

Effect of modified atmosphere packaging conditions on the quality of the fibre-rich pork sausages incorporated with natural antioxidant blends

Mehak Jandyal¹, O P Malav², Nitin Mehta³, Rajesh V Wagh⁴

¹Assistant Professor, Department of Livestock Products Technology, College of Veterinary Sciences, Rampuraphul, Punjab, India

²Associate Professor, Department of Livestock Products Technology, College of Veterinary Sciences, RampuraPhul, Punjab, India

³Professor and Head, Department of Livestock Products Technology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

⁴Assistant professor, Department of Livestock Products Technology, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

Abstract

The present investigation examined the combined effect of a plant-based antioxidant cocktail and modified atmosphere packaging (MAP) on the refrigerated storage stability of fibre-enriched pork sausages. Sausages were formulated with a phyto-extract blend (0.30%) comprising *Moringa oleifera*, *Ribes nigrum*, *Ampelopsis grossedentata*, and *Fagopyrum esculentum*, and compared with a control (no antioxidant) and a synthetic antioxidant treatment (100 ppm BHT). Products were stored under MAP at 4 ± 1 °C and evaluated periodically for physicochemical traits, lipid oxidation indices, instrumental colour parameters, microbiological quality and sensory acceptability. The combined application of MAP and the phyto-extract blend effectively curtailed lipid oxidation, microbial proliferation, and colour loss, showing significantly ($P < 0.05$) better results. Treated sausages remained microbiologically safe and sensorially acceptable up to 35 days of refrigerated storage. The findings highlight the potential of combining MAP with plant-derived antioxidants as a clean-label strategy for extending the shelf-life of functional pork products.

Keywords: Functional meat, oxidative stability, plant polyphenols, MAP

Introduction

Meat products, particularly comminuted formulations such as pork sausages, are highly vulnerable to lipid oxidation and microbiological spoilage during refrigerated storage, which compromises colour, flavour and overall acceptability and reduces commercial shelf-life (Moawad *et al.*, 2020)^[10]. Modified atmosphere packaging (MAP) has become a cornerstone technology in meat preservation by reducing oxygen availability and thereby slowing oxidative and aerobic microbial processes; however, MAP alone does not always prevent quality deterioration during extended chilled storage and can present limitations related to colour stability and growth of anaerobic or CO₂-tolerant psychrotrophs. Concurrently, plant-derived antioxidant extracts rich in polyphenols, flavonoids and other bioactive phytochemicals have demonstrated strong radical-scavenging and antimicrobial effects in meat matrices, and are increasingly explored as “clean-label” alternatives to synthetic antioxidants. Evidence from recent studies and reviews indicates that phyto-extracts (e.g., extracts from *Moringa oleifera*, black currant, vine tea and buckwheat) can retard lipid oxidation, improve colour stability and reduce microbial loads in processed meats when applied at appropriate levels. Combining MAP with natural antioxidant interventions offers a promising hurdle-technology approach: MAP reduces oxygen transfer, while phyto-extracts scavenge residual free radicals and exert antimicrobial activity, producing a synergistic effect that can extend shelf-life more effectively than either strategy alone (Yan *et al.*, 2024)^[18] Recent reviews and experimental reports emphasise this synergy and discuss integration

strategies (e.g., direct incorporation, active packaging, or coatings), yet they also note important knowledge gaps concerning optimal extract combinations, concentrations, and their effects specifically in fibre-enriched, comminuted pork matrices.

Within this framework, the study assessed the efficacy of a 0.30% (w/w) polyphenol-rich blend derived from *Moringa oleifera*, *Ribes nigrum*, *Ampelopsis grossedentata*, and *Fagopyrum esculentum*, under modified atmosphere packaging (MAP), to preserve quality and extend the refrigerated storage life of fibre-enriched pork sausages. The work aims to (i) quantify effects on physicochemical and oxidative stability indices, (ii) assess microbial dynamics and instrumental colour retention, and (iii) determine sensory shelf-life under MAP conditions. Accordingly, the study examined how a selected mixture of plant extracts, together with MAP, contributes to quality preservation and shelf-life extension of pork sausages during refrigeration.

