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Lactobacillus buchneri and molasses can alter the physicochemical properties of cassava leaf silage

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ABSTRACT

In developing countries where feed resources are scarce, cassava leaves can be used as feed for animals. However, the use of cassava leaves is limited mainly because of their high fibre content and overall acceptability by animals. The resolution to this problem is to process the cassava leaves by ensiling and using additives. Therefore, the objective of the study was to determine the effects of including different inclusion levels of molasses and bacteria concentration on the physicochemical properties of cassava leaf silage. Molasses was added at inclusion levels of 0, 3, 5 and 7 g/100g of the chopped cassava leaves, and *Lactobacillus buchneri* was mixed with chopped cassava leaves at different concentrations of 0, 3.1×10^6 cfu/ml, 3.1×10^8 cfu/ml and 3.1×10^{10} cfu/ml. The effects of inclusion level of molasses on the colour, smell and texture of cassava leaf silage were significant ($P < 0.05$). Inclusion of bacteria concentration also influenced the smell of silage ($P < 0.05$). Effects of the inclusion level of molasses and bacteria concentration resulted in decreased pH, crude protein and crude fibre of silage ($P < 0.05$). There was a quadratic relationship between Ca and K with inclusion level of molasses in cassava leaf silage ($P < 0.05$). A positive linear relationship was observed between Mg and molasses inclusion levels in cassava leaf silage ($P < 0.05$). Using principal component analysis (PCA), molasses had a strong positive correlation with PCA 1, whereas crude fibre, pH and crude protein had a positive correlation with PCA 2. The inclusion level of bacterial concentration was negatively correlated to Ca, CP, P and CF. From the study, the use of molasses and *L. buchneri* can greatly improve the physicochemical qualities of cassava leaf silage.

1. Introduction

Leaf meals have been used for a long time as an alternative feed source for animals. However, they are fibrous, so there are conflicting reactions to their use as feed for some livestock species. For example, ruminants have microbes in their gut that break down the fibres to form volatile fatty acids; hence, the fibrous feed can provide valuable nutrients for the animal [1,2]. On the other hand, non-ruminant animals like pigs and poultry lack these microbes [3]. Therefore, feeding fibrous feed to non-ruminants can be a

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challenge. Over the past years, technological changes have produced exogenous enzymes for breaking down some plant-fibrous feed materials. However, plants have complex fibre matrices, which make enzymes less efficient in breaking them down [4]. Another drawback in their use is that they are specific depending on their goal [4]. For example, to our knowledge, most enzymes have been specifically manufactured to break down specific fibre matrices in grain and not in leaf meals. Since leaf meals are now becoming a popular animal feed ingredient in the resource-constrained world, it is essential to investigate other ways of processing them so that non-ruminant animals can efficiently utilise them.

Various techniques for breaking down plant fibres have been reported in numerous investigations. One technique to break down the fibrous feed material is fermentation and/or ensiling [5]. Some plant materials are highly fermentable, whereas others are not because they have different physicochemical characteristics. This has presented a challenge to farmers, researchers, and feed compounders. For instance, fermentation is limited in some plant materials because they contain low water-soluble carbohydrates [6]. Therefore, to promote fermentation, farmers in developing countries have resorted to mixing legumes and grasses, that is, plants with high water-soluble carbohydrates and plants with low water-soluble carbohydrates, to improve fermentation [7].

Some examples of additives used to improve the fermentation process include molasses and probiotics [8]. Molasses is a byproduct of sugar processes and has been found to increase the water-soluble carbohydrates for the fermentation process. Up to 7 % can be used for ensiling plant materials as feed materials [9]. Probiotics are now in use in most countries. For example, *Lactobacillus buchneri* is a probiotic currently used to improve silage aerobic stability [10]. Hence, it prevents spoilage. In combination, molasses and *Lactobacillus* can improve the silage fermentation process. It is envisaged that the bacterial species will also break down the plant fibrous feed material during the fermentation process.

Cassava (*Manihot escutenta*) is now considered an important crop in the tropics to reduce hunger. Cassava is used in most tropical developing countries where people consume the tuber. The human population hardly use the leaves, except in a few countries where it is used as a vegetable [11]. An estimated yield of about ten thousand tonnes of dry cassava leaves can be produced per hectare [12]. Hence, plenty of leaves can be used as feed for livestock. Bakare et al. [13] investigated the use of leaves in chickens. Up to 10 % inclusion level can be used in broiler diets. Several studies have also reported using the leaves to be limited in non-ruminant animals [14–16]. The authors postulated that high fibre content in the leaves limited the use of higher inclusion levels in the diets of chickens. Ensiling the leaves might reduce fibre levels, lower antinutritional factors, and improve the plant material's chemical composition and physical characteristics. The study's objective was to determine the effects of inoculating molasses and bacteria concentrations on the physicochemical properties of cassava leaf silage.

