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USE OF METEROXYLON SAGU AS A STABILIZING AGENT IN YOGHURT

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A B S T R A C T

Yoghurt is popular fermented milk product with higher nutritional value and significant health beneficial effects. The formulation of yoghurt with optimum texture, appearance, consistency and stability to synersis, and shelf life is major challenge to dairy industries. Usually, syneresis is reduced by either increasing the total solids of yoghurt or by using stabilizers. The main objective of the present study was to use *Metroxylon sagu* as stabilizing agent in yoghurt and to evaluate its influence on the composition, shelf life, texture and sensory characteristics of yoghurt. Enrichment of yoghurt with *Meteroxylon sagu* (Sagudana) at different levels was studied for physicochemical (pH, acidity, fat, synersis, viscosity, protein, water holding capacity), sensory evaluation (flavour, body and texture, acidity, appearance and container closure) and microbiological analysis (TVC and Colliform). Yoghurt prepared by incorporation of *Meteroxylon sagu* (@ of 0.1%, 0.25% and 0.5%) was compared for these characteristics to the yoghurt containing stabilizer gelatin (0.5% w/w). These attributes were significantly affected by the use of stabilizer and its rate of incorporation. Use of *Meteroxylon sagu* produced better results in terms of lowering syneresis Y0 (0.43mL), Y1(0.83 mL) and improving appearance, body and texture. The decrease in water holding capacity for controlled and treated samples was 2.84%, 18.47%, 10.64% and 18.24% for Y0, Y1, Y2 and Y3 respectively. Addition of *Meteroxylon sagu* upto 0.5% did not influence taste and overall acceptability. *Meteroxylon sagu* @ 0.25% gave best results for overall sensory acceptability and yoghurt shelf life was increased upto 30 days.

Keywords: Yoghurt, *Meteroxylon sagu,* synersis, shelf life.

INTRODUCTION

Fermented milk products are very popular products and new varieties are regularly entering in the consumer market. Most commonly used fermented dairy products are the sour cream, butter milk, ropy milk, acidophilus milk, cheese and yoghurt. Yoghurt is the most popular and ideal food representing pleasant aromatic flavor, thick creamy consistency and several health benefits (Huma *et al*., 2003). It is made in variety of composition (fat and dry matter content), either plain or with added substances such as fruits, sugar and gelling agents. The essential flora of yoghurt consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *Bulgaricus*. For a satisfactory flavour to develop, approximately equal numbers of both species should be present. Volatile compounds produced by the yoghurt bacteria include small amounts of acetic acid, diacetyl and most importantly acetaldehyde (Walstra *et al.,* 1985). The therapeutic properties associated with yoghurt have increased both its production and consumption all over the world. Many health benefits like protection against gastrointestinal upsets, lowering cholesterol, improved lactose digestion and enhanced immune response are due to live bacteria present in yoghurt. It has also been recognized as a healthy food, due to the beneficial action of its high level of protein and calcium contents (Pedrigon *et al.,* 2002; Tamime and Robinson, 1999).

There are some common problems in the production of yoghurt such as synersis, improper texture, lower shelf life, sourness, acidity and hardness (Debrabandere and Debaerdemaeker, 2002; Lee and Lucey, 2010). Synersis (separation of water from product) is the major problem which reduce the shelf life of yoghurt and it might be reduced by increasing the casein content of the milk, reducing the incubation temperature ((Lucey, 2001; Anwer *et al*., 2013). and rate of acidification (Fiszman *et*

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al., 1999), or by adding stabilizers which interact with the casein network.

