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Comparative study of the physico-chemical and functional properties of *Ashwagandha* root extract enriched *Shrikhand* and control *Shrikhand* during storage

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Abstract
During storage, the color and appearance score of control *Shrikhand* (CS) samples were significantly high ($p<0.05$) as compared to *Ashwagandha* root extract enriched *Shrikhand* (AS). The flavor score decreased ($p<0.05$) as the storage periods increases in both types of *Shrikhand* (AS and CS). The differences in the values of flavor score between the groups recorded at various intervals were significant ($p<0.05$). The texture score decreased ($p<0.05$) after 14th day in AS and 7th day of storage in CS Samples. The overall texture score of the samples AS were higher than CS during storage. Irrespective of the groups, the sweetness score of the samples decreased ($p<0.05$) as the storage periods increased. The overall acceptability score was inversely proportionate ($p<0.05$) to the storage periods. The overall acceptability score of CS samples were significantly higher ($p<0.05$) than AS samples, except at 21st day of storage. The protein, fat, ash percentage increased ($p<0.05$) in both types of samples (AS and CS) during storage except moisture percentage of the samples. As the storage periods prolonged, the pH decreased and acidity content increased ($p<0.05$) in both types of *Shrikhand*. During entire storage periods the counts of LB (10^7cfu/g), ST (10^7cfu/g) and SPC (10^7cfu/g) were significantly high ($p<0.05$) in samples AS than CS. The counts of LB and ST decreased ($p<0.05$) in all the samples upto 21st day of storage, but thereafter it increased ($p<0.05$). The Yeast and Mould were not observed upto 7th day, but at 14th day it augmented significantly ($p<0.05$) in both the groups (AS and CS). The coliform were also not observed in the samples during the storage. With increasing of number of days the DPPH and ABTS also decreased significantly ($p<0.05$) from 81.40 to 25.65 and 73.25 to 20.50 respectively.

Keywords: *Shrikhand, Ashwagandha,* flavor, texture, coliform, DPPH, ABTS

Introduction

Shrikhand is semi solid soft, sweetish sour fermented dairy product. It is a popular delicacy in Gujarat, Maharastra and Karnataka. It is consumed as a desert. It is prepared from cow, buffalo or mixed milk. Lactic fermented curd is obtained by the associative action of microorganisms on the milk constituents. Health benefits of milk include good bone health, robust skin, good immune system, prevention of illness such as hypertension, dental decay, dehydration, respiratory problems, obesity, osteoporosis and even some forms of cancer. Many animals do provide us with this vital health substances but cow's milk is considered the best wholesome supplements for children as well as adults.

It is prepared from lactic acid fermentation. It is made with *Chakka* (strained yoghurt/curd) which is finely mixed with sugar and flavoring agents. It has the nutritive goodness of fermented milk products. Like *Dahi* (curd), it is very refreshing particularly during summer months. It is popular because of its characteristic flavor, taste, palatable nature and possible therapeutic value (Nigam *et al.*, 2009) [5].

Functional foods are very important in maintaining the health and fulfilling the nutritional requirement of the body. Functional components could be incorporated in food items to increase their demand and acceptability among the consumers. Many functional foods have been developed as *Satavari* bread, herbal *Ghee* (*Arjuna Ghee*), herbal yoghurt, herbal milk, herbal *Sandesh*, herbal *Rasogolla*, Functional snacks, *Spirullina* biscuits etc. These products have been found to be with medicinal properties such as anti-cancerous, anti-diabetic, dietary fiber enriched and protein enriched. The medicinally important herbs in food products cannot be directly accepted due to their bitter flavor and undesirable appearance. Incorporation of whole herb in food products may result in deteriorative changes in food products. The incorporation of herbal extract possessing active ingredients may be a better option to design the nutraceutical foods.

The roots of *Ashwagandha* have a lot of functionally important active constituents that are helpful in tumor treatment, immunomodulation memory enhancing capacities, neuroprotective power etc.

Milk and milk products may act as carrier for the beneficial effects of herbs. *Shrikhand* is a fat and sugar rich product, which allows the higher incorporation of herbal extracts and gets more acceptable sensory value than other dairy products.

Material and Methods

Keeping in view the beneficial and medicinal values of *Ashwagandha* and *Shrikhnad*, it was planned to develop herbal *Shrikhand* by incorporating the root extract of *Ashwagandha*. The optimization of ingredients of herbal *Shrikhand* has been done. Then the comparative study of the physico-chemical and functional properties of *Ashwagandha* root extract enriched *Shrikhand* (AS) *and* control *Shrikhand* (CS) has been done.

Results and Discussion

Changes in physico-chemical characteristics during storage

The physico-chemical characteristics of the samples were studied upto 28th days of storage at 10±1 ºC (Table 4.10 and 4.11). The results of the samples in respect of color and appearance, flavor, texture, sweetness, overall acceptability, fat, protein, ash, TS, moisture, pH and acidity content are discussed here under the following heads:

4.3.2.1 Effect of storage periods on color and appearance

The color and appearance score of *Shrikhand* was varied from 8.46±0.0058 to 8.25±0.0058 for AS and from 8.81±0.0033 to 8.41±0.0033 for CS samples (Table 1; Fig. 1). The highest color and appearance score for sample AS was 8.46±0.0058 followed by 8.41±0.0058, 8.36±0.0058, 8.31±0.0058 and 8.25±0.0058 found at 0, 7th, 14th, 21st and 28th days of storage. The values presented in table 4.8 clearly indicates that the highest color and appearance score for sample CS was 8.81±0.0033 followed by 8.72±0.0033, 8.68±0.0033, 8.59±0.0033 and 8.41±0.0033 found at 0, 7th, 14th, 21st and 28th days of storage. The values clearly indicates that color and appearance in CS sample was significantly high ($p<0.05$) as compared to AS samples at all the stages of storage. The values in both the groups significantly ($p<0.05$) decreased as the periods of storage prolonged. This may be due to growth of micro-oganisms responsible for spoilage. Kumar *et al.* (2011) [3] reported that apple pulp and *Celosia argentea* fortified *Shrikhand* show decrease in color and appearance score significantly ($p<0.05$) during storage periods. In confirmity with our findings, Nigam *et al.* (2009) [5] have also reported that the incorporation of papaya pulp in the manufacture of *Shrikhand* shows decline in color and appearance score during storage.

