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Antioxidant, physicochemical, and sensory properties of buffalo meat patties incorporated with roselle (*Hibiscus sabdariffa* L.), wolfberry (*Lycium barbarum* L.), and beetroot (*Beta vulgaris* L.) purées

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Abstract

The present work evaluated the antioxidant, physicochemical, and sensory properties of buffalo meat patties incorporated with 2% roselle (*Hibiscus sabdariffa* L.), wolfberry (*Lycium barbarum* L.), or beetroot (*Beta vulgaris* L.), and chill-stored (4°C) for 11 days. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 2-thiobarbituric acid reactive substances (TBARS), shrinkage, cooking yield, water holding capacity, pH, colour, textural properties, and sensory evaluation of the patties were examined. Patties incorporated with roselle, wolfberry, and beetroot had increased scavenging activity, thus decreasing oxidative activity in the patties during storage. Cooking yield was improved in all treatments with significant decrease in pH in both cooked and uncooked roselle-incorporated patties. No changes were observed for the texture of all samples, while roselle-incorporated patties maintained the redness after the 11th day of storage. Sensory attributes of the modified patties were acceptable to all panellists. In conclusion, the incorporation of roselle in buffalo meat patties showed more beneficial effects than the other purées tested in improving the quality of the patties while maintaining their sensory properties.

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Introduction

Along with the increase in global consumption and production of meat and meat products, there are also increase in the demands for healthier meat and meat products (Jiménez-Colmenero *et al.*, 2001). However, the process of producing meat products such as grinding meat to produce patties damages the stable formation of the muscle membrane, thus creating a larger surface area that eventually promotes lipid oxidation and microbial growth in stored meat products, which in turn reduce the nutritional, sensory, and safety qualities of meat products (Aguirrezábal *et al.*, 2000; Hawashin *et al.*, 2016). Meanwhile, although the fat content in meat products contributes to its organoleptic characteristics and emulsification stability, the saturated fat content is detrimental to human health as it is a risk factor for cardiovascular diseases. Moreover, the presence of polyunsaturated fats in meat and meat products could also decrease their oxidative stability, thus leading to an increase in oxidation. Therefore, several approaches to reduce oxidative damage have been proposed such as optimising storage temperature which would prevent microbial spoilage and meat deterioration due to lipid oxidation (Hur *et al.*, 2009; Ab Aziz *et al.*, 2020), and through the use of natural antioxidants (Abdel-Khalek, 2013).

The replacement of animal fat with plant-based fat, and the addition of natural antioxidants improve the nutritional quality of meat products by decreasing the level of saturated fatty acids (Jung and Joo, 2013). It has been scientifically proven that plants are rich in phenolic compounds which serve as antioxidative agents (Choe and Min, 2009). Several studies have demonstrated that the incorporation of fruits and vegetables in meat and meat products contribute to improved nutritional quality. From previous studies, the shelf life of ground meat and its colour stability were improved and enhanced by the addition of some

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natural antioxidants such as plum purée (Yildiz-Turp and Serdaroglu, 2010) and *ulam raja* (*Cosmos caudatus* Kunth) leaves (Reihani *et al.*, 2014).

Roselle (*Hibiscus sabdariffa* L.) has been widely used in drinks, herbaceous medicines, and as a colouring agent in foods (Jung and Joo, 2013). The flower extracts are rich in phytochemicals containing anthocyanin used as a food colouring, which also has antimicrobial activity (Jung *et al.*, 2013; Ojulari *et al.*, 2019). Wolfberry (*Lycium barbarum* L.) also contains polysaccharides, phenolic compounds, carotenoids, and vitamins (Potterat, 2010; Dong *et al.*, 2012), and has been widely used as a supplement in animal feeds because of its antioxidative characteristics (Abdel-Khalek, 2013). Beetroot (*Beta vulgaris* L.) possesses antioxidant properties as it contains carotenoids and flavonoids, as well as the red pigment called betalains which have been used as a natural food colour in meat and dairy products (Jasmitha *et al.*, 2018). Betalains are also reported to remain unharmed in their antioxidative properties even in gastrointestinal conditions, thus proving their utilisation as a functional food (Frank *et al.*, 2005).

