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ORIGINAL PAPER

Microbial, Nutritional, and Antioxidant Stability of Fruit and Vegetables Discards Treated with Sodium Metabisulfite During Aerobic and Anaerobic Storage

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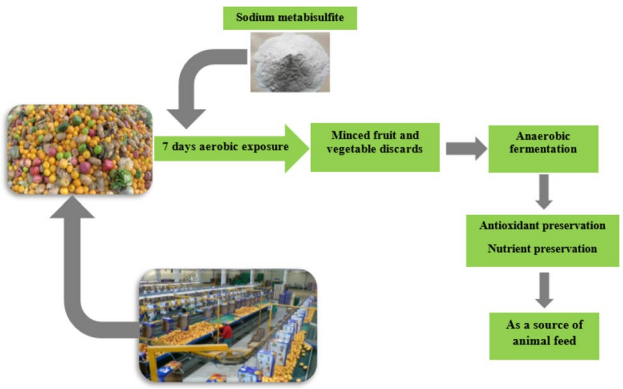
Abstract

Fruits and vegetables are a rich source of natural antioxidants; therefore their discards can be viewed as a functional feed ingredient in animal nutrition. The aim of present study was to examine the effects of sodium metabisulfite (SMB) on microbial, nutritional, and antioxidant stability of fruit and vegetable discards (FVD) under laboratory- and large-scale conditions. Initially, FVD were mixed without or with 6 g SMB/kg biomass, aerobically challenged for 7 days, and then stored anaerobically up to 28 days. Under both aerobic and anaerobic conditions, negligible loss of the nutrient constituents was evident in SMB-treated FVD. Conversely, the rapid rise in the microbial population of FVD (without SMB) resulted in biomass deterioration and substantial dry matter loss and sugar exhaustion. Although the prolonged storage of SMB-treated FVD resulted in the moderate loss of carotenoids, total phenolics and DPPH radical scavenging activity slightly changed. Overall, a series of laboratory- and large-scale experiments demonstrated the effectiveness of SMB in conserving the nutrient constituents and the antioxidant capacity of FVD under aerobic and anaerobic storage, which might enable a viable route to the effective utilization of these discards as a functional ingredient for animal feed applications.

Graphic Abstract



Keywords Antioxidant capacity · Carotenoid · Fruit and vegetable waste · Nutrient composition · Phenolics

Statement of Novelty

This study presents the findings of a series of laboratory and large-scale experiments that investigated the effectiveness of sodium metabisulfite on microbial, nutritional, and

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antioxidant properties of fruit and vegetable discards for animal feed applications. Sodium metabisulfite was an effective preservative under both aerobic and anaerobic conditions, as its application to fruit and vegetable discards resulted in a negligible loss of nutrient and antioxidant constituents. The findings of this study might provide a feasible route for the effective utilization of fruit and vegetable discards as a functional feed ingredient for animals.

Introduction

Essentially, fruits and vegetables are an important source of vitamins, minerals, and phytonutrients in the human diet [1]; thus, the use of fruit and vegetable discards (FVD) in animal diets may provide benefits beyond a simple feed ingredient. For example, the findings of Angulo et al. [2] and Romero-Huelva et al. [3] confirmed the benefits of FVD as a functional feed ingredient in the diet of dairy cows and goats.

The high biodegradable organic matter and moisture contents in FVD accelerate the growth and proliferation of undesirable microorganisms [4, 5]. Moreover, owing to their high biochemical and chemical oxygen demand, improper treatment of FVD can result in water and air pollution [6, 7]. A recent survey by our research team in a commercial large-scaled packing house revealed FVD accumulation inside the facility usually for a week until their transportation to a recycling center. FVD spoilage is accelerated during this accumulation period, especially during the hot months of the year, which necessitates the development of an on-site preservation technology for their safe use.

The ingredient composition of FVD is highly variable, which presents difficulties for their standardization as a steady and continuous feed ingredient [8]. This problem may be avoided by the on-site storage of a large quantity of FVD under anaerobic conditions. However, one of the main obstacles with the direct ensiling of high-moisture biomass, typically less than 25% dry matter (DM), such as FVD is the excessive energetic cost expressed in loss of DM and nutrients, in addition to the potential risk of clostridial fermentation [9–11]. Generally, silage losses are associated with the production of gaseous volatile fatty acids and alcohols that contribute to the degradation of air and water quality [12]. This has resulted in the enforcement of strict regulations by governmental air and water regulatory agencies in an attempt to maintain cleaner water and air [13]. Therefore, the use of additives that suppress the generation of these substances would be favorable from the economic and environmental standpoints [14].

Recently, we successfully used sodium metabisulfite (SMB) for preserving FVD under aerobic conditions so they can be used as an animal feed ingredient [5]. Our extensive investigations confirmed that 6 g SMB/kg biomass (fresh basis) was the minimum amount that could effectively preserve the nutrient constituents of FVD under aerobic condition for a week [5]. However, less is known about the possible protective or destructive effects of SMB on the antioxidant activity and bioactive components of FVD under aerobic and anaerobic conditions. SMB is a white, granular solid that is produced from the reaction of sulfur dioxide with sodium carbonate, and is Generally Recognized as Safe (GRAS 182.3766) as a food preservative in FDA's GRAS list [15]. Earlier studies confirmed the benefits of using SMB as an additive on silage fermentation patterns as well as animal performance [16–18]. Moreover, the use of SMB as a preservative can contribute to the sulfur requirements of animals. However, to the authors' knowledge no information exists on how SMB would affect the antioxidant capacity of FVD that have been aerobically challenged prior to their ensiling. Therefore, this study aimed at examining time course changes in the conservation indices and antioxidant capacity of FVD as a high-moisture biomass under laboratory- and large-scale conditions.

