediate for preparing other soy products (Gatade et al., 2009 and Mark et al., 2006).

Despite many advantages, the use of soybean and its products has been limited because of its off-flavor i.e. beany flavour generated during processing and storage (Torres-Penaranda and Reitmeier, 2001). In general, beany flavor is resulted due to peroxidation of polyunsaturated fatty acids or esters catalyzed by lipoxygenase. Soybean fat consists 85% of unsaturated fatty acid and hence it gets easily hydroperoxidized by lipoxygenase as well as auto oxidation. The hydroperoxidized lipid which is unstable and easily gets cleaved enzymatically or non-enzymatically resulted in generating off-flavor such as n-hexanal and n-hexanol (Mizutani and Hashimoto, 2004 and Mattick and Hand, 1969).

Traditionally soymilk is prepared by overnight soaking of soybeans, grinding vigorously, heating and filtering to obtain soymilk (Lo et al., 1968). The soaking and grinding processes carried out at room temperature which leads to peroxidation resulted in formation of off-flavor in soymilk. So, it is important to prevent lipid peroxidation to reduce off-flavor (Mizutani and Hashimoto, 2004; Wilkens et al., 1967). Many researchers were attempted to control off-flavor like heating at high temperature, grinding the soybean at high temperature and use of flavoring substances to mask the off-flavor of soymilk.

The soymilk produced traditionally is found to be having thin consistency and to improve it thickening agent can be use (Wang et al., 2001). Sodium carboxymethyl cellulose (Na-CMC), a natural polymer is water soluble derivative of biologically degradable natural polysaccharide cellulose. In aqueous solution it represents a complex rheological system, since it forms aggregates and associations, and hence higher level structures. It is nontoxic and widely available at low costs (Florjancic et al., 2002). It is generally used to improve

sensory and rheological quality of the food products like ice-cream and other liquid food products (Moore and Shoemaker, 2006).

Therefore, the focus of present investigation was to reduce off-flavour and increase the acceptability of the soymilk by improving sensory quality i.e. addition of stabilizer and favour. Also the storage stability of the flavored soymilk was studied during storage.

#### MATERIAL AND METHODS

## Materials

Soybeans of JS-335 cultivar were procured from local market of Kolhapur and used for preparation of soymilk. Sucrose, mango essence and sodium carboxymethyl cellulose (Na-CMC, E 466) of food grade quality were purchased from local store and analytical grade chemicals were used for analysis.

#### Preparation of soymilk

Soymilk samples were prepared by three different methods, viz. control (traditional), microwave and autoclave treatments. The control sample was prepared by following the method suggested by **Deshpande** *et al.* (2008), whereas other two samples were prepared with slight modification in the procedure of control method

In microwave treated method, the soybeans were soaked for overnight and washed and treated with microwave at 2450 MHz for 5 min (**Chauhan and Chauhan, 2008**). These microwave treated soybeans were used for preparation of soymilk by following the remaining steps (grinding and filtering) same as that of control sample.

In autoclave treatment method the soybeans after soaking and washing were autoclaved at 121°C temperature and 15 psi pressure for 15 min (**Gatade** *et al.*, **2009**). The treated soybeans were then washed in hot water ( $\approx 70^{\circ}$ C) and cold water ( $\approx 25^{\circ}$ C) for 2 to 3 times. After washing the soybeans were used for grinding followed by filtration. The residue was again ground and filtered for 2 to 3 times. The grinding was performed with hot water ( $85^{\circ}$ C  $\pm$  1°C) to maintain the temperature ( $70^{\circ}$ C  $\pm$  5°C) (**Wilkens, 1967**). The flow diagram of autoclaving method is given in figure 1.

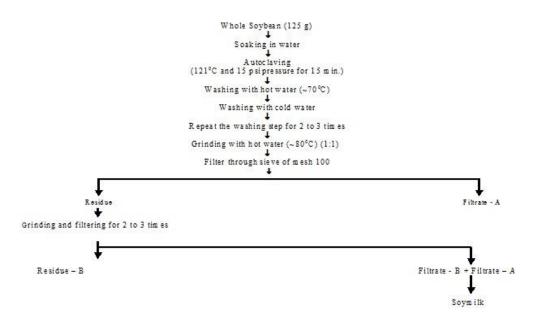


Figure 1 Flow chart for preparation of soymilk by autoclaving treatment

#### Effect of process parameters on quality of soymilk

The soymilk samples prepared by different methods (Control, microwave and autoclave) were evaluated for their quality with respect to oxidative stability (TBA value), proximate composition, rheological property (Viscosity) and sensory characteristics.