Although the aforementioned plants have antioxidative properties, the effects of their supplementation in meat products have yet to be clarified, particularly regarding the quality and sensory evaluation. Therefore, the present work investigated the antioxidant, physicochemical, and sensory properties of buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées.

Materials and methods

Preparation of roselle, wolfberry, and beetroot purées

Dried roselle calyces, dried wolfberry, and fresh beetroot were soaked in distilled water (1:2, roselle/wolfberry/beetroot:distilled water) for 48 h at 4°C, then ground using a hand blender (Philips, United Kingdom) for 5 min until purées were obtained.

Formulation of buffalo patties

The production of buffalo meat patties was performed following the method of Ismail *et al.* (2021) with slight modifications. Post-rigor topside buffalo meats consisting of different types of muscle were obtained from Sri Ternak, Seri Kembangan, Selangor. The buffalo meat was minced using a meat mincer (HOBART 4812, USA), and mixed with salt, sodium tripolyphosphate (STPP), sugar, black pepper, and crushed fresh garlic using a mixer (GRT-BF, Taiwan) for 5 min at 135 rpm until all the ingredients were homogeneously distributed in the mixer bowl. Then, roselle, wolfberry, or beetroot purées were added based on the formulation described in Table 1, and mixed for another 10 min. The homogenous mixture (90 g) was transferred into a manual burger mould (OMAS BT10, Italy), and shaped before storage at 4°C. All the analyses were carried out on day 1, colour on day 1 and 11, while DPPH and TBARS on day 1, 2, 4, 7, and 11.

Table 1. Formulation of buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées.

Ingredients (%)	C (%)	PWR (%)	PWW (%)	PWB (%)
Buffalo meat	76	74	74	74
Fat	10	10	10	10
Salt	1.5	1.5	1.5	1.5
Sodium tripolyphosphate (STPP)	0.5	0.5	0.5	0.5
Sugar	0.5	0.5	0.5	0.5
Crushed fresh garlic	1.0	1.0	1.0	1.0
Black pepper	0.5	0.5	0.5	0.5
Iced water	10	10	10	10
Purée	0	2	2	2

C = control, PWR = patties with roselle, PWW = patties with wolfberry, and PWB = patties with beetroot.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay

Samples were prepared, and the DPPH radical scavenging activity was determined following the method of Soriano *et al.* (2018) with slight modifications. Briefly, 3 g of the chill-stored patty was weighed using an analytical balance (Shimadzu AY220, Japan), and homogenised in 6 mL of methanol:water (80:20, v/v) using a homogeniser (Heidolph DIAX 900, Germany) at 10,000 rpm for 1 min. The mixture was then centrifuged at 9,840 *g* for 10 min using a microrefrigerated centrifuge (Kubota 3740, Japan), and the supernatant was filtered through Whatman filter paper No 1. An aliquot (200 µL) of the supernatant was added to 800 µL of distilled water and 1 mL of 0.2 mM methanolic DPPH solution, then vortexed using a test tube shaker (Vibromix 104 EV, India) at high speed for 2 min. Next, the mixture was left in the dark for 20 min before the absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV Mini 1240, Japan). The percentage of DPPH radical scavenging activity was calculated using Eq. 1:

Radical Scavenging Activity =
[(Absorbance Control – Absorbance Sample) /
Absorbance Control] × 100

(Eq. 1)

2-thiobarbituric acid reactive substances (TBARS) analysis

Lipid oxidation was determined following the method of Zeb and Ullah (2016). Firstly, 5 g of chilled sample was homogenised in 50% glacial acetic acid using a homogeniser at high speed, and filtered through Whatman filter paper No. 4. Then, 5 mL of the filtrate was transferred into 5 mL of 4 mM TBA prepared in glacial acetic acid, and heated in a 95°C water bath for 60 min. The test tubes were left to cool at room temperature before the absorbance was measured at 532 nm using a UV-Vis spectrophotometer. TBARS were calculated using the MDA-TBA calibration curve and expressed as µM/g.