Materials and Methods

Chemicals and Reagents

Methanol, ascorbic acid, 2, 2-diphenyl-1-picrylhydrazil (DPPH), Folin–Ciocalteu reagent, aluminum trichloride, gallic acid, quercetin, and all other solvents/chemicals (analytical grade) were obtained from Sigma Chemical Co. (Saint Louis, MO, USA). Food-grade SMB ($Na_2S_2O_5$; purity = 97.6%) was obtained from Cathay Chemical Works Co. (Taiwan).

Sample Preparation

The detailed methods for sample preparation and preservation process have previously been described [5, 8]. In both laboratory- and large-scale experiments, the quantity of major individual ingredients discarded during each month constituting more than 90% of total discards were surveyed from the input and output data in the packing house. The proportion of individual ingredients in the total discard used in laboratory- or large-scale experiment is provided in Supplementary Table S1. In laboratory-scale experiment, FVD (about 200 kg) were collected from a large-scaled commercial packing house (E-mart Fresh Center, Icheon city, Korea). The discards were roughly sliced (30–40 mm pieces), mixed, and divided at random into two allotments (each 100 kg). Metabisulfite powder was not added to one bucket, but was applied at 6 g/kg FVD to the other bucket. Each allotment was mixed thoroughly for 10 min using a horizontal feed mixer (DDK-801 M, Daedong Tech. Co.,

Korea). Each allotment was sub-divided into 15 portions (5 kg each; five replicate buckets per treatment). This experiment produced a total of 30 buckets (8 L capacity). The buckets were placed outdoor at ambient temperatures for 7 days of aerobic exposure. The mean temperature and relative humidity for the duration of the aerobic storage were 29.8 °C and 74.7%, respectively. Next, five buckets per treatment were randomly selected, and the content of each bucket was thoroughly mixed and representative subsamples were collected for analyses. For anaerobic storage, the aerobically challenged FVD was ground by using an electronic meat grinder and transferred into a 5 L glass jar. A lid with a rubber ring fitted with four clamps provided airtight conditions. Five jars were used per treatment. The jars were opened after 7, 14, and 28 days, and the representative samples were obtained for microbiological and antioxidant analyses. The samples collected for the analysis of antioxidant activity and bioactive compounds were stored at − 80 °C until being freeze-dried.

In large-scale experiment, approximately 1,600 kg of FVD were obtained from the same packing house on 3 consecutive days. Similar to laboratory-scale experiment, FVD were roughly sliced into 30–40 mm pieces, and then divided into four portions (each 400 kg), where each portion was thoroughly mixed for 10 min with 2.4 kg of SMB using the aforementioned feed mixer. After mixing, 400 kg allotments were transferred into 600 L buckets. The buckets were placed outdoor under aerobic exposure for 7 days. The mean temperature and relative humidity for the duration of the aerobic storage were 27.3 °C and 78.2%, respectively. After 7 days, the aerobically challenged discards were ground using a meat mincer and transferred into 2-layered polybags embedded inside a 600 L capacity bucket, and stored outdoor in sealed conditions for 14 and 28 days. Each polybag contained 400 kg FVD. The mean temperature and relative humidity for the duration of the anaerobic storage were 29.6 °C and 68.6%, respectively. At specified sampling times, representative samples were obtained from different locations (bottom: 55–70 cm deep; middle: 25–40 cm deep; and surface: 0–5 cm deep) for analyses of chemical composition, microbiological profile, and antioxidant properties.

Microbiological and Analytical Procedures

The extract for quantification of metabolites and enumeration of microbial populations was prepared as described previously [5]. For extract preparation, 20 g of fresh or ensiled sample was mixed with 120 mL of distilled, autoclaved water for 5 min. The suspension was filtered through four layers of medical gauze. The pH of the extract was immediately measured with an HI9321 pH meter (Hanna Instrument, Portugal). The filtrate was centrifuged (10,000 g; 4 °C for 10 min) for the analysis of water-soluble carbohydrates (WSC) using the procedure of Dubois et al. [19] and NH_3–N [20]. The spread-plating method was used for microbial enumeration after construction of appropriate dilutions. The total viable colonies of bacteria and lactic acid bacteria (LAB) were counted after spread-plating and incubation (36 °C ± 1 °C; 48 h) on plate count agar and MRS agar, respectively. The viable colonies of yeast and mold were enumerated on yeast extract glucose chloramphenicol agar (Difco Laboratories Inc. Detroit, MI, USA), and incubated at 25 °C ± 1 °C. The incubation continued until 3 days for yeast and 5 days for mold. Yeast and mold were discriminated using the colony appearance appraisal.