#### Oxidative stability and proximate analysis

The oxidative stability of soymilk samples were determining by thio-barbituric acid (TBA) value test (Ronald et al., 1999). Also, the proximate composition like total solid, protein, fat, ash and total carbohydrate were analyzed using standard procedure suggested by Ranganna (2000).

#### Rheological characteristic

Rheological characteristic of samples were determined by evaluating viscosity using Brookfield viscometer (Model: LVDV-II pro). The temperature of soymilk samples was maintained constant  $(25^{\circ}C \pm 1^{\circ}C)$  which was achieved by placing the sample cup in water bath. The standard procedure described by **Ranganna** (2000) was followed for determination.

### Effect of CMC on quality of soymilk

On basis of sensory evaluation results autoclaved sample was selected to study the effect of CMC. To improve the sensory quality with respect to mouth feel (body) and consistency of soymilk, CMC was added at different levels (l.e. 0, 0.1, 0.2, 0.3, 0.4 and 0.5%).

#### Preparation of Mango flavored soymilk

The autoclave treated sample added with optimized concentration of stabilizer (i.e. 0.2%) was flavored by addition of different concentration of mango emulsion and sucrose (Table 1). All these ingredients were blended with soymilk using high speed blender (5000 rpm) for 5 min and subjected to sensory analysis.

Table 1 Formulation of mango flavored soymilk

Sample code	Soymilk (ml)	Sodium CMC (g)	Sucrose (g)	Mango essence (ml)
$M_1$	100	0.2	10	0.05
$M_2$	100	0.2	15	0.05
$M_3$	100	0.2	20	0.05
$M_4$	100	0.2	10	0.10
$M_5$	100	0.2	15	0.10
$M_6$	100	0.2	20	0.10
$M_7$	100	0.2	10	0.20
$M_8$	100	0.2	15	0.20
$M_9$	100	0.2	20	0.20

#### Sensory analysis

All the samples were evaluated for sensory attributes like color, flavor, taste, mouth feel (body) and overall acceptability, using 9 point hedonic scale by panel of 10 semi-trained judges (**Ranganna**, 2000).

#### Storage study of mango flavored soymilk

The best samples was selected on the basis of sensory results and subjected to storage stability. The samples were filled in 200 ml pre-sterilized glass bottles and were pasteurized at  $85\pm5^{\circ}\mathrm{C}$  for 15 min. The prepared sample bottles were stored at room temperature ( $30\pm2^{\circ}\mathrm{C}$ ) and refrigeration temperature ( $12\pm1^{\circ}\mathrm{C}$ ) for 60 days. These samples were analyzed for microbial and sensorial quality at regular interval of 5 days. Total plate count (TPC) was carried out by pour plate method (**Cappuccino and Sherman, 2005**) and sensory analysis was done after determining the total plate count (TPC).

#### Statistical analysis

The significant difference of mean values was assessed with one-way analysis of variance (ANOVA). Tukey test was carried out using Instat software to determine whether there was any significant difference at level of (P < 0.05).

#### RESULTS AND DISCUSSION

# Effect of treatments on oxidative stability and chemical composition of soymilk

The samples prepared with three different methods (i.e. Traditional, microwave and autoclave treatment) were subjected to determination of oxidative stability, and proximate analysis and obtained results are presented in table 2. The oxidative stability was determined by using TBA value test as it is one of the most widely used because it is rapid and responsive method. Significant difference was observed between autoclaved treated sample and other two samples (p<0.001). No significant difference was observed between sample A and sample B. The soymilk sample prepared by autoclave treatment method showed reduced TBA value as compared to control and microwave treated samples. The reduction in TBA value might be due to heat inactivation of lipoxygenase enzyme during autoclaving and hot grinding process (Wang et al., 2001). Lipids in cracked, dehulled, soybeans rapidly oxidized after the lipoxidase system was activated by increasing moisture content to 20% and wet heat was found an effective method for inactivation of lipoxygenase enzyme (Mustakas et al., 2011).

Proximate analysis of soymilk samples showed that the composition of autoclave treated sample was having total solid (11.4%), protein (4.9%), fat (3.4%), ash (0.6%) and total carbohydrate (2.5%). There was significant difference (p<0.001) between sample C and other two samples. The total solid and protein content of autoclave treated sample was higher as compared to other samples. This might be due to heat inactivation of antinuritional factors, which retards the availability of protein (Kin-Chor and Keshavan, 2007). There was no any significant difference in composition of control and microwave treatment sample. The compositions of control and microwave treatment sample are in close agreement with those reported by **Deshpande** *et al.*, (2008).