Shrinkage

Shrinkage of the buffalo meat patties was determined by Vernier callipers using Eq. 2 (Serdaroglu and Degirmencioglu, 2004):

Shrinkage (%) =
[(raw thickness – cooked thickness) +
(raw diameter – cooked diameter)] /
(raw thickness + raw diameter)] × 100

(Eq. 2)

Cooking yield

Buffalo meat patties were cooked using a non-sticky pan for 4 min on each side, with the weight recorded before and after cooking to calculate the percentage cooking yield using Eq. 3 (Kahar *et al.*, 2021):

Cooking Yield (%) =
(weight of cooked patty / weight of raw patty) × 100

(Eq. 3)

Water holding capacity (WHC)

The WHC of the buffalo meat patties were determined following the method of Alemán *et al.* (2016) with slight modifications. A sample of buffalo patty was weighed (1.5 g) and placed in a centrifuge tube with a filter paper (Whatman No 1). The sample was centrifuged for 15 min using a microrefrigerated centrifuge (Kubota 3740, Japan) at 20°C and 4,000 *g*. The WHC was calculated as the amount of water retained in the sample per 100 g of water before centrifuging in triplicate.

pH

The pH of the raw and cooked patties was determined following the method of Kahar *et al.* (2021) with slight modifications. Briefly, 5 g of buffalo meat patty was homogenised in 45 mL of distilled water at high speed for 1 min, and the pH was measured using a pH meter (Sartorius PB10, USA).

Texture profile analysis

The textural properties of the cooked buffalo meat patties were determined following the method of Reihani *et al.* (2014) using a texture analyser (TA-XT2i Stable MicroSystems, London). Seven textural parameters were assessed: hardness (g), springiness (mm), cohesiveness, adhesiveness (g.s), gumminess (g), resilience, and chewiness (g.mm).

Colour

The colour of the surface of the raw buffalo meat patties was measured using a chromameter (Konica Minolta CR-410, Japan). The colour coordinates in L^*, a^*, and b^* were recorded. The apparatus was fixed and standardised against a white plate before each measurement (Ismail *et al.*, 2021).

Sensory evaluation

Sensory analysis of cooked buffalo meat patties was performed using a 9-point Hedonic scale (1 = extremely dislike, 9 = extremely like; Ismail *et al.*, 2021). Thirty untrained panellists evaluated the sensory attributes of the four samples of cooked buffalo meat patties namely appearance, aroma, flavour, colour, texture, and overall acceptability of the randomly coded buffalo patties.

Statistical analysis

One-way analysis of variance (ANOVA) with Tukey's test was used to analyse the data individually without considering the interaction. Minitab software version 19 (Penn State, USA) was used for statistical analysis.

Results

DPPH and TBARS assays

The antioxidant activities of PWR, PWW, and PWB were determined by measuring the amount of free radical scavenging activity using the DPPH assay on day 1, 2, 4, 7, and 11 of storage at 4°C; the higher the free radical scavenging activity, the higher the antioxidant activity of the sample. The initial free radical scavenging activity measured on day 1 of storage varied significantly ($p < 0.05$) among treatments. PWR showed a significantly ($p < 0.05$) higher scavenging activity as compared to other treatments; however, all treatments, except the untreated meat patties, showed significant increases in the scavenging activity between the 1st and 7th day of storage (Table 2). This showed that the incorporation of roselle, wolfberry, and beetroot purées into the buffalo meat patties could have increased the scavenging activity, thus decreasing the oxidative activity in the meat patties during storage.