Table 1: Effect of storage on sensory attributes *Ashwagandha* root extract enriched *Shrikhand* (AS) and control *Shrikhand* (CS) samples

Storage	Color and Appearance		Flavor		Texture		Sweetness		Overall Acceptability	
	AS	CS	AS	CS	AS	CS	AS	CS	AS	CS
0 Day	8.46 ± 0.0058^{a}	8.81 ±0.0033^{a}	8.45 ±0.0058^{aA}	8.75 ±0.0033^{a}	7.60±0^{aA}	7.58 ±0.0033^{abAB}	8.75 ±0.0058^{aA}	8.77 ±0.0058^{aA}	8.30±0^{aA}	8.76 ±0.0033^{a}
7th Day	8.41 ±0.0058^{b}	8.72 ±0.0033^{b}	8.14 ±0.0088^{b}	8.36 ±0.0033^{bA}	7.59 ±0.0033^{aA}	7.56 ±0.0033^{bB}	8.14 ±0.019^{bB}	8.22 ±0.0088^{b}	8.033 ±0.033^{b}	8.41 ±0.0058^{b}
14th Day	8.36 ±0.0058^{c}	8.68 ±0.0033^{c}	7.62 ±0.013^{cB}	7.97 ±0.006 7^{c}	7.58±0^{aAB}	7.42 ±0.0033^{c}	7.70 ±0.052^{c}	8.16 ±0.0088^{cB}	7.80 ±0.012^{c}	8.26 ±0.0088^{cA}
21st Day	8.31 ±0.0058^{d}	8.59 ±0.0033^{d}	6.90 ±0.058^{d}	7.63 ±0.0067^{dB}	7.11 ±0.0067^{bC}	7.10±0^{dC}	6.43 ±0.012^{d}	7.20 ±0.0033^{d}	7.57 ±0.010^{dB}	7.53 ±0.019^{dB}
28th Day	8.25 ±0.0058^{e}	8.41 ±0.0033^{e}	4.97 ±0.067^{e}	6.43 ±0.067^{e}	6.83 ±0.033^{c}	6.80±0^{e}	5.24 ±0.015^{e}	6.02 ±0.020^{e}	7.25 ±0.0058^{e}	7.30 ±0.0033^{e}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, $P<0.05$)
Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, $P<0.05$)
CS=Control *Shrikhand* samples,
AS= *Ashwagandha* root extract enriched *Shrikhand* (optimised level)

Table 2: Effect of storage on chemical characteristics of *Ashwagandha* root extract enriched *Shrikhand* (AS) and control *Shrikhand* (CS) samples

Storage	Fat		Protein		Moisture		TS		Ash		pH		Acidity	
	AS	CS	AS	CS	AS	CS	AS	CS	AS	CS	AS	CS	AS	CS
0 Day	8.62 ±0.017^{aAB}	8.59 ±0.0033^{aB}	10.14 ±0.01^{aA}	8.51 ±0.016^{aB}	39.74 ±0.075^{aA}	41.66 ±0.0058^{aB}	60.26 ±0.075^{aA}	58.34 ±0.0058^{aB}	0.80± 0^{aA}	0.78± 0^{aB}	4.39± 0^{aA}	4.68 ±0.0033^{aB}	1.28 ±0.0033^{aA}	1.21 ±0.0033^{aB}
7th Day	8.73 ±0.017^{bC}	8.64 ±0.0066^{bA}	10.23 ±0.017^{bC}	8.64 ±0.010^{bD}	39.42 ±0.0067^{bC}	41.05 ±0.065^{bD}	60.58 ±0.0067^{bC}	58.95 ±0.065^{bD}	0.80± 0^{aC}	0.78± 0^{aD}	4.36 ±0.0033^{bC}	4.60 ±0.0033^{bD}	1.30 ±0.0033^{bC}	1.23 ±0.0033^{bD}
14th Day	8.82 ±0.017^{cD}	8.69 ±0.0058^{cE}	10.33 ±0.017^{cE}	8.75± 0^{cF}	39.32 ±0.0088^{bE}	40.37 ±0.012^{cF}	60.68 ±0.0088^{bE}	59.63 ±0.012^{cF}	0.81± 0^{aE}	0.79± 0^{aF}	4.34 ±0.0033^{cE}	4.54 ±0.0033^{cF}	1.34 ±0.0033^{cE}	1.26± 0^{cF}
21st Day	8.90± 0^{dF}	8.73 ±0.012^{dG}	10.43 ±0.016^{dG}	8.8 ±0.0033^{cH}	39.083 ±0.044^{cG}	49.88 ±0.0058^{dH}	60.92 ±0.044^{cG}	60.12 ±0.0058^{dH}	0.81± 0^{aG}	0.80± 0^{aH}	4.31 ±0.0033^{dG}	4.42 ±0.0033^{dH}	1.38 ±0.0058^{dG}	1.29 ±0.0033^{dC}
28th Day	8.93 ±0.017^{dH}	8.83 ±0.0058^{eI}	10.57 ±0.033^{eI}	8.91 ±0.0033^{dJ}	38.68± 0^{dI}	39.46 ±0.0033^{eC}	61.35 ±0^{dI}	60.54 ±0.0033^{eC}	0.81± 0^{aI}	0.80± 0^{aJ}	4.30 ±0.0033^{eI}	4.38 ±0.0058^{eA}	1.41 ±0.0033^{eH}	1.35 ±0.0058^{eI}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, $P<0.05$)
Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, $P<0.05$)
CS=Control *Shrikhand* samples,
AS= *Ashwagandha* root extract enriched *Shrikhand* (optimised level)