Table 2 Oxidative stability and proximate analysis of soymilk samples

Sample sta	Oxidative stability (TBA	Proximate	Proximate Analysis (%)					
	Value)	Total Solids	Protein	Fat	Ash	Total Carbohydrate		
A	4.7ª	10.2ª	3.7ª	2.8a	0.4ª	3.3ª		
В	$4.6^{a}$	10.5 <sup>a</sup>	3.6 <sup>a</sup>	$2.6^{a}$	$0.5^{ab}$	3.8 <sup>a</sup>		
C	2.2 <sup>b</sup>	11.3 <sup>b</sup>	$4.9^{b}$	$2.9^{b}$	$0.6^{b}$	$2.9^{b}$		

A: Control; B: Microwave treatment; C: Autoclave treatment. The values are the mean of 3 independent observations. The values with different superscripts in a column differ significantly (p < 0.05).

#### Effect of treatments on rheological (Viscosity) and sensory parameters of soymilk

The treated and control samples were subjected to rheological and sensorial analysis and obtained results are reported in table 3. The results denote that viscosity of autoclave treated sample was high as compared to other two samples. This increase in viscosity may be due to increase in total solid content of soymilk. The autoclave treatment makes the soybean soft and fascinates the grinding and filtration steps, which results in increase in total solid content (Kin-Chor and Keshavan, 2007). Significant difference was observed between sample B and sample C (p<0.05).

The results of sensory analysis showed that the autoclave treated sample obtained maximum sensory score with respect to flavor, taste, mouth feel and overall acceptability as compared to other samples. The sensory panel members rejected control sample due to its off flavor, whereas microwave treated sample got slightly higher scores than the control samples. The autoclave treated sample scored maximum score with respect to flavor than other two samples. No significant difference was obtained among all samples with respect to color (p<0.05). The samples were significantly different with each other with respect to flavor, taste and overall acceptability (p<0.001). This may be due to reduction in activity of lipoxygenase enzyme, which is responsible for off-flavor development. Flavor reduction in autoclave sample was also confirmed by TBA value, which was found low in case of autoclave treated sample (Tab 1). The results obtained were well comparable with those earlier reported (Gatade et al., 2009 and Kin-Chor and Keshavan, 2007).

 Table 3 Rheology (Viscosity) and Sensory analysis of treated soymilk samples

Sample at	Viscosity	Sensory	Sensory parameters					
	at 25°C (Cp)	Color	Flavor	Taste	Mouth Feel (body)	Overall Acceptability		
A	13.8 <sup>ab</sup>	$8.0^{NS}$	5.0 <sup>a</sup>	6.0 <sup>a</sup>	$6.0^{a}$	5.5 <sup>a</sup>		
В	13.6 <sup>a</sup>	$8.0^{NS}$	$6.0^{b}$	6.5 <sup>b</sup>	$6.0^{a}$	6.0 <sup>b</sup>		
C	14.2 <sup>b</sup>	$8.0^{NS}$	$7.0^{c}$	$8.0^{\rm c}$	$8.0^{b}$	$8.0^{\circ}$		

The values with different superscripts in a column differ significantly (p < 0.05)and NS are not significant. The values are the mean of 3 independent observations for viscosity and 10 independent observations for sensory parameters.

#### Effect of CMC on rheological (Viscosity) and sensorial properties of soymilk

The autoclave treated sample obtained maximum sensory score with respect to flavor and taste, the consistency was low which affected the sensory quality with respect to consumer's acceptability. Hence, to improve the quality with respect to viscosity and mouth feel, Na-CMC (Sodium - Carboxymethyl cellulose) as a thickening agent was used at different concentration and the prepared samples were subjected for viscosity and sensory analysis (Table 4). The results denotes that the increase in concentration of CMC affects significantly (p<0.05) the viscosity of soymilk. Sensory analysis results showed that the sensory score get increased with increase in concentration upto 0.2% level and above 0.2% level it gets decreased. Colour was not affected by the addition of CMC . There was no any significant difference in flavor upto 0.3% addition of CMC. Also, above 0.2% CMC concentration significant difference was observed with respect to taste. The results for mouth feel and overall acceptability revealed that the quality of soymilk increases significantly upto 0.2% CMC. Above 0.2% CMC concentration the quality get decreased significantly (p<0.001). The addition of thickening agent increases viscosity of soymilk (Wang et al., 2001).