The lipid oxidation of uncooked PWR, PWW, and PWB was determined using the TBARS assay on day 1, 2, 4, 7, and 11 of storage in 4°C; the higher the TBARS, the higher the lipid oxidation. The TBARS values increased for all treatments, suggesting an increase in lipid oxidation during storage. The PWR consistently and significantly ($p < 0.05$) showed the lowest lipid oxidation (as indicated by the lowest TBARS value) of all treatments during storage. Also, PWW and PWB had less lipid oxidation as compared to the untreated patties. This suggested that incorporating roselle, wolfberry, and beetroot purées into buffalo meat patties could have decreased the lipid oxidation of the meat patties, thus retaining the quality characteristics of the meat patties (Table 2).

Table 2. Free radical scavenging activity and TBARS value of raw buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées on different storage days at 4°C.

Analysis	Sample	Storage duration (day)				
		1	2	4	7	11
DPPH	C	8.98 ± 1.09^{dA}	7.07 ± 0.77^{dAB}	6.94 ± 0.45^{dAB}	6.13 ± 0.68^{dB}	5.32 ± 0.90^{dB}
	PWR	45.42 ± 0.45^{aB}	50.53 ± 1.12^{aA}	46.62 ± 0.84^{aB}	52.24 ± 0.56^{aA}	51.55 ± 0.30^{aA}
	PWW	36.61 ± 1.56^{bB}	37.34 ± 2.05^{bB}	40.02 ± 1.45^{bAB}	41.46 ± 1.41^{bA}	40.01 ± 0.27^{bAB}
	PWB	17.03 ± 0.87^{cB}	17.24 ± 2.33^{cB}	20.01 ± 1.69^{cAB}	23.63 ± 1.35^{cA}	17.50 ± 1.38^{cB}
TBARS	C	0.67 ± 0.02^{aD}	0.72 ± 0.01^{aCD}	0.87 ± 0.09^{aBC}	0.98 ± 0.01^{aB}	1.42 ± 0.11^{aA}
	PWR	0.46 ± 0.01^{dC}	0.55 ± 0.04^{cB}	0.53 ± 0.01^{bB}	0.59 ± 0.02^{cB}	0.74 ± 0.03^{cA}
	PWW	0.57 ± 0.02^{cC}	0.63 ± 0.03^{bC}	0.77 ± 0.02^{aB}	0.85 ± 0.05^{bB}	1.11 ± 0.04^{bA}
	PWB	0.62 ± 0.01^{bD}	0.70 ± 0.01^{aCD}	0.82 ± 0.02^{aBC}	0.89 ± 0.05^{bB}	1.14 ± 0.09^{bA}

C = control, PWR = patties with roselle, PWW = patties with wolfberry, and PWB = patties with beetroot. Values are mean ± SD (standard deviation). Means followed by different lowercase superscripts in the same column are significantly different between different treatments within the same analysis. Means followed by different uppercase superscripts in the same row are significantly different between different storage times.

Shrinkage, cooking yield, WHC, and pH

Table 3 shows the shrinkage, cooking yield, WHC, and pH values of the buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purée as compared to the control. PWR, PWW, and PWB showed significantly higher ($p < 0.05$) cooking yields as compared to the untreated patties, with PWW purée showing the highest cooking yield, followed by PWB and PWR. However, there was no significant difference in the shrinkage between untreated and treated patties, with more shrinkage occurring in the untreated patties.

There was no significant difference in the WHC of the treated patties, with an increasing trend in the WHC of PWR, PWW, and PWB as compared to the untreated patties, thus suggesting that the incorporation of roselle, wolfberry, and beetroot purées could have improved the ability of the meat patties to retain water.

The pH of the raw PWR and PWW was significantly ($p < 0.05$) lower than the PWB and untreated patties. However, the pH of the cooked patties increased significantly ($p < 0.05$) as compared to the raw buffalo meat patties, with only cooked PWR having a significantly ($p < 0.05$) lower pH as compared to other treatments.

