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Research article

Effects of brewer's spent yeast inclusion level and ensiling duration on fermentative, fungal load dynamics, and nutritional characteristics of brewer's spent yeast-based silage

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A R T I C L E I N F O

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A B S T R A C T

Objective: This study was conducted to evaluate the effect of brewer's spent yeast (BSY) inclusion level and ensiling duration (ED) on fermentative, fungal load dynamics, and nutritional characteristics of brewer's spent-yeast based silage.
Materials and methods: To prepare the silages materials, 4 BSY inclusion levels (0, 10, 20, and 30%) to replace BSG and 3 ED (2,4 and 6 weeks) were arranged in 4 × 3 factorial combination using a completely randomized design (CRD) in 5 replications. The ratio of brewery spent grain (BSG) to wheat bran (WB) used majorly as protein and energy sources, respectively was 30:69 with a 1% salt addition. Parameters measured include observation for surface spoilage, yeast and mold colony count, silage temperature, pH, total dry matter loss (TDML), major proximate, detergent fractions and permanganate lignin, in-vitro organic matter digestibility (IVOMD) and estimated metabolizable energy (EME) values.
Results: The study revealed that at any BSY inclusion level and ED, extensive mold growths and discolorations were not observed. However, slightly higher values of 6.5, 5.7, and 12.2 colonies forming unit (CFU)/g DM yeast, mold, and total fungal counts (TFC), respectively were recorded only at the 6 weeks of the fermentation period with 30% BSY inclusion level. Brewer's spent yeast inclusion level and ED had a significant ($P < 0.05$) effect on silage temperature (mean = 18.05 °C) and pH (mean = 4.16). Among proximate and detergent values, crude protein (mean CP g/kg DM = 204.5), neutral detergent fiber (mean NDF g/kg DM = 552.9), and acid detergent fiber (mean ADF g/kg DM = 115.9) responded significantly ($P < 0.05$) to both BSY inclusion levels and ED.
Conclusion: Among nutritional quality, CP, IVOMD, and EME of silage samples were subjected to substantial improvements when silage masses were prepared from 20% BSY inclusion levels and when the same silage materials were allowed to ferment for four weeks. In addition, the lab-based experiment should be supported with additional silage quality parameters like volatile fatty acid content of the silage materials and supplementation of ruminant livestock under both on-station and on-farm conditions using either a pilot and/or target animals.

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1. Introduction

In developing countries, livestock production and productivity are mainly constrained by a shortage of feed and the costly price of commercial concentrates [1]. Feeding commercial concentrate would be possible if and only if farmers in these countries have access to such expensive concentrate feeds [2]. As a result, the poor availability of high-quality feeds is particularly becoming a major concern to smallholder dairy farmers in these countries. In Ethiopia, feed price instability and fluctuations in the supply of local raw materials are some of the challenges that feed processing industries are currently confronting. Consequently, most dairy farms and consumers are challenged by the ever-rising cost of processed animal feeds and dairy products, respectively [3,4].

Smallholder dairy farmers living around the brewery factories have been utilizing the cheap by-products obtained from local industries. The most common livestock feed obtained from the production of beer includes BSG (brewery-spent grain) and BSY (brewery-spent yeast) [5]. Brewer's spent yeast, the second major by-product of the brewing industry is the yeast that is left after the brewing process is over. It is a fermented product containing inactive yeast cells and other metabolites formed during fermentation [6]. Due to its rapid growth during the fermentation stage, the yeast mass can multiply about 3–5 times, generating a production surplus and becoming the second-largest waste from breweries [7]. It is estimated that 15 to 18 tons of surplus yeast can be produced per 10,000 hL (hl) of the finished beer. It is an excellent source of protein, non-starch polysaccharides, B-complex vitamins, minerals, and other unidentified growth factors but the disposal of this by-product is a problem for the breweries [8,9]. The use of sewer lines and landfills as a method of BSY disposal is expensive and unsustainable [5]. Therefore, the most practical and commonly used outlet for the disposal of BSY in other countries is as a constituent of animal feeds, especially as a cheap source of protein as well as a source of minerals and B vitamins [10]. Similarly, in Ethiopia, liquid brewer's spent yeast (LBSY) is used as a common protein source to replace costly but conventionally used agro-industrial by-product feeds. About 360,758 hl of LBSY is produced every year but a small proportion of this by-product is used as dairy cattle feed and a large volume is seen accumulated at production sites causing disposal and public health problems [3,11].