Dry matter (DM) content was determined after the samples (180–200 g) were dried at 65 °C for 72 h, and then ground (particle size = 1 mm) for the analysis of crude protein (N × 6.25; AOAC method 990.03). The weight loss (DM basis) was calculated from the difference between the initial (day 0) and final target weight, based on the DM content of the silage mass. The loss of volatile compounds during oven drying was considered for the correction of DM values, with 100% volatilization for ammonia [21], 8% for lactic acid, and 95% for acetic acid [22]. Lactic acid was quantified using a colorimetric method [23]. The commercial kits supplied from Megazyme (Megazyme International Ireland Ltd., Bray, Co., Wicklow, Ireland) were used for quantification of ethanol (K-ETOH; ACS Manual Format), acetic acid (K-ACET; ACS Manual Format), and free sulfite (K-SULPH; ACS Manual Format). Free sulfite was only quantified in samples treated with SMB.

Methanolic Extraction

The modified procedure of Chandrasekara and Shahidi [24] was used for the methanolic extraction of FVD. Before extraction, the freeze-dried samples were pulverized using a pestle and mortar under dark conditions. The homogenized ground FVD (1 g) was mixed with 10 mL of a methanol–water mixture (80:20, v/v) and shaken by using a rotary shaker at 250 rpm for 1 h at 30 °C. The supernatant was harvested after centrifugation of the suspension (4000 g for 10 min at 4 °C). The residual solids were re-extracted three more times with the same procedure. The volume of the collected supernatant reached up to 50 mL using methanol (80%). The supernatant was kept at – 80 °C until analysis.

Total Phenolics

The original procedure of Singleton and Rossi [25] as modified by Silva and Sirasa [26] was used for deteminination of total phenolics. In brief, after dilution with distilled water (1:10 v/v), Folin–Ciocalteu reagent was mixed with the supernatant (1:10). After 5-min equilibration, 2 mL of sodium carbonate (7.5%, w/v) was added to the mixture.

After room-temperature incubation for 2 h, absorbance was read at 765 nm using an S-1100 UV spectrophotometer (Scinco, Korea). A standard curve was established using gallic acid dissolved in 80% methanol (0.03–0.50 mg/mL).

Total Carotenoids

The modified procedure of de Carvalho et al. [27] was used for quantification of total carotenoids. Briefly, 8 mL of acetone was added to 1 g of freeze-dried sample under dark conditions. The paste was pulverized with a pestle and mortar and then filtered under vacuum using a sintered funnel (5 µm). The extraction procedure was repeated three times until the filtrate became colorless. Next, the filtrate was transferred to a separatory funnel containing petroleum ether (25 mL) and mixed well. Acetone was removed through the addition of ultrapure water (Milli-Q, Millipore; Darmstadt, Germany). The procedure was repeated several times until an acetone-free extract was obtained. Excess water was removed with the addition of sodium sulfate (7 g). A final volume of the petroleum ether layer containing the carotenoids was reached at 50 mL using additional petroleum ether. Petroleum ether was used as the blank. Total carotenoid content was computed using the following equation:

$$\text{Total carotenoid content } (\mu g/\text{g}) = \left[\text{A} \times \text{V (mL)} \times 10^4\right] / \left[\text{A}_{1\text{cm}}^{1\%} \times \text{W (g)}\right] \quad (1)$$

where A = absorbance at 450 nm; V = extract volume; W = dry weight; $A_{1cm}^{1\%} = 2592$ (β-carotene extinction coefficient in petroleum ether).

DPPH Assay

The modified procedure of Brand-Williams et al. [28] was used for determination of the DPPH free radical-scavenging activity. The supernatant (100 µL) or ascorbic acid standard (0.06–0.5 mg/mL) was mixed with 3.9 mL of the methanolic solution of DPPH (0.06 mM). After incubation at room temperature for 30 min, absorbance was read at 515 nm. The results were expressed as mg ascorbic acid equivalents (ASE) per g dry FVD, according to the following equation [29]:

$$\text{SE (mg AA/g)} = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}} - \text{A}_{\text{AA}}} \times \text{AA concentration (mg/mL)} \times \text{supernatant volume (50 mL)} \times \text{g dry FVD}, \quad (2)$$

where A = absorbance, AA = ascorbic acid.

Total Flavonoids

The procedure of Kim et al. [30] was used for quantification of flavonoids. Briefly, a 1 mL aliquot of supernatant or standard solution of quercetin was mixed with 4 mL of deionized water. At the beginning, this mixture was added to 300 µL of 5% $NaNO_2$ (w/v), and allowed to equilibrate for 5 min. Then, 300 µL of aluminum trichloride (10%) was added to the mixture, and incubated for 1 min at room temperature. Thereafter, to the mixture were added 1 M NaOH (2 mL) and 2.4 mL of deionized water and vortexed immediately. Absorbance was read at 510 nm.

Data Analysis

Data were analyzed in a completely randomized design using the PROC MIXED of SAS [31], and the difference between the least-squares means was identified by using the Tukey's multiple test range at the 0.05 probability level. The linear response of storage length from the initial preservation day to the end of anaerobic storage was estimated using polynomial contrasts. In either experiments, the replicate bucket or glass jar was considered as the experimental unit.