Table 3. Shrinkage, cooking yield, water holding capacity, and pH values of buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées.

Sample	Shrinkage (%)	Cooking yield (%)	WHC (%)	pH (raw)	pH (cooked)
C	27.37 ± 0.16^{a}	65.17 ± 1.12^{c}	76.21 ± 0.89^{a}	5.74 ± 0.04^{aA}	5.97 ± 0.06^{aB}
PWR	25.26 ± 1.56^{a}	75.75 ± 1.60^{b}	80.11 ± 4.33^{a}	5.52 ± 0.02^{bA}	5.82 ± 0.02^{bB}
PWW	21.11 ± 4.25^{a}	80.15 ± 0.27^{a}	78.93 ± 0.99^{a}	5.48 ± 0.01^{bA}	6.00 ± 0.02^{aB}
PWB	21.69 ± 2.41^{a}	78.50 ± 0.62^{ab}	79.22 ± 2.15^{a}	5.72 ± 0.03^{aA}	5.97 ± 0.04^{aB}

C = control, PWR = patties with roselle, PWW = patties with wolfberry, and PWB = patties with beetroot. Values are mean ± SD (standard deviation). Means followed by different lowercase superscripts in the same column are significantly different between different treatments. Means followed by different uppercase superscripts in the same row are significantly different in pH values between raw and cooked patties.

Texture profile analysis (TPA)

The TPA results showed that there was no significant difference in hardness, gumminess, chewiness, adhesiveness, springiness, resilience, and cohesiveness of all treated patties, thus suggesting that the textural properties were unaffected by the incorporation of the purées (Table 4).

Colour

The instrumental colour values of L^*, a^*, and b^* which indicated lightness, redness, and yellowness, respectively, were evaluated for each of the chilled-stored buffalo meat patties on day 1 and 11. There was no significant difference in L^* and a^* among treatments on day 1, whereas b^* significantly ($p < 0.05$) decreased in PWR as compared to the untreated patties. However, after storage for 11 days, only a^* significantly ($p < 0.05$) decreased in all treated patties except PWR, which maintained a^*, while b^* was observed to significantly ($p < 0.05$) decrease in both PWW and PWB throughout storage as compared to untreated patties. On the 11th day of storage, the PWR showed a significantly ($p < 0.05$) lower b^* as compared to the untreated patties (Table 5).

Sensory evaluation

There was no significant difference in the sensory evaluation of the colour, aroma, flavour, appearance, and overall acceptability of the treated and untreated buffalo meat patties, with only the texture of PWW showing a significant ($p < 0.05$) increase as compared to PWR (Table 6).

Discussion

Roselle, wolfberry, and beetroot had antioxidative effects on meat patties during storage, which could have been due to the presence of phenolic compounds. The present work demonstrated

Table 4. Texture profile analysis of cooked buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées.

Samples	Hardness (g)	Gumminess (g)	Chewiness (g.mm)	Adhesiveness (g.s)	Springiness (mm)	Resilience	Cohesiveness
C	5503.52 ± 693^{a}	4402.11 ± 249.8^{a}	3734.64 ± 137.6^{a}	-2.13 ± 0.19^{a}	0.85 ± 0.02^{a}	0.33 ± 0.03^{a}	0.80 ± 0.00^{a}
PWR	6949.76 ± 637^{a}	5424.95 ± 587.5^{a}	4652.11 ± 508.9^{a}	-2.54 ± 0.22^{a}	0.86 ± 0.00^{a}	0.30 ± 0.02^{a}	0.78 ± 0.01^{a}
PWW	4709.14 ± 1353^{a}	3677.68 ± 1007.6^{a}	3045.40 ± 908.04^{a}	-2.14 ± 0.34^{a}	0.82 ± 0.02^{a}	0.32 ± 0.04^{a}	0.78 ± 0.04^{a}
PWB	7475.32 ± 1897^{a}	5444.25 ± 649.1^{a}	4313.87 ± 484.8^{a}	-4.20 ± 1.58^{a}	0.79 ± 0.07^{a}	0.29 ± 0.02^{a}	0.73 ± 0.05^{a}

C = control, PWR = patties with roselle, PWW = patties with wolfberry, and PWB = patties with beetroot. Values are mean ± SD (standard deviation). Means followed by different lowercase superscripts in the same column are significantly different between different treatments.