2. Materials and methods

2.1. Harvesting and study area

Leaves were harvested while green from the Fiji National University (FNU) farm. The farm is located at a latitude of 15–20° S and an altitude of 175–182° E. First, the stalk from the leaves was removed. Thereafter, they were chopped using a chaff cutter. The resultant chopped leaves were between 1 and 3 cm in size. The crude protein content of the fresh leaves used in the study was 21.4 %.

2.2. Experimental design

Two additives, that is, molasses [MS] and *Lactobacillus buchneri*, were used in the study. The additives were mixed with chopped cassava leaves at three different levels/concentrations. First, molasses was added at inclusion levels of 0, 3, 5 and 7 g/100g of the chopped cassava leaves. The lactic acid bacteria species, *Lactobacillus buchneri* (Bio-way Technology Co., Ltd., Shanghai, China), was mixed with chopped cassava leaves at different concentrations of 0; 3.1×10^6 cfu/ml, 3.1×10^8 cfu/ml and 3.1×10^{10} cfu/ml. Approximately 100 mL of inoculant was added to 25 kg fresh forage. Forty-eight samples were prepared, with three replicate samples from each treatment. Samples treated with different additives were vacuum-packed in strong polythene bags in triplicate and stored in the laboratory at room temperature between 27 °C–31 °C for 28 days [17]. We expect all fermentation phases to occur within the first 28 days of ensiling.

Table 1
Score chart for physical characteristics and pH of the silage quality.

	Score scale (0–5)					
Observation	0 (Very bad)	1 (Bad)	2 (Going bad)	3 (Moderate)	4 (Good)	5 (Excellent)
Appearance	Very dark	Dark	Brown	Pale green	Light green	Green
Smell	Offensive	Poor	Almost pleasant	Fairly pleasant	Pleasant	Very pleasant
Texture	Slimy	Very soft	Soft	Firm but wet	Firm	Very firm
pH	>6.5	6.1–6.5	5.6–6.0	4.6 - 5.5	4.0–4.5	<4.0

2.3. Analyses of samples

2.3.1. Physical characteristics

The physical characteristics of the ensiled samples were assessed after 28 days by three experienced technicians. The observed physical characteristics were appearance, smell, texture, and pH. Table 1 shows a description of the physical characteristics of silage. The physical characteristics were scored on a scale of 0–5 (Table 1). pH was measured using a method described by Bernardes et al. [18]. First, the silage samples were macerated. Then, 25 g of macerated silage was mixed with 100 mL of water, and 15 min of standing time was adopted before pH was recorded with a pH meter (PL-700PVS, Dogger Science, Taipei, Taiwan).

2.3.2. Chemical characteristics

Silage samples were analysed for dry matter (DM), crude protein (CP), crude fibre (CF), calcium, phosphorus, potassium and magnesium. The DM content of cassava leaf silage was determined by drying the samples in an oven at 65 °C until a stable weight was obtained [19]. Samples were weighed before and after drying, and DM was calculated using the formula described by Chimphepo et al. [20]. The dried samples were ground to pass through a 2 mm sieve prior to analyses of CP, CF, Ca, P, K and Mg. Crude protein was determined using the Kjheldal method [21]. The total nitrogen obtained was multiplied by a conversion factor of 6.25 to obtain the crude protein content. Crude fibre content was determined by Weende's method [22]. The molybdenum blue method was used to determine phosphorus [23]. Potassium (K), calcium (Ca) and magnesium (Mg) concentrations were determined with atomic absorption spectrophotometer [24]. Sampling for nutritional analysis was done on day 28 after ensiling. The samples were analysed at Koronivia Research Station (Department of Chemistry), Ministry of Agriculture, Fiji.

2.4. Statistical analyses

Using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc), data were analysed as a 4 x 4 factorial arrangement based on a completely randomised design. A significant level was detected at 5 % in the statistical model with the effects of molasses and LAB inclusion and potential interaction as independent variables. Means were separated using the pdiff option of SAS (SAS Institute Inc). Orthogonal polynomial contrasts were used to assess the significance of linear or quadratic models to describe the relationship between the response and independent variables. Principal component analysis (PCA) was used to determine relationships among response variables using JMP IN statistical software (JMP IN version 3.2.1, SAS Institute Inc).