Results and Discussion

Laboratory-Scale Experiment

The time-course of weight loss (DM basis) during the 7-day aerobic storage and 28-day anaerobic storage in the presence or absence of SMB in the laboratory-scale experiment is presented in Fig. 1. After aerobic storage, approximately 18% of mass in the control FVD was lost, and this loss further continued during the silage fermentation and increased to 29.1% after 28 days of ensiling. Conversely, the weight loss was negligible in SMB-treated FVD, with only 4.1% loss after the 35-day storage. Essentially, during ensiling, the weight loss usually occurs even in well-fermented silages [32], the extent of which is largely dependent on the initial sugar content of the biomass and the pre-ensiling microflora of the biomass, as the consumption of fermentable carbohydrates with other microorganisms rather than homofermentative LAB contributes to the excessive DM reduction during ensiling.

Nutritional, microbiological, and antioxidant properties of FVD treated without or with SMB, arranged by the length of storage (aerobic and anaerobic) in laboratory-scale experiment, are presented in Table 1. Contrary to the noticeable differences in the nutrient composition and microbial profile of SMB-free FVD, a narrow difference was seen in those parameters of SMB-treated FVD after 7 days of aerobic storage. For example, 17.4% of WSC was lost after

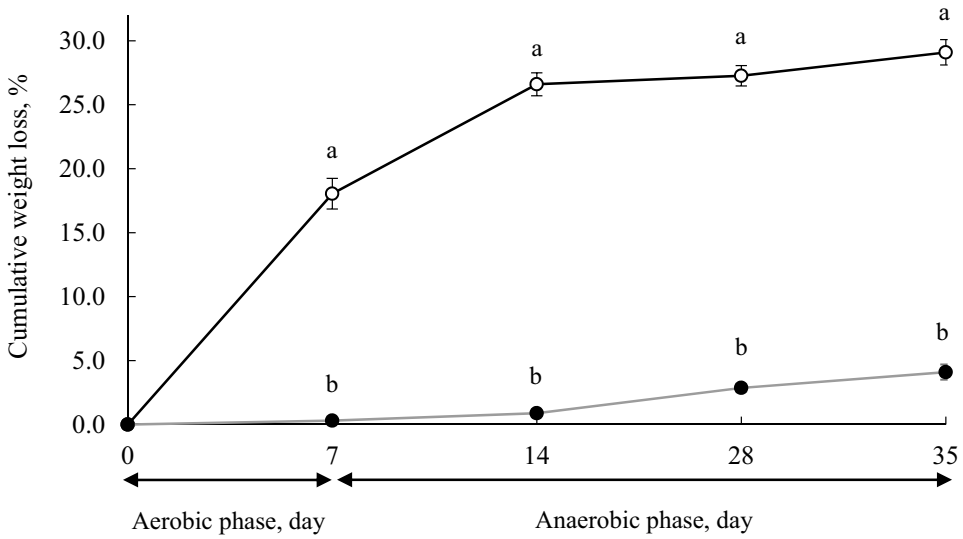

Fig. 1 Time course of weight loss during 7-day aerobic storage and 28-day anaerobic storage in the presence or absence of sodium metabisulfite in laboratory-scale experiment. The black line represents control and the gray line represents sodium metabisulfite treatment. The error bars at each point represent the standard deviation. At each time point, means with different superscripts differ

7 days of storage in the control FVD. The numbers of LAB, yeast, and mold increased in the control FVD; however, their numbers were below the detection limit in the aerobically challenged FVD treated with SMB. After 7-day aerobic storage of control FVD, the marked increase in lactic acid production (from the negligible value of 0.45 g/100 g DM to 3.08 g/100 g DM) was mirrored in the pH decline of 0.61. Likewise, the initial biomass had negligible amounts of acetic acid, ethanol, and ammonia-N; however, their contents increased substantially after the aerobic exposure. These metabolites were produced in smaller extents in the SMB-treated FVD, which was expected as SMB treatment led to the substantial suppression of mold, LAB and yeast, as well as a logarithmic reduction of 1.41 log cfu/g fresh mass in the number of bacteria.

Similar to the aerobic storage, considerable changes occurred in the nutrient constituents and microbial profile of control FVD under anaerobic storage. With silage fermentation, ethanol concentration of the control silage was rapidly exhausted. In support of this observation, earlier studies reported the oxidation of ethanol in corn silage over a day; however, the oxidation rate is accelerated when the silage spoilage is more rapid, resulting in significant declines in ethanol content over hours [12, 33]. The WSC content declined by 54% after ensiling for 28 days, which resulted in the rapid accumulation of lactic acid content as silage fermentation was initiated. This was expected as the rapid consumption of WSC by silage microbial community, notably homofermentative LAB, produces large quantities of lactic acid [32]. The exposure of FVD to aerobic stress, in addition to the high moisture content of FVD, is likely the contributory factor that accelerated the loss of DM and sugars during silage fermentation. Recently, Brüning et al. [34] reported that a 4-day delay (aerobic stress) in sealing of corn silage negatively affected the silage quality with respect to the increase in yeast number that resulted in the loss of DM (11%) and WSC (65%). Likewise, the ensiling losses of fresh orange peels (18% DM content) constituted about one-third of the fresh peel DM content, with lactic acid, and acetic acid being the major fermentation products and lactobacilli and yeasts being the dominant microbial populations [35].