Appearance and physical characteristics are important quality parameters of yoghurt (Amatayakul *et al*., 2006). The stabilizers are used to improve the consistency, viscosity and reduce the synersis rate (Lucey, 2002). Gelatin, starches, pectin, alginate, carrageenan, derivatives of methylcellulose, gum arabic, tragacanth, karaya, locust bean gum (LBG), guar and xanthan gums are the compounds used as stabilizers in yoghurt (Tamime and Robinson, 1985). These stabilizers may improve the consistency and dispersion properties of spray-dried yoghurt powder in water (Ramirez-Figueroa *et al*., 2002). The primary stabilizers such as carboxymethyl cellulose (CMC), LBG, alginate, or guar gum can be used as a thickener in conjunction with a secondary stabilizer such as carrageenan to reduce synersis (Hansen, 1993). Use of soluble fibers as stabilizer has some advantages due to their beneficial effects for human health (Labell, 1990). Coronary disease, hypertension, diabetes, hypercholesterolemia and gastrointestinal disorders may decrease or be prevented by consuming fiber in the diet (Dello Staffolo *et al*., 2004). Some stabilizers not only stabilize the product but also used as fat replacers like malodextrin which gives better taste, appearance and also reduce synersis (Parveen, 2004).

Sagu powder is principally used in the food industry as a thickening and stabilizing agent. Sagu palm which is mostly grown in the islands of Malaysia and Indonesia and contributed almost 70% of all sagu production. Sagu is also being produced in Pakistan but the data regarding its scale of production is not available. Nutritionally, it is rich in carbohydrates (starches), low in fat and high in dietary fiber and minerals (calcium and iron). It is also inexpensive, economic and readily available food (Morin *et al*., 1979; Walter and Sam, 2002). However, there is no study in the literature concerning the use of *Meteroxylon sagu* as a stabilizing agent in yoghurt. Being a water-soluble hydrocolloid, it may functions to provide water control by thickening and gelling. Keeping in view the hydration property, it was planned to use *Metroxylon sagu* as stabilizing agent in yoghurt to evaluate its influence on the composition, shelf life, texture and sensory characteristics of yoghurt. Besides that, the objective was also to introduce a cheaper and local source of stabilizer and to meet the concept of HALAL source of stabilizer. Being an Islamic state Haram source is not acceptable, as in case of gelatin that is mostly extracted from pig skin and bones.

MATERIALS AND METHODS

Procurement of raw material: Buffalo milk for yoghurt manufacturing was procured from Dairy farm, University of Agriculture, Faisalabad. Food grade stabilizers, gelatin and *Meteroxylon sagu* were obtained from local market, commercial freeze dried starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was obtained from local distributor of Christ Hansen and skim milk powder was purchased from local market.

Experimental plan: Fresh milk used for yoghurt preparation was first standardized for fat (3%) and total solids (15%). After standardization, milk was divided into four equal portions and stabilizers were added. *Meteroxylon sagu* was added @ 0.1%, 0.25% and 0.5% while gelatin @ 0.5% as a controlled sample. The product was manufactured by heating buffalo milk to 95°C for 5 min, homogenized at 20-25 MPA/15 minutes, cooling to 45°C, inoculating with 2.5% starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in equal proportions), packed into food grade plastic cups, holding till pH 4.6 was attained, cooled to 15°C and then resulted product stored to 4°C.

Physiochemical analysis: Yoghurt samples were tested for pH by using pH meter (WTW series pH-720) and acidity was determined according to the method No. 947.05 (AOAC 2000). Viscosity was determined by using a DV-E viscometer with spindle No. 4 at 60 rpm (Brookfield, model LVDVE 230, serial number E5896). For the measurement of water holding capacity (WHC) in the experimental yoghurt samples, 5 g of yogurt was centrifuged at 4500 rpm for 30 min at 10 ^{0}C. After centrifugation, the supernatant was removed and the pellet was collected and weighed. The WHC was calculated as follows:

WHC (%) = $[1-W_t/W_i] \times 100$

Where wt is weight (g) of the pellet and wi is initial weight (g) of the sample (Wu *et al.,* 2000). The syneresis in yoghurt samples was measured as; 5mL of yoghurt was centrifuged at 5000 rpm for 20 minutes at 4^{0}C and separated whey was measured after 1 minute. Whey separation amount was expressed as volume of separated whey per 100 ml of yoghurt (Rodarte *et al*., 1993).

