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(Original Article)

Sustainable Preservation of Beef Burgers Using Blueberry

Abeer M. Fathy*; Asmaa M. Abdel-Rahman; El-sayed A. Ramadan and Soumia M.I. Darwish

Food Science and Technology Department, Faculty of Agriculture, Assiut University, Assiut.

*Corresponding author email: soumiadarwish123@hotmail.com
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Abstract

Consumer demand for natural preservatives in processed meat products has prompted research into alternative options that can maintain health and safety standards. In this study, the chemical composition and antioxidant activity of blueberries (*Vaccinium corymbosum* L.) (BB) were investigated, and four beef burger formulas were (control beef burger) (C), a beef burger with 0.01% butylated hydroxytoluene (BHT) (T1), a beef burger with 1% BB (T2), and a beef burger with 1.5% BB (T3). The results revealed that BB contained high levels of total phenolics (435 mg GAE/100g sample, flavonoids (59.76mg Quercetin/ 100g sample), and anthocyanins (173.3mg/ 100g.), making it a promising candidate for use as a natural preservative. Furthermore, incorporating BB into the beef burgers improved all sensory parameters when compared with control, and significantly increased cooking yield, while decreasing cooking loss ($p< 0.05$). The inclusion of BB also resulted in a clear decrease in the levels of thiobarbituric acid reactive substances (TBARS) and peroxide values ($p<0.05$), indicating its potential as a functional ingredient with preservative ability during meat product processing. Therefore, the use of blueberries as a natural preservative in meat products is recommended as a sustainable alternative to synthetic preservatives.

Keywords: *Blueberry, Vaccinium corymbosum L., Antioxidant activity, Beef burger, Sustainable.*

Introduction

Fresh and processed meat products are an integral part of the human diet owing to their high protein, fat, and mineral content (Lorenzo & Pateiro, 2013). Nevertheless, the elevated proportion of unsaturated fatty acids and cholesterol present in these items contributes to an escalated oxidation process, notably when cooking and chopping (Domínguez *et al*., 2016) Oxidation has detrimental effects on meat quality, impacting its color, taste, texture and nutritional value. Free radicals are the primary initiators of oxidation, which occurs through a complex series of reactions involving oxygen, fats, pigments, proteins and vitamins, all of which are critical components of animal flesh and its processed food items (Johnson and Decker, 2015). The use of combinations of Phenolic antioxidants such as butylated hydroxytoluene (BHT), can delay the oxidation process by scavenging peroxyl radicals or inhibiting free radical formation (Kumar *et al*.,

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2015). Nonetheless, the utilization of commercial antioxidants may result in adverse effects on human health, which demands for using of the natural antioxidants in meat and meat product manufacture (Fernandes *et al*., 2018).

Blueberries (*Vaccinium corymbosum*) contain high levels of phytochemicals includingflavonoids, and polyphenolic compounds (Zeng *et al*., 2021) that act as potent antioxidants, lowering the occurrence of oxidative stress-induced damage (Skrovankova *et al*., 2015)

Although previous studies have used berry extracts in the production of processed animal-based food items (Tamkutė *et al*., 2019 and Babaoğlu *et al*., 2022) Therefore, this study aims to evaluate the impact of adding blueberries on the physical, chemical and sensory properties of beef burgers during storage.

Materials and Methods

Materials

Three kilograms of blueberry fruits were obtained from the Saudi market branch in Nasr City, Cairo, Egypt. A local butcher shop in Assiut City, Egypt supplied fifteen kilograms of boneless ground beef meat. Other ingredients of the beef burger (wheat flour, salt, dry onion, dry garlic, spices, natural flavors) were obtained from the local market, in Assiut, Egypt. Furthermore, all chemicals used in this study were purchased from EL-Gomhouria for Trading Chemicals and Drugs Co., Assiut City, Egypt, Gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Folin Ciocalteu's phenol reagent gets it from Sigma-Aldrich Chemie Gmbh Munich, Germany. Furthermore, butylated hydroxytoluene (BHT) was obtained from PIOCHEM, Egypt.

Methods

Preparation of Blueberries fruits

The blueberries were washed, then lyophilized at the National Agriculture Research Center in Cairo, Egypt.

Preparation of beef burger samples

Beef burgers were prepared according to Heinz and Hautzinger (2007), with some modifications. The control formulation for beef burgers comprised 80% meat and 10% rehydrated texturized soy. (1g soy: 2ml water), 4% onions, 2% Rusk, 1.5% salt and 2.5% spices mix. The mixture was served as a control (C) (without any preservatives). While sample with synthetic antioxidant butylated hydroxytoluene (BHT) were added (0.01%) coded with T1, other samples of beef burgers were prepared as the same previous method with blueberries powder (BB) addition (as natural antioxidants) at different concentrations (1% and 1.5%) and coded with T2 and T3, respectively. All formulations were packaged aerobically in foam plates, enclosed with polyethylene bags, and then stored under refrigeration (4 ± 1°C) for 9 days. Samples were analysis during storage and subjected to physical, and chemical analysis.

Analytical methods

Proximate composition analysis

Moisture, crude fibers, crude fat, ash and protein contents were determined in blueberry and beef burger samples according to the methods described in the AOAC (2000). Total carbohydrates were calculated by difference according to Turhan *et al*., (2005), while the caloric content was determined using Livesy (1995).