As anaerobic fermentation commenced, LAB proliferated rapidly and dominated the microbial community of control silage (no SMB), peaking at 8.39 log cfu/g biomass after 7 days of ensiling. However, as fermentation progressed, the viable numbers of LAB declined. This observation was expected as a negative correlation exists between silage fermentation length and the viability of LAB [36]. The mold count of control silage remained below the detection limit (< 2.8 log cfu/g silage), which is possibly related to the low oxygen concentration of ensiling environment that inhibited the fungal growth [32]. Yeasts also dominated the microbial community in control silage; however, as ensiling period progressed, their numbers gradually declined. This high population of yeasts in control silage is possibly responsible for the extensive loss of DM and nutrients [32]. Borreani et al. [14] suggested that the predominance of microorganisms other than LAB in the silage microbial community is usually associated with significant DM loss in the form of CO_2. The high number of yeasts in control silage, despite the high lactic acid content, can possibly be explained by the mild antimicrobial properties of lactic acid that was not able to suppress the growth and proliferation of yeasts [32]. More specifically, the initial high concentrations of fermentable carbohydrates are associated with high numbers of yeasts, which are known to convert WSC into carbon dioxide and alcohols [32].

Table 1 Nutritional, microbiological, and antioxidant properties of fruit and vegetable discards without or with sodium metabisulfite (SMB) according to the storage length in the laboratory-scale experiment

Items	Length of aerobic storage, day		Length of anaerobic storage, day			SEM	*P*-value	
	0	7	7	14	28		Storage	Linear
Control (no SMB added)								
pH	4.17	3.56	3.55	3.55	3.52	0.02	< 0.01	< 0.01
Dry matter (DM), %	14.9	12.3	8.90	8.52	8.37	0.07	< 0.01	< 0.01
Crude protein, g/100 g DM	10.4	9.47	13.1	12.8	14.2	0.18	< 0.01	< 0.01
Water-soluble carbohydrates, g/100 g DM	52.2	43.1	26.1	23.1	19.8	0.73	< 0.01	< 0.01
Ammonia-N, µg/g DM	0.36	3.88	4.45	14.1	15.7	0.16	< 0.01	< 0.01
Ethanol, mg/g DM	1.41	4.38	0.57	0.63	0.57	0.07	< 0.01	< 0.01
Acetic acid, mg/g DM	0.05	9.28	4.84	5.33	4.79	0.23	< 0.01	< 0.01
Lactic acid, g/100 g DM	ND	3.08	10.0	9.92	11.8	0.54	< 0.01	< 0.01
Total bacteria, $\log_{10}$ cfu/g fresh mass	5.96	8.32	8.60	8.36	7.48	0.09	< 0.01	< 0.01
Lactic acid bacteria, $\log_{10}$ cfu/g fresh mass	5.86	7.89	8.39	8.03	7.03	0.08	< 0.01	< 0.01
Yeasts, $\log_{10}$ cfu/g fresh mass	3.78	6.59	6.92	6.58	5.43	0.08	< 0.01	< 0.01
Molds, $\log_{10}$ cfu/g fresh mass	4.48	7.21	< 2.8	< 2.8	< 2.8	0.09	< 0.01	< 0.01
Antioxidant properties								
Total phenolic content, mg GAE/g DM[a]	9.63	10.4	13.7	13.8	14.9	0.16	< 0.01	< 0.01
Total carotenoid content, µg/g DM	116	154	271	254	249	2.22	< 0.01	< 0.01
DPPH, mg ASE/g DM[b]	4.63	4.89	6.06	6.03	6.64	0.13	< 0.01	< 0.01
SMB treatment (6 g SMB/kg biomass)								
pH	3.99	4.01	3.98	4.01	4.04	0.01	0.28	0.06
Dry matter (DM), %	15.6	15.1	14.8	14.4	14.2	0.04	< 0.01	0.05
Crude protein, g/100 g DM	9.97	9.51	6.25	6.64	7.03	0.09	< 0.01	< 0.01
Water-soluble carbohydrates, g/100 g DM	51.5	52.0	50.6	48.2	46.7	0.69	0.15	0.02
Ammonia-N, µg/g DM	0.32	2.01	1.97	7.49	5.37	0.08	< 0.01	< 0.01
Ethanol, mg/g DM	1.18	1.23	0.39	0.17	0.15	0.01	< 0.01	< 0.01
Acetic acid, mg/g DM	0.06	ND	0.42	0.23	0.28	0.01	< 0.01	< 0.01
Lactic acid, g/100 g DM	ND	ND	ND	ND	ND	–	–	–
Total bacteria, $\log_{10}$ cfu/g fresh mass	5.42	4.16	3.94	4.43	4.22	0.05	< 0.01	< 0.01
Lactic acid bacteria, $\log_{10}$ cfu/g fresh mass	5.14	< 2.8	< 2.8	< 2.8	< 2.8	0.42	< 0.01	< 0.01
Yeasts, $\log_{10}$ cfu/g fresh mass	3.47	< 2.8	< 2.8	< 2.8	< 2.8	0.17	< 0.01	< 0.01
Molds, $\log_{10}$ cfu/g fresh mass	4.20	< 2.8	< 2.8	< 2.8	< 2.8	0.50	< 0.01	< 0.01
Antioxidant properties								
Total phenolic content, mg GAE/g DM	10.8	11.2	11.9	12.1	11.5	0.09	0.01	< 0.01
Total carotenoid content, µg/g DM	103	98.2	99.1	89.5	86.4	1.38	0.05	< 0.01
DPPH, mg ASE/g DM	4.76	4.68	4.72	4.63	4.21	0.08	0.41	0.11