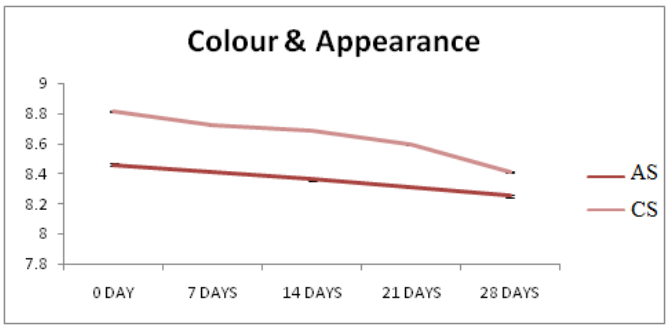

Fig 1: Changes in color and appearance score of *Shrikhand* during storage

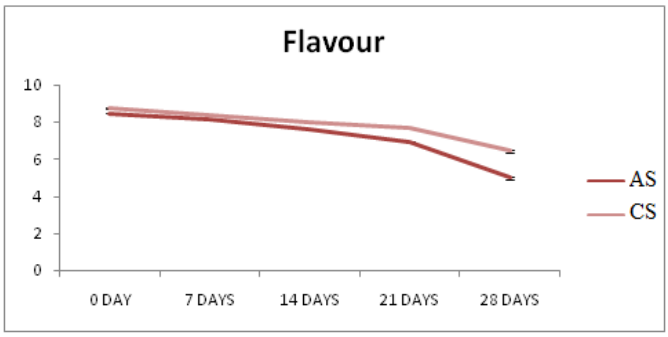

Fig 2: Changes in flavor score of *Shrikhand* during storage

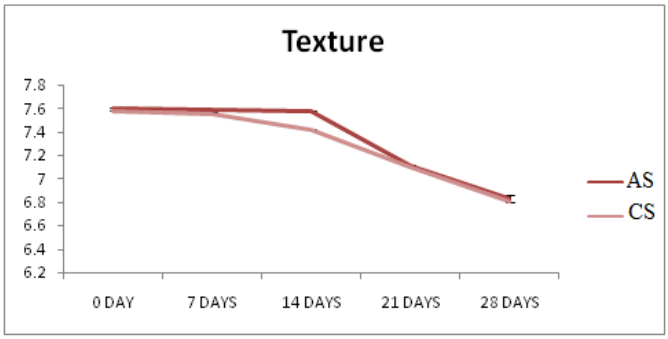

Fig 3: Changes in texture score of *Shrikhand* during storage

Effect of storage period on flavor

Flavor (i.e. taste and smell) is the most important factor for the acceptance of the products. The flavor score of AS sample ranged from 8.45±0.0058 to 4.97±0.0058 and for CS samples from 8.75±0.0033 to 6.43±0.067 (Table 1; Fig. 2). As the storage periods of both (AS and CS) samples increases the flavor score was decreased ($p<0.05$). The decrease in the flavor score between the groups were not significant up to 21st day of storage but thereafter significant ($p<0.05$).

From the data (Table 1) an inverse relationship between the flavor score and storage period were noted. The decrease in flavor score during storage periods may be due to growth of spoilage micro-oganisms resulting which acidity increased and bitter taste developed. The flavor score of AS was not acceptable at 28th day of storage. This may be due to increased in acidity and bitter taste. *Shrikhand* prepared with fortification of papaya pulp (Nigam *et al.*, 2009) [5] and apple pulp and *Celosia argentea* (Kumar *et al.*, 2011) [3] also showed decreased in flavor score ($p<0.05$) during storage periods.

Effect of storage period on texture

The effect of different storage periods on texture score of *Ashwagandha* root extract enriched *Shrikhand* (AS) against control (CS) samples are clearly depicted in table 1. The score for AS samples ranged between 7.60±0 and 6.83±0.033 and for CS between 7.58±0.0033 and 6.80±0 (Table 1; Fig. 3). The decrease in texture score was recorded significant ($p<0.05$) after 14th day of storage in AS sample and 7th day of storage in CS Samples. The rest of the values were not significant.

The texture score of AS was higher than CS samples during all the storage intervals. This may be due to decrease in moisture content of the samples during storage. In confirmity with our findings, Nigam *et al.* (2009) [5] have also reported that the texture score decreased during storage periods in papaya pulp fortified *Shrikhand*. Kumar *et al.* (2011) [3] also reported a similar decline in texture score during storage when apple pulp and *Celosia argentea* were added in *Shrikhand*.

Effect of storage periods on sweetness

The highest sweetness score for sample AS was 8.75±0.0058 followed by 8.14±0.019, 7.70±0.052, 6.43±0.012 and 5.24±0.015 at 0, 7th, 14th, 21st and 28th days of storage, respectively. The values presented in the table 1 (Fig. 4) clearly indicates that the highest sweetness score for sample CS was found to be 8.77±0.0058 followed by 8.22±0.0088, 8.16±0.0088, 7.20±0.0033 and 6.02±0.020 found at 0, 7th, 14th, 21st and 28th days of storage, respectively. The sweetness score ranged from 8.75±0.0058 to 5.24±0.015 for AS and from 8.77±0.0058 to 6.02±0.020 for CS samples during storage. As the storage periods of both the samples (AS and CS) increased the sweetness score significantly ($p<0.05$) decreased.

The values in table 1 clearly depicts that the differences in the sweetness score between AS and CS samples were non significant ($p>0.05$) at 7th day of storage in AS and at 14th day of CS samples. The differences between AS and CS samples were non significant ($p<0.05$) at 0 day of storage. The sweetness score of AS was lower than CS at all storage intervals. This may be due to lack of sweetening agent in soy and increased microbial activity during storage. Nigam *et al.* (2009) [5], Kumar *et al.* (2011) [3] and Sonawane *et al.* (2007) [10] were at par with the results found in the present investigation.

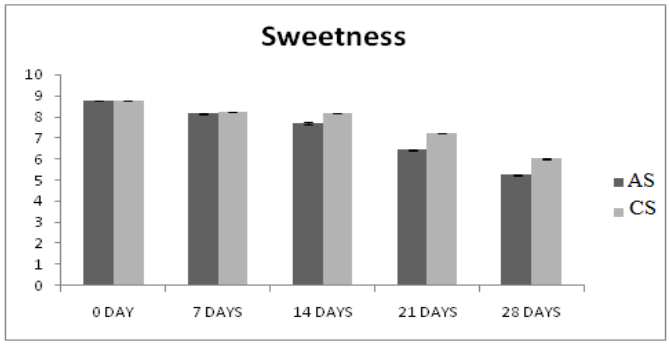

Fig 4: Changes in sweetness score of *Shrikhand* during storage

Effect of storage periods on overall acceptability

The overall acceptability depends on color and appearance, flavor, texture and sweetness score of the products. The overall acceptability score (Table 1; Fig. 5) ranged from 8.30±0 to 7.25±0.0058 for AS and from 8.76±0.0033 to 7.30±0.0033 for CS samples. The highest overall acceptability score for sample CS was 8.76±0.0033 followed by 8.41±0.0058, 8.26±0.0088, 7.53±0.019 and 7.30±0.0033 at 0, 7th, 14th, 21st and 28th days of storage, respectively. The values presented in the table 4.10 clearly indicates that the highest

overall acceptability score for sample AS was 8.30±0 followed by 8.033±0.033, 8.16±0.0088, 7.80±0.012, 7.57±0.010 and 7.25±0.0058 at 0, 7th, 14th, 21st and 28th days of storage periods, respectively. The difference between AS and CS sample was non significant (p>0.05) at 0 day of storage in AS and at 14th day of CS sample. The difference in the values between both the samples were also non significant (p<0.05) at 21st day of storage.

