RESEARCH ARTICLE

Storage Potential and Chemical Composition of Wet Distillers Grains with Solubles (WDGS) Treated with Different Additives

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Abstract

To study the keeping quality of Wet Distillers Grains with Solubles (WDGS) during 12 days of the storage period, WDGS was treated with salt, citric acid, lactic acid, propionic acid and sulfuric acid at 1% of DM. No surface spoilage was observed up to 6 days in any of the treatments and up to 12 days in propionic and sulfuric acid treated WDGS. Mean pH was significantly lower ($p<0.01$) in 1% sulfuric acid treated WDGS. Mean NH3-N (mg/100g) concentration increased ($p<0.01$) from 0 day to 6th day and then decreased towards 12th day of storage among treatments with higher mean value in 1% sulfuric acid (100.32) and lower value in 1% propionic acid (67.26) treated WDGS. Yeast count increased towards 12th day of storage in all the treatments with higher counts in 1% salt treated WDGS. From 0 day to 12th day of storage, mean CP values were decreased significantly ($p<0.01$) with higher ($p<0.01$) mean CP value of 37.18% in propionic acid treated WDGS. Based on the overall results, it was concluded that WDGS can be stored up to 6 days without any additive, further with 1% propionic acid (DMB) treatment it can be stored up to 12 days in good quality.

Keywords: Additives, Chemical composition, Keeping quality, WDGS.

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.3.17

Introduction

India possess huge livestock population including about 193.6 million cattle and 111.8 million buffaloes (FAO, 2022), necessitating the use of alternative feed resources like wet distillers grain with solubles (WDGS). The increased ethanol production from grain in recent years has resulted in increased availability of wet distillers grains with solubles (WDGS). WDGS is the main by-product of brewing industry, accounting about 85% of total by-products released (Mussatto *et al.,* 2006). WDGS is rich in protein with 1.8 times greater rumen escape protein value of soybean meal (Zentek *et al.,* 2014) and 130% the energy value of maize (Klopfenstein *et al.,* 2008). However, WDGS contains high moisture with 30-35% DM (Buckner *et al.,* 2011) resulting in storage issues. Shelf-life of WDGS depends on exposure to oxygen and ambient temperature (Christensen *et al.,* 2010) and the nutritional quality of distillers' grain rapidly declines due to mould and yeast growth (Balamurugan *et al.,* 2018). In this regard the present study was undertaken to know the keeping quality of WDGS with different additives.

Materials and Methods

In order to suggest the storage practice to farmers, the WDGS was subjected to different treatments such as addition of salt or acids such as citric, lactic, propionic and sulphuric acid at 1% of dry matter. WDGS without any additive was used as control. After procuring WDGS, on first day 20 kg of WDGS was mixed thoroughly with respective additive @ 1% DM, and 2 kg of the treated WDGS was stored at room temperature of 25 to 30°C in separate plastic jars in duplicate and covered with lids. Samples were collected from each jar of each treatment on 0, 3, 6, 9 and 12th day to analyse for pH, NH_3-N and yeast and mould count. A separate set of jars

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How to cite this article: Kavitha, P., Ravi, A., Srinivasa Rao, D., Ravindra Reddy, Y., Padmaja, K., & Chaitanya, R. K. (2025). Storage Potential and Chemical Composition of Wet Distillers Grains with Solubles (WDGS) Treated with Different Additives. Ind J Vet Sci and Biotech, 21(3), 85-90.

Source of support: Nil

Conflict of interest: None

Submitted 01/02/2025 **Accepted** 02/03/2025 **Published** 10/05/2025

containing each treatment was kept undisturbed for surface spoilage study during the storage period of 12 days. The remaining quantity of WDGS of each treatment was kept in plastic buckets and about 1 kg of WDGS was collected on 0, 3, 6, 9 and 12 day of storage and the samples were dried in hot air oven at 60°C for 48 h in aluminium trays and the dried samples were ground in a Wiley mill, stored in plastic bottles for chemical analysis (AOAC, 2005) and mycotoxin estimation.

Surface Spoilage and pH of WDGS

The samples stored for different days were examined for spoilage by observing for growth of moulds, fungus and colour change.