The end of the preservation period corresponds to 0-d of anaerobic storage

SEM standard error of mean; *ND* no detection; *DPPH* 2, 2-diphenyl-1-picrylhydrazyl

[a]Expressed as milligrams of gallic acid (GAE) equivalents per g dry weight

[b]Expressed as milligrams of ascorbic acid (ASE) equivalents per g dry weight

Contrary to control silage, SMB treatment was highly effective in the conservation of nutrient constituents and the suppression of the undesirable microbial population during the 28 day anaerobic storage. The population of LAB remained below the detection limit in connection with the low number of the undesirable microorganisms (yeast and mold), which resulted in the very negligible or undetectable concentration of lactic acid. Predictably, the losses of DM and nutrient were minor and highlighted the preservative potency of metabisulfite ion as compared with lactic acid, which is an important organic acid preservative in normal silage fermentation [32]. The mechanism underlying the inhibitory effect of sulfites on microbial viability is not thoroughly understood; however, it has been suggested that the reaction of sulfite with cofactors and coenzymes, amino acids, pyrimidines, and nucleosides may result in microbial

cell death. Moreover, sulfites are known to contribute to the rapid depletion of ATP prior to cellular death in yeast. This series of events may collectively disturb the microbial energy metabolism, ultimately resulting in cell death [37].

The time-course of free sulfite concentration in SMB-treated FVD during the 7-day aerobic storage and 28-day anaerobic storage (laboratory-scale experiment) is illustrated in Fig. 2. After aerobic exposure, an average of 11% free sulfite was lost, which slowed during silage fermentation, with a free sulfite loss of about 10% during the 28 day fermentation. SMB provides preservation primarily through the bisulfite ion, an active form of SMB that retards the microbial decay of the product [38]. The reversible bond formation between free sulfite and certain compounds such as carbonyl compounds (mainly acetaldehyde and pyruvic acid) may have contributed to the decline of free sulfite level. The bound sulfite is known to have a weak preservative function in preventing the product decay [39, 40].

Total phenolics, carotenoids, and antioxidant capacity of FVD without or with SMB in relation to storage lengths in laboratory-scale experiment, are presented in Table 1. Before presenting the data, it should be noted that no correction factor was used for the interfering substances in determination of total phenolics. This may have resulted in overestimated total phenolic values, because various compounds such as reducing sugars, inorganic ions, and proteins in food extracts may also be reactive with Folin–Ciocalteu reagent [41]. Generally, as the storage duration was prolonged, phenolics and carotenoids accumulated in FVD biomass that was not treated with SMB, which was reflected in increased antioxidant capacity. This can be explained by the excessive loss of DM and nutrients and thus the accumulation of bioactive constituents in FVD biomass, which were possibly less sensitive to degradation during storage. Additionally, a mounting body of evidence suggests that wounding of fruits and vegetables promotes the synthesis and accumulation of phenolics with antioxidant properties [42]. Wounding stress is believed to affect the physiology of fruits and vegetables, activating the responses that may induce the biosynthesis and accumulation of phenolic compounds or other secondary metabolites [43]. Reyes et al. [44] observed that wounding stress increased the antioxidant capacity of the fruits and vegetables; however, this response was tissue dependent. The authors reported that the changes in phenolic content ranged from a decrease of 26% up to a rise of 191%, which was reflected in the changes in antioxidant capacity ranging from a 51% decline to an increase of 442%. Similarly, Reyes and Cisneros-Zevallos [45] found that wounding stress induced the accumulation of total phenolics and increased antioxidant capacity (85%) in purple-flesh potato.

In contrast to the control FVD, as the storage period prolonged in SMB-treated FVD, total carotenoid content declined moderately; however, total phenolic content and the DPPH radical-scavenging activity did not change. This suggests substantial contributions of polyphenols to the antioxidant capacity of FVD, which agrees with earlier studies that found a linear correlation between total polyphenols and antioxidant activity (DPPH assay) [46, 47]. In support of our findings, pretreatment with metabisulfite protected phenolic compounds in mango [48] and pumpkin flour [49]. In their study, Aydin and Gocmen [49] found that the metabisulfite-treated pumpkin flour exhibited a slightly higher DPPH radical-scavenging activity, which was linked to the higher retention of their phenolics. Contrary to control silage, the loss of DM and nutrient was negligible in SMB-treated FVD, which may not have caused the accumulation of polyphenols, thereby resulting in slight changes in antioxidant activity of SMB-treated FVD. Presently, it is probable that