The overall acceptability score of AS samples were lower than CS at all days of storage. An inverse relationship (p<0.05) between overall acceptability and storage periods were recorded in the samples. This may be due to increased activity of spoilage micro-oganisms in the samples during storage. Patel *et al.* (1993) [6] reported that the overall acceptability score of *Chakka* decreased with increase in storage periods due to deterioration of flavor. Verma (2013) [11], Kumar *et al.* (2011) [3], Nigam *et al.* (2009) [5] and Sonawane *et al.* (2007) [10] were also having similar opinion (decline in the sensory parameters) when various dairy products were stored for longer duration at refrigerated temperature.

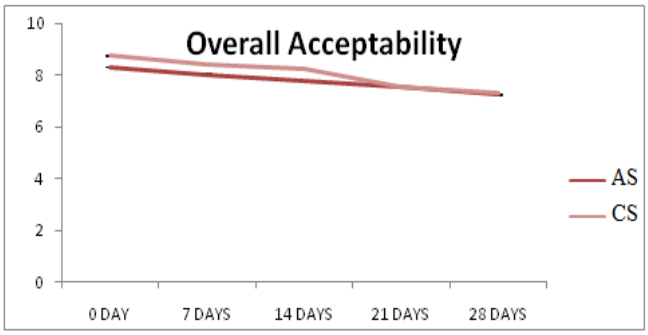

Fig 5: Changes in overall acceptability score of *Shrikhand* during storage

Effect of storage on protein

The values presented in table 2 (Fig. 6) clearly depicts that as the storage periods increases the protein content also increased (p<0.05) in both type of samples. The highest protein content for sample AS was 10.57±0.033 followed by 10.43±0.016, 10.33±0.017, 10.23±0.017 and 10.14±0.01; for sample CS it was 8.91±0.0033 followed by 8.8±0.0033, 8.75±0, 8.64±0.010 and 8.51±0.016 at 28th, 21st, 14th, 7th and 0 day of storage, respectively. The protein content of samples CS increased significantly (p<0.05) during storage except at 14th and 21st day. The protein content was higher in the sample AS than CS (Fig. 6), which may me due to presence of *Ashwagandha* root. The similar findings have also been reported by Nigam *et al.* (2009) [5], Kumar *et al.* (2011) [3] and Verma (2013) [11] when studied the effect of storage period on protein content in various dairy products.

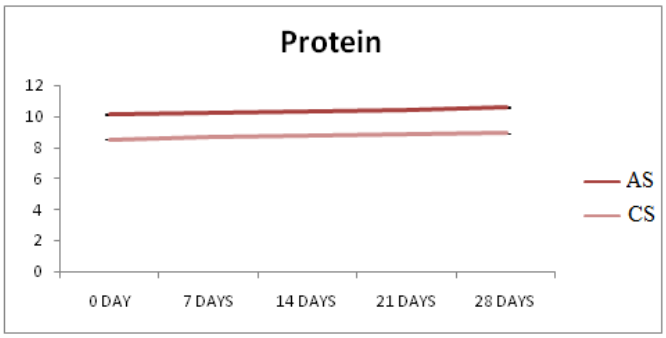

Fig 6: Changes in protein percentage of *Shrikhand* during storage

Effect of storage periods on fat

The fat per cent varied from 8.62±0.017 to 8.93±0.017 in samples AS and from 8.59±0.003 to 8.83±0.0058 in CS samples (Table 2; Fig. 7). The fig. 4.30 clearly indicates that as the storage periods increased of the samples AS the fat per cent increased significantly (p<0.05) upto 21st days of storage, but thereafter the increase were non-significant (p>0.05). It is clear from the data (Table 2) that as the storage periods prolonged, the fat per cent also increased (p<0.05) in the CS samples. The difference at 0 day of AS with CS sample (at 0 and 7th day) was found not significant (p>0.05). The increase in the levels of fat may be due to loss of moisture content in the samples during storage. The findings of Nigam *et al.* (2009) [5] was at par with the result investigation when papaya fortified *shrikhand* was stored at refrigerated temperature. The studies conducted on the effect of refrigerated storage on chemical characteristics of apple pulp and *Celosia argentea* fortified *shrikhand* (Kumar *et al.*, 2011) [3] reported similar increase in fat content during storage. Verma (2013) [11] found that moisture content in soy cake fortified *Burfi* was reduced during storage. On contarary to our findings, Kumar (2013) [3] reported that fat content of herbal ice cream decreased during storage.

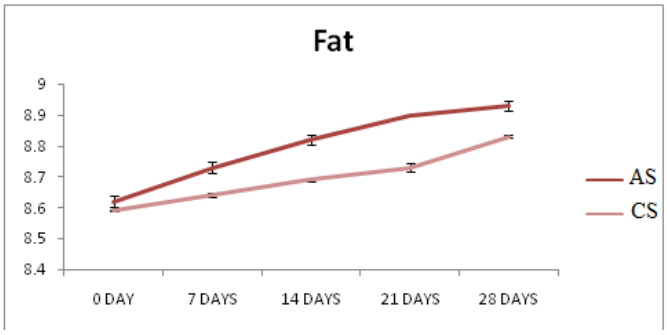

Fig 7: Changes in fat percentage of *Shrikhand* during storage

Effect of storage on ash

The average ash content in *Shrikhand* samples at 0 day was 0.80±0.0% for AS and 0.78±0.0% for CS samples (Table 2; Fig. 8). The difference in the values were significant (p<0.05). These values slightly increased as storage periods prolonged, but the differences in those values were not significant. The ash content in AS group was 1.2% higher than CS group at 28th day of storage whereas the difference in the value at 0 day was 2.6 per cent. The findings of Nigam *et al.* (2009) [5], Kumar *et al.* (2011) [3], Kumar (2013) and Verma (2013) [11] were inconfirmity with our findings when studied the effect of refrigerated storage on various dairy products.