Total phenolic content, flavonoids, and antioxidant activity:

Total phenolics: According to the method of Abdel-Gawad (1982) total phenolic

compounds extraction of samples was done via alkali hydrolysis followed by extractionthe phenolics using diethyl acetate at pH 3.5, then removing the trace of water by dehydration with anhydrous sodium sulfate. The extracted residue was purified by removing any trace of diethyl acetate under vacuum. The purified residue was then dissolved in methanol and subjected to the Folin-Ciocalteu method for the estimation of phenolic compounds using a spectrophotometer. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of sample on a dry weight basis, as described by Singleton and Rossi (1965).

The total flavonoid content was estimated using the method of Jia *et al*. (1999) with minor modifications. A 500 µl of sample extract (prepared for total phenolic compounds) was mixed with 2 ml of distilled water and subsequently with 0.15 ml NaNO2 (5%). After 6 min, 0.15 ml AlCl3 (10%) was added and allowed to stand a further 6 min., after that, 2 ml NaOH (4%) was added to the mixture. Immediately, distilled water was added to make the final volume up to 5 ml. Then, the mixture was mixed and allowed to stand for 15 min. The absorbance was measured by spectrophotometer against a blank at 510 nm. The results were reported as milligrams of catechin equivalents / 100 g of dry sample weight (mg CE/ 100g sample).

Antioxidative activity was evaluated using the procedure described by Hangun-Balkir, and McKenney, (2011).. A known weight (0.010 g) of DPPH was dissolved in 100 mL ethanol (80%). The extract was prepared as follow: 5 g of blueberry was mashed, and it was stirred in 80% ethanol (a minimum amount) for about 15 minutes. The antioxidant solution was centrifuged at 5000 rpm for 3 minutes. As a sample liquid the supernatant was kept. Individual solutions of antioxidants were prepared at concentrations varying from 1 to 10 mg/mL in 80% ethanol. Two of DPPH solution was added to a 2 mL sample solution, then the mixture was incubated for 30 minutes at room temperature after it shaken well. 2 mL DPPH mixture with 2 mL of 80% ethanol was used as a control measurement. The absorbance of the sample solutions and control was measured at 517 nm by using CARY-100 UV-Vis spectrophotometer. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition percentage was computed using the following formula:

I % = [(AB - AS)/AB] x 100

As I=DPPH radical inhibition %; AB= control sample absorbance; AS = absorbance of a tested sample at the end of the reaction.

Each assay was carried out in triplicate. The inhibitory concentration (IC50) was used to express the oxidative capacity of the fruits. This inhibitory concentration is the antioxidant concentration responsible for inhibiting 50% of free radical activity.

Determination of total carotenoids content from berries fruit

Carotenoids of the blueberry were determined according to the methods described by Holm (1954); Wetsttein (1957). In dark bottles, 30 ml of 85% acetone was mixed with 5 grams of each sample and left for 15 hours at room temperature. Then, on glass wool, the sample is filtered into a 100 mL volumetric flask and made up to volume with acetone solution 85%.

Absorbance for the prepared extracts was read on the spectrophotometer at wavelengths of 662, 644 and 440 nm. A blank experiment using acetone (85%) was carried out. The contents of total carotenoids and chlorophylls were calculated using the following equations:

Chlorophyll A (mg/L) = (9.784 × A662) – (0.99 × A664).

Chlorophyll B (mg/L) = (21.426 × A664) – (4.65 × A662).

Chlorophyll A+B (mg/ L) = (5.134 × A662) + (20.436 × A644).

Total carotenoids (mg/L) = 4.695 ×A440 – 0.268× (Chlorophyll A+B).

Determination of Anthocyanins in berries fruit

Anthocyanin was determined according to AOAC (2006), (Not found in the references) by preparing two extracts from each sample, one containing a potassium chloride (KCl) buffer solution (pH 1.0), and the other containing a sodium acetate buffer solution (pH 4.5). The absorbance was measured by using a spectrophotometer at 530 nm and 700 nm. The results are expressed as mg cyanidin-3- glucoside equivalent (cyd-3-glu E)/ 100g sample.

Quantification of Ascorbic acid (Vitamin C) content

Ascorbic acid (Vitamin C) was determined by the method described by AOAC (1990). The dye solution was created by dissolving 2,6-dichlorophenol indophenol (DCPI) (0.025%) in hot water that contained sodium bicarbonate (NaHCO3). and the final solution was stored at 3°C. Dye molarity was determined by using ascorbic acid with conservation solution (15 gm oxalic acid + 40 ml acetic acid 10%, The final volume was made up to 500 mL with distilled water. The volume of DCPI, which was used in titration was recorded and used in the calculation of Vitamin C (%) according to the following equation:

$$\text{Vitamin C (\%)} = \frac{\text{Dye volume used in titration x dye molarity}}{\text{Sample volume}} \text{ x } 100$$

Quantification of Tannins

Tannins were determined in the samples according to the method described by Earp et al. (1981). 0.2 g of blueberries were macerated in 50 ml of 1% HCl in methanol for 24 hrs. at ambient temperature. Then, one ml of sample extract was added to 5 ml of vanillin reagent (which was prepared by adding vanillin (4%) to 8% hydrochloric acid in CH_3OH (1:1)). The absorbance was measured at 500 nm after 20 minutes.