Material and Methods

Collection and Preparation of Plant Material

Phyto-extracts, i.e. *Moringa oleifera* leaves extract (MLE), *Ribes nigrum* (Black currant extract-BCE), *Fagopyrum esculentum* (Buckwheat powder-BWE), were procured from Sv Agro, Mumbai, India and *Ampelopsis grossedentata* (Vine tea extract -VTE) was procured from Suvar. Power.Sdn.Bhd, Malaysia.

Hydroethanolic extracts of *Moringa oleifera*, *Ribes nigrum*, *Fagopyrum esculentum*, and *Ampelopsis grossedentata* were prepared using a standardised solvent extraction method. A hydroethanolic solution (70%, v/v) was used to extract the

dried plant matter at a 1:10 (w/v) ratio while agitated continuously for 24 h at room temperature. The extracts were filtered, concentrated under reduced pressure at 40 °C, and freeze-dried to obtain powdered extracts. The dried extracts, procured from certified commercial suppliers, were reconstituted and blended in predetermined proportions to formulate the phyto-extract cocktail (CPE) used in sausage preparation. The pork sausages were formulated into three treatment groups: T1 (P+CPE), consisting of sausages incorporated with a 0.30% phyto-extract blend; T2 (P), serving as the control without the addition of any antioxidant; and T3 (P+BHT), containing 100 ppm butylated hydroxytoluene (BHT) as a synthetic antioxidant.

Preparation of pork sausage

The fibre-enriched pork sausages were prepared following the procedure described previously by Jandyal *et al.* (2022) [6].

Physicochemical analysis of pork sausages

Determination of pH and Water Activity

The pH of pork sausages (n = 6) was determined by means of a digital pH meter (model SAB 5000) manufactured by Lab India, Mumbai. For sausage samples, A 10 g portion of the sample was blended with 50 mL of distilled water for 1 min, after which pH was measured using a glass electrode. In the storage study, the pH of the sausage was measured directly. Water activity was measured with a portable water activity meter (Hygro.Palm AW1, Rotronic). Finely ground samples were placed in moisture-free sample cups, and readings were taken in duplicate according to the manufacturer's instructions.

Peroxide Value

Peroxide value was determined following the method of Koniecko (1979) [7] with minor modifications. A 5 g sample was extracted using chloroform in the presence of anhydrous sodium sulphate, after which filtration was carried out. A measured portion of the extract was allowed to react with glacial acetic acid (2ml) and potassium iodide, after which distilled water and starch indicator were introduced. The liberated iodine was titrated against 0.1 N sodium thiosulfate, and the peroxide value was expressed as meq/kg of sample.

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS values were determined following the method of Witte *et al.* (1970) [17] with minor modifications. Approximately 10 gm of cooked sausage was homogenised in chilled 20% trichloroacetic acid prepared with 2 M orthophosphoric acid, then brought to volume with distilled water and filtered. An aliquot of the filtrate was reacted with thiobarbituric acid reagent and incubated in the dark. The absorbance value was determined at a wavelength of 532 nm with the aid of a UV-Vis spectrophotometer, and TBARS results were calculated as mg malondialdehyde per kg of sample using a conversion factor of 5.2.

Instrumental Color Evaluation

Instrumental color parameters (L^* , a^* , b^*) were measured using a calibrated Lovibond RT-300 reflectance tintometer (UK) under D65 illumination. Lightness, redness, and yellowness values were recorded from three distinct locations on the sausage surface, and the mean of these

readings was used for analysis. For storage studies, colour readings were taken on the freshly cut internal surface immediately after package opening to minimise exposure to light and oxygen.