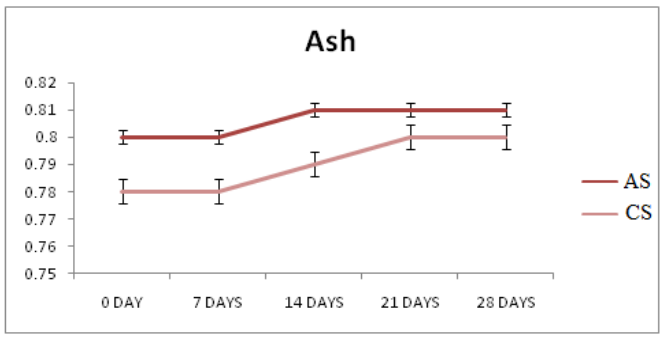

Fig 8: Changes in ash percentage of *Shrikhand* during storage

Effect of storage on moisture

The moisture content varied from 39.74±0.075 to 38.68±0 in

AS samples and from 41.66±0.005839 to 0.46±0.0033 in the CS samples during storage (Table 2; Fig. 9). The moisture content was the highest at 0 day (39.74±0.075%) followed by 7th (39.42±0.0067%), 14th (39.32±0.0088%), 21st (39.083±0.044%) and 28th (38.68±0%) days of storage intervals, respectively.

In CS group, the highest moisture content was 41.66±0.0058 followed by 41.05±0.065, 40.37±0.012, 49.88±0.0058 and 39.46±0.0033 per cent found at 0, 7th, 14th, 21st and 28th days, respectively. The moisture content of the sample CS decreases significantly ($p<0.05$) during storage. The fig. 9 clearly depicts that the average moisture content was higher in the samples CS than AS. The difference in the values between AS and CS samples were not significant ($p>0.05$) except at 7th day for AS and 28th day for CS samples. There was an inverse relationship between moisture content and storage periods. The findings of Sonawane *et al.* (2007) [10], Nigam *et al.* (2009) [5], Kumar *et al.* (2011) [3] and Verma (2013) [11] were at par with the results of present investigation.

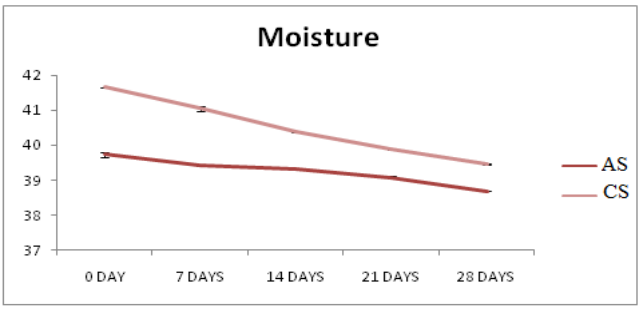

Fig 9: Changes in moisture percentage of *Shrikhand* during storage

Effect of storage on total solid (TS)

A perusal of data presented in the table (Table 2; Fig. 10) clearly depicts that as the storage periods in AS sample increased the TS content also increased ($p<0.05$) except at 7th and 14st days. The highest TS content for the sample SS was 61.35±0 followed by 60.92±0.044, 60.68±0.0088, 60.58±0.0067and 60.26±0.075 at 28th, 21st, 14th, 7th and 0 day of storage, respectively. The highest TS content in sample CS was 60.12±0.0058 followed by 60.54±0.0033, 59.63±0.012, 58.95±0.065 and 58.34±0.0058 at 28th, 21st, 14th, 7th and 0 days of storage, respectively. The TS content in the samples CS increases significantly ($p<0.05$) as the storage duration increased. The per cent TS was varied from 60.26±0.075 to 61.35±0% for AS and from 58.34±0.0058 to 60.54±0.0033% for CS samples during storage (upto 28th days) at refrigeration temperature. The difference between AS and CS sample was significant ($p<0.05$) except in between at 7th day of AS and 28th day of CS sample during storage (Fig. 10). The present results corroborates the findings of Nigam *et al.* (2009) [5], Kumar *et al.* (2011) [3] and Verma (2013) [11] when dairy products were stored for longer duration.

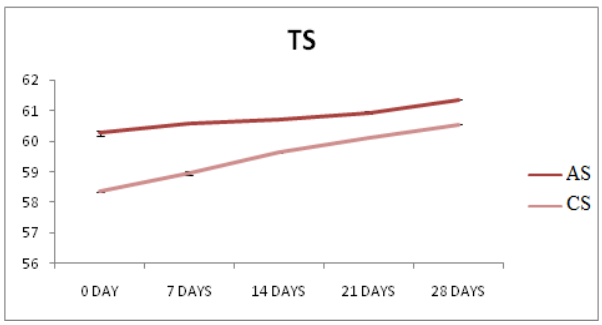

Fig 10: Changes in TS content of *Shrikhand* during storage

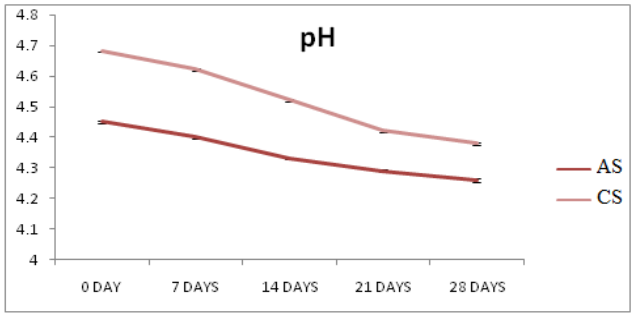

Fig 11: Changes in pH of *Shrikhand* during storage

Effect of storage on pH

The pH of the samples was the highest when tested at 0 day in both the groups (AS and CS) (Table 2; Fig. 11). The recorded values were 4.39±0, 4.36±0.0033, 4.34±0.0033, 4.31±0.0033 and 4.30±0.0033 found in AS group and 4.68±0.0033, 4.60±0.0033, 4.54±0.0033, 4.42±0.0033 and 4.38±0.0058 in CS group at 0, 7th, 14th, 21st and 28th days of storage, respectively. These values clearly depicts that the pH content of both product (AS and CS) were decreasing significantly ($p<0.05$) as the storage periods increased. The differences in the intensity of pH deterioration between AS and CS samples were significant ($p<0.05$) except at 0 day of storage in AS and at 28th day of CS sample. This may be due to increase in microbial activity during storage. Nigam *et al.* (2009) [5], Kumar *et al.* (2011) [3], Kumar (2013) [4] and Verma (2013) [11] have also reported similar views when dairy products were stored.