Determination of lipid oxidation of prepared beef burger

Quantification of thiobarbituric acid (C4H4N2O2S) reactive substances (TBARS): TBARS values were determined in beef burger samples at 0, 3, 6 and 9 days of storage at 4±1˚C. According to the method of method was used to assess the effectiveness of natural additives as antioxidants. Forty ml. of trichloroacetic acid (7.5%) was added to twenty grams of sample, then leave the mixture for 30 minutes after homogeneity for one minute. The mixture was filtered through Whatman No. 1 filter paper. 5 mL of sample extract was taken and added to 5 mL of TBA (thiobarbituric acid) solution (0.2883 g TBA/ 100 ml water). For the plank sample, it is 5 ml of TBA solution and 5 ml of distilled water. After covering the tubes, they are heated for 40 minutes in a boiling water bath. The absorbance of the samples was measured at 538 nm using ultraviolet visible scanner Spectrophotometer (LKB 4054 Cambridge, England) after rapid cooling of the samples in an ice bath. By multiplying the absorption values in the number 7.8, the value of thiobarbituric acid is calculated, which is expressed in milligrams of malonaldehyde per 1000 grams of sample.

Quantification of peroxide value: Peroxide value is a measure of the amount of peroxides present in a sample, which can indicate the extent of lipid oxidation. It was determined according to the method of AOAC (2006), and the value of peroxide was calculated according to the following equation:

Peroxide value = ((N×V×1000)/W)

Where: N = Normality of sodium thiosulfate solution

V = Volume of sodium thiosulfate solution used for titration (mL)

W = Weight of sample (g)

Assessment of the sensory attributes of the beef burger:

By ten staff members in the Food Science and Technology Department, Faculty of Agriculture, Assiut University, the sensory attributes (texture, flavor, taste, color and overall acceptability) of beef burgers were evaluated according to the method of Lindley *et al*., (1993).

Microbiological analysis of prepared beef burger

Preparation of the sample: 90 ml of sterile peptone solution (9gm peptone/1L distilled water) was mixed with ten grams of each sample in a blender, under sterile

conditions to give 1/10 dilution. For counting several types of bacteria, the prepared serial dilutions were used.

Determination of total plate bacterial counts: The total number of bacterial colonies was assessed using the plate counting technique on nutrient agar medium, following the procedures outlined by APHA (1976) and Difco (1984). The plates were incubated at 37°C for 48 hours.

Determination of yeast and mold counts (YMC): The YMC was determined using Bacto yeast malt (Y.M) agar medium, following the methods described in the Difco Manual (1998). The plates were incubated at 30 ±2°C for 5-7 days.

Physical characterization

Cooking loss: cooking loss value was determined according to the method of Lee et al. (2008)by using the following equations

$$\text{Cooking loss (g/ 100g)} = \frac{Wr - Wc}{Wr} \times 100$$

Where: Wr: the weight of raw burger (g); Wc: the weight of cooked burger (g).

Cooking yield: The cooking yield values of beef burger samples were determined by subtracting cooking loss from 100 according to El-Nemr (1979).

Color measurement: The L*, a*, and b* values of the samples were measured using the International Commission on Illumination (CIE) color space system. at three different points of the sample with a Chroma meter (Minolta CR 400, Minolta Camera, Co., Osaka, Japan). On a standard white ceramic plate (a = - 0.13, b = - 0.30, L = 95.97) the calorimeter has been calibrated before reading. Corresponding a* value (degree of redness (0 to 60) or greenness (0 to -60); b* values (yellowness (0 to 60) or blueness (0 to - 60) and L* value (lightness of color from zero (black) to 100 (white) were measured for all burger samples. Hue is a color attribute by which red, yellow, green and blue are identified, while Chroma distinguishes between dull and vivid colors were calculated according to the following equation:

(a2+b2)0.5 (Abonyi *et al*., 2002).

PH value: In a homogenate prepared with a 10 g sample and distilled water (90 ml) pH values of the studied beef burger samples were measured using pH meter (Turhan *et al*., 2005).

Water holding capacity (WHC): the WHC (water holding capacity) was determined by using the centrifugation method as described by Hamm (1960), \ Through the difference between the weight of the sample before and after centrifugation, the E.F (expressible fluid) was determined.

Statistical analysis

The ANOVA (analysis of variance) was performed using SPSS software (version 16.0 for Windows, SPSS Inc., Chicago, IL) to determine significant differences among means. Duncan's multiple comparisons were used for mean difference testing.

Results and Discussion

Blueberry analysis

Chemical composition

The chemical composition of blueberry was present in Table 1. The obtained results for blueberry analysis showed that moisture, ash, fat, protein, fiber, and total carbohydrates recorded 84.09, 0.243, 0.332, 0.86, 2.96 and 11.50 g/ 100g samples, respectively; which was in the same line with that reported by USDA (2013) which illustrated that moisture, ash, crude fat protein, and carbohydrates in blueberry were 87.7, 0.08, 0.74, 0.48, and 11.54g/ 100g, respectively. Likewise, Hidalgo and Almajano (2017) found that fat, protein and carbohydrates in blueberries were 0.33, 0.74 and 14.49%, in each sample. Additionally, the caloric value of blueberry samples was determined to be 52.44 kcal per 100 grams.

Table 1. Gross chemical composition and caloric value of blueberries

Parameters%	Blueberry
Moisture%	84.09±0.078
Ash*	0.243±0.003
Crude fat*	0.332±0.008
Protein*	0.86±0.03
Crude fiber*	2.96±0.115
Total carbohydrates**	11.50±0.167
Caloric value	52.44±0.647

*Means± SD (standard deviation) of duplicate (g/ 100g on dry weight basis); ** Total carbohydrates calculated by differences.