The pH was measured using a pH meter (Cyberscan pH 510, Eutech instruments) immediately after sampling. Each sample was mixed thoroughly with distilled water in 1:10 ratio. The pH of the distilled water was noted first, then the pH of the treatment was checked.

Estimation of NH_3-N, Yeast and Mould Count

The WDGS juice was extracted with a 1:10 ratio of physiological saline to sample (fresh weight) to assess the concentration of ammonia nitrogen (Sastry *et al*., 1999).

The yeast and mould count in the WDGS of different treatments was estimated on 0, 3, 6, 9 and 12th day of preservation by pour plate method using HiCrome OGYE agar with Oxytetra selective supplement by serial dilution process (Elzbieta *et al*., 2005).

Estimation of Mycotoxins

For estimation of multi-mycotoxins, samples were sent to Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Namakkal, Tamil Nadu.

Statistical Analysis

The data was subjected to analysis through software (version 23.0; SPSS, 2015) by applying analysis of variance and the treatment means were ranked using Duncan's multiple range test with a significance at $p<0.05$ (Snedecor and Cochran, 1994).

Results and Discussion

No surface spoilage was seen up to 6 days of storage in any of the treatments, except in 1% salt treated WDGS. No visible spoilage was observed with 1% propionic acid and 1% sulphuric acid treatments up to 12 days of storage and heavy spoilage (extensive fungal growth with discoloration) was seen in samples stored without any preservative by the 12th day of storage (Fig. 1). Likewise, Kitaw *et al.* (2022) noticed no surface spoilage at 15°C until 6th day, at 20°C until 4th day of storage and with considerable changes at 25°C with duration. Allen *et al.* (1975) also observed reduced sub-surface spoilage of WDGS stored for 14 days with the addition of propionic acid at 0.40% of wet brewers' grain weight.

In the present study the pH (Table 1) of control, 1% salt, 1% citric acid and 1% lactic acid treated WDGS increased significantly ($p<0.01$) from 0 day to 6th day of storage and then decreased to 12th day of storage. The increase in pH from 0 day to 6th day of storage correlated with the results of McClurkin and Ileleji (2015), who reported increased ($p<0.05$) pH from 0 day to 7th day of storage. The pH of 1% propionic acid and 1% sulfuric acid treated WDGS increased ($p<0.01$) from 0 day to 12th day of storage in the present study. Mean pH of 1% sulfuric acid treated WDGS was lower ($p<0.01$) than other treatments. As well, lowest pH in WDGS ensiled with 0.5% sulfuric acid compared to other treatments was noted by Kazemi *et al.* (2014). The changes in pH of stored WDGS with different additives in the present study may be attributed to the changes in the NH_3-N content in stored WDGS.

The NH_3-N concentration in WDGS shows the degree of protein degradation. Mean NH_3-N (mg/100g WDGS) (Table 2) concentration in WDGS increased significantly ($p<0.01$) from 0 day (70.79) to 6th day (92.76) and decreased towards 12th day of storage (67.39) across treatments. In consideration with the microbial growth and surface spoilage it may be ascertained that the increased number of microbes utilize the released NH_3-N and resulted in lower concentrations after 6 days of storage. Overall mean NH_3-N (mg/100g WDGS) concentration was significantly ($p<0.01$) higher in 1% H_2SO_4 (100.32) and lower in 1% propionic acid (67.26) treated WDGS. In contrast Kazemi *et al.* (2014) reported lowest NH_3-N in WDGS ensiled with 0.5% sulfuric acid. Increased NH_3-N concentration in 1% H_2SO_4 treated DDGS might be due to protein degradation by adding H_2SO_4 (Eslamian *et al.,* 2013). Lower NH_3-N concentration in 1% propionic acid treated WDGS might be due to decreased proteolytic activity because of its preservative effect.