As the feed costs for small dairy farmers in Ethiopia represent more than 70% of total costs [12], it is therefore, essential to reduce the cost of feeding by utilizing agricultural and industrial by-products like BSY with relatively lower prices than those of commercial concentrate. Consequently, there is a need to develop appropriate ways of using this by-product as livestock feed with higher nutritional value but higher water content that hinders its transportation and storage [10]. According to Ref. [13], a total mixed ration (TMR) silage made by mixing wet brewery by-products with other feed ingredients helps to omit the time of mixing before feeding, minimizes the risk of effluent production, and avoids self-selection of feeds by animals. Thus, making silage mixing wheat bran (WB), BSG, and LBSY can be developed in areas where flour and beer manufacturing factories are located. However, information on the optimal BSY inclusion level and ensiling duration of brewer's spent yeast-based silage has not been studied yet. Therefore, the objective of this study was to evaluate the effects of BSY inclusion levels and ensiling duration (ED) on fermentative, fungal load dynamics, and nutritional characteristics of BSY-based silage.

2. Materials and methods

2.1. Experimental location, sampling procedures and measurements

The study was conducted at Holetta Agricultural Research Center (HARC) animal nutrition, dairy microbiology, and soil laboratories. The center is located at 9°03′28.82″ E latitude and 38°30′17.59″ E longitude at an elevation of 2,400 m above sea level. The average annual rainfall in the area is 1144 mm, while the average daily temperature ranges from 6 to 21 °C and with an average relative humidity of 60.6%. Freshly produced BSY and BSG samples were obtained from the Heineken brewery factory and transported in air-tight jar cans and plastic bags, respectively to HARC. Wheat bran (WB), the other major input for silage preparation was purchased from a nearby animal feed processing plant. Both BSY and BSG samples were stored below −20 °C until they were used for experimental silage preparation. Brewery spent grain and BSY samples were then removed from the deep freezer for thawing under sun drying for a period of 6 h. The ratio of BSG as a protein source and WB as an energy source in the on-station formulated base/control concentrate diet was 30:69 with 1% salt (NaCl) addition (Table 1). Brewer's spent yeast slurry was added to a control concentrate diet at the rate of 0, 10, 20, and 30% to replace the protein source i.e., BSG on a fresh matter basis (W/W). The resultant mix after adding the WB (energy source) was then subjected to ensiling using three ensiling periods (2, 4, and 6 weeks). The inclusion levels of BSY suggested for the present study were directly adopted from previous research recommendations [2, [14]. Completely randomized design with a 4 × 3 factorial combination each treatment combination replicated five times was used. The mixed feed ingredients were

Table 1
Experimental protocol used to formulate the treatments of BSY-based silage diets.

Treatment	Inclusion level of BSY slurry to BSG (%,W/W)		Inclusion level of other ingredients (total % DM basis)		Ensiling period (weeks)	Replications
	BSY	BSG	WB	Salt		
T1	0	100	69	1	2, 4 and 6	5X
T2	10	90	69	1		
T3	20	80	69	1		
T4	30	70	69	1		

BSG = brewery spent grain, BSY = brewer's spent yeast, DM = dry matter, WB = wheat bran; W/W = weight by weight basis.

then filled into a mini plastic bucket silo of 4 L capacity, the inside of which, have been lined with a plastic sheet. All plastic buckets were filled at a similar packing density (2000g per plastic bucket on a fresh matter basis) and inverted upsides down to allow some effluents out. The final silage mixes for each treatment was compacted simultaneously by hand and periodic tamping was made with a wooden stick. The tightly packed mini silos were immediately closed and sealed to ensure air-tight conditions during the course of fermentation for the respective durations. Finally, experimental silage diets were incubated at room temperature (∼20 °C) after placing a heavy load over the top of each silo to exclude remaining air from the silage masses.

2.2. Sensory evaluation of brewer's spent yeast-based silage

Panelists containing an aggregate of five experienced professionals from the HARC Feeds and Nutrition Research Department rated the quality of experimental BSY-based silage diets by measuring surface mold occurrences as judged by sensory parameters that included the color, texture, odor, and extent of mold cover for each silage replicates. One day of training was given to all panelists and informed consent was obtained from all participants before the start of the evaluation process. The panel evaluation was converted into a numeric scale from 0 to 5, where 0 = no visible spoilage; l = slight mold growth; 2 = mold growth + discoloration; 3 = mold growth, discoloration + surface collapse; 4 = mold growth, discoloration, surface collapse + slight odor; 5 = mold growth, discoloration, surface collapse + offensive odor [15].

2.3. Yeast and mold colony counts of brewer's spent yeast-based silage

Yeasts and molds colony counts were determined by direct plate count after pouring and plating 25 g of ground dried BSY-based silage samples that dissolved in 225 ml of peptone water onto potato dextrose agar medium injected with 1 ppm per each 100 ml of agar with chloramphenicol and streptomycin to restrict bacterial growth [16]. Plates were incubated aerobically at 28 ± 1 °C for 3–5 days after which growing molds and yeast colonies were directly counted [17].