Phenolics, flavonoids content and antioxidant activity of blueberries

Data in Table 2 showed that the total phenolic content was 435 mg GAE/ 100g sample. The obtained results are in agreement with those reported by Bunea *et al*. (2011) as they reported that the total phenolic content ranged between 424.84 and 819.12 mg GAE/100 g in blueberries. Whereas lower total phenolic content was found by Vasco *et al*. (2008), as they reported that the total phenolic content in blueberry was 305.38 mg GAE/100 g. The difference in the total phenolics content may be attributed to environmental factors, such as temperature, light, genetic variation and agronomic practices of the berries according to Bunea *et al*. (2011).

Results in this study showed that flavonoid content of blueberry was 59.76 g CE/100g sample. The obtained results are in agreement with those reported by Okan, *et al*. (2018), they found that total flavonoid content ranged between 30.44 and 91.69 mg QE/100 mg in blueberries. Furthermore, Samec and Zegarac (2011) found lower content of total flavonoids in blueberry (47.53 mg CE/100g), when compared with this study.

Furthermore, Table 2 shows that the blueberry recorded inhibition rate reaches 88.45% and IC50 was 0.805 mg/ mL. Which illustrated the great importance of blueberries in free radicals eliminating. The result obtained is

partially similar with Hangun-Balkir *et al*. (2012), as they found that IC50 in blueberry was 0.70.

Table 2. Evaluation of Phenolic Compounds, Flavonoids, and Antioxidant Activity in Blueberries

Parameters%	Blueberry
Total Phenolics (mg GAE/100 g) *	435±14.849
Total flavonoids (mg CE/100 g) *	59.76±0.00
DPPH**	88.45±0.026
IC50	0.805
Total carotenoids**(mg /100 g)	1.363±0.002
Total anthocyanin** (Cyd-3-glu E)/ 100g)	173.3±0.373
Ascorbic acid (mg/ 100g) *	74.7±0.566
Total tannins (mg/ 100g) *	494.55±0.00

*Means± SD (standard deviation) of duplicate (g/ 100g on a dry weight basis); ** Means± SD (standard deviation) of triplicate

Total carotenoids

The amount of total carotenoids found in blueberries was measured to be 1.363 mg per 100 g of the fruit (as indicated in Table 2), and this result is slightly higher than that indicated by Bunea *et al*., (2012), where they indicated that the main carotenoids in blueberries are lutein, with average total carotenoids of 0.266 milligrams per 100 grams .The variation in carotenoid content could be attributed to variances in the cultivars of blueberries and the surrounding environmental conditions.

Total anthocyanin

The total anthocyanin content in blueberry was found to be 173.3mg/100g. This result is consistent with the findings of Okan *et al*. (2018), who reported that the total anthocyanin content in blueberry varied from 50.60 to 322 mg malvidin–3–glucoside/100g.

Ascorbic acid content

Table 2 shows that the blueberry contains 74.7 mg ascorbic acid/100g sample. This result was compatible with those obtained by Karadeniz *et al*. (2011), they reported that ascorbic acid content in blueberry was 73.21 mg/100g.

Tannins content

Tannin content in blueberry in this study was recorded at 494.55 mg/100g. Heinonen (2007) reported that the total tannins in blueberry were 741 mg/100g, which was higher than the data obtained in this study.

Beef burger manufacturing

Sensory evaluation

Sensory evaluations of beef burgers without and with blueberry addition (at different levels) and their appearance before cooking are shown in Figures 1 and 2, respectively.

Based on the data collected from the panelists, it was found that the sensory scores of the grilled samples indicated a significant improvement in taste, texture, and overall acceptability with the addition of blueberries to the burger, compared to the control sample ($p < 0.05$). Whereas sample T3 (with 1.5% blueberry) recorded the highest scores among all studied samples. Furthermore, data in Fig.1 showed that the color parameter did not influence by blueberry addition. These findings were in accordance with those obtained by Turan and Şimşek (2021) when they used black and blue berries extract during making beef patties.

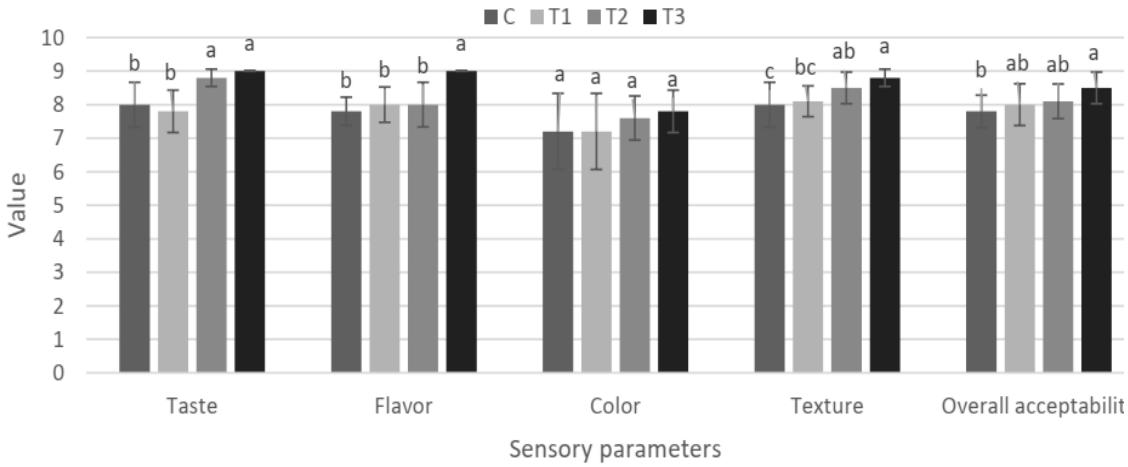

Fig. 1. Sensory evaluation of beef burgers without and with blueberry addition (at different levels).