Microbiological Analysis

Microbial status of the sausages was evaluated through enumeration of standard plate counts, psychrotrophic bacteria, and coliforms by the pour-plate technique. Serial decimal dilutions were prepared in sterile diluent, and duplicate plates were inoculated. Plate Count Agar was used for determining standard plate count and psychrotrophic count, whereas Violet Red Bile Agar served for coliform estimation. SPC plates were incubated at $35-36 \pm 2$ °C for 24 h. For psychrotrophic organisms, incubation was carried out at 4 ± 1 °C for 10–14 days, while coliform plates were maintained at $35-36 \pm 2$ °C for 24 h. Only plates bearing 30 to 300 colonies were considered for enumeration, and microbiological data were presented as logarithmic values of colony-forming units (\log_{10} . CFU) per gram of sausage sample.

Sensory Evaluation

Sensory assessment was conducted by a semi-trained panel comprising seven members. Visual colour and odour of pork sausage were assessed using a 5-point descriptive scale, while pork sausages were evaluated using an 8-point descriptive scale. Panellists were briefed before evaluation, and samples were scored for colour intensity and odour acceptability using defined descriptive anchors. All treatments were analyzed in three replicates, resulting in 21 observations overall (n = 21).

Statistical Analysis

Experimental data were analysed using Design-Expert® software for response surface methodology and IBM SPSS Statistics (version 20.0). All measurements were conducted in duplicate and repeated three times (n = 6). Sensory data were obtained from seven panellists with 21 total observations. The data were analysed using a two-way ANOVA to determine the influence of treatment and storage duration. Differences among means were separated by Duncan's multiple range procedure at $P < 0.05$. Findings are presented as mean \pm standard error.

Results and discussion

Physicochemical Properties

As illustrated in Table 1, pH values did not vary significantly ($P > 0.05$) among treatments on day one, suggesting that neither antioxidant incorporation nor packaging influenced the initial values. A significant ($P < 0.05$) decline in pH was recorded in the control samples until day 14 of storage, after which a gradual rise was observed. Such pH dynamics are commonly associated with early fermentation activity and subsequent proteolytic spoilage during extended refrigeration. Following day 14, a significantly lower ($P < 0.05$) pH was recorded in antioxidant-treated sausages than in the control samples. Among treatments, fibre-enriched sausages stored under modified atmosphere packaging (MAP) consistently exhibited lower pH values than aerobically packaged samples throughout storage. This effect is primarily attributed to the dissolution of CO_2 in the aqueous phase of meat, leading to carbonic acid formation and suppression of

spoilage microorganisms, thereby delaying alkaline metabolite accumulation. The pH of sausages containing 0.30% phyto-extract blend was comparable to that of samples treated with P+BHT, with no significant difference ($P < 0.05$). The comparable trend might be due to plant-derived bioactive constituents that modulated the structure and activity of the microbial community. Kumar (2015) [8] reported this trend in MAP-packaged pork patties, while a similar observation was made in enrobed goat meat patties. This finding is comparable to that of Zhai *et al.* (2018) [19], who investigated pH variations in MAP salted ducks during storage.

Water activity decreased significantly ($P < 0.05$) with increasing storage time across all treatments. Until day 7, a_w values remained similar ($P > 0.05$) among treatments; however, a more pronounced reduction was observed in sausages treated with phyto-extracts or BHT under MAP compared to the control. The greater decline in a_w may be attributed to the combined effects of MAP and antioxidant incorporation, which collectively restrict moisture availability and microbial growth, thereby enhancing storage stability. These findings corroborated with Kumar *et al.* (2015) [8] observations in pork patties and Wang (2000) [15] in sausages. Peroxide values (PV) increased progressively in all samples during refrigerated storage Table 2, reflecting ongoing primary lipid oxidation. At every storage interval, the control sausages exhibited significantly higher ($P < 0.05$) PV compared to antioxidant-treated samples. The P+CPE group consistently recorded the lowest PV at the end of storage, while the control showed