2.4. Evaluation of fermentative characteristics of brewer's spent yeast-based silage

The silage temperature was measured immediately after opening the silos using a laboratory thermometer inserted into the center of the silo. The ambient temperature (mean = 15.4 °C) and relative humidity (RH) (mean = 84%) were recorded throughout the silage incubation periods. Silage pH determination was carried out by taking and mixing 20 g of silage sample from the top, middle, and bottom parts of the mini silo per treatment and subjecting the samples to dissolve in 100 ml distilled water inside a graduated beaker. The samples were continuously blended using a glass stirrer and allowed to settle for an hour before filtering with filter paper. Silage pH was measured directly from the extract using a conventional digital pH meter (Hanan Benchtop pH meter), calibrated with buffer solutions at pH 4 and 7 [18]. Experimental silos were weighed at the beginning of the experiment and end of the ED to determine total dry matter loss (TDML) by difference (DM of fresh sample–DM of silage)/DM of fresh sample*100) [19].

2.5. Determination of chemical composition and in-vitro digestibility of brewer's spent yeast-based silage

Representative samples from fresh BSG, BSY, WB, and silage test diets taken at 2, 4, and 6 weeks of the ensiling periods were used for laboratory evaluation of proximate and detergent fractions. All samples were dried in a forced-air oven at 55 °C for 72 h and ground to pass through a screen size of 1 mm sieve in a Wiley mill and were stored in polyethylene bags until ready for lab analysis. All samples were then analyzed for proximate fractions of DM, ash, organic matter (OM), and CP according to Ref. [20] procedure. Detergent fractions of NDF, ADF, and saturated potassium permanganate lignin (PmL) were determined according to the procedures outlined by Ref. [21]. Two-stage *in-vitro* digestibility technique was employed to analyze the IVOMD of the samples [22]. The rumen fluid was collected from the three rumen cannulated steers before the morning feeding that was fed natural pasture hay ad-libitum and supplemented with about 2 kg of concentrate mixture per steer/day. The liquor from three steers was mixed on a volume basis and filtered through cheesecloth to carry out *in-vitro* digestibility technique. Estimated metabolizable energy (EME) content was estimated from IVOMD as EME (MJ/kg) = 0.16*g IVOMD/kg [23].

2.6. Statistical design and data analyses

The model for CRD using a 4 × 3 factorial arrangement of the lab BSY based silage trial was

$$Yijk = \mu + Ci + Lj + CxLij + eijk;$$

Where; Yijk = response variables; μ = Overall mean; Ci = Effect of ED (2, 4, 6 weeks), Lj = Effect of BSY inclusion level (0, 10, 20, 30%); CxL (ij) = Interactional effect for both factors and; eijk = Random error. Laboratory data were subjected to analysis of variance using the general linear model (GLM) procedures of R software, version 4.2.1 [24]. Duncan's Multiple Range Test at P ≤ 0.05 was used for mean separation.

3. Results

3.1. Chemical composition of experimental diets

The chemical compositions of feed ingredients used for the study are presented in Table 2. Accordingly, FBSY had the least DM and fiber constituents of NDF, ADF, and lignin but was found to be higher in ash, CP, IVOMD and EME contents compared to the other protein source, BSG and the energy source (WB) used to formulate the base concentrate diet. This is almost equivalent to having 45.02 and 19.46% more CP and EME while still 87.93, 88.51, and 64.78% less NDF, ADF, and lignin contents, respectively for BSY over that of BSG.

3.2. Sensory evaluation of brewer's spent yeast-based silage

The extent of surface spoilage of brewer's spent yeast-based silages that ensiled under the different incubation periods is presented in Table 3. No surface spoilage has been rated at all levels of BSY inclusion of the silage samples incubated up until the 4th -week storage. However, silage deterioration as rated by slight mold growth and silage discoloration was noted for test BSY-based silages in which the BSY inclusion level exceeded ≥20% and that incubated for six weeks of ED. Generally, at any given BSY level and ED, silage deterioration as witnessed by surface spoilage ratings of 3, 4, and 5 was not observed.

The combined analysis of BSY level and ED on fermentative, fungal load dynamics, and chemical composition of FBSY-based silage is presented in Table 4. Accordingly, the interaction effect was significant for silage temperature ($P<0.05$), pH ($P<0.0001$), TDML ($P<0.05$), yeast ($P<0.01$), mold ($P<0.05$), and TFC ($P<0.01$). Similarly, interactional effect for certain chemical composition parameters including silage CP ($P<0.0001$), NDF ($P<0.01$), ADF ($P<0.05$), IVOMD ($P<0.05$), and EME ($P<0.05$) was found to be significant. Permanganate lignin (PmL) and fresh silage DM responded ($P < 0.05$) to the independent effects of both BSY and ED. Absolute DM ($P < 0.0001$) responded only to the main factor effect of ED while ash contents ($P < 0.01$) were influenced by BSY inclusion level. Organic matter contents of the silages were noted to be the only nutritional parameter that didn't respond to both main and interactional effects.