Effect of storage on acidity (% Lactic Acid)

The acidity of *Shrikhand* samples varied from 1.28±0.0033 to 1.41±0.0033 in AS and from 1.21±0.0033 to 1.35±0.0058 in CS groups during storage (Table 2; Fig. 12). The acidity content in both the groups significantly ($p<0.05$) inceased as the storage periods increased (Fig. 12). The average acidity content were 1.28±0.0033, 1.30±0.0033, 1.34±0.0033, 1.38±0.0058 and 1.41±0.0033 in samples AS and 1.21±0.0033, 1.23±0.0033, 1.26±0, 1.29±0.0033 and 1.35±0.0058 in samples CS at 0, 7th, 14th, 21st and 28th days of storage, respectively. The fig. 12 clearly depicts that the acidity content for both product (AS and CS) was increasing significantly ($p<0.05$) during refrigerated storage. The differences in the values between AS and CS samples during storage were significant ($p<0.05$) except at 7th day in AS and at 21st day in CS samples. This may be due to growth of microorganisms responsible for spoilage of milk and milk products. Similar observations were also recorded by Patel *et al.* (1993) [6], Jain (2003) [2], Sonawane *et al.* (2007) [10], Nigam *et al.* (2009) [5], Bhat *et al.* (2010) [1], Kumar *et al.* (2011) [3], Kumar (2013) and Verma (2013) [11] when various dairy products were stored for longer duration on refrigeration temperature.

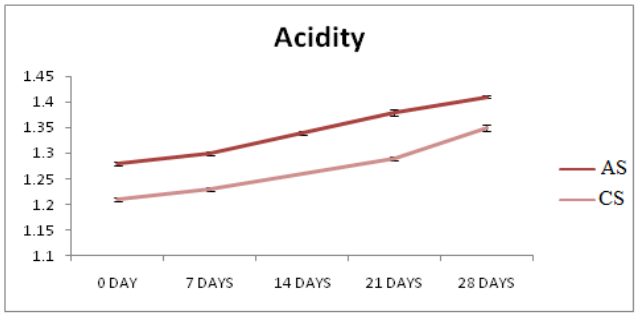

Fig 12: Changes in acidity of *Shrikhand* during storage

Changes in microbial characteristics of *Ashwagandha* root extract enriched *Shrikhand* during storage

The microbial count of the samples stored upto 28th days of storage at 10±1ºC in respect of *Lactobacillus bulgaricus* (LB), *Streptococcus thermophilus* (ST), standard plate count (SPC), yeast and mould count (YMC) and coliform count were studied and results are discussed here under:

Effect of storage on LB, ST and standard plate count (SPC)

During storage, one or more food characteristics can reach an undesirable state and as a consequence the consumer may reject the product or it can even cause detrimental health. At this moment, it is considered that the food has reached the end of its shelf life (Singh *et al.*, 2011) [5]. The effect of storage on LB, ST and SPC values (10^7cfu/g) for AS and CS samples are depicted in table 3.

The highest LB count (10^7cfu/g) for sample AS was 78.00±0.64 followed by 75.07±0.37, 69.20±0.64, 68.47±0.37 and 65.53±0.37 found at 0, 7th, 14th, 28th and 21st days of storage, respectively. The values presented in the table 3 (Fig. 13) clearly indicates that the highest LB (10^7cfu/g) for sample CS was 45.00±0.64 followed by 40.27±0.94, 33.37±0.37, 32.33±0.27 and 30.67±0.37 found at 0, 7th, 14th, 28th and 21st days of storage, respectively. The values presented in the table clearly indicates that as the storage increases the LB count in both the samples (AS and CS) decreased ($p<0.05$) except in between 14th and 28th day of storage. This may be due to adverse effect of storage on viability of LB after 21st day.

The highest ST (10^7cfu/g) for sample AS was 70.63±0.73 followed by 67.70±0.64, 63.33±0.61, 59.73±0.37 and 56.43±0.37 found at 0, 7th, 14th, 28th and 21st days of storage, respectively. The values presented in the table 3 (Fig. 14) clearly indicates that the highest ST (10^7cfu/g) for sample CS was 29.50±0.64 followed by 27.37±0.37, 26.12±0.37, 25.47±0.37 and 23.27±0.37 found at 0, 7th, 14th, 28th days of storage,respectively

Table 3: Effect of storage on microbial characteristics of *Ashwagandha* root extract enriched *Shrikhand* (AS) and *control Shrikhand* (CS) samples

Storage	LB (10^7 cfu/g)		ST (10^7 cfu/g)		SPC (10^7 cfu/g)		YMC (10^1 cfu/g)		Coliform	
	AS	CS	AS	CS	AS	CS	AS	CS	AS	CS
0 Day	78.00 ±0.64^{aA}	45.00 ±0.64^{aB}	70.63 ±0.73^{aA}	29.50 ±0.64^{aB}	161.00± 0^{aA}	109.33 ±0.67^{aB}	0.00± 0^{aA}	0.00± 0^{aA}	ND	ND
7th Day	75.07 ±0.37^{bC}	40.27 ±0.94^{bD}	67.70 ±0.64^{bC}	27.67 ±0.37^{bD}	162.67 ±0.67^{bC}	112.33 ±0.33^{bD}	0.00± 0^{aA}	0.00± 0^{aA}	ND	ND
14thDay	69.20 ±0.64^{cE}	33.37 ±0.37^{cF}	63.33 ±0.61^{cE}	26.12 ±0.37^{cF}	164.66 ±0.33^{cE}	115.33 ±0.33^{cF}	1.00± 0^{bB}	1.00± 0^{bB}	ND	ND
21thDay	65.53 ±0.37^{dG}	30.67 ±0.37^{dH}	56.43 ±0.37^{dG}	23.27 ±0.37^{dH}	166.33 ±0.33^{dG}	119.00 ±0.57^{dH}	2.33±0.33^{cC}	2.00± 0^{cC}	ND	ND
28thDay	68.47 ±0.37^{eI}	32.27 ±0.37^{eJ}	59.73 ±0.37^{eI}	25.47 ±0.37^{eJ}	171.67 ±0.88^{eI}	125.00 ±0.58^{eJ}	5.67±0.33^{d}	3.00± 0^{d}	ND	ND