the highest, with a marked acceleration in PV accumulation between days 21 and 35. This trend aligns with recent observations that MAP systems with elevated CO_2 levels can suppress oxidative deterioration in meat products by limiting the availability of oxygen and slowing the formation of hydroperoxides (Wang *et al.*, 2022) [16]. Thiobarbituric acid reactive substances (TBARS), which indicate secondary lipid oxidation, were similar among treatments on day one but increased significantly ($P < 0.05$) over time. Compared to control and BHT samples, sausages containing phyto-extracts consistently showed significantly lower TBARS values over the storage period, demonstrating enhanced inhibition of oxidative breakdown. Both treatment and storage time had significant ($P < 0.05$) effects on TBARS levels, consistent with recent research showing that bioactive compounds combined with advanced packaging systems can effectively mitigate lipid oxidation during chilling (Abdullah *et al.*, 2024) [1]. A significant ($P < 0.05$) rise in FFA content was observed during refrigeration, reflecting progressive breakdown of lipids. Nevertheless, compared with the control, antioxidant-incorporated sausages exhibited significantly ($P < 0.05$) decreased FFA contents. FFA values of P+CPE and BHT groups were comparable during early storage but diverged moderately at later stages, indicating that the phyto-extract blend provided sustained protection against lipid degradation. These observations concur with those of Rahman *et al.* (2021) [12], who noted significantly higher ($P < 0.05$) free fatty acid contents in control beef patties than in patties treated with BHA and black cumin extract during refrigerated storage.

Table 1: Physicochemical changes in fibre-enriched pork sausages treated with a phyto-extract blend during refrigerated storage under MAP (4 ± 1 °C) (Mean \pm S.E.) *

Treatments/Days	Day 1.	Day 7.	Day 14.	Day 21.	Day 28.	Day 35.
pH						
T1 (P+CPE)	5.92 \pm 0.04 ^b	5.85 \pm 0.02 ^{Bc}	5.70 \pm 0.04 ^{Bd}	5.85 \pm 0.02 ^{Bc}	5.92 \pm 0.02 ^{Bb}	6.15 \pm 0.02 ^{Ba}
T2 (P)	5.98 \pm 0.03 ^b	5.93 \pm 0.01 ^{Ac}	5.87 \pm 0.01 ^{Ad}	5.92 \pm 0.01 ^{Ac}	6.05 \pm 0.05 ^{Ab}	6.50 \pm 0.02 ^{Aa}
T3 (P+BHT)	5.95 \pm 0.03 ^b	5.85 \pm 0.03 ^{Bc}	5.70 \pm 0.02 ^{Bd}	5.89 \pm 0.03 ^{Bb}	5.96 \pm 0.06 ^{Bb}	6.30 \pm 0.02 ^{Ca}
a_w						
T1 (P+CPE)	0.992 \pm 0.03 ^{Ba}	0.990 \pm 0.02 ^{Bb}	0.984 \pm 0.01 ^{Bc}	0.852 \pm 0.02 ^{Bc}	0.848 \pm 0.01 ^{Bd}	0.835 \pm 0.01 ^{Be}
T2 (P)	0.998 \pm 0.04 ^{Aa}	0.991 \pm 0.01 ^{Ab}	0.987 \pm 0.03 ^{Ac}	0.881 \pm 0.01 ^{Ad}	0.875 \pm 0.01 ^{Ac}	0.870 \pm 0.01 ^{Af}
T3 (P+BHT)	0.990 \pm 0.04 ^{Ba}	0.982 \pm 0.02 ^{Bb}	0.980 \pm 0.01 ^{Bb}	0.876 \pm 0.01 ^{Cc}	0.851 \pm 0.02 ^{Cd}	0.840 \pm 0.02 ^{Ce}

*Mean \pm S.E. Means bearing different superscripts within a row (lowercase) and within a column (uppercase) differ significantly ($P < 0.05$). n = 6. T1 (P + CPE) = pork sausages containing 0.30% cocktail phyto-extracts; T2 (P) = pork sausages without phyto-extracts; T3 (P + BHT) = pork sausages containing 100 ppm BHT.