No mould count was observed in the present study in any of the treatments during the 12 days of storage period. In contrast Kitaw *et al.* (2022) found increased mould count with storage duration at 25°C. Yeast count (Table 3) increased towards 12th day of storage in all the treatments with higher counts in 1% salt treated WDGS. Similarly, McClurkin and Ileleji (2015) and Alvarez *et al.* (2016) found increased yeast count from 0 day to 7-10 days of storage period. Increased yeast count and considerable visual fungal growth with discoloration in 1% salt treated WDGS is in accordance with the findings of Hatungimana and Erickson (2019), who reported that wet brewers' grains (WBG) treated with 3.8% salt had more yeast count than treated with 0.15% commercial preservative. Lowest yeast count was observed with 1% propionic acid treatment during the storage period in the present study, indicating its capacity to preserve the material. All the treatments at different days of storage were negative for multi-mycotoxins. This finding is not in agreement with the results of McClurkin and Ileleji (2015) and Simas *et al.* (2007), who reported presence of mycotoxins in stored WDGS.

Mean values of proximate composition of WDGS with different treatments at different days of storage are presented

in Table 4. DM content did not vary within the treatment during 12 days of storage. Mean DM content was significantly ($p<0.05$) higher in 1% salt treated WDGS. Salt addition decreases water activity due to its osmolaric effect and resulted in increased DM content as stated by Balamurugan *et al.* (2018). CP content (Fig. 2) was decreased ($p<0.05$) with duration of storage in control, 1% salt, 1% lactic acid and 1% H_2SO_4 treated WDGS. Mean CP values of WDGS decreased significantly ($p<0.01$) with the duration of storage from 0 day to 12th day and the loss in CP was about 9.06% by 12 day of storage. No changes ($p>0.05$) were observed in CP content of 1% propionic acid treated WDGS (Fig. 2) up to 12 days of storage. Mean CF was significantly ($p<0.01$) lower in 1% H_2SO_4 treated WDGS (Fig, 2). In harmony with our results, Eslamian *et al.* (2013) reported decreased ($p<0.05$) CP, NDF and ADF contents with the addition of 2% sulphuric acid in ensiled wet brewers grain for 60 days compared to control and molasses treated WBG and stated decrease in CP might be due to increased protein degradation. EE content in different treatments did not differ significantly in different treatments with duration of storage, except in 1% citric acid treated WDGS. This can be strengthened by the statement given by Moriel *et al.* (2015) that the extent of microbial utilization of crude fat was less compared to other nutrients in stored WDGS. Differences in NFE and TA might be due to relative changes in other nutrients (Santos *et al.,* 2010).