Table 5. Colour attributes of raw buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées on day 1 and 11 of storage at 4°C.

Samples	Day 1			Day 11		
	L*	***a****	***b****	***L****	***a****	***b****
C	48.65 ± 1.92^{aA}	15.57 ± 3.42^{aA}	10.98 ± 0.99^{abA}	40.21 ± 5.68^{aA}	8.21 ± 1.59^{aB}	9.08 ± 0.83^{aA}
PWR	47.19 ± 1.573^{aA}	9.98 ± 1.11^{aA}	7.33 ± 1.55^{cA}	36.75 ± 9.86^{aA}	8.44 ± 0.19^{aA}	6.31 ± 1.66^{bA}
PWW	46.98 ± 1.23^{aA}	14.46 ± 3.52^{aA}	13.36 ± 0.15^{aA}	37.78 ± 5.68^{aA}	7.71 ± 0.71^{aB}	8.71 ± 0.52^{abB}
PWB	46.25 ± 1.16^{aA}	14.56 ± 1.55^{aA}	9.20 ± 0.83^{bcA}	34.52 ± 11.21^{aA}	7.52 ± 1.97^{aB}	6.55 ± 0.66^{abB}

C = control, PWR = patties with roselle, PWW = patties with wolfberry, and PWB = patties with beetroot. Values are mean ± SD (standard deviation). Means followed by different lowercase superscripts in the same column are significantly different between different treatments. Means followed by different uppercase superscripts in the same row are significantly different between different storage times within the same measurement.

Table 6. Sensory evaluation attributes of cooked buffalo meat patties incorporated with either roselle, wolfberry, or beetroot purées.

Samples	Attributes					
	Colour	**Aroma**	**Flavour**	**Texture**	**Appearance**	**Overall Acceptability**
C	6.43 ± 1.25^{a}	6.97 ± 1.00^{a}	6.87 ± 1.41^{a}	6.23 ± 1.74^{ab}	6.30 ± 1.50^{a}	6.83 ± 1.12^{a}
PWR	6.37 ± 1.56^{a}	6.40 ± 1.57^{a}	6.21 ± 1.57^{a}	5.83 ± 1.60^{b}	6.43 ± 1.43^{a}	6.33 ± 1.37^{a}
PWW	6.77 ± 1.10^{a}	6.50 ± 1.22^{a}	6.43 ± 1.83^{a}	6.90 ± 1.19^{a}	6.57 ± 1.28^{a}	6.63 ± 1.65^{a}
PWB	6.47 ± 1.43^{a}	6.60 ± 1.28^{a}	6.70 ± 1.18^{a}	6.73 ± 1.34^{ab}	6.60 ± 1.48^{a}	6.93 ± 0.87^{a}

C = control, PWR = patties with roselle, PWW = patties with wolfberry, and PWB = patties with beetroot. Values are mean ± SD (standard deviation). Means followed by different lowercase superscripts in the same column are significantly different between different treatments.

the antioxidative activity of PWR, PWW, and PWB, with the highest scavenging activity observed in PWR. This is in line with a previous study by Mohd Esa *et al.* (2010) which reported free radical scavenging activity in methanolic extracts of roselle calyces. Moreover, the higher free radical scavenging activity obtained might have been due to a large number of polyphenols in roselle calyces as they play a vital role in antiradical and antioxidant activities. According to Mohammed *et al.* (2007), phytosterols (β-sitosterol) and vitamin E (γ-tocopherol) in roselle can donate hydrogen, thus reducing and decolourising DPPH. The DPPH results were also in line with the TBARS values of the treated patties. The increase in the scavenging activity of PWR associated with the lowest value of TBARS as compared to other treated and untreated patties during storage was reflected in the decreased amount in lipid oxidation of PWR.