Table 2: Oxidative Stability of Fibre-Enriched Pork Sausages Treated with a Phyto-Extract Blend during Refrigerated Storage in MAP (4 ± 1 °C) (Mean \pm S.E.) *

Treatments/Days	Day 1.	Day 7.	Day 14.	Day 21.	Day 28.	Day 35.
Peroxide value (meq/Kg)						
T1 (P+CPE)	3.60 \pm 0.05 ^{Bf}	3.36 \pm 0.05 ^{Be}	3.84 \pm 0.07 ^{Cd}	4.09 \pm 0.04 ^{Cc}	4.27 \pm 0.03 ^{Cb}	5.35 \pm 0.03 ^{Ca}
T2 (P)	3.95 \pm 0.05 ^{Af}	4.14 \pm 0.08 ^{Ae}	4.27 \pm 0.09 ^{Ad}	4.48 \pm 0.01 ^{Ac}	5.03 \pm 0.04 ^{Ab}	6.09 \pm 0.04 ^{Aa}
T3 (P+BHT)	3.15 \pm 0.07 ^{Bf}	3.25 \pm 0.06 ^{Be}	3.68 \pm 0.01 ^{Bd}	4.01 \pm 0.01 ^{Bc}	4.37 \pm 0.05 ^{Bb}	5.55 \pm 0.03 ^{Ba}
TBARS (mg malonaldehyde/Kg)						
T1 (P+CPE)	0.395 \pm 0.001 ^{Be}	0.410 \pm 0.002 ^{Ce}	0.445 \pm 0.001 ^{Cd}	0.508 \pm 0.002 ^{Bc}	0.610 \pm 0.007 ^{Bb}	0.724 \pm 0.009 ^{Ca}
T2 (P)	0.430 \pm 0.002 ^{Ae}	0.449 \pm 0.001 ^{Ae}	0.485 \pm 0.001 ^{Ad}	0.528 \pm 0.002 ^{Ac}	0.752 \pm 0.005 ^{Ab}	1.45 \pm 0.005 ^{Aa}
T3 (P+BHT)	0.376 \pm 0.002 ^{Bf}	0.420 \pm 0.001 ^{Be}	0.454 \pm 0.001 ^{Bd}	0.515 \pm 0.003 ^{Bc}	0.612 \pm 0.003 ^{Bb}	0.732 \pm 0.003 ^{Ba}
Free fatty acid (%)						
T1 (P+CPE)	0.156 \pm 0.03 ^{Bf}	0.166 \pm 0.03 ^{Be}	0.189 \pm 0.01 ^{Cd}	0.201 \pm 0.03 ^{Cc}	0.225 \pm 0.05 ^{Cb}	0.272 \pm 0.07 ^{Ca}
T2 (P)	0.176 \pm 0.03 ^{Af}	0.193 \pm 0.02 ^{Ae}	0.213 \pm 0.01 ^{Ad}	0.329 \pm 0.02 ^{Ac}	0.404 \pm 0.03 ^{Ab}	0.484 \pm 0.03 ^{Aa}
T3 (P+BHT)	0.116 \pm 0.05 ^{Bf}	0.173 \pm 0.04 ^{Be}	0.197 \pm 0.04 ^{Bd}	0.210 \pm 0.04 ^{Bc}	0.251 \pm 0.06 ^{Bb}	0.345 \pm 0.05 ^{Ba}

*Mean \pm S.E. Means bearing different superscripts within a row (lowercase) and within a column (uppercase) differ significantly ($P < 0.05$). n = 6. T1 (P + CPE) = pork sausages containing 0.30% cocktail phyto-extracts; T2 (P) = pork sausages without phyto-extracts; T3 (P + BHT) = pork sausages containing 100 ppm BHT.