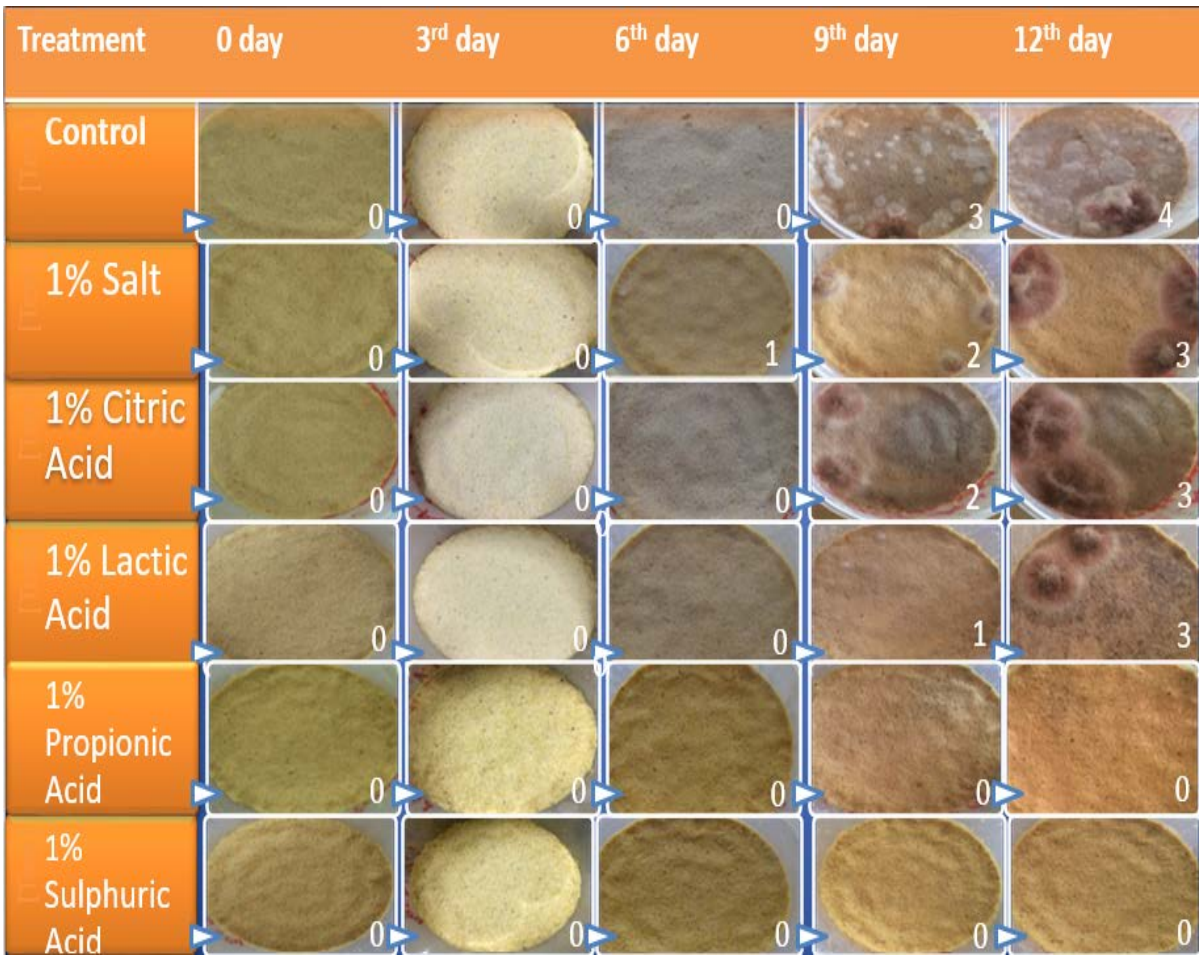

Fig. 1: Surface spoilage of WDGS with different treatments during 12 days of storage period.Note: Number on day wise photograph shows rating of spoilage on 0-4 scale. 0 = no visible spoilage; 1 = slight fungal growth; 2 = moderate fungal growth with discoloration; 3 = considerable fungal growth with discoloration; 4 = Extensive fungal growth with discoloration

Table 1: Effect of different additives on pH of WDGS during storage

Treatment	Storage days					Mean**
	0	3	6	9	12	
Control**	$3.41^{d}\pm0.04$	$3.59^{c}\pm0.01$	$4.45^{a}\pm0.01$	$3.98^{b}\pm0.06$	$3.91^{b}\pm0.03$	$3.87^{A}\pm0.10$
1% Salt**	$3.47^{d}\pm0.02$	$3.58^{c}\pm0.03$	$4.14^{a}\pm0.03$	$3.68^{b}\pm0.01$	$3.64^{bc}\pm0.02$	$3.70^{C}\pm0.06$
1% Citric acid**	$3.12^{c}\pm0.11$	$3.98^{b}\pm0.06$	$4.42^{a}\pm0.05$	$3.77^{b}\pm0.07$	$3.98^{b}\pm0.07$	$3.85^{A}\pm0.12$
1% Lactic acid**	$3.23^{c}\pm0.07$	$3.43^{c}\pm0.05$	$4.06^{a}\pm0.11$	$3.73^{b}\pm0.06$	$3.87^{ab}\pm0.03$	$3.66^{C}\pm0.08$

1% Propionic acid**	3.33^{d}±0.02	3.35^{d}±0.03	4.03^{b}±0.04	3.88^{c}±0.04	4.29^{a} ±0.06	3.77^{B}±0.10
1% H_2SO_4 **	2.63^{d}±0.05	3.07^{c}±0.03	3.43^{b}±0.02	3.41^{b}±0.04	3.62^{a}±0.03	3.23^{D}±0.09
Mean**	3.20^{e}±0.07	3.50^{d}±0.06	4.09^{a}±0.08	3.74^{c}±0.05	3.88^{a}±0.06	--

^{abcd}Values in a row not sharing common superscripts differ significantly ** ($p<0.01$).
^{ABCD}Values in a column not sharing common superscripts differ significantly ** ($p<0.01$).