Sensory evaluation: The sensory evaluation of

Meteroxylon sagu enriched yoghurt was done by a panel of judges from selective faculty members and students at University of Agriculture, Faisalabad. Judges were trained and familiar for yoghurt's sensory attributes like flavour, body and texture, acidity, container, appearance and over all acceptability. These attributes were given score as flavour 45, body and texture 33, acidity 10, appearance 10 and container 02 (IDF, 1987). Yoghurt samples were coded and then presented to panel of judges for evaluation and water was also provided for mouth wash.

Microbiological analysis: Total viable count for yoghurt was tested by the methods determined by Cappuccino and Sherman (1996) and Colliform was also tested by the methods determined by Cappuccino and Sherman (1996).

Statistical analysis: The data was analyzed by applying two factor completely randomized design. Experimental treatments, with stabilizer addition were checked by ANOVA at 1% and 5% level of significance. Tukey's HSD test was used to conclude statistically different treatments (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Physiochemical analysis: Results of physiochemical attributes of four types of yoghurts are presented in Table. 1. Highly significant results of pH were found for storage period (p≤0.05). The pH values of yoghurt samples were decreased as storage time increased. The decrease in pH is due to the conversion of lactose into lactic acid during storage (Kamaruzzaman *et al.,* 2000; Anjum *et al*., 2007). Acidity also showed highly significant results for storage of yoghurt. Maximum increase in acidity was 0.883% for Y2 and minimum increase is 0.833% for Y0, it shows that Y0 has more ability to resist against changes. Previous studies (Bilal, 1995; Anwer *et al*., 2013) reported that acidity increased during storage mainly due to microbial activity and production of lactic acid and formic acid.

Table 1. Effet of "*Metroxylon sagu*" on the pH and acidity(%) of yoghurt during storage.

Storage Days		Treatments				
		Y0	**Y1**	**Y2**	**Y3**	**Mean**
pH	**0**	5.15±0.01	4.84±0.02	4.83±0.02	4.88±0.01	**4.95[A]**
	04	4.79±0.04	4.54±0.02	4.58±0.01	4.58±0.01	**4.62[B]**
	07	4.63±0.02	4.51±0.03	4.51±0.01	4.56±0.03	**4.55[C]**
	10	4.60±0.01	4.45±0.02	4.48±0.03	4.51±0.02	**4.51[D]**
	13	4.39±0.01	4.39±0.01	4.44±0.02	4.50±0.03	**4.43[E]**
	16	4.37±0.02	4.38±0.04	4.43±0.01	4.49±0.02	**4.42[F]**
	23	4.31±0.02	4.36±0.01	4.32±0.01	4.42±0.02	**4.35[G]**
Mean		**4.61[A]**	**4.49[D]**	**4.51[C]**	**4.58[B]**	
Acidity (%)	**0**	0.36±0.01	0.45±0.02	0.41±0.01	0.38±0.02	**0.40[E]**
	04	0.68±0.02	0.77±0.02	0.86±0.01	0.72±0.02	**0.76[D]**
	07	0.74±0.01	0.92±0.03	0.88±0.02	0.86±0.01	**0.85[C]**
	10	0.98±0.02	0.99±0.02	0.97±0.04	0.95±0.03	**0.97[B]**
	13	0.99±0.02	0.99±0.01	1.00±0.03	0.97±0.01	**0.99[AB]**
	16	1.02±0.03	0.99±0.01	1.02±0.02	0.98±0.01	**1.00[AB]**
	23	1.06±0.01	1.05±0.01	1.04±0.02	1.03±0.01	**1.05[A]**
Mean		**0.83[C]**	**0.88[A]**	**0.88[A]**	**0.84[B]**	

Significant difference is marked with different letters in the same column.