3.3. Yeast and mold colony counts of BSY-based silages

The interaction effect of BSY level and ED on yeast, mold, and TFC for BSY-based silage is presented in Table 5. The fungal count was significantly affected ($P<0.01$) by the interaction effect with yeast, mold, and TFC observed to have been occurring within the range of 3.32–6.51, 3.19–5.72, and 6.51–12.23 CFU/g DM, respectively. Higher (6.51 CFU/g DM) and lower (3.32 CFU/g DM) yeast count ($P < 0.01$) was observed at the sixth and second week of the ED and at the 30 and 0% of BSY inclusion levels, respectively. A similar trend ($P < 0.05$) was observed for mold and TFC as with the yeast, the highest (5.72 CFU/g DM) and the lowest (3.19 CFU/g DM) values still being recorded for the same level of BSY inclusion level and ED.

3.4. Fermentative characteristics of BSY-based silage

The effect of BSY level and ED on fermentative characteristics as measured through silage temperature, pH, and TDML of BSY-based silage are presented in Table 6. Silage temperature, pH, and TDML were greatly influenced ($P < 0.05$) by the associative factors effects of BSY level and ED. Silage temperature, in general, did not show any predictable trend both with BSY inclusion level and ED except the higher temperature recorded in the 4th weeks of the ED and which was observed at all levels of BSY inclusions. On the other hand, the pH of the silage ranged between 3.90 and 4.37 with the lowest silage pH value ($P < 0.0001$) at all but 20% of BSY inclusion levels being recorded for silage samples incubated at the sixth weeks from initial dates of the ED. The total dry matter loss of silage in the present study was in the range of 0.84–9.99% with the lowest and highest values ($P < 0.0001$) at all lengths of the ED being recorded for silage samples the BSY inclusion level was set at nil (0%) and at the maximum levels (30%), respectively. The result from the present study revealed that TDML showed an increasing trend ($P < 0.0001$) with an increase in both BSY inclusion level and ED.

3.5. Chemical composition and in-vitro organic matter digestibility of BSY-based silage

The interaction effect of BSY inclusion level and ED for some chemical composition parameters are indicated in Table 7 while those

Table 2
Chemical composition, *in-vitro* organic matter digestibility (g/kg DM) and ME (MJ/kg DM) of experimental feed ingredients.

Item	DM	Ash	OM	CP	NDF	ADF	PmL	IVOMD	EME
FBSY	124.0	54.0	946.0	379.0	73.0	22.0	20.0	761.0	12.2
BSG	256.0	49.0	952.0	209.0	603.0	195.0	57.0	613.0	10.0
WB	935.0	52.0	948.0	140.0	472.0	113.0	37.0	692.0	11.1

BSG = brewery spent grain; FBSY = brewer's spent yeast; WB = wheat bran; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; PmL = permanganate lignin; IVOMD = in-vitro organic matter digestibility; EME = estimated metabolizable energy.

Table 3
Ratings of surface spoilage of BSY-based silage ensiled for different duration (week) and BSY inclusion level (%) (Mean daily temperature = 15.4 °C and RH = 84%).

ED (week)	BSY inclusion level from protein source (%)			
	0	10	20	30
2	0	0	0	0
4	0	0	0	0
6	0	0	1	2

Ratings: 0 = no visible spoilage; l = slight mold growth; 2 = mold growth + discoloration; 3 = mold growth, discoloration + surface collapse; 4 = mold growth, discoloration, surface collapse + slight odor; 5 = mold growth, discoloration, surface collapse + offensive odor.

Table 4
Combined analysis of BSY level (%) and ED (week) for fermentative, fungal load dynamics and chemical composition of BSY-based silage (Mean daily temperature = 15.4 °C and RH = 84%).

Parameters	Mean	Max	Min	Effects		
				ED (weeks)	BSYL (%)	ED*BSYL
Temp (^{0}C)	18.05	18.32	17.74	P < 0.0001	P < 0.01	P < 0.05
pH	4.16	4.37	3.90	P < 0.0001	P < 0.0001	P < 0.0001
TDML (%)	5.08	9.99	0.84	P < 0.0001	P < 0.0001	P < 0.0001
Yeast (log10 CFU/g DM)	5.34	6.51	3.32	P < 0.0001	P < 0.0001	P < 0.01
Mold (log10 CFU/g DM)	4.89	5.11	4.68	P < 0.0001	P < 0.0001	P < 0.05
TFC (log10 CFU/g DM)	11.26	11.71	10.80	P < 0.0001	P < 0.0001	P < 0.01
DM (g/kg DM)	935.19	951.76	918.88	P < 0.0001	NS	NS
DM* (g/kg DM)	386.97	461.08	333.64	P < 0.0001	P < 0.0001	NS
Ash (g/kg DM)	59.11	63.50	55.62	NS	P < 0.01	NS
OM (g/kg DM)	939.83	944.38	932.00	NS	NS	NS
CP (g/kg DM)	204.54	224.50	179.90	P < 0.01	P < 0.0001	P < 0.0001
NDF (g/kg DM)	552.98	643.10	494.50	P < 0.01	P < 0.0001	P < 0.01
ADF (g/kg DM)	115.93	125.56	101.20	P < 0.01	P < 0.0001	P < 0.05
PmL (g/kg DM)	40.52	49.60	31.00	P < 0.01	P < 0.0001	NS
IVOMD (g/kg DM)	643.08	689.40	607.42	NS	P < 0.0001	P < 0.05
EME (MJ/kg DM)	10.28	11.03	9.71	P < 0.05	P < 0.05	P < 0.05