Table 2: Effect of different additives on NH_3- N (mg/100g) of WDGS during storage

Treatment	Storage days					Mean**
	0**	3**	6**	9**	12**	
Control**	64.26^{e}±0.59	76.25^{b}±0.63	103.30^{a}±0.95	72.73^{c}±0.37	69.86^{d}±0.38	77.28^{C}±4.53
1% Salt**	63.92^{d}±0.06	72.08^{c}±0.20	112.13^{a}±0.78	75.82^{b}±0.42	65.0^{d} ±0.50	77.79^{C}±5.91
1% Citric acid**	65.21^{d}±0.34	78.55^{c}±0.50	85.14^{b}±0.22	115.60^{a}±0.61	79.44^{c}±0.69	84.79^{B}±5.58
1% Lactic acid**	64.26^{bc}±0.63	61.95^{c}±0.97	66.69^{b}±0.69	76.19^{a}±0.98	58.95^{d}±0.15	67.26^{D}±1.94
1% Propionic acid**	64.38^{c}±0.94	71.83^{a}±0.78	68.67^{b}±0.55	62.95^{c}±0.13	56.63^{d}±0.26	64.89^{E}±1.74
1% H_2SO_4	102.69^{b}±0.94	117.65^{a}±0.40	117.11^{a}±0.17	89.69^{c}±0.83	74.47^{d}±0.09	100.32^{A}±5.52
Mean**	70.79^{d}±4.31	80.51^{c}±5.14	92.76^{a}±5.81	82.16^{b}±5.09	67.39^{e}±2.44	--

^{abcd} Values in a row not sharing common superscripts differ significantly **($p<0.01$).
^{ABCD} Values in a column not sharing common superscripts differ significantly ** ($p<0.01$).

Table 3: Effect of different additives on yeast count (cfu/g) of WDGS during storage

Treatment	Storage days				
	0 day	3rd day	6th day	9th day	12th day
Control	0	$2x10^4$	$2.3x10^5$	$2x10^6$	$9x10^6$
1% Salt	0	0	$8.2x10^5$	$3.2x10^6$	$2x10^7$
1% Citric acid	0	$2x10^4$	$1x10^5$	$6x10^5$	$1.7x10^7$
1% Lactic acid	0	$1x10^4$	$4.2x10^5$	$4x10^6$	$5.25x10^6$
1% Propionic acid	0	0	$6x10^3$	$1x10^4$	$1x10^4$
1% Sulfuric acid	0	0	$4x10^4$	$1.2x10^5$	$6x10^5$

Table 4: Overall effect of additives and storage duration on chemical composition of WDGS (on DM bases)

	Proximate composition (%)					
	DM	CP	CF	EE	NFE	TA
Overall effect of treatment						
Control	27.87^{b}±0.22	35.55^{bc}±0.53	7.60^{ab}±0.11	6.75^{b}±0.16	45.20^{ab}±0.54	4.90^{d}±0.06
1% Salt	29.23^{a}±0.36	35.62^{bc}±0.46	7.57^{ab}±0.23	6.67^{b}±0.18	44.49^{b}±0.38	5.65^{a}±0.11
1% Citricacid	28.21^{b}±0.26	36.27^{b}±0.41	7.74^{a}±0.14	7.17^{a}±0.18	43.35^{c}±0.27	5.48^{b}±0.23
1% Lactic acid	28.26^{b}±0.26	35.12^{c}±0.62	7.22^{b}±0.04	7.14^{a}±0.12	45.48^{ab}±0.64	5.06^{c}±0.06
1% Propionic acid	28.32^{b}±0.36	37.18^{a}±0.21	7.43^{ab}±0.07	7.40^{a}±0.17	43.04^{c}±0.18	4.96^{cd}±0.07
1% Sulfuric acid	28.48^{b}±0.21	34.95^{c}±0.57	6.49^{c}±0.17	6.78^{b}±0.12	46.31^{a}±0.71	5.47^{b}±0.10
SS	*	**	**	**	**	**
Overall effect of storage duration						
0 day	28.33 ± 0.03	37.60^{a}±0.31	7.23±0.02	7.07^{b}±0.01	43.11^{c}±0.22	5.07^{b}±0.01
3rd day	28.52± 0.37	36.49^{b}±0.33	7.23±0.10	6.74^{c}±0.10	44.08^{bc}±0.41	5.41^{a}±0.14
6th day	28.82±0.32	35.54^{c} ±0.29	7.28±0.17	6.84^{bc}±0.17	44.95^{ab}±0.48	5.39^{a}±0.21
9th day	27.95±0.21	35.16^{c} ±0.33	7.33±0.19	6.89^{bc}±0.15	45.33^{a}± 0.56	5.35^{a}±0.10
12th day	28.37±0.30	34.19^{d}±0.45	7.63±0.27	7.38^{a}±0.21	45.75^{a}±0.63	5.04^{b}±0.11
SS	NS	**	NS	**	**	**