Y0 = control (0.5% Gelatin), Y1 = 0.1% *Metroxylon sagu*, Y2 = 0.25% *Metroxylon sagu*, Y3 = 0.5% *Metroxylon sagu.*

Highly significant results were found for synersis (mL) during storage (P≤0.01) and also for different concentrations of stabilizers (Fig.01). It was indicated that synersis in yoghurt for controlled and treated samples decreased with the passage of time. Decrease in synersis was 1.87 to 0.45 mL, 2.25 to 0.83 mL, 1.96 to 0.92 mL and 1.99 to 1.25 mL for treatments Y0 to Y3 respectively. However, both Y0 and Y1 showed best results in case of reduction in synersis. Previous findings of Yuceer (2006) and Guven *et al.* (2005) are also in accordance with present investigation.

The reduction in synersis may be due to increasing concentration of adsorbing polymer, the stabilization–destabilization mechanisms undergo transitions from

(1) bridging flocculation, to (2) steric stabilization, to (3) depletion flocculation by unadsorbed polysaccharide in the serum phase, to (4) colloidal aggregates trapped in a viscous polymer network. There was reduction in water holding capacity (%) of yoghurts during storage as shown in Fig.02. Highly significant results were also found for different concentrations of stabilizers. The decrease in water holding capacity for controlled and treated samples was 2.84%, 18.47%, 10.64% and 18.24% for Y0, Y1, Y2 and Y3 respectively. The treatment Y1 represented that water holding capacity of *Metroxylon sagu* treated samples remained higher as compared to gelatin treated sample. The interaction between casein aggregates and polysaccharides stabilizers became weaker due to lactic acid formation (Schorsch *et al.,* 1999: van Vliet *et al.,* 1991).

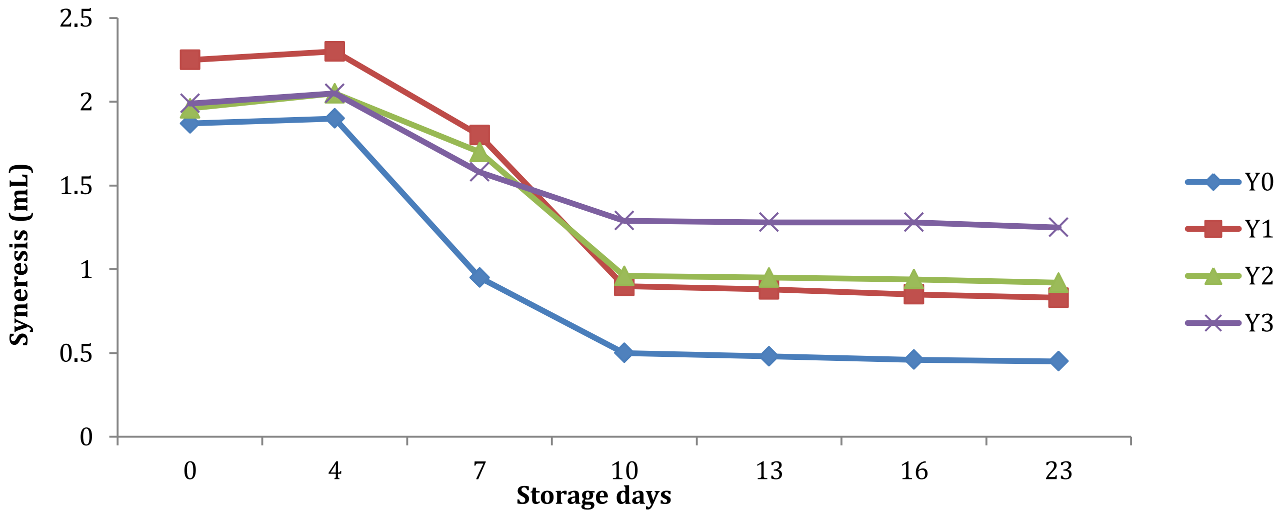

Figure 1. Effect of "*Metroxylon sagu*" on the synersis in yoghurt during storage.
(Y0: Gelatin 0.5%, Y1: *Metroxylon sagu* 0.1%, Y2: *Metroxylon sagu* 0.25%, Y3: *Metroxylon sagu* 0.5%).