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, P<0.05)
Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, P<0.05)
CS=Control *Shrikhand* samples,
AS= *Ashwagandha* root extract enriched *Shrikhand* (optimised level)
ND is abbreviated as Not Detected

The values presented in the table 3 (Fig. 15) clearly indicates that the highest SPC (10^7cfu/g) was recorded at 28th day of storage for both (AS and CS) samples. As the storage increases the SPC count also increased ($p<0.05$) for both (AS and CS) samples. The difference between AS and CS sample was found significant ($p<0.05$) during storage. The increase in SPC count may be due to growth of undesirable microorganisms during storage.

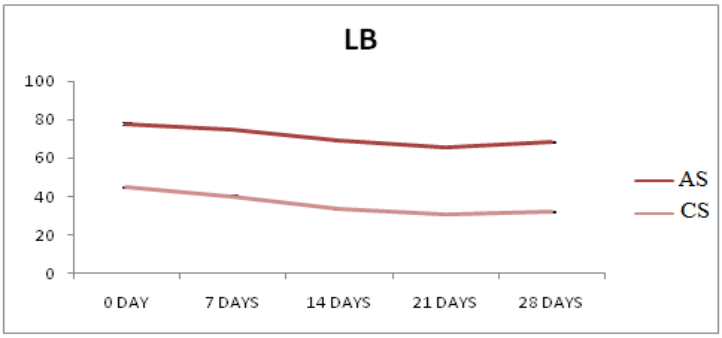

Fig 13: Changes in LB count of *Shrikhand* during storage

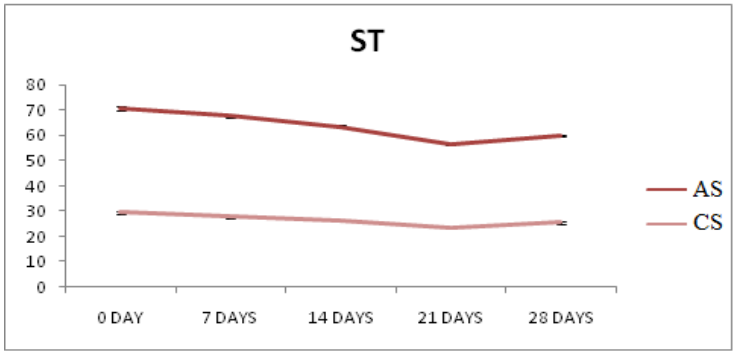

Fig 14: Changes in ST count of *Shrikhand* during storage

The table 3 clearly depicts that the viable count of LB is higher than ST which may be due to production of high lactic acid by LB. The SPC contains high viable count than both microorganisms *viz*., LB and ST which may be due to presence other microorganisms. The fig. 15 also depicts that the viable count of AS is higher than CS during storage. This may be due to presence of oligosaccharides, a prebiotic, in AS (Scalabrini *et al.*, 1998; Singh and Singh, 2013) [8]. The viability of LB and ST decreased during storage which may be due to death of cells. In conformity with our findings, Kumar (2009) [4] also reported that the viability of LB and ST in frozen yoghurt decreased during storage.

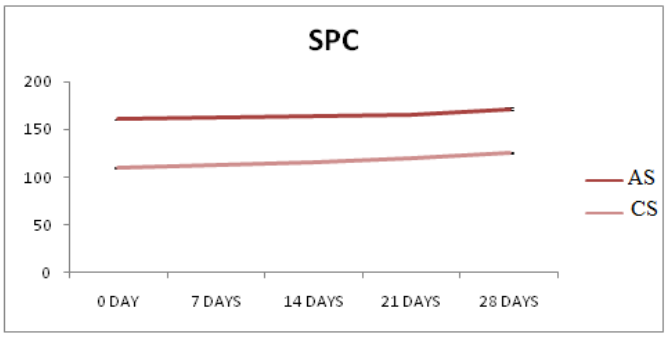

Fig 15: Changes in SPC of *Shrikhand* during storage

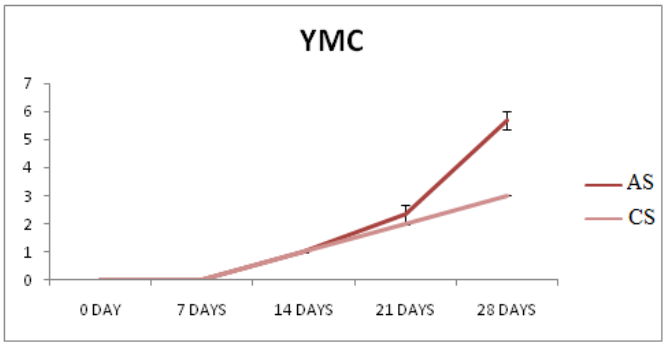

Fig 16: Changes in YMC of *Shrikhand* during storage

Effect of storage on YMC and coliform count

The table 3 (Fig. 16) clearly depicts that upto 7th day the YMC was absent but it augmented to 5.67±0.33x10^{1}cfu/g for AS and 3.0±0x10^{1}cfu/g for CS groups upto 28th day of storage. The differences in the value of YMC in both AS and CS groups were non-significantly increased ($p>0.05$) with increase in storage period upto 21th days, but further enhancement in storage period indicates increased ($p<0.05$) in YMC. The coliform count was absent during storage in both (AS and CS) samples. Patel *et al.* (1993) [6], Jain (2003) [2], Sonawane *et al.* (2007) [10], Nigam *et al.* (2009) [5], Bhat *et al.* (2010) [1], Kumar *et al.* (2011) [3], Kumar (2013) [4] and Verma (2013) [11] also reported similar decline in the YMC of various dairy products during refrigerated storage.

Loss in DPPH and ABTS during storage

The table 4 clearly shows the result of loss in percentage of DPPH (1, 1 – Diphenyl-2-picrylhydrazyl) Inhibition and ABTS (2, 2- Azinobis- 3- ethylene benzoline-6 sulphonic acid) from 0 day of storage to 28 days of storage of AS. There was not significant decrease ($p<0.05$) in DPPH and ABTS during first 7 days of storage. Fig. 17 and 18 clearly shows the losses in DPPH and ABTS after 7 days and upto 28 days of storage.