3. Results

Table 2 shows summary statistics for the effects of inclusion level of molasses and bacteria concentration on the physicochemical properties of cassava leaf silage. For physical properties, the effects of inclusion level of molasses on the colour, smell and texture of cassava leaf silage were significant ($P < 0.05$). Likewise, the effects of inclusion level of bacteria concentration on smell of silage were significant ($P < 0.05$). The interaction of molasses and bacteria concentration levels on physical properties was not significant. Effects of inclusion level of molasses on pH, DM, CP, CF, Ca, K, and Mg were significant ($P < 0.05$). Only pH, DM, CP and CF were significant for the effects of inclusion levels of bacteria concentration ($P < 0.05$). In this study, significant factors will be described in detail.

The inclusion of molasses affected the colour, smell and texture of cassava leaf silage (Fig. 1 a, b, c; $P < 0.05$). The sensory score for colour and smell decreased linearly with increased levels of molasses in cassava leaf silage (Table 3; $P < 0.05$). On the other hand, the sensory score for smell increased with the inclusion level of molasses (Table 3; $P < 0.05$). A positive linear relationship between bacterial concentration and sensory score for smell was also observed (Table 3; $P < 0.05$).

Effects of the inclusion level of molasses resulted in a decrease in the pH of silage (Fig. 2a; $P < 0.05$). A high pH value of about 5.5 was observed in silage with no molasses. pH started to decrease with inclusion level of molasses. Low pH values were observed in silage with 3, 5 and 7 g/100g inclusion molasses. There was a negative linear relationship between the inclusion level of molasses and pH in cassava leaf silage (Table 4; $P < 0.05$). The same trend was observed for pH in silage inoculated with *L. buchneri* ($P < 0.05$). High pH values were observed for silage with no bacteria compared to silage with 3.1×10^8 cfu/ml and 3.1×10^{10} cfu/ml inclusion of bacteria concentration (Fig. 2b). There was a negative linear relationship between the inclusion of bacteria concentration and pH in cassava leaf silage (Table 4; $P < 0.05$). There was an inconsistent pattern of DM content in silage inoculated with molasses and bacteria concentration. A quadratic relationship was observed for the inclusion level of molasses and bacteria (Table 4; $P < 0.05$).

Inclusion level of molasses resulted in a linear decrease in CP content in cassava leaf silage (Table 5; $P < 0.05$). A high CP content of

Table 2
Summary statistics for effects of inclusion of molasses and bacteria concentration on physicochemical properties of cassava leaf silage.

	Physical properties			Chemical properties							
Source	CLR	Smell	TXR	pH	DM	CP	CF	Ca	P	K	Mg
Molasses	*	*	*	*	*	*	*	*	NS	*	*
Bacteria	NS	*	NS	*	*	*	*	NS	NS	NS	NS
Molasses*Bacteria	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

CLR – colour; TXR – texture; DM – dry matter; CP – crude protein; CF – crude fibre; Ca – calcium; P – phosphorus; K – Potassium; Mg - magnesium.
* Significant ($P < 0.05$); NS – not significant ($P > 0.05$).