The storage interval and stabilizers showed highly significant effect on viscosity of yoghurt (Fig. 03). The initial and final values of viscosity from 0 to 23rd day of storage were 4634.00 to 4568.00 (cps), 5794.00 to 3523.00 (cps), 4834.00 to 6768.00 (cps) and 4812.00 to 4610.00 (cps) for Y0, Y1, Y2 and Y3 respectively. For all the treatments, viscosity was increased from 1st to 4th day, maximum increase was observed in Y3 sample, and then there was decrease in viscosity from 4th to 7th days of storage for all the treatments and maximum decrease was observed in Y1 treatment. From 7th to 23rd of storage, again increase in viscosity was observed and maximum increase was observed for Y2 treatment.

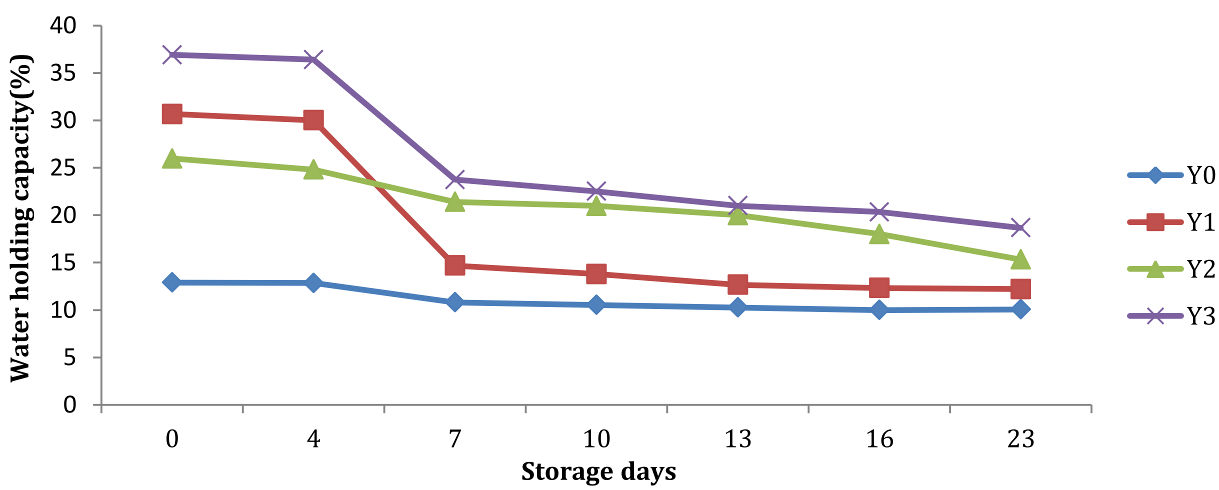

Figure 2. Effect of "*Metroxylon sagu*" on the water holding capacity (WHC) in yoghurt during storage.
(Y0: Gelatin 0.5%, Y1: *Metroxylon sagu* 0.1%, Y2: *Metroxylon sagu* 0.25%, Y3: *Metroxylon sagu* 0.5%).