Table 4: Loss in % DPPH and %ABTS during storage

Days	% DPPH Inhibition	%ABTS
0 day	81.40 ± 0^{aA}	73.25 ± 0.67^{aB}
7th day	80. 10 ± 0.67^{bC}	70.85 ± 0.33^{bD}
14th day	67.87 ± .33^{cE}	55.45 ± 0.33^{cF}
21st day	45.54 ± 0.33^{dG}	32.80 ± 0.57^{dH}
28th day	25.65 ± 0.88^{eI}	20.50 ± 0.58^{eJ}

Values bearing different small superscripts (a, b, c) in a column differ significantly (Duncan test, $p<0.05$)
Values bearing different capital superscripts (A, B, C) in between column differ significantly (Duncan test, $p<0.05$)

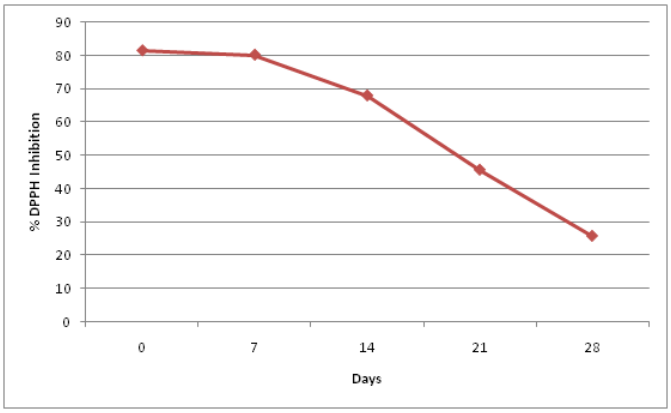

Fig 17: Changes in %DPPH inhibition of *Ashwagandha* root extract enriched *Shrikhand* during storage

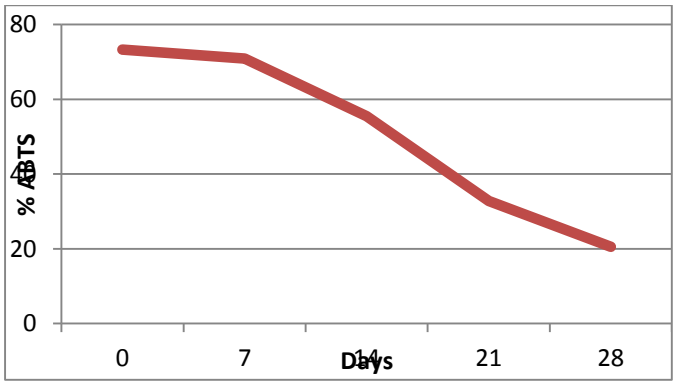

Fig 18: Changes in %ABTS of *Ashwagandha* root extract enriched *Shrikhand* during storage

Conclusion

Ashwagandha root extract enriched *Shrikhand* was significantly rich ($p<0.05$) in protein, ash, total solid, acidity and fat content but lowest ($p<0.05$) in moisture and pH and is comparable with control *Shrikhand*. During storage, the color and appearance scores of control *Shrikhand* (CS) (8.81±0.0033 to 8.41±0.0033) were significantly high ($p<0.05$) as compared to *Ashwagandha* root extract enriched *Shrikhand* (AS) (8.46±0.0058 to 8.25±0.0058) samples. The values in both the groups decreased ($p<0.05$) as the storage periods increased. The flavor score decreased ($p<0.05$) as the storage periods increases in both types of *Shrikhand* (AS and CS). The difference in the values between the groups recorded at various intervals were significant ($p<0.05$). The texture ranged from 7.60±0 to 6.83±0.033 in AS and 7.58±0.0033 to 6.80±0 in CS samples. The texture score decreased significantly ($p<0.05$) at the storage after 14th day in AS and 7th day in CS Samples. The overall texture score of the samples AS were higher than CS during storage. Irrespective of the groups, the sweetness score of the samples decreased ($p<0.05$) as the storage periods increased. From 7th day

onwards, the sweetness score in CS samples were significantly high ($p<0.05$) than AS samples. The overall acceptability score was inversely proportionate ($p<0.05$) to the storage periods. The overall acceptability score of CS samples were significantly higher ($p<0.05$) than AS samples, except at 21st day of storage.

The highest protein level in groups AS (10.57± 0.033%) and CS (8.91± 0.0033%) was recorded at 28th day of storage. The increase in protein was high ($p<0.05$) in both types of samples during storage except from 14th to 21st days in CS samples. The protein content was significantly ($p<0.05$) higher in the samples AS than CS, except at 14th day of storage. The fat per cent increased ($p<0.05$) in both types of *Shrikhand* except from 21st to 28th day of AS samples. At 0 day, the difference in the values between AS and CS samples were not significant. The ash content in all the samples apparently increased as storage periods increased. The ash content in the AS samples were very high ($p<0.05$) than CS. The moisture per cent was significantly ($p<0.05$) high in samples CS than AS during storage. The moisture content decreased ($p<0.05$) in both the groups as the storage periods increased except from 7th to 14th days in AS samples. An inverse relationship was recorded between moisture content and total solids in the samples. As the storage periods prolonged, the pH decreased and acidity content increased ($p<0.05$) in both types of *Shrikhand*. The differences in the values between AS and CS samples were significant ($p<0.05$).

During entire storage periods, the counts of LB (10^7cfu/g) and ST (10^7cfu/g) were significantly high ($p<0.05$) in samples AS than CS. These counts decreased ($p<0.05$) in all the samples up to 21st day of storage, but thereafter it increased ($p<0.05$). The SPC count increased ($p<0.05$) as the storage periods increases in both the groups. The differences in the values between the groups were high ($p<0.05$). The Yeast and Mould counts (YMC) were not observed upto 7th day, but at 14th day it augmented significantly ($P<0.05$) in both the groups (AS and CS). The coliform counts (10^1 cfu/g) were not observed in any sample during storage. With increasing of number of days the DPPH and ABTS also decreased significantly ($p<0.05$) from 81.40 to 25.65 and 73.25 to 20.50 respectively.

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