The lowest antioxidative activity was measured in the untreated patties which can be regarded as the benchmark in determining the activity in meat. In contrast with other treatments, the scavenging activity of untreated patties showed a constant decreasing trend from day 1 until 11, which might have been due to the degeneration of muscle and the occurrence of lipid oxidation during storage. Lipid oxidation commonly occurs when meat is stored in a refrigerator due to higher temperature (4°C) as compared to freezing temperature (> -20°C), thus causing faster meat deterioration (Vaithiyanathan *et al.*, 2011).

Treated samples showed the highest scavenging activity on the 7th day, then started to deplete until the 11th day, thus suggesting that seven days of storage could be the optimum storage time as the decreasing DPPH scavenging activity on subsequent days would affect the shelf life of the treated patties. These results agree with Hawashin *et al.* (2016) who reported that the peak DPPH scavenging activity of destoned olive cake in raw beef burger occurred on the 7th day. It is also possible that the polymerisation of the polyphenols reduces the availability of oligomers for charge delocalisation, which might contribute to the increased antioxidative activity during the initial storage. However, antiradical capacity will then decrease after a few days of storage as polymerisation reaches and exceeds its critical value due to high molecular complexity and steric hindrance reducing the presence of hydroxyl groups to react with the DPPH radicals (Pinelo *et al.*, 2004). On the 11th day of storage, the DPPH and TBARS values of PWR were significantly different ($p < 0.05$) from the other treatments and untreated patties. These results showed the capability of roselle to decrease lipid oxidation in buffalo meat patties, which could have been due to the antioxidative bioactive compounds found in roselle calyces (Mohd Esa *et al.*, 2010).

Cooking yield is an important factor for the meat industry in the determination of product behaviour during cooking. The treated patties had a higher cooking yield percentage than the untreated patties, thus suggesting that roselle, wolfberry, and beetroot purées could have improved the moisture retention in the treated patties. This is in line with Shyamala and Jamuna (2010) who reported that beetroot has a high-water retention capacity that could be utilised to enhance the moistness of a product. Ergezer *et al*. (2014) also reported that the incorporation of 10% and 20% potato purée in meatballs increased the cooking yield by 5.53 and 19.77%, respectively. Meanwhile, Serdaroglu and Degirmencioglu (2004) reported that fat and moisture loss during cooking could result in a low cooking yield. The untreated patties showed the lowest cooking yield percentages, which could have been due to the lower fat retention caused by the fat loss during cooking following the reduction in matrix density of the patties and loss of fat stability (Ergezer *et al.*, 2014). This also suggested that the patties incorporated with roselle, wolfberry, and beetroot purées could have improved fat retention by improving the interaction with matrix in the patties, thereby increasing the cooking yield. Cooking heat also contributes to the denaturation of protein, thus promoting the release of chemically bound water from the meat (Vieira *et al.*, 2009). Therefore, it could also be suggested that the treated patties had a protective effect on the protein-water interaction, thereby reducing the amount of water released from the patties. The improvement of cooking yield in the patties could also have been due to the water retention ability of the dietary fibre that was found in those three ingredients (Mercado-Mercado *et al.*, 2015; Ma *et al.*, 2019; Mirmiran *et al.*, 2020). It has been reported that the dietary fibre incorporated into meat products will increase emulsion stability, thus resulting in a higher cooking yield as well as improving water binding capacity of the products (Biswas *et al.*, 2011; Mehta *et al.*, 2015). However, the present work demonstrated that the treatments did not improve the WHC of the patties, which might suggest that the treatments did not protect the free water movement from the patties by gravity. This observation is not in line with other studies by Gómez-Cortés *et al.* (2018) and Yildiz-Turp and Serdaroglu (2010) which showed improved WHC, which in turn could have been due to the different mechanisms of treatment in affecting WHC, for instance heat, pressure, and gravity, as well as the fibre types, length, particle size, and porosity of the dietary fibres in the treatments, thus contributing to the free water movement from the patties (Kim and Paik, 2012).