DM = Dry matter; * = Fresh silage DM; ED = ensiling duration; PmL= Permanganate lignin; BSYL = brewer's spent yeast level; TDML = total dry matter loss; CFU = colony forming unit; TFC = total fungal count; Temp = temperature; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; PmL = permanganate lignin; IVOMD = in-vitro organic matter digestibility; EME = estimated metabolizable energy.

Table 5
Interaction effect of BSY level (%) and ED (week) on yeast, mold and TFC of BSY-based silage (Mean daily temperature = 15.4 °C and RH = 84%).

ED (weeks)	BSYL (%)	Fungal count (log10 CFU/g DM of BSY-based silage)		
		Yeast	Mold	TFC
2	0	3.32^{f}	3.19^{c}	6.51^{f}
4	0	4.68^{e}	3.41^{c}	8.09^{e}
6	0	5.21^{cd}	3.59^{c}	8.81^{e}
2	10	5.01^{de}	4.59^{b}	9.61^{d}
4	10	5.45^{cd}	4.29^{b}	9.75^{cd}
6	10	5.60^{bc}	4.71^{b}	10.31^{bcd}
2	20	5.30^{cd}	4.48^{b}	9.78^{cd}
4	20	5.44^{cd}	4.39^{b}	9.83^{cd}
6	20	5.60^{bc}	4.79^{b}	10.39^{bcd}
2	30	5.98^{b}	4.72^{b}	10.70^{b}
4	30	6.04^{b}	4.52^{b}	10.56^{bc}
6	30	6.51^{a}	5.72^{a}	12.23^{a}
	SEM	0.162	0.167	0.271
	P-value	<0.01	<0.05	<0.01

BSYL = brewer's spent yeast level; CFU = colony forming unit; ED = ensiling duration; TFC = total fungal count; SEM = standard error of mean; Means with in a column with different superscripts differ at p < 0.05.

parameters influenced by main factor effects are indicated in Table 8. The CP showed incremental changes (P < 0.0001) with BSY inclusion level and ED compared to that recorded for the control silages. However, the trend among the intervention silage groups remained consistent only for up to 20% level of BSY inclusion. In general, higher CP contents were noted when BSY was included at the rate of 20% and for silage samples incubated for an ED of ≥4 weeks. Similarly, compared to the control silage groups, fiber constituents

Table 6
Interaction effect of BSY level (%) and ED (week) on fermentative characteristics of fresh BSY-based silage (Mean daily temperature = 15.4 °C and RH = 84%).

ED (weeks)	BSYL (%)	Parameters		
		Temperature (^{0}C)	pH	TDML (%)
2	0	17.76^{c}	4.37^{a}	0.84^{l}
4	0	18.28^{a}	4.22^{c}	1.13^{j}
6	0	17.74^{c}	3.92^{e}	2.96^{i}
2	10	17.80^{c}	4.29^{b}	0.95^{k}
4	10	18.32^{a}	4.27^{b}	3.47^{g}
6	10	17.98^{bc}	4.28^{b}	6.77^{d}
2	20	18.06^{ab}	4.02^{d}	3.32^{h}
4	20	18.24^{a}	4.15^{c}	6.47^{e}
6	20	17.98^{bc}	3.90^{e}	9.56^{c}
2	30	18.16^{ab}	4.16^{c}	5.74^{f}
4	30	18.24^{a}	4.24^{b}	9.82^{b}
6	30	18.12^{ab}	4.00^{d}	9.99^{a}
	SEM	0.081	0.020	0.009
	P-value	<0.05	<0.0001	<0.0001

BSYL = brewer's spent yeast level; ED = ensiling duration; TDML = Total dry matter loss, SEM = standard error of mean; Means with in a column with different superscripts differ at $p < 0.05$.

Table 7
Interaction effect of BSY level (%) and ED (week) on chemical composition, IVOMD and EME of FBSY-based silage (Mean daily temperature = 15.4 °C and RH = 84%).