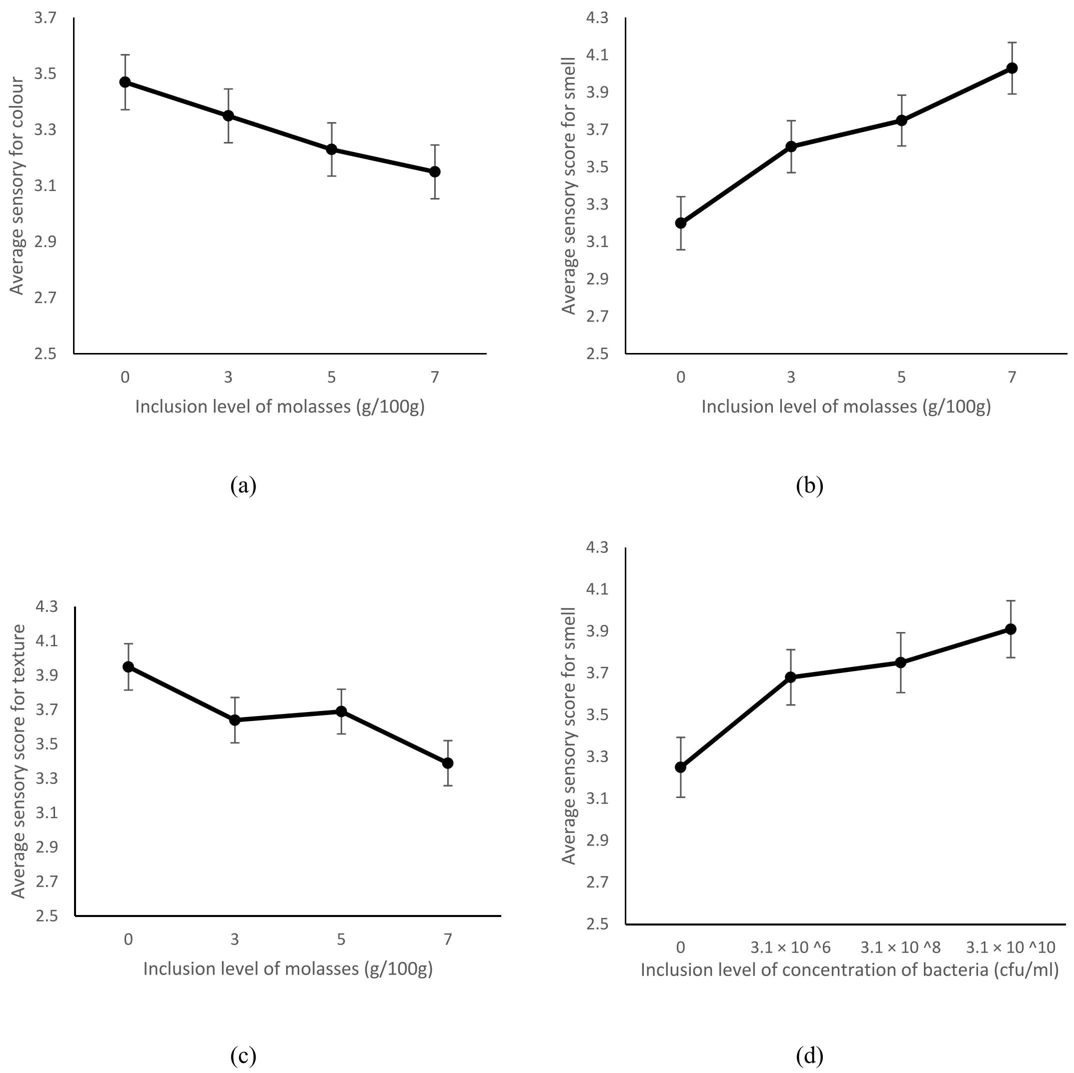

Fig. 1. Effects of inoculation of molasses and Lactobacillus buchneri on physical properties of cassava leaf silage.

Table 3
Regression equations relating inclusion level of molasses and bacteria concentration to physical properties (Colour, Smell and Texture) of cassava leaf silage.

Linear	Quadratic	Parameter	Equations	R^2	RMSE
*	NS	Colour	Colour = 3.44 - 0.041*Molasses	0.038	0.538
*	NS	Smell	Smell = 3.22 + 0.117*Molasses	0.119	0.822
*	NS		Smell = 3.24 + 0.068*Bacteria	0.084	0.838
*	NS	Texture	Texture = 3.92 - 0.068*Molasses	0.055	0.730

about 19.8 % was observed in silage with no molasses, whereas a low CP content of about 17.8 % was observed in silage with 7g/100g molasses (Fig. 3a). There was an inconsistent pattern of CP content in silage inoculated with different bacteria concentrations (Fig. 3b). There was neither a linear nor quadratic relationship between CP content and the inclusion level of bacteria concentration in cassava leaf silage. Crude fibre content decreased with inclusion level of molasses and bacteria ($P < 0.05$). There was a linear relationship between CF content and the inclusion level of molasses and a quadratic relationship between CF content and the inclusion of bacteria concentration (Table 5; $P < 0.05$).

Fig. 4 shows the effects of inclusion level of molasses in cassava leaf silage on Ca, K and Mg content. Potassium and magnesium content increased with inclusion level of molasses in cassava leaf silage ($P < 0.05$). Conversely, Ca decreased with inclusion level of molasses in cassava leaf silage. There was a quadratic relationship between Ca and K with inclusion level of molasses in cassava leaf silage (Table 6; $P < 0.05$). A positive linear relationship was observed between Mg and molasses inclusion levels in cassava leaf silage