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB.

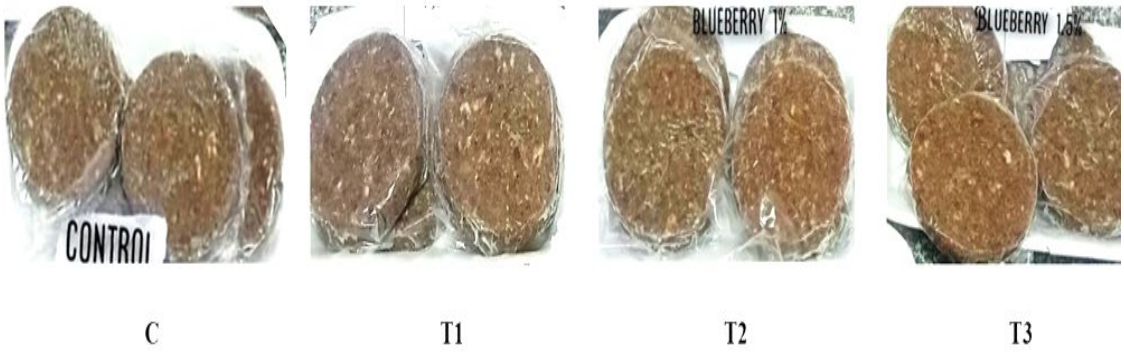

Fig. 2. Appearance of burger samples without and with using blueberry.

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB.

Physical properties

Table 3 presents the data for cooking yield and cooking loss. The results showed that incorporating blueberries in the beef burgers led to a significant increase in cooking yield and a decrease in cooking loss ($p< 0.05$) when compared to the control sample. The enhancement of cooking yield could be attributed to the ability of BB fibers to keep water (Anderson and Berry, 2001). Whereas the

decrease in cooking loss values after blueberry addition could be attributed to the high content of phenolics in BB; as polyphenolic compounds prevent protein deterioration during cooking (Trujillo-Mayol *et al*., 2021)

Table 3. Cooking yield and cooking loss of beef burger samples

Burger samples	Cooking yield	Cooking loss
C	85.66^{c}±0.208	14.25^{a}±0.220
T1	87.77^{a}±0.023	12.22^{c}±0.023
T2	87.73^{a}±0.012	12.26^{c}±0.012
T3	86.44^{b}±0.012	13.56^{b}±0.012

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB; a-c Means of triplicate ±SD (standard deviation) with different small letters in the same column differ significantly at $p<0.05$.

Chemical composition

The chemical analysis of beef burgers fortified with different concentrations of blueberry fruit at different concentrations (1% and 1.5%) is illustrated in Table 4. The addition of BB increased the moisture content and thus improved the juiciness of burger samples. This increase could be attributed to the ability of blueberry fibers to bind water and the enhancement of the water holding capacity of beef burgers.

Table 4. Effect of blueberry addition on chemical composition (on a dry weight basis %) in beef burgers.

Burger samples	Moisture	Fat	Protein	Ash
C	67.74b±0.012	24.95b±0.016	66.31b±0.316	6.91c±0.352
T1	66.94c±0.015	26.79a±0.188	60.19c±0.275	6.92c±0.073
T2	67.86a±0.026	25.07b±0.126	66.96a±0.151	7.59b±0.152
T3	67.92a±0.070	25.17b±0.074	67.43a±0.449	8.01a±0.110

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB.; a-c Means of triplicate ±SD (standard deviation) with different small letters in the same column differ significantly at $p<0.05$.

Furthermore, sample T1 (beef burger with 0.01% BHT) recorded the highest content of crude fat, and this result is consistent with those mentioned by Maria Gracileide de Alencar *et al.* (2022) when adding Grape skin as an antioxidant in beef burger. Whereas addition of BB during processing burgers caused a non-significant ($p>0.05$) increase in fat content, when compared with control.

On the other hand, Table 4 shows that BB addition during processing burgers increased protein and ash contents significantly ($p <0.05$), when compared to the control.

Microbiological analysis

According to the literature, the beef burger is a medium suitable for the growth of many microbes and microorganisms, due to the high percentage of moisture, protein, and fermentable carbohydrates, as well as the degree of acidity (Zafar *et al*., (2016)). The results in Fig. 3 showed that beef burger samples contained 1.5% of blueberries (sample T3) and had the lowest number of bacteria

(5.878 log CFU/ g). Besides non-significant ($p>0.05$) differences were found between sample T3 and sample T1 (beef burger with 0.01% BHT) in microbial load, which referred to the strong efficacy of blueberries in reducing the bacterial growth, thus prolonging storage period and improving burger quality.

On the other hand, yeast and molds were not detected in all samples under investigation.