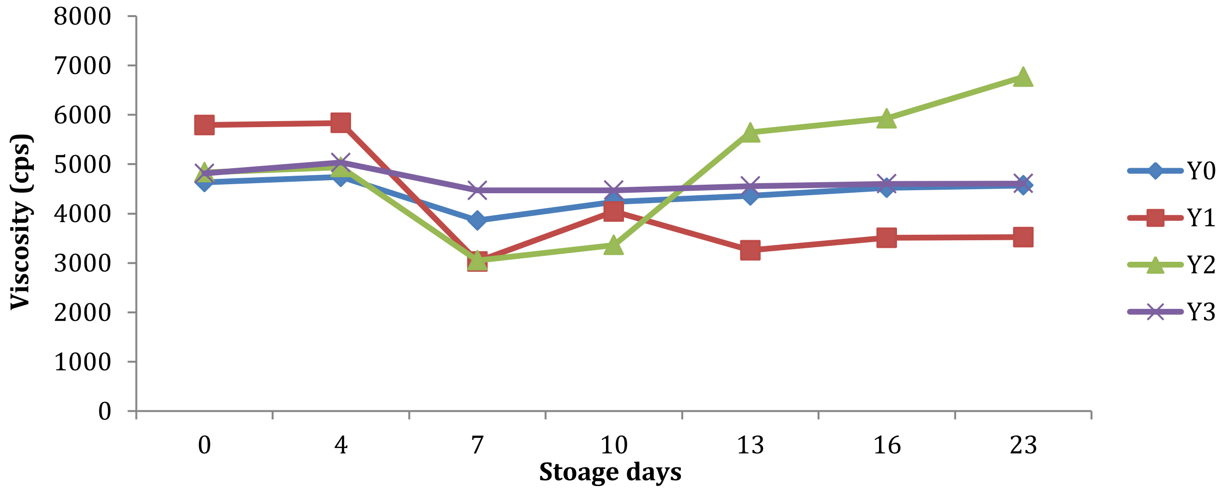

Figure 3. Effect of "*Metroxylon sagu*" on the Viscosity of yoghurt during storage.
(Y0: Gelatin 0.5%, Y1: *Metroxylon sagu* 0.1%, Y2: *Metroxylon sagu* 0.25%, Y3: *Metroxylon sagu* 0.5%).

Table 2. Effet of "*Metroxylon sagu*" on the sensory attributes of yoghurt during storage.

Storage Days		Treatments				
		Y0	**Y1**	**Y2**	**Y3**	**Mean**
Flavor	**0**	40.0±0.09	39.0±0.05	40.0±0.07	39.0±0.05	**39.50A**
	04	9.0±0.09	39.0±0.02	40.0±0.03	39.0±0.07	**39.25AB**
	07	36.0±0.08	38.0±0.04	40.0±0.04	39.0±0.09	**38.25B**
	10	34.0±0.07	34.0±0.04	39.0±0.03	39.0±0.05	**36.50C**
	13	32.0±0.07	33.0±0.05	39.0±0.05	38.0±0.03	**35.50C**
	16	30.0±0.08	32.0±0.05	37.0±0.05	36.0±0.04	**33.75D**
	23	29.0±0.09	30.0±0.07	37.0±0.03	36.0±0.05	**33.00D**
Mean		**4.61A**	**4.49D**	**4.51C**	**4.58B**	
Body and Texture	**0**	28.0±0.20	27.0±0.08	30.0±0.09	28.0±0.07	**28.25B**
	04	29.0±0.09	29.6±0.09	30.0±0.11	29.0±0.02	**29.42AB**
	07	30.0±0.08	29.0±0.17	32.0±0.15	30.0±0.08	**30.25A**
	10	28.0±0.30	29.0±0.18	32.0±0.19	29.0±0.05	**29.50A**
	13	27.0±0.15	27.0±0.19	30.0±0.12	29.0±0.05	**28.25B**
	16	27.0±0.18	27.0±0.09	30.3±0.08	29.0±0.19	**28.33B**
	23	25.0±0.20	26.0±0.06	31.0±0.08	30.0±0.16	**28.00C**
Mean		**27.71C**	**27.81C**	**30.76A**	**29.14B**	
Sensory Acidity	**0**	7.67±0.02	7.33±0.02	8.00±0.01	7.33±0.02	**7.58A**
	04	8.00±0.01	7.50±0.04	8.00±0.02	7.50±0.01	**7.75A**
	07	8.00±0.01	7.00±0.02	9.00±0.01	8.00±0.01	**8.00A**
	10	6.00±0.02	6.00±0.03	8.00±0.01	8.00±0.02	**7.00AB**
	13	4.00±0.03	5.00±0.01	8.00±0.03	7.00±0.02	**6.00BC**
	16	3.00±0.02	4.00±0.01	8.00±0.05	7.00±0.03	**5.50C**
	23	3.00±0.01	4.00±0.04	7.00±0.03	7.00±0.01	**5.25C**
Mean		**5.67B**	**5.83B**	**8.00A**	**7.41A**	