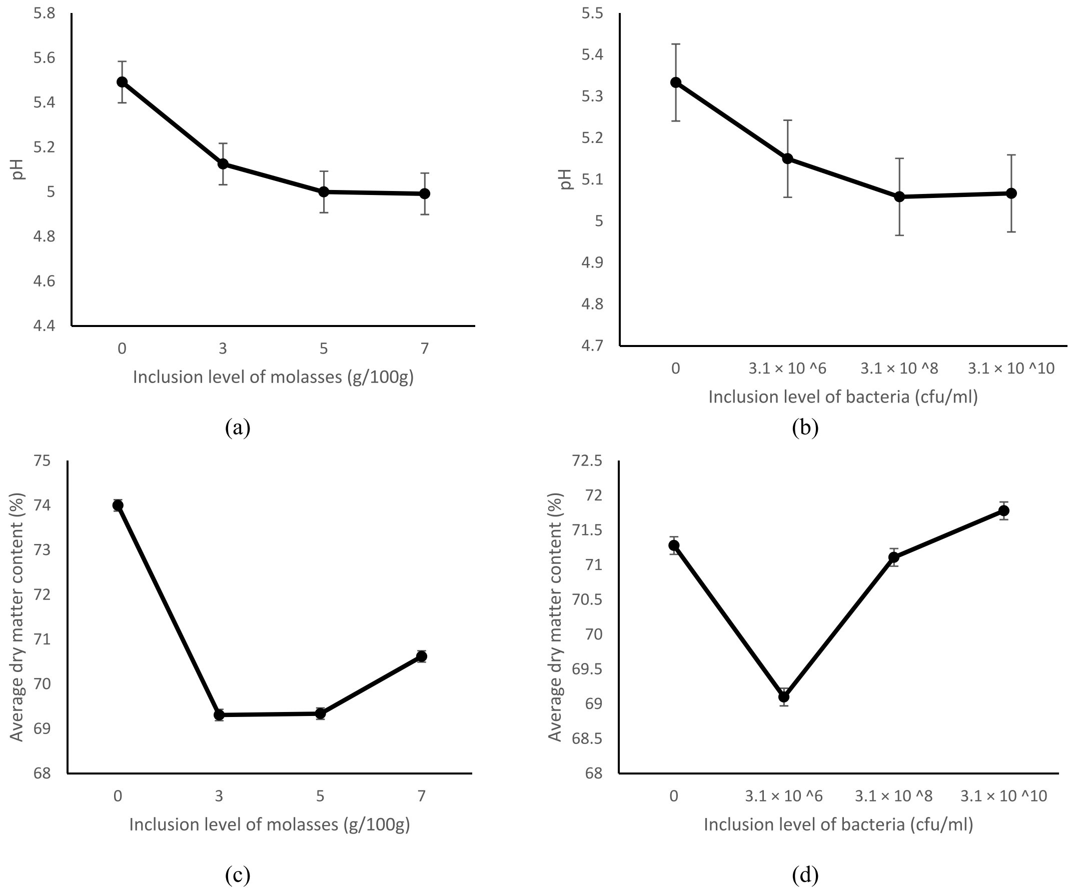

Fig. 2. Effects of inclusion level of molasses and *Lactobacillus buchneri* concentration on pH (a)(b) and dry mater content (c)(d) of cassava leaf silage.

Table 4
Regression equations relating inclusion level of molasses and bacteria concentration to pH and dry matter content of cassava leaf silage.

Linear	Quadratic	Parameter	Equations	R^2	RMSE
*	NS	pH	pH = 5.33 - 0.029*Bacteria	0.083	0.367
*	NS	pH	pH = 5.427 - 0.073*Molasses	0.256	0.331
NS	*	DM	DM = 71.24 - 0.826*Bacteria +0.091*$Bacteria^2$	0.148	2.344
*	*	DM	DM = 73.96 - 2.247*Molasses + 0.255*$Molasses^2$	0.623	1.559

Table 5
Regression equations relating inclusion level of molasses and bacteria concentration to crude protein and crude fibre content of cassava leaf silage.

Linear	Quadratic	Parameter	Equations	R^2	RMSE
*	NS	CP	19.92–0.255Molasses	0.233	1.222
*	*	CF	12.34 + 0.668*Bacteria-0.096*$Bacteria^2$	0.258	2.346
*	NS	CF	13.89 - 0.623*Molasses	0.387	2.096

(Table 6; $P < 0.05$).

Principal component analysis was used to determine relationships among the variables (inclusion levels of molasses, inclusion level of bacteria concentration, Ca, Mg, CP, P, K, DM and CF). Fig. 5 shows that PC 1 and PC 2 accounted for 76.1 % of the variation. Molasses had a strong positive correlation with PCA 1, whereas crude fibre, pH and crude protein had a positive correlation with PCA 2. The inclusion level of bacterial concentration was negatively correlated to Ca, CP, P and CF.

4. Discussion

In the study, molasses and lactic acid-producing bacteria (*L. buchneri*) were the additives used to improve the ensiling process.