^{ab}Values in column bearing different superscripts differ significantly.*$p<0.05$;**$p<0.01$; NS-Non significant

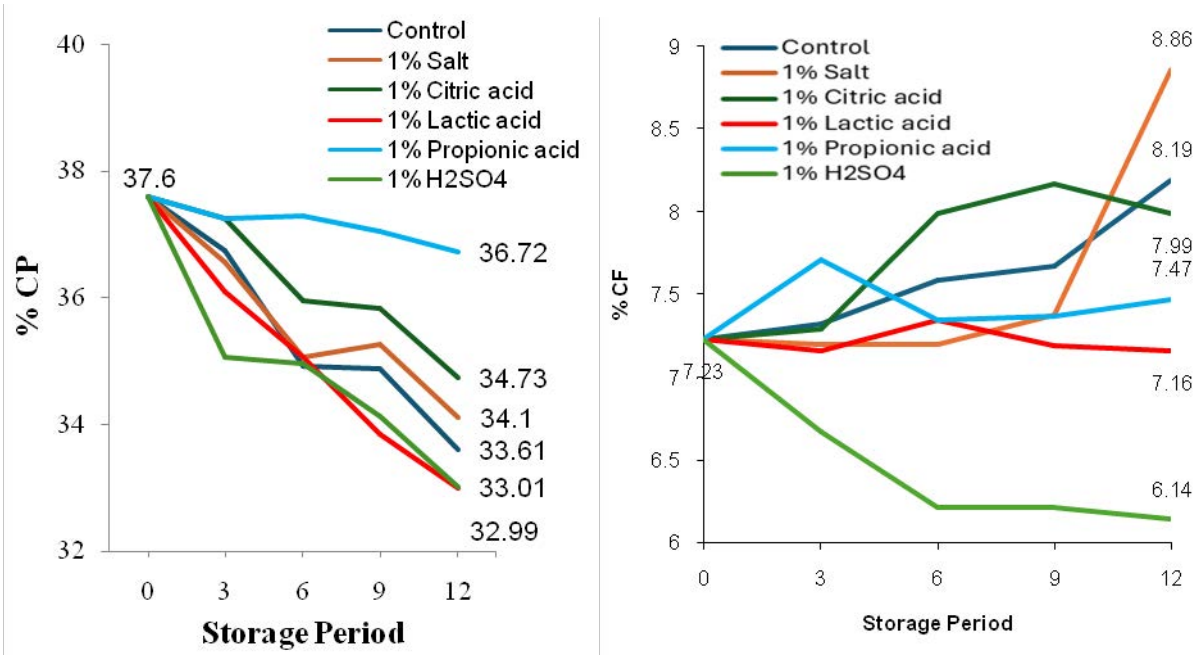

Fig. 2: Effect of additives on percent CP and CF of WDGS during storage

Conclusion

Based on the overall results of the study it was concluded that WDGS can be stored up to 6 days without any additive, further with 1% propionic acid (DMB) treatment it can be stored up to 12 days in good quality.

Acknowledgement

The authors are grateful to Sri Venkateswara Veterinary University for providing the necessary facilities and financial support to carry out the research work.

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