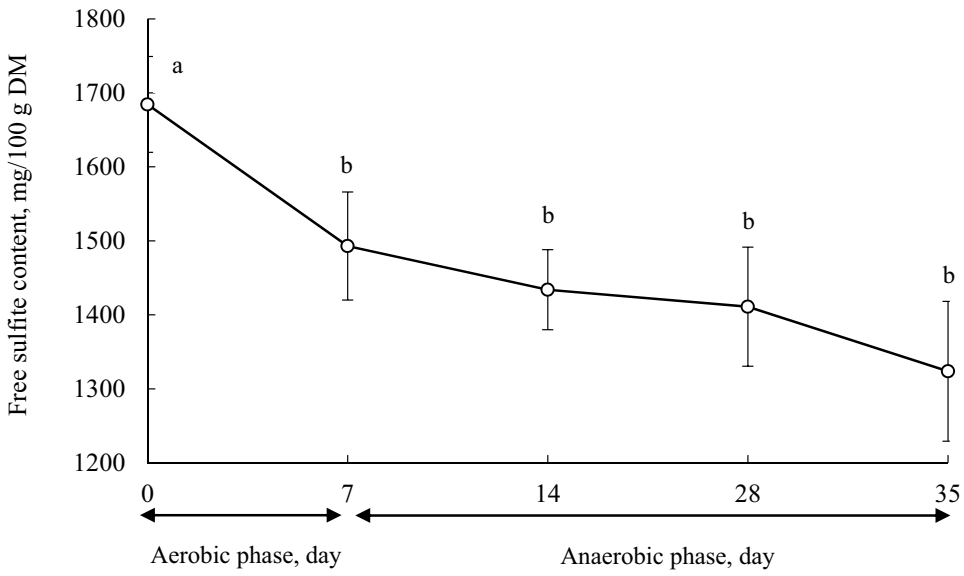

Fig. 2 The time course of free sulfite concentration in sodium metabisulfite-treated fruit and vegetable discards during 7-day aerobic storage and 28-day anaerobic storage in laboratory-scale experiment. The error bars at each point represent the standard deviation. Means with different superscript(s) differ

the SMB suppression of the enzymes involving in the biosynthesis of phenolics in wounded FVD, was reflected in the lack of difference in phenolic content of SMB-treated FVD. The slight tendency to a declining DPPH scavenging activity can be explained by the destruction of some bioactive compounds with antioxidant capacity, which is dependent on moisture content, oxygen, and temperature [50]. Our study showed that after a 7-day aerobic exposure, FVD were stored under anaerobic conditions where oxygen presence was very low. This anoxic condition may have contributed to the retention of phenolics and antioxidant capacity of SMB-treated FVD during the ensiling storage. Moreover, the presence of SMB in silage fermentation consumes large quantities of oxygen during the initial phase of ensiling [51], which provides the strict anaerobic medium that possibly protected the bioactive constituents susceptible to oxidation.

Earlier studies showed that the treatment of fruits and vegetables with bisulfite solution before drying contributed to the retention of color, carotenoids, ascorbic acid, as well as antioxidant activity [48, 52]. Past studies investigating the use of metabisulfite as a silage additive also reported the effective retention of carotene in the bisulfite-treated silage relative to control silage [53, 54]. In support of our findings that prolonged storage resulted in the moderate loss of carotenoids, Zhao and Chang [55] found a 20% loss of total carotenoids in carrot samples treated with 0.2% sodium bisulfite after 4 months of storage. Chen et al. [48] reported that compared with 1% ascorbic acid solution, soaking of mango with 1% sodium bisulfite solution before their drying contributed to the higher retention of carotenoids. Gardner et al. [56] studied the antioxidant potential of a range of fruit juices and found the slight contribution of carotenoids to their antioxidant capacity. This explanation may provide evidence as to why despite a reduction in carotenoid content over the storage time in SMB-treated samples, the antioxidant capacity declined slightly. The suggested mode of action of metabisulfite in the retention of carotenoids was explained by the oxygen-scavenging action of radical SO_2 that prevented the oxidative deterioration of carotenoids [57]. Hymavathi and Khader [58] reported that a 72–75% retention of total carotene in mango powder for up to 2 months of storage. In their study, the authors found a positive correlation between the β-carotene content and the SO_2 amount, which suggested the protective effect of SO_2 on the retention of carotenoids. However, owing to their high number of double bonds, carotenoids are susceptible to oxidation and isomerization [59, 60]. Rodriguez-Amaya [61] suggested that enzymatic and non-enzymatic oxidations were the major cause of carotenoid loss during processing and storage of foods. This oxidation was known to be dependent on the availability of oxygen and the carotenoid structure, which is stimulated by light, heat, enzymes, and peroxides [61]. These series of events may explain the moderate degradation of carotenoids in SMB-treated FVD. In agreement, Baloch et al. [62] studied the stability of carotenoids in dried tomato powder over a 90 day storage, and observed that potassium metabisulfite substantially decreased the rate of carotenoid loss, but this loss increased with storage, with losses of nearly 50% after 45 days of storage. Generally, the total loss of carotenoids during both the preservation and storage of forages ranges from 20–80% of the initial content [60].

Large-Scale Experiment

As explained earlier, the laboratory-scale experiment showed that although the rapid domination of LAB contributed to the substantial lactic acid production in control silage, the silage fermentation was not successful, which was accompanied by a putrid smell associated with excessive losses of DM and nutrients. In contrast, metabisulfite application was very effective in the retention of nutrients and silage with desirable organoleptic properties. This was the motivation of the follow-up experiment to demonstrate the production of metabisulfite silage at a larger scale.