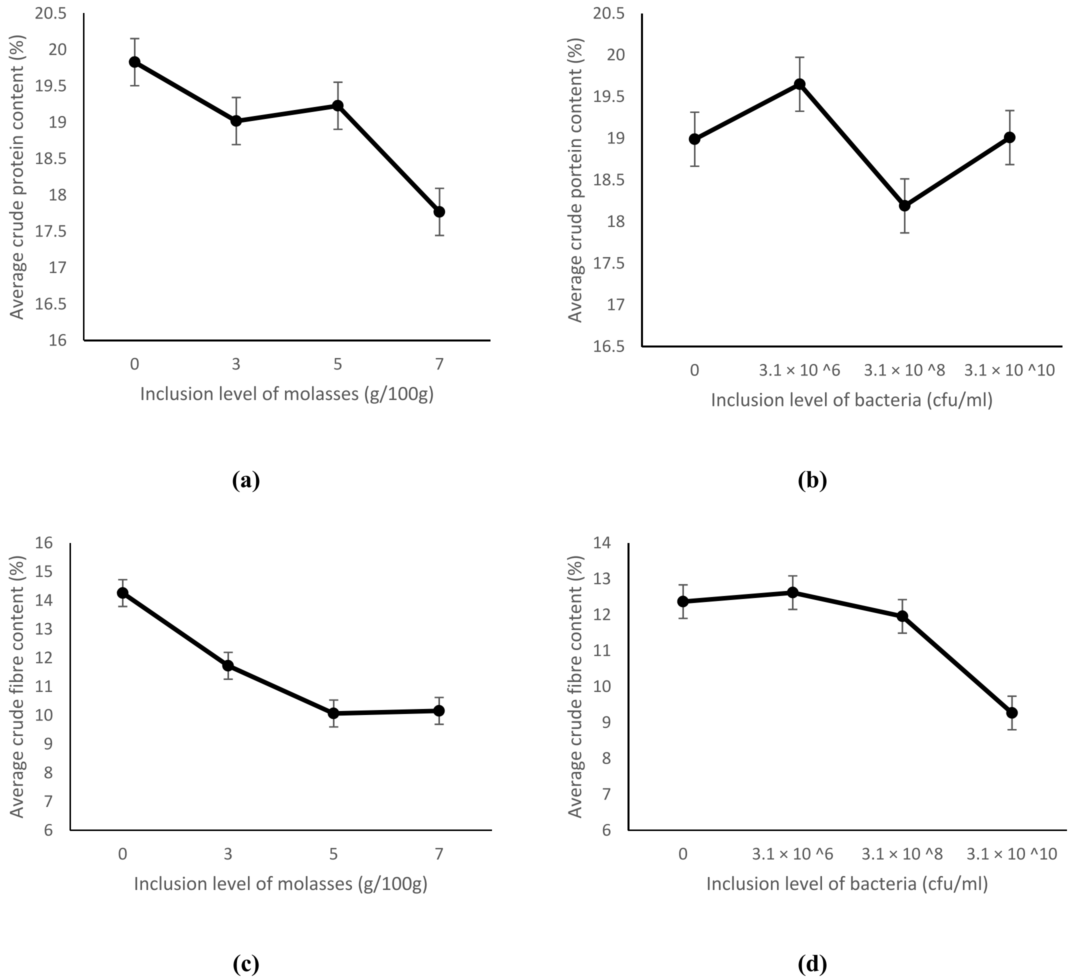

Fig. 3. Effects of inclusion level of molasses and *Lactobacillus buchneri* concentration on crude protein (a)(b) and crude fibre content (c)(d) of cassava leaf silage.

Molasses increases the water-soluble carbohydrates (WSC) of forage and *L buchneri* helps to stabilise and ferment the forage. Hence, we expected a normal fermentation process to occur during the ensiling of cassava leaves. Good silage with a mild, slightly acidic and fruity smell was produced in the study. Furthermore, mould was not detected in all the silage treatments.

According to Babayemi [25], maintaining a green colour of leaves provides good silage. When the colour changes from green to brown or very dark, it indicates that the silage has gone bad. In the current study, colour was assessed by observers trained to score the physical characteristics of silage, and scores showed silage with light and pale green colour, indicating moderate to good silage. The inclusion level of molasses might have influenced the colour of the silage. According to Handriati et al. [26], adding molasses might have changed the colour of the silage. The blackish-brown colour of molasses can cause the discolouration of the cassava leaf silage, resulting in a deviation from the normal greenish colour. McDonald [27] also reported the importance of storage temperature in controlling the colour of silage. The lower the temperature (<30 °C), the less likely the colour will change. Conversely, temperatures above 30 °C result in dark yellow or closer to brown silage due to the caramelisation of sugars in the forage. The storage temperature range in the study was between 28 °C and 31 °C; hence, this might have caused a slight departure from the typical green colour of good silage.