Table 4 Effect of addition of CMC on Rheological and Sensorial quality of

Sample Code	Viscosity	Sensorial Parameters				
	at 25°C (Cp)	Color	Flavor	Taste	Mouth Feel	Overall acceptability
$V_0$	14.2 <sup>a</sup>	$9.0^{NS}$	$8.0^{a}$	$9.0^{a}$	$8.0^{a}$	8.5 <sup>a</sup>
$V_1$	21.3 <sup>b</sup>	9.0	$8.0^{a}$	$9.0^{a}$	8.5 <sup>d</sup>	8.5 <sup>a</sup>
$V_2$	$37.0^{\circ}$	9.0	$8.0^{a}$	$9.0^{a}$	$9.0^{\rm e}$	$9.0^{d}$
$V_3$	50.4 <sup>d</sup>	9.0	$8.0^{a}$	8.5 <sup>b</sup>	$8.0^{a}$	8.5 <sup>a</sup>
$V_4$	$62.0^{\rm e}$	9.0	7.5 <sup>b</sup>	$8.0^{\rm c}$	7.5°	$8.0^{\rm c}$
$V_5$	$92.8^{f}$	9.0	7.5 <sup>b</sup>	7.5 <sup>d</sup>	$7.0^{b}$	7.5 <sup>b</sup>

 $(V_0 = 0\% \text{ CMC}, V_1 = 0.1\% \text{ CMC}, V_2 = 0.2\% \text{ CMC}, V_3 = 0.3\% \text{ CMC}, V_4 = 0.4\% \text{ CMC}$  and  $V_{\rm c} = 0.5\%$  CMC)

The values with different superscripts in a column differ significantly (p < 0.05) and  $^{NS}$  are not significant. The values are the mean of 3 independent observations for viscosity and 10 independent observations for sensory parameters.

#### Sensory analysis of mango flavored soymilk

The soymilk sample containing 0.2% CMC was selected on the basis on sensory score for preparation of mango flavored soymilk. The mango flavored soymilk was subjected to the sensory panel members for sensory analysis and obtained results are reported in table 5. The results indicate that the addition of sucrose and flavor is desirable in improving the sensory characteristics of the soymilk. The significant difference was observed for flavor between the samples containing different concentration of flavor. Among all formulations sample M4, M5 and M6 scored maximum sensory score with respect to flavor and overall acceptability. But, sample M5 scored maximum sensory score with respect to taste than M4 and M<sub>6</sub>. Further increase in sucrose and flavor decreases quality significantly with respect to taste beyond what was achieved with 15% sucrose and 0.1% flavor concentration.

**Table 5** Sensory analysis of mango flavored soymilk

Sample	Color	Flavor	Taste	Mouth feel (Body)	Overall acceptability
$M_1$	9.0 <sup>NS</sup>	8.0 <sup>a</sup>	8.0ª	9.0 <sup>NS</sup>	8.5ª
$M_2$	9.0	$8.0^{a}$	8.5 <sup>b</sup>	9.0	8.5 <sup>a</sup>
$M_3$	9.0	$8.0^{a}$	8.5 <sup>b</sup>	9.0	8.5 <sup>a</sup>
$M_4$	9.0	$9.0^{\rm c}$	8.5 <sup>b</sup>	9.0	$9.0^{b}$
$M_5$	9.0	$9.0^{\rm c}$	$9.0^{\rm c}$	9.0	$9.0^{b}$
$M_6$	9.0	$9.0^{\rm c}$	8.5 <sup>b</sup>	9.0	$9.0^{b}$
$M_7$	9.0	8.5 <sup>b</sup>	$8.0^{a}$	9.0	8.5 <sup>a</sup>
$M_8$	9.0	8.5 <sup>b</sup>	$8.0^{a}$	9.0	8.5 <sup>a</sup>
$M_9$	9.0	8.5 <sup>b</sup>	$8.0^{a}$	9.0	8.5 <sup>a</sup>

The values are the mean of 10 independent observations. The values with different superscripts in a column differ significantly (p < 0.05) and  $^{\rm NS}$  are not significant.

#### Effect of storage conditions on microbial and sensory quality of mango flavored sovmilk

Table 6 Effect of storage conditions on microbial count of flavored soymilk

C4	C+ D:- 1 (1)	TPC
Storage Temperature	Storage Period (days)	(CFU/gm)
	05	ND
	10	ND
	15	ND
Room	20	ND
	25	ND
Temperature (31°C)	30	$2.4X10^{3}$
(31 C)	35	$5.8X10^{3}$
	40	Sample discarded
	05	ND
	10	ND
	15	ND
	20	ND
	25	ND
Refrigeration	30	ND
Temperature	35	ND
(12°C)	40	ND
	45	ND
	50	ND
	55	ND
	60	ND
ND: Not Detected		