ED (week)	BSYL (%)	Chemical composition and IVOMD (g/kg DM) and EME (MJ/kg DM)				
		CP	NDF	ADF	IVOMD	EME
2	0	182.6^{f}	643.1^{a}	120.9^{abc}	614.2^{c}	9.8^{c}
4	0	179.9^{f}	602.2^{b}	122.3^{ab}	610.7^{c}	9.8^{c}
6	0	185.3^{f}	638.5^{a}	124.2^{a}	607.4^{c}	9.7^{c}
2	10	210.8^{cd}	565.4^{c}	125.5^{a}	661.1^{ab}	10.6^{ab}
4	10	214.5^{bc}	559.1^{cd}	114.4^{bc}	659.5^{ab}	10.6^{ab}
6	10	207.0^{de}	540.7^{de}	113.3^{c}	642.1^{bc}	10.3^{bc}
2	20	201.0^{e}	494.9^{g}	119.0^{abc}	624.2^{bc}	10.0^{bc}
4	20	224.5^{a}	504.9^{fg}	117.4^{abc}	689.4^{a}	11.0^{a}
6	20	220.1^{ab}	494.5^{g}	118.2^{abc}	688.2^{a}	11.0^{a}
2	30	208.8^{cd}	550.7^{cd}	113.0^{c}	637.7^{bc}	10.0^{bc}
4	30	209.8^{cd}	517.1^{f}	101.6^{d}	642.2^{bc}	10.3^{bc}
6	30	210.1^{cd}	524.5^{ef}	101.2^{d}	639.9^{bc}	10.0^{bc}
	SEM	0.160	0.667	0.271	1.257	0.201
	P-value	<0.0001	P < 0.01	<0.001	<0.05	<0.05

DM = Dry matter; CP=Crude protein; NDF=Neutral detergent fiber; ADF = Acid detergent fiber; IVOMD= In-vitro organic matter digestibility; EME = Estimated metabolizable energy; SEM = standard error of mean; BSYL = brewer's spent yeast level; ED = ensiling duration; Means within a column with different superscripts differ at $P < 0.05$.

(NDF and ADF) of the silages were substantially improved ($P < 0.01$) with interaction effects of BSY inclusion and ED. On average this was a 13.1, 26.1 and 18.3% reduction in NDF and a 4.0, 3.6 and 16.3% reduction in ADF over the control for BSY inclusion rate of 10, 20, and 30%, respectively. Among the intervention groups (BSY-based silages), maximum average NDF reduction was indicated for 20% BSYL while this goes up to 30% BSY inclusion level for ADF constituents of the silage mass. Both IVOMD and EME contents were considerably improved ($P < 0.05$) over the control silage (silage to which no BSY was included). The average values for IVOMD were 611, 654, 667, and 640 g/kg DM while the recorded values for EME were 9.8, 10.5, 10.7, and 10.2 MJ/kg DM for the control, 10, 20, and 30% BSY inclusions levels, respectively. Compared with control values there was close to having 6.6, 8.5, and 4.6% more IVOMD and 6.6, 8.4, and 4.5% more EME for silage made from 10, 20, and 30% inclusion rates, respectively. Even though substantial improvements were observed over the control silages, it should be noted that both IVOMD and EME values didn't respond to interactional effects when BSY was added at the rate of 30% inclusion and incubated for the various length of ED. The result from the current study indicated that silage made with BSY inclusion levels of 20% and that was subjected to ≥4 weeks of ensiling periods proven to have markedly higher ($P < 0.05$) IVOMD and EME values over all remaining silage treatments (exceptions are those silage materials made at 10% BSY inclusion level and that ensiled for 2 to 4 weeks).

Fresh silage dry matter, ash, and PmL responded better to the main effect factors (Table 8). Accordingly, fresh silage dry matter and PmL contents significantly decreased ($P < 0.0001$) with incremental changes in the level of inclusion rates of BSY in the silages. Higher values ($P < 0.0001$) for both constituents were recorded for silages with no BSY inclusions (control silages) while the least values ($P < 0.0001$) were noted for silages prepared from higher BSY inclusions level (30%). Similarly, DM was found to have declined with ED with silage samples ensiled for 2 weeks having 7.86% more DM over that ensiled for 6 weeks of ED (Table 8). The result from the same

Table 8
The main effect of BSY level (%) and ED (week) on fresh silage DM and chemical composition of fresh BSY-based silage (Mean daily temperature = 15.4 °C and RH = 84%).

BSYL (%)	Chemical composition (g/kg DM)		
	DM*	Ash	PmL
0	443.8[a]	58.1[b]	48.7[a]
10	393.4[b]	57.1[b]	44.3[b]
20	365.0[c]	59.0[b]	35.7[c]
30	345.6[d]	62.0[a]	33.3[d]
SEM	0.311	0.082	0.068
P-value	<0.0001	<0.01	<0.0001
ED (week)			
2	404.5[a]	61.7	41.6[a]
4	383.6[b]	62.7	41.6[a]
6	372.7[c]	61.0	38.3[b]
SEM	0.26	0.07	0.05
P-value	<0.0001	0.068	<0.01

DM* = fresh silage dry matter; PmL = permanganate lignin; SEM = standard error of mean; BSYL = brewer's spent yeast level; ED = ensiling duration; Means within a column with different superscripts differ at $P < 0.05$.

table showed that while ash values remained consistent ($P > 0.05$) over ED, PmL values showed lower values ($P < 0.01$) when silage samples were only ensiled for an extended period of 6 weeks.