Significant difference is marked with different letters in the same column.
Y0 = control (0.5% Gelatin), Y1 = 0.1% *Metroxylon sagu*, Y2 = 0.25% *Metroxylon sagu*, Y3 = 0.5% *Metroxylon sagu*.

The fluctuation, protein rearrangement and protein–protein contact may increase viscosity with the passage of time (Abu-Jdayil and Mohameed, 2002; Ozer *et al.,* 1998).

Sensory analysis: In sensory evaluation, flavor of the product is one of most important factor to estimate the consumer's response towards product acceptance. It is revealed from results (Table 02) that Y2 treatment got highest points 40/45 as compared to other treatments. However, only 3 points reduction was observed during 23 days of storage in this treatment.

All the treatments remained in acceptable range due to addition of stabilizer and also stored at low temperature. Yoghurt samples treated with *Meteroxylon sagu* represented best results for flavor. The decrease in flavor of yoghurt during storage was observed in previous studies (Kamaruzzaman *at al.,* 2002; Shukla and Jain, 1991).

There was 3.0 points decrease in score of body and texture for Gelatin that was used as controlled, for Y0 only 1.0 point decrease in value while there was increase in value for Y2 and Y3. However, Y2 got highest points for body and texture; there was least decrease in body and texture for Y2. It shows that Y2 that containing 0.25% of stabilizer, represented best results. These results are in accordance with previous findings (Bilal, 1995; Basset, 1983). However, it was observed in present investigation that there was maximum decrease in acidity in gelatin treated yoghurt, comparatively less decrease was observed in Y1, and while in Y2 and Y3 acidity values remained same. It was also observed that there was increase in acidity from 4th to 10th day then it was either remained same or decreased with storage. This change in acidity might be due to activity of microbes and conversion of lactose into lactic acid.

Effect of different concentration of stabilizers and storage intervals are highly significant for appearance and container closure (Table 03). *Meteroxylon sagu* treated samples that contained 0.25% and 0.5% *Meteroxylon sagu* were regarding best for appearance. These results were according to findings of Bilal (1995), Mehanna and Mehna (1989). Panel awarded points indicated that container closure suitability remained same throughout the research.

Table 3. Effet of "*Metroxylon sagu*" on the container closure and appearance of yoghurt during storage.

Storage Days		**Treatments**				
		Y0	**Y1**	**Y2**	**Y3**	**Mean**
Container Closure	**0**	1.00	1.00	1.00	1.00	**1.00**
	04	1.00	1.00	1.00	1.00	**1.00**
	07	1.00	1.00	1.00	1.00	**1.00**
	10	1.00	1.00	1.00	1.00	**1.00**
	13	1.00	1.00	1.00	1.00	**1.00**
	16	1.00	1.00	1.00	1.00	**1.00**
	23	1.00	1.00	1.00	1.00	**1.00**
Mean		**1.00**	**1.00**	**1.00**	**1.00**	
Appearance	**0**	8.44±0.05	7.85±0.03	9.06±0.02	8.91±0.02	**8.57^{A}**
	04	8.52±0.02	7.79±0.03	8.97±0.02	8.80±0.01	**8.52^{A}**
	07	8.46±0.02	7.01±0.05	8.79±0.03	8.61±0.03	**8.22^{AB}**
	10	6.50±0.03	6.12±0.04	8.28±0.01	8.16±0.03	**7.26^{BC}**
	13	6.57±0.04	6.44±0.02	8.21±0.07	8.25±0.02	**7.37^{BC}**
	16	5.33±0.02	6.48±0.04	8.55±0.04	7.44±0.02	**6.95^{C}**
	23	5.28±0.02	5.36±0.03	7.59±0.03	7.52±0.05	**6.44^{C}**
Mean		**7.01^{B}**	**6.72^{B}**	**8.49^{A}**	**8.24^{A}**	