ND: Not Detected

The mango flavored soymilk sample ( $M_5$ ) was selected on the basis of sensory analysis to evaluate the shelf life. The samples were divided into two different lots, out of which one lot stored at room temperature ( $30 \pm 2^{0}$ C) and other lot at refrigeration temperature ( $12 \pm 2^{0}$ C). The samples stored at both conditions were analyzed for microbial and sensorial quality at the regular interval of 5 days. The Obtained results are summarized in tab 6 and 7. The results of microbial analysis denote that the total plate count of flavored milk stored in both conditions did not show any growth upto 25 days of storage. After 25 days of storage the samples stored at room temperature showed the growth in total plate count. The samples stored at room temperature showed very high growth ( $5.8 \times 10^{3}$  CFU/ml) after 35 days of storage, so that the sample stored at room temperature was discarded. In case of sample stored at low temperature did not show any growth even after 60 days of storage. These results revealed that shelf life of sample stored at room temperature was about 30 days and that stored at refrigeration temperature was about 60 days.

The sensory analysis was carried out after microbial analysis and results of sensory analysis are reported in tab 7. The quality of mango flavored soymilk get decreased significantly (p<0.001) with respect to color, taste and overall acceptability after 10 days of room temperature storage. Flavor quality was reduced significantly (p<0.001) after 5 days of room temperature storage. The mouth feel was affected significantly (p<0.001) after 15 days of storage. The sensory analysis of room temperature stored sample after 30 days was not conducted as it was not acceptable from microbial point of view. For refrigeration temperature stored sample there was no any significant difference in sensorial quality upto  $60^{th}$  day. The favor was affected significantly (p<0.001) after 45 days of refrigeration storage. Hence, pasteurization at  $100^{\circ}$ C for 15 min coupled with refrigeration storage was adequate to increase the shelf life of mango flavored soymilk (Wang et al., 2001).

Table 7 Effect of storage conditions on sensory analysis of flavored soymilk

Storage	Storage Period	Sensory parameters				
Temperature	in days	Color	Flavor	Taste	Mouth feel (Body)	Overall acceptability
	5	9.0ª	9.0 <sup>e</sup>	9.0ª	9.0ª	9.0ª
	10	$9.0^{a}$	8.5 <sup>d</sup>	$9.0^{a}$	$9.0^{a}$	$9.0^{a}$
	15	8.5 <sup>e</sup>	$8.0^{\rm c}$	8.5 <sup>b</sup>	$9.0^{a}$	8.5 <sup>b</sup>
Room Temperature	20	$8.0^{d}$	$7.0^{b}$	8.5 <sup>b</sup>	8.5°	$8.0^{\circ}$
(31°C)	25	7.5°	$6.0^{a}$	$7.0^{c}$	$8.0^{b}$	$7.0^{\rm d}$
	30	$7.0^{b}$	$6.0^{a}$	$6.0^{d}$	$8.0^{\rm b}$	$6.0^{\rm e}$
	35	Sample discarded	Sample discarded	Sample discarded	Sample discarded	Sample discarded
	05	9.0 <sup>NS</sup>	9.0°	$9.0^{NS}$	9.0 <sup>NS</sup>	9.0 <sup>NS</sup>
	10	9.0	$9.0^{\rm a}$	9.0	9.0	9.0
	15	9.0	$9.0^{\rm a}$	9.0	9.0	9.0
	20	9.0	$9.0^{a}$	9.0	9.0	9.0
<b>5</b> 01 1	25	9.0	$9.0^{a}$	9.0	9.0	9.0
Refrigeration	30	9.0	$9.0^{a}$	9.0	9.0	9.0
Temperature (18°C)	35	9.0	$9.0^{\rm a}$	9.0	9.0	9.0
	40	9.0	$9.0^{a}$	9.0	9.0	9.0
	45	9.0	$9.0^{a}$	9.0	9.0	9.0
	50	9.0	8.5 <sup>b</sup>	9.0	9.0	9.0
	55	9.0	8.5 <sup>b</sup>	9.0	9.0	9.0
	60	9.0	8.5 <sup>b</sup>	9.0	9.0	9.0

The values are the mean of 10 independent observations. The values with different superscripts in a column differ significantly (p < 0.05) and  $^{NS}$  are not significant.

# CONCLUSION

Based on results it was concluded that use of autoclave treatment and hot water grinding for preparation of soymilk was best method for preparation of soymilk with reduced off-flavor and increased acceptability. Addition of CMC (0.2%), sucrose (15%) and mango flavor (0.1%) will give additional benefits with respect to sensory quality. Also, pasteurization at 100°C for 15 min coupled with good sanitation procedure and stored at refrigeration temperature make the product stable for consumption for about 60 day. Hence, the study will be helpful to improve acceptability and increase the consumption of soymilk for nutritional and economical benefits.

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