4. Discussion

4.1. Chemical compositions of experimental diets

The dry matter content of FBSY reported in this experiment was comparable with [9] (10–15%) and [2] (12%). The CP value of FBSY was 379 g/kg DM and much lower than earlier studies by Ref. [25] (638 g/kg) but, comparable with [26] who reported the CP contents of FBSY at 380 and 395 g/kg, respectively. The reported value for the CP content of FBSY in the present study can also be compared with other protein sources like soybean meal with a CP content of 380 g/kg [8] implying that FBSY can potentially substitute other protein sources from the diet of animals. The Metabolizable energy content of FBSY from the present study was about 12.2 MJ/kg DM and thus goes comparable with an earlier report by Ref. [2]. Brewery-spent grain in the present study had a DM content of 256 g/kg which was more or less in accordance with earlier values of 200–250 g/kg reported by Ref. [27], but lower than the value of 292 g reported by Ref. [28], and higher than the value 242 g/kg reported by Ref. [4]. The CP contents of wet brewery spent grain (WBSG) in the present study were lower than earlier reports [4] (265 g/kg) and [28] (244 g/kg) but comparable with [27] who reported the CP contents of WBSG was 190 g/kg. The ME (MJ/kg DM) contents of WBSG was 9.8 MJ/kg DM which was in agreement with the 10.7 MJ, ME value previously reported [29]. The variations in chemical compositions of FBSY and WBSG contents in the present study and previously reported values were associated with several factors including malt grains type, conditions for cultivation and time of harvesting, malting and mashing process, amount and type of the adjuncts added in mixture with the barley malt during the process of wort production, period of fermentation, processing techniques, and analytical procedures [30]. Generally, BSY had a comparable nutritional value with BSG which made it a very good candidate protein supplementary feed to replace the most commonly used and costly protein sources in the country. This, however, requires to be verified under *in-vivo* trials involving pilot and/or target animals.

4.2. Sensory evaluation of brewer's spent yeast-based silage

Surface spoilage was not encountered at the 2nd and 4th weeks of the ensiling periods. Similarly, at 6th weeks of ED, no visible spoilage was observed except slight mold growth at 20%, and mold growth and discoloration at 30% of the BSY inclusion level was observed. According to Ref. [31], aerobic deterioration of silage material stored at high temperatures and longer durations could be major factors responsible for high losses in nutritional components and reduction in DM digestibility. Generally, at any given BSY level and ED, spoilage rating of 3, 4, and 5 was not observed due mainly to the low ambient temperature (15.4 °C) recorded during the ensiling period. Other studies by Ref. [32] also reported that extensive mold growth and discoloration of silage materials was observed only for longer storage duration and at the higher temperatures. On the other hand [30], reported that when WBSG stored under aerobic conditions for more than two days at temperatures above 20 °C showed visible changes, including an unpleasant odor, surface cracking, and color changes, which could have been caused by the rapid proliferation of yeast and molds. At 20 and 30% of the BSY inclusion level that was ensiled for 6 weeks of ED, relatively higher TDML of the silage sample was observed as a result of mold growth and discoloration as compared to the control group with no BSY. Generally, the result from the current trial further implies that it would be safe to feed a BSY-based silage diet to dairy cattle provided that the concentrate diet containing the yeast slurry at levels not exceeding 30% is ensiled for a storage duration of 4 weeks.

4.3. Yeast and mold colony counts of brewer's spent yeast-based silage

In the present study, the fungal count dynamics were significantly affected by the interactional effect of BSY level and ED. The relatively highest (6.51 CFU/g DM) and the lowest (3.32 CFU/g DM) yeast count was observed at the 6th and 2nd weeks of the ED when BSY was applied at the rate of 30 and 0%, respectively. A similar trend was observed for mold colony count with the highest (5.72 CFU/g DM) and the lowest (3.19 CFU/g DM) values being still recorded at BSY inclusion levels of 30 and 0%. By comparison [31], has reported lower yeast (2.7–4.9 CFU/g DM), and mold (2.1–3.7 CFU/g DM) values for BSG that were stored using different conservation techniques. Generally, the microbial characteristics of liquid feed during fermentation are very dependent on the time of incubation and incubation temperature [33]. The presence of mold in a stored feed greater than 5.0 CFU/g DM, is considered undesirable leading to higher losses of DM and other essential nutrients [34]. In the present study, 9.99% TDML was witnessed only for 30% BSY-based silage that was subjected to the 6th week of the ED. However, all other samples from the current study were below 5.0 CFU/g DM which further indicated the fact that the silage can be safely used for animal feeding.