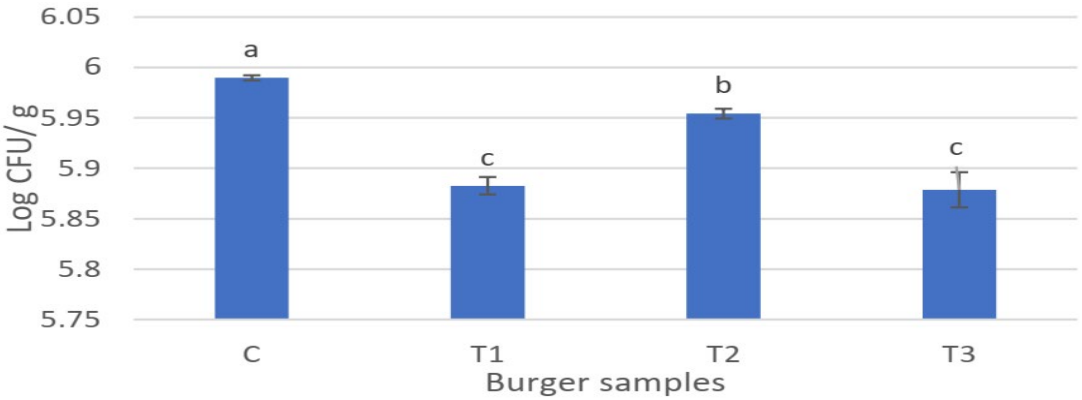

Fig. 3. Total bacterial count (TBC) of burger samples without and with blueberry addition.

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB; a-c Means of triplicate ±SD (standard deviation) with different small letters differ significantly at $p<0.05$

Storage of beef burger samples

Physical properties

Color values

Fig. 4 illustrates that adding blueberry (sample T2) increased the L* value of beef burger, while increasing the level of BB addition decreased L* values, when compared to the control sample. Likewise, Martín-Mateos *et al*. (2022) found a similar trend when they added different levels of sweet cherry to burgers. Moreover, the storage period decreased the L* value in all studied samples. A similar observation was found by Babaoğlu *et al*., (2022), when they used berry pomace water extracts in making beef patties.

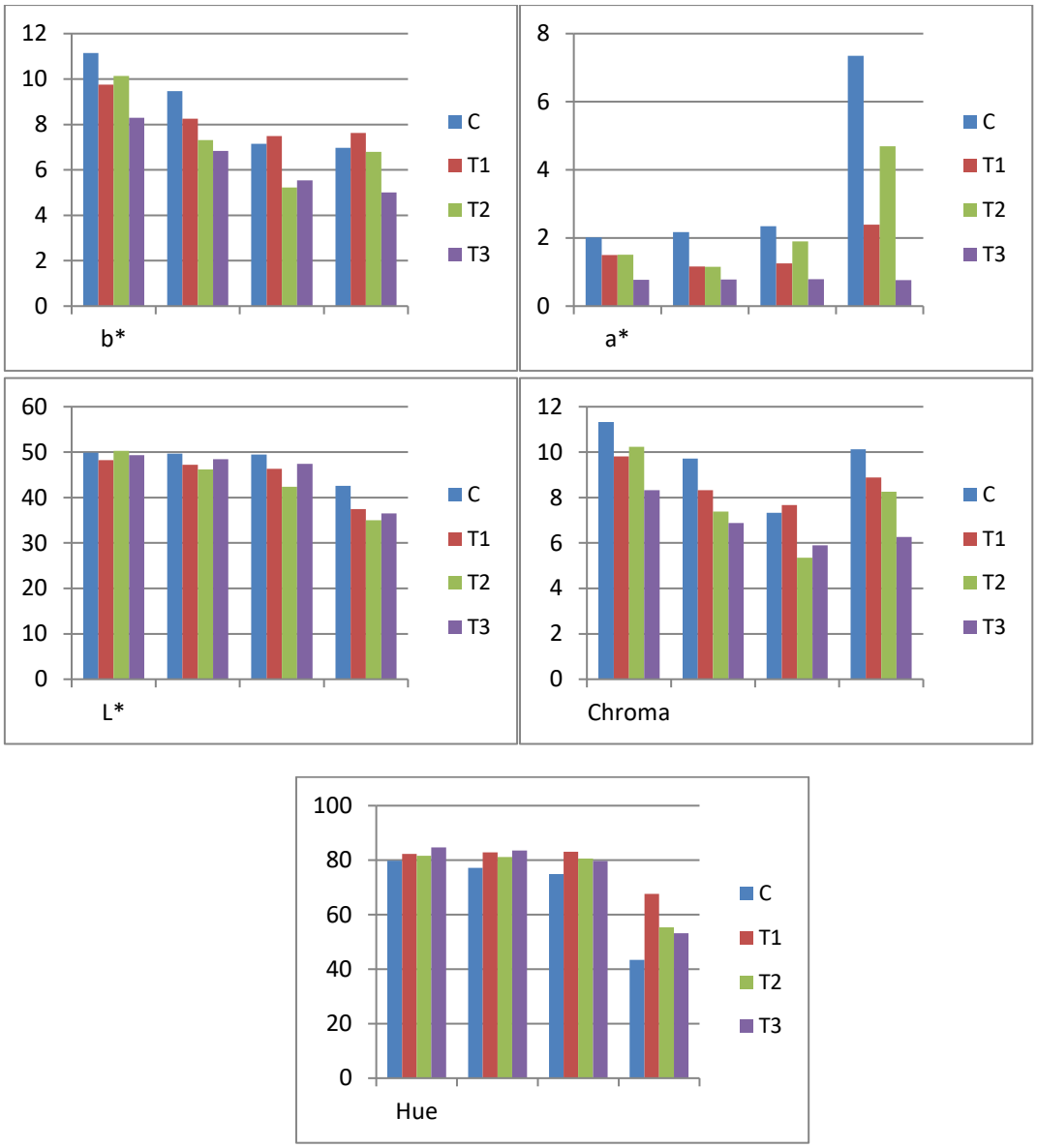

Fig. 4. colour parameters of beef burgers without and with blueberry addition.
C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB

Fig. 4 shows that BB addition decreased a* value from 2.02 in control sample to 1.51 in sample T2, while sample T3 recorded 077. Babaoğlu *et al*. (2022) found a similar trend when used blueberry pomace water extracts in making beef patties. Whereas storage of beef burgers for up to 9 days caused an increase in a* value, and the control sample recorded the highest value (7.35), followed by sample T2 (with 1% BB addition). This referred to the increase in burgers' palatability, as the higher positive a* value implies more redness, fresh meat shows less light and red appearance during display according to Cardoso *et al*., (2023). On contrary, Babaoğlu *et al*., (2022) recorded a decrease in a* values of beef patties with storage.

On the other hand, the b* value decreased with BB addition, while the control sample recorded the highest value (11.15). Furthermore, storage affected negatively on b* value in all studied samples.

Regarding the chroma values of beef burgers, there was a trend to decrease of chroma values associated with BB addition. Besides, storing beef burgers for nine days decreased chroma values in all studied samples.

Meanwhile, Fig.4 reveals that the Hue value increased with the increase of BB addition. Furthermore, the storage period decreased Hue values in all studied samples.

pH and water holding capacity (WHC)

Table 5 shows the changes in pH values of the prepared beef burger without and with blueberries addition. The statistical analysis of the mean of groups showed that the control sample recorded the highest pH value, while the addition of blueberry during making beef burger decreased pH values significantly ($p<0.05$). These results are consistent with Turan and Şimşek (2021).

Table 5 shows the significant effect (P<0.05) of treatments, storage time and their interactions on pH values and WHC.

Item	Beef burger samples	Storage time (days)				Mean of groups
		0	3	6	9	
pH	C	8.5±0.1	6.11±0.006	6.24±0.021	6.95±0.031	6.95^{a}
	T1	7.56±0.153	5.87±0.017	5.32±0.021	5.26±0.208	6.01^{c}
	T2	8.3±0.173	5.95±0.015	5.92±0.021	6.83±0.03	6.75^{b}
	T3	7.46±0.153	5.85±0.012	5.24±0.01	5.22±0.038	5.94^{c}
	Mean	7.96^{A}	5.95^{C}	5.68^{D}	6.06^{B}	
WHC	C	65.73±0.031	56.82±0.030	48.33±0.038	39.55±0.021	52.61^{b}
	T1	66.56±0.021	57.30±0.305	49.47±0.115	39.40±0.265	53.18^{a}
	T2	65.52±0.021	53.48±0.032	41.28±0.021	35.26±0.021	48.88^{c}
	T3	66.95±0.021	49.22±0.031	42.36±0.021	36.24±0.021	48.69^{d}
	Mean	66.19^{A}	54.20^{B}	45.35^{C}	37.61^{D}	

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB; Means of triplicate ±SD (standard deviation); $^{A-D}$ Different the capital letters in the same row means significant difference ($p<0.05$) between storage periods; $^{a-d}$ Different the small letters in the same column means significant difference ($p<0.05$) between treatments.

On the other hand, storage of beef burger samples decreased pH value to its lowest value after six days of storage, while increasing the storage period up to the ninth day increased the pH value.

Changes in water holding capacity (WHC) (% bound water) of the freshly prepared beef burger and during refrigerated storage (4 ± 1°C) for 9 days are presented in Table 5. The obtained data showed that the addition of blueberries to the beef burger led to a significant ($p< 0.05$) decrease in the water holding capacity, these results are consistent with those indicated by Hafssa *et al*. (2015) when they

used pomegranate peels as a natural antioxidant during processing beef sausage. Furthermore, an increase in the storage period caused a significant (P<0.05) decrease in WHC.

Table 5.1: Mean squares and P-values (in parentheses) for pH values and water holding capacity

Factor	df	Mean Square (P-values)	
		pH	WHC
Treatment[1]	3	3.16 (0.000*)	68.18 (0.000*)
Storage time[2]	3	13.06 (0.000*)	1808.16 (0.000*)
treatment × storage time	9	0.370 (0.000*)	13.57 (0.000*)

[1]Treatments: C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB

[2] Storage time: 0,3, 6 and 9 days; df: degrees of freedom; *Statistically significant at P<0.05

Chemical properties

Values of Thiobarbituric acid ($C_4H_4N_2O_2S$) reactive substances (TBARS) were measured:

Lipid oxidation is one of the main factors limiting the acceptability and quality of meat and meat products. The TBARS (thiobarbituric acid reactive substances) values (mg malondialdehyde/kg) are used as an indicator to measure the oxidative rancidity that occurs in meat products during storage. (Ammar and Aboalfa, 2017).