Smell is one criterion used for determining the quality of the silage produced [28]. The observation that smell was influenced by the inoculation of bacteria and molasses was expected. Molasses might have given a pleasant smell in the silage. This is consistent with the findings of Mahala and Khalifa [29]. The pleasant smell of cassava leaf silage might also be due to a drop in pH, which indicates normal fermentation process. A drop in pH is a result of the production of different volatile fatty acids [30]. Depending on the volatile fatty acid produced, different smells are imparted, which determine the quality of the silage [31]. Volatile fatty acids in the study were, however, not determined. Bacteria also influenced the smell of silage. Our results conform to other studies conducted by Mahgoub et al. [32] and Saeed et al. [33]. The fermentation process played a role in determining the texture. In the study, the texture of the silage had a score within the range of 3.3–3.9, indicating good and/or moderate silage.

Hydrogen potential (pH) is an important element influencing silage quality [32]. The drop in pH values varies depending on the

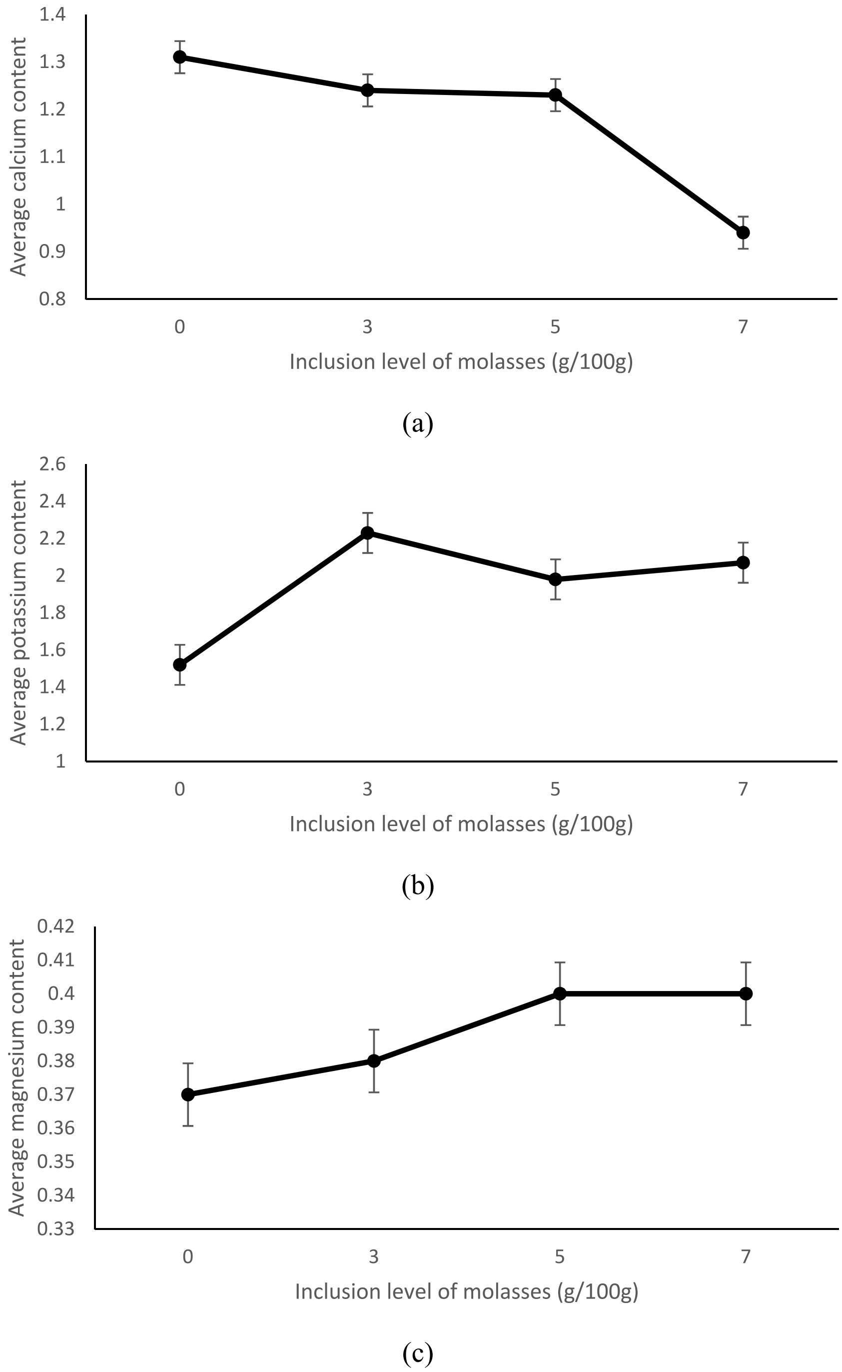

Fig. 4. Effects of inclusion level of molasses on calcium (a), potassium (b) and magnesium (c) concentration in cassava leaf silage.