Nutritional, microbiological, and antioxidant properties of FVD treated with SMB, arranged by the length of storage (aerobic and anaerobic) in large-scale experiment, are presented in Table 2. In general, the conservation profile of SMB-treated FVD was comparable to those observed in the laboratory-scale experiment with respect to the minor loss of nutrients, microbiological stability, and the diminishment of free sulfite. However, when compared to the laboratory experiment, storage in ton bags resulted in a greater loss of DM and sugars, which indicates that the storage stability of FVD silage is influenced by the storage environment. For example, a 13.2% decline in WSC content was seen after 35 days of storage (aerobic and anaerobic); whereas, the same storage period in the laboratory-scale experiment resulted in only 9.3% loss of WSC.

Overall, the findings of the large-scale experiment indicate that FVD treated with SMB can be stored satisfactorily under sealed conditions where the oxygen-limiting environment of a silo, as well as metabisulfite ion, provide protection against undesirable microflora damage and preserve the nutrient constituents of FVD without the need for lactic acid fermentation, which is necessary in conventional types of silage. Metabisulfite treatment of FVD may enable the maintenance of the nutritional quality of FVD for prolonged periods, thereby contributing to the economics and environmental sustainability of preservation systems.

Generally, total phenolic content and antioxidant capacity of FVD in this experiment was greater than values observed in the laboratory experiment, while total carotenoids were found to be lower in the large-scale experiment, which is possibly related to the difference in the individual ingredient composition between the two experiments. Loss of

Table 2 Nutritional, microbiological, and antioxidant properties of fruit and vegetable discards treated with sodium metabisulfite according to the storage length in the large-scale experiment

Items	Length of aerobic storage, day		Length of anaerobic storage, day		SEM	*P*-value	
	0	7	14	28		Storage	Linear
Conservation parameters							
pH	4.07	4.03	3.95	3.92	0.02	0.02	< 0.01
Dry matter (DM), %	14.9	14.2	13.9	12.8	0.15	< 0.01	< 0.01
Crude protein, g/100 g DM	10.6	10.9	9.29	8.81	0.21	< 0.01	< 0.01
Water-soluble carbohydrates, g/100 g DM	43.3	40.6	38.2	37.1	0.62	0.01	< 0.01
Ammonia-N, µg/g DM	0.67	7.54	10.6	12.3	1.05	< 0.01	< 0.01
Ethanol, mg/g DM	0.34	0.91	0.72	0.69	0.05	0.02	0.08
Acetic acid, mg/g DM	0.07	0.08	0.25	0.31	0.02	< 0.01	< 0.01
Free sulfite, g/100 g DM	1.53	1.39	1.04	0.94	0.06	< 0.01	< 0.01
Total bacteria, $\log_{10}$ cfu/g fresh mass	6.03	5.47	5.05	5.39	0.12	0.02	< 0.01
Lactic acid bacteria, $\log_{10}$ cfu/g fresh mass	4.65	< 2.8	< 2.8	< 2.8	0.45	< 0.01	< 0.01
Yeasts, $\log_{10}$ cfu/g fresh mass	4.13	3.81	< 2.8	< 2.8	0.37	< 0.01	< 0.01
Molds, $\log_{10}$ cfu/g fresh mass	3.85	< 2.8	< 2.8	< 2.8	0.45	< 0.01	< 0.01
Antioxidant properties							
Total phenolic content, mg GAE/g DM[a]	12.3	11.7	11.5	12.0	0.13	0.27	0.36
Total flavonoid content, mg QE/g DM[b]	8.46	8.05	7.84	7.22	0.14	0.02	< 0.01
Total carotenoid content, µg/g DM	63.3	61.3	56.3	54.6	0.94	0.01	< 0.01
DPPH, mg ASE/g DM[c]	6.72	6.39	6.19	6.01	0.10	0.33	0.08

The end of the preservation period corresponds to 0-d of anaerobic storage

SEM standard error of mean; *DPPH* 2, 2-diphenyl-1-picrylhydrazyl

[a]Expressed as milligrams of gallic acid (GAE) equivalents per g dry weight

[b]Expressed as milligrams of quercetin (QE) equivalents per g dry weight

[c]Expressed as milligrams of ascorbic acid (ASE) equivalents per g dry weight

carotenoids was lower than that observed in the laboratory experiment (16.5 versus 9.3% after 35 days of storage), which may be explained by the greater loss of DM and nutrients during the storage of FVD under large-scale condition. Similar to laboratory-scale experiment, total phenolics narrowly changed during the storage period. However, a loss of 14.7% in total flavonoids and a tendency to a linear reduction ($P = 0.08$) was seen in the DPPH scavenging activity during the 35 days of the storage. More recently, Nadeem et al. [63] reported that as the length of storage increased (90 days), total phenolics, flavonoid content, and antioxidant activity of a carrot and grape juice blend treated with 1% potassium metabisulfite gradually decreased.

Conclusions

Our initial hypothesis was to determine if antioxidant capacity of fruit and vegetable discards could be preserved over time with the addition of sodium metabisulfite. In addition to providing microbiological stability and preserving the nutrient constituents of the discards using metabisulfite, our findings suggested the protective function of metabisulfite on the antioxidant capacity of discarded fruits and vegetables under aerobic and anaerobic storage. However, as the time of storage lengthened, total carotenoids declined modestly. Overall, the use of discards as a feed is a relatively new class of ingredient with functional properties that may increase animal productivity. Experiments are in progress in laboratory to investigate the applicability of metabisulfite-treated fruit and vegetable discards as a functional feed ingredient for ruminant.

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