Significant difference is marked with different letters in the same column.

Y0 = control (0.5% Gelatin), Y1 = 0.1% *Metroxylon sagu*, Y2 = 0.25% *Metroxylon sagu*, Y3 = 0.5% *Metroxylon sagu.*

Microbiological analysis: It is evident from results (shown in Table. 4) that there was increase in total viable count (cfu/ml) during storage interval while there was non-significant effect due to different concentrations of stabilizers. These results were in the agreement with that of Younis *et al,*(2000), Al-Hadethi *et* *al.,*(1992), Dave *et al.,* (1992). Colliform (cfu/100mL) was absent in milk and it remained absent throughout the storage interval, It may be due to production of acidity in yoghurt, good storage condition plus good quality of packaging and raw material.

Table 04. Effet of "*Metroxylon sagu*" on the microbiological attributes of yoghurt during storage.

Storage Days		**Treatments**				
		Y0	**Y1**	**Y2**	**Y3**	**Mean**
TVC	**01**	8.25x106	8.19x106	8.175x106	8.17x106	**8.19x106**^{A}
(cfu/ml)	**04**	1.75x107	1.70x107	1.71x107	1.77x107	**1.76x107**^{B}
	07	2.20x107	2.19x107	2.13x107	2.30x107	**2.20x107**^{BC}
	18	2.49x108	2.40x108	2.43x108	2.48x108	**2.48x108**^{CD}
TVC	**01**	8.25x106	8.19x106	8.175x106	8.17x106	**8.19x106**^{A}
(cfu/ml)	**04**	1.75x107	1.70x107	1.71x107	1.77x107	**1.76x107**^{B}
	07	2.20x107	2.19x107	2.13x107	2.30x107	**2.20x107**^{BC}
Mean		**3.67x107**^{A}	**2.09x107**^{D}	**1.92x107**^{C}	**1.93x107**^{B}	
Coliform	**0**	−ve	−ve	−ve	−ve	**−ve**
(cfu/100mL)	**04**	−ve	−ve	−ve	−ve	**−ve**
	07	−ve	−ve	−ve	−ve	**−ve**
	18	−ve	−ve	−ve	−ve	**−ve**
Coliform	**0**	−ve	−ve	−ve	−ve	**−ve**
(cfu/100mL)	**04**	−ve	−ve	−ve	−ve	**−ve**
	07	−ve	−ve	−ve	−ve	**−ve**
Mean		**−ve**	**−ve**	**−ve**	**−ve**	

Significant difference is marked with different letters in the same column.

Y0 = control (0.5% Gelatin), Y1 = 0.1% *Metroxylon sagu*, Y2 = 0.25% *Metroxylon sagu*, Y3 = 0.5% *Metroxylon sagu*.

CONCLUSION

It was concluded from present investigation that *Metroxylon sagu* @ 0.25% represented best results for overall sensory acceptability. It was also observed that yoghurt could be produced up to shelf life of 30 days by modifying storage and specially packaging condition at storage temperature 4^{0}C. The yoghurt with 0.25% concentration of *Metroxylon sagu* contributes to firm body and texture, decrease in whey separation, increase in viscosity and ultimately there was increase in shelf life of yoghurt.

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