According to Bertram *et al*. (2004), meat shrinkage is initiated at 60°C as myosin is denatured and precipitated at 40°C, thus leading to collagen shrinkage at 50°C. Meanwhile, Du and Sun (2005) claimed that low shrinkage of a meat product would result in a high cooking yield and *vice versa*, due to the loss of fluid and fat during cooking, which also leads to weight and dimension losses of the meat. Despite there was no significant effect of the treatments on the shrinkage of the patties, the untreated control showed a higher shrinkage percentage as compared to the treated patties, thus indicating the potential improved interaction of fluid and fat with the matrix in the patties with the incorporation of roselle, wolfberry and beetroot.

Colour contributes to the palatability, wholesomeness, and freshness of the product. PWR had lower yellowness as compared to PWW and PWB, with the redness and yellowness of PWR were not significantly different even after storage, while both characteristics were reduced in PWW and PWB. This might have been due to natural colour pigments, anthocyanins, that are commonly found in roselle. Anthocyanins produce colour ranges from red, purple, and blue based on their pH (Zhang *et al.*, 2019), hence, producing darker-coloured patties as compared to the other treatments. Meanwhile, lipid oxidation also affected the colour by increasing the formation of metmyoglobin during storage, which might have been due to the reduction in metmyoglobin-reducing enzyme activity, thus contributing to meat discolouration (Viana *et al.*, 2017; Augustyńska-Prejsnar *et al.*, 2018). In the present work, roselle was demonstrated to maintain the redness and yellowness of PWR, which could be reflected by reduced metmyoglobin formation, thus indicating the effect of roselle in slowing the rate of lipid oxidation as compared to the other treatments.

PWR and PWW had the lowest pH due to their acidic characteristics (Amagase *et al.*, 2009; Mikulic-Petkovsek *et al.*, 2012; Balarabe, 2019), which is in line with a previous study by Jung and Joo (2013) who reported a lower pH with an increasing amount of roselle extract in pork patties. Raw PWB had a pH close to control samples due to beetroot's characteristic, which is not acidic. According to Pérez and Pérez (2009), the pH of beetroot juice is 6.34, which might explain why raw PWB almost had the same pH as the untreated control patties. However, the pH of buffalo patties changed significantly when exposed to heat, which is in line with Reihani *et al.* (2014), who reported a higher pH of cooked frozen beef patties treated with *ulam raja* leaves. The pH rises as the meat is cooked due to protein denaturation which could result in a tender and juicier product (Penny, 1969; Roberts and Lawrie, 1974). Meanwhile, the pH of cooked PWR was significantly the lowest among the cooked patties due to its acidity. Meat with a high pH is more tender and juicier in texture than meat with a lower pH (Penny, 1969), as evidenced by the panellists' evaluations which showed that cooked PWR had a significantly lower texture score as compared to PWW. The texture profile analyses were not significantly different; however, the concentration of the treatment should also be taken into account as it could affect the analysis of the sensory attributes (Jung and Joo, 2013).

Conclusion

The incorporation of roselle, wolfberry, and beetroot purées were beneficial in inhibiting and delaying lipid oxidation in buffalo meat patties as they had significant increase in scavenging free radical activity, increase in cooking yield percentages, decrease in TBARS values, and the panellists' acceptability of the formulated patties was similar to the control patties. Roselle was the best treatment, showing higher potential in reducing lipid oxidation during storage with the same eating quality as evaluated by the panellists.

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