4.4. Fermentative characteristics of BSY-based silage

The temperature of the mini silo silage was significantly ($P < 0.05$) influenced by the interactional effect of BSY inclusion level and ED. Consequently, at the 4th week of the ED, the mini silo silage temperature was higher for all levels of BSY as compared to other storage duration and BSY levels. In line with this, a similar result was reported by Ref. [35], where the temperature of the silage measured upon opening the silo/plastic bags, was ranged from 16.62 to 19.52 °C. The same source also indicated that, as the fermentation period increases, the difference in temperature became small. The relatively smaller temperature of the silo in the present study could possibly be associated with the lower ambient temperatures (15.4 °C) during the ED. According to Ref. [36], the silage temperature of small silos is similar to the ambient temperature or just a few degrees warmer than normal temperature and if properly packed and sealed immediately, the average temperature should not increase to more than 5–8 °C above the ambient temperature.

The pH of the silage in the present study was found to range between 3.90 and 4.37. In the present study, all silage samples had a pH value of ≤4.37, which was within the acceptable range for quality silage [23], which can be attributed to the presence of threshold soluble carbohydrate found in wheat bran that could trigger lactic acid production during the fermentation period. Similarly, according to the [2] report, the pH of silage material prepared from a mixture of liquid BSY and cassava pulp was within a range of 3.59–3.91 and 3.70–3.84 for different BSY levels and ED, respectively.

Total dry matter loss of silage in the present study was within the range of 0.84–9.99%. It has also been observed that the TDML of silage was increasing as the BSY level and ED increased. In agreement with the present study [35] reported 7.55% dry matter loss for maize stover silage during the fermentation period of eight weeks. Likewise, a dry matter loss that stenches over 8.6% to 9.6% for wet distiller grains stored under aerobic and anaerobic storage conditions in bunker silos were also reported [37].

4.5. Chemical composition and in-vitro organic matter digestibility of brewer's spent yeast-based silage

The CP content of ensiled BSY-based silage in the present study was noted to be markedly affected by associative effects of BSY level and ED. Thus, a relatively higher CP value was observed at 4th week of the fermentation period when BSY was used at an inclusion rate of 20%. Therefore, the results indicated that as the fermentation period and BSY inclusion level increased, the CP content of the silage tended to have also increased up to 20% of BSY level and up until the 4th week of the ED, then showed a decreasing trend. Similarly [2], reported that the CP content of liquid BSY mixed with cassava pulp was significantly improved as the BSY level increased from 0 to 30% in the silage. Contrary to the present findings, relatively higher (265 g CP/kgDM) content was reported by Ref. [30] for WBSG ensiled for 42 days. The increasing BSY level in the silage material fevered microorganism development, the bacteria present during silage fermentation are protein in nature with more than 75% of their cell mass in the form of the true protein [38]. Therefore, if silage materials are ensiled properly, efficient fermentation and stability will be carried out and natural bacteria present in the medium will have no chance to act on the product except by becoming part of the medium.

The NDF content of BSY-based silage was in the range of 495–643 g/kg DM which somehow aligned with earlier findings [2] who declared a reduction in the NDF contents as the level of BSY increases in the silage materials. In another but related study, when WBSG was ensiled alone for 42 days, the NDF value was observed to have reached up to 610 g/kg DM [31]. The ADF content from the present study was within the range of 101–126 g/kg DM and when substitution levels of BSY for BSG increase, the ADF content was noted to be decreasing with the minimum ADF value observed during the 4th and 6th weeks of the ED at 20 and 30% of BSY inclusion level. Similarly [2], reported decreasing trend of ADF values with the level of BSY increments in the silage materials. The IVOMD and EME of silage material in the present study were in the range of 607–689 g/kg DM, and 9.7–11.0 MJ/kg DM, respectively with relatively higher IVOMD and EME values in the present study being reported for 20% of BSY level and that ensiled for 4 to 6 weeks. In a related study by Ref. [2] the inclusion of BSY level in cassava pulp by up to 30% substantially improved IVOMD and EME of silage material within 4 weeks from the initial date of the ensiling period.

5. Conclusion and recommendations

Brewery spent yeast had a comparable nutritional value with BSG which made it a very good candidate protein supplementary feed to replace the most commonly used and costly protein sources. It can effectively replace BSG to make good quality silage when it can be used at levels ≤20% and the ED is set at 4 weeks without any compromise in the nutritional quality of the resultant silage feed.

However, to draw comprehensive conclusions and recommendations about the benefits of BYS-based silage, the lab-based findings need to be supplemented with the evaluation of volatile fatty acids and verified under both on-station and on-farm conditions using either a pilot and/or target animals.

Declarations

Consent for publication

Not applicable.

Ethics approval

Not applicable.

Written Consent for publication

Not applicable.

Availability of data

Original research data of this manuscript is available from the corresponding author on reasonable request.

Code availability

Not applicable.

Author contribution statement

Endale Yadessa, Berhan Tamir, Getu Kitaw,and Mesfin Dejene: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gebremaryam Terefe: Performed the experiments; Analyzed and interpreted the data.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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