As shown in Table 6, the addition of blueberry during processing burgers decreased TBARS values significantly (P<0.05), while the control sample recorded the highest TBARS value (mean of the group was 0.883). Indeed, the phenolic compounds in blueberries may react with oxygen radicals and therefore inhibit lipid oxidation (Chang *et al*., 2002). The results are in agreement with previous studies that illustrated the natural antioxidant efficiency in delaying lipid oxidation in meat products (Jia *et al*., 2012; Rodríguez-Carpena *et al*., 2019; Babaoğlu *et al*., 2022). Furthermore, storage of beef burgers for nine days increased TBARS significantly (p< 0.05) as shown in Table 6

On the other hand, peroxide values in beef burger samples were estimated. Data in Table 6 showed that using blueberries during making beef burgers reduced peroxide values significantly (p< 0.05), and the control sample recorded the highest peroxide value. likewise, Kowalczyk *et al*. (2023) found that the addition of 0.05 % and 0.1 % of chokeberry leaf extract inhibited lipid oxidation significantly (p< 0.05) in the veal burgers. Furthermore, using cranberry fruit extract powder during making cheese decreased peroxide values (Khalifa and Wahdan, 2015). Whereas storage of beef burgers for 9 days increased peroxide values significantly.

Table 6.1 shows the significant effect (P<0.05) of treatments storage time and their interactions on TBARS values and peroxide values.

Table 6. Changes in thiobarbituric acid reactive substances (TBARS) values (mg malonaldehyde/ kg of the sample) and peroxide value (miliequivalents of active oxygen/ kg of the sample) of the beef burgers that were prepared and stored under refrigeration (4 ± 1°C) for 9 days.

Item	Beef burger samples	Storage time (days)				Mean of groups
		0	3	6	9	
TBARS	C	0.450±0.00	0.949±0.00	1.025±0.002	1.107±0.00	0.883a
	T1	0.204±0.00	0.206±0.00	0.208±0.000	0.212±0.00	0.207c
	T2	0.233±0.00	0.240±0.00	0.305±0.003	0.314±0.00	0.273b
	T3	0.204±0.00	0.207±0.00	0.209±0.000	0.212±0.00	0.208c
	Mean	0.273D	0.400C	0.437B	0.461A	
Peroxide value	C	3.026±0.064	4.43±0.208	5.62±0.005	6.43±0.208	4.88a
	T1	1.66±0.22	2.03±0.063	2.6±0.2	3.03±0.057	2.33c
	T2	1.7±0.2	2.09±0.176	2.99±0.01	3.26±0.153	2.51b
	T3	1.53±0.115	1.99±0.01	2.4±0.2	3.03±0.061	2.24c
	Mean	1.98D	2.64C	3.40B	3.94A	

C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB; Means of triplicate ±SD (standard deviation); $^{A-D}$ Different the capital letters in the same row means significant difference ($p<0.05$) between storage periods; $^{a-d}$ Different the small letters in the same column means significant difference ($p<0.05$) between treatments.

Table 6.1. Mean squares and P-values (in parentheses) for thiobarbituric acid (TBA) and peroxide value.

Factor	df	Mean Square (P-values)	
		TBA	Peroxide value
Treatment[1]	3	1.29 (0.000*)	19.18(0.000*)
Storage time[2]	3	0.084 (0.000*)	8.88(0.000*)
treatment × storage time	9	0.061 (0.000*)	0.557(0.000*)

[1] Treatments: C: control beef burger; T1: beef burger with 0.01% butylated hydroxytoluene (BHT); T2: beef burger with 1% blueberry (BB); T3: beef burger with 1.5% BB

[2]Storage time: 0,3, 6 and 9 days; df: degrees of freedom; *Statistically significant at $P<0.05$

Conclusion

The results of this study demonstrate that blueberry powder can serve as a sustainable and natural source of various beneficial compounds, such as phenolic compounds, flavonoids, total carotenoids, anthocyanins, and ascorbic acid. Moreover, the addition of blueberry powder to beef burgers can significantly improve their quality characteristics, as well as inhibit lipid oxidation during refrigerated storage, similar to the synthetic antioxidant BHT. In particular, burgers with 1.5% blueberry powder (sample T3) exhibited the highest sensory parameters, indicating that blueberry could be a promising and sustainable alternative to synthetic antioxidants in meat products. These findings highlight the potential of blueberry as a sustainable ingredient to enhance the quality and extend the shelf-life of meat products, while reducing the reliance on synthetic antioxidants.

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الحفاظ المستدام على برجر اللحم باستخدام التوت الازرق

عبير ممدوح فتحى*، اسماء محمد عبد الرحمن، السيد عبد النبي رمضان، سوميه محمد ابراهيم درويش

قسم علوم وتكنولوجيا الاغذية، كلية الزراعة، جامعة اسيوط.

الملخص

وفقًا لنتائج الدراسة، يمكن أن يكون مسحوق التوت الأزرق مصدرًا طبيعيًا ومستدامًا للعديد من المواد الصحية، بما في ذلك المركبات الفينولية والفلافونويد والكاروتينات الكلية والأنثوسيانين وحمض الأسكوربيك. بالإضافة إلى ذلك، فإن إضافة مسحوق التوت الأزرق إلى برجر اللحم البقري يمكن أن يعزز صفاته النوعية ويمنع أكسدة الدهون أثناء التخزين المبرد، مثل مضادات الأكسدة الاصطناعية BHT. تم العثور على أعلى المقاييس الحسية في البرغر الذي يحتوي على 1.5% من مسحوق التوت الأزرق (عينة T3)، مما يشير إلى أن العنب البري يمكن أن يكون بديلاً واعدًا ومستدامًا لمضادات الأكسدة الاصطناعية في منتجات اللحوم. توضح هذه النتائج إمكانات العنب البري كمكون مستدام لتحسين جودة منتجات اللحوم ومدة الصلاحية مع تقليل الاعتماد على مضادات الأكسدة الاصطناعية.