Table 6
Regression equations relating inclusion level of molasses to calcium, potassium and magnesium concentration in cassava leaf silage.

Linear	Quadratic	Parameter	Equations	R^2	RMSE
NS	*	Ca	$Ca = 1.29 + 0.033*Molasses - 0.011*Molasses^2$	0.519	0.135
NS	*	K	$K = 1.55 + 0.259*Molasses - 0.0278*Molasses^2$	0.221	0.455
*	NS	Mg	$Mg = 0.365 + 0.00549*Molasses$	0.155	0.034

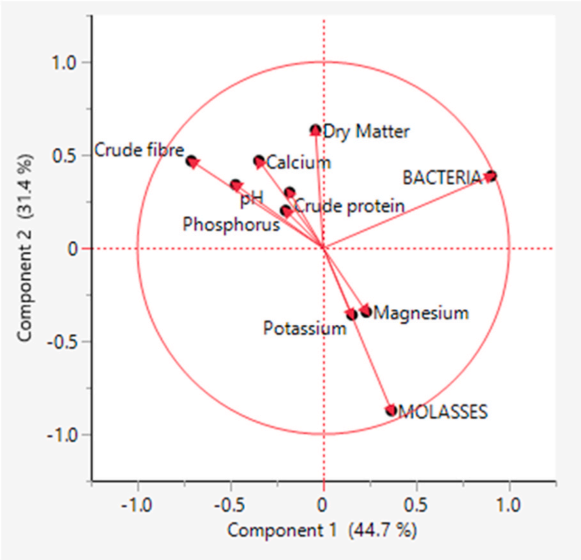

Fig. 5. Loading plot showing data for correlations among physicochemical properties and inclusion levels of additives (molasses and *Lactobacillus buchneri*) chemical and sensory properties (principal component[component]1 vs [component]2).

plant species. Legume silages generally have a higher pH (4.7–5.0) than grass silages (4.3–4.7) [34]. Cassava leaves in the study had high CP content, which might have resulted in high buffering capacity and, consequently, high pH values of 4.9 and 5. Buffering capacity was, however, not determined in the study. High DM content also results in an increase in pH. This occurs because when silage DM rises, the amount of metabolic water available for the growth of lactic acid bacteria becomes limited [35]. Nevertheless, the decrease in pH in the cassava leaf silage is an indication of lactic acid production, which is important for the preservation of forage [36]. The pH values in the study are still close to the appropriate range, according to Zeleke [37]. The inclusion of molasses in the study decreased pH. This conforms to a study conducted by McDonald et al. [38], who reported the pH to decrease with the addition of 2 % of molasses during ensiling. High metabolic activity of *L. buchneri* led to high pH in the wheat, sorghum and maize silages.

Crude fibre is important in characterizing the nutritional value of forage for animals. High fibrous forages result in reduced palatability and digestibility. In the study, it was envisaged that the addition of LAB would reduce the fibres during fermentation. Both additions of molasses and *L buchneri* influenced fibre in silage. Molasses is a substrate that can be used by fibre-decomposing microbes to degrade fibre during silage fermentation. A plethora of studies conform with our study Ribas et al. [39] and Lynch et al. [40].

Mineral content in silage is mainly affected by plant species and edaphic factors [41,42]. Since cassava leaves used in the study are from the same species and region with the same edaphic factors, it is expected that not many changes in the mineral composition will occur, even with the inclusion levels of molasses and bacteria. The observed decrease in the amounts of calcium in silage with inclusion levels of molasses might be attributed to the use of organic matter by microorganisms or percolation during the fermentation process [43,44]. Conversely, the observed increase in K and Magnesium might be attributed to the inclusion of molasses for initiating the fermentation process.

5. Conclusion

From the study, the use of molasses and *L. buchneri* can greatly improve the physicochemical qualities of cassava leaf silage. The additions of the additives will prevent nutrient losses during ensiling and help with the preservation process. It is advised to inoculate cassava leaf silage with molasses and *L. buchneri* at concentrations of 5–7g/100g and 3.1×10^{10} cfu/ml, respectively.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

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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