Instrumental color profile parameters

Table 3 presents the L^* , a^* , and b^* values of pork sausages during storage under MAP. All colour attributes exhibited significant ($P < 0.05$) changes during refrigerated storage. On day one, sausages treated with CPE and BHT exhibited significantly ($P < 0.05$) lower L^* values than the control, and lightness decreased steadily in every treatment as storage progressed. The decrease in L^* values was more pronounced in antioxidant-treated samples, with CPE-treated sausages exhibiting the lowest lightness at the end of storage.

The observed decrease in L^* values during refrigerated storage may be attributed to oxidation of muscle pigments and to interactions between plant-derived phenolic compounds and meat proteins, which can influence pigment stability and light reflectance. Recent research highlights the increasing use of plant-based compounds, including phenolic-rich extracts, to enhance quality and shelf life in meat systems by mitigating oxidative deterioration and preserving sensory attributes, including colour (Olvera-Aguirre *et al.*, 2023; Bai *et al.*, 2025) [3, 11]. This aligns with findings that plant extracts can provide antioxidant activity and delay oxidative changes in meat matrices, thereby affecting colour parameters retained during chilled storage (Zhou *et al.*, 2023) [20]. Similar reductions in lightness following the incorporation of rosemary, green tea, and sea

buckthorn extracts in meat products have been reported previously (Fernández-López *et al.*, 2005; Salejda *et al.*, 2014) [13]. Although a general decline in lightness was evident, MAP conditions appeared to moderate the extent of colour deterioration by limiting oxidative reactions. A marked reduction ($P < 0.05$) in redness (a^*) was observed in every group from the beginning of storage to day 14. Although redness continued to decline during extended storage, sausages incorporated with CPE consistently maintained higher a^* values than control and BHT-treated samples after 21 days of storage. This improved redness stability might result from the antioxidant potential inherent in the phyto-extracts and the protective effect of MAP in inhibiting metmyoglobin formation, thereby preserving the desirable red colour of pork sausages. Comparable findings were reported by Lorenzo *et al.* (2014) [9], who observed higher redness values in pork patties treated with grape seed extract during refrigerated storage compared to untreated controls. The increase in b^* values indicate a gradual enhancement of yellowness in pork sausages, which may be attributed to the natural pigments present in the phyto-extract cocktail and their synergistic interaction with meat components. Similar increases in b^* values following the addition of plant-based extracts to meat products have been reported and are often linked to the colour contribution of phenolic compounds.

Table 3: Instrumental Colour Changes in Pork Sausages Treated with a Phyto-Extract Blend during Refrigerated Storage in MAP (4 ± 1 °C) (Mean \pm S.E.)

Treatments/ Days	Day 1.	Day 7.	Day 14.	Day 21.	Day 28.	Day 35.
L^* (Lightness)						
T1 (P+CPE)	53.38 \pm 0.28 ^a	51.81 \pm 0.17 ^{Bb}	50.71 \pm 0.19 ^{Bc}	49.10 \pm 0.20 ^{Cd}	48.11 \pm 0.26 ^{Be}	47.11 \pm 0.12 ^{Bf}
T2 (P)	53.54 \pm 0.32 ^a	52.25 \pm 0.25 ^{Ab}	51.73 \pm 0.21 ^{Ac}	50.61 \pm 0.13 ^{Ad}	49.32 \pm 0.23 ^{Ae}	48.25 \pm 0.15 ^{Af}
T3 (P+BHT)	53.49 \pm 0.39 ^a	52.00 \pm 0.10 ^{Ab}	51.25 \pm 0.24 ^{Ac}	49.81 \pm 0.15 ^{Bd}	49.13 \pm 0.17 ^{Ae}	48.13 \pm 0.13 ^{Af}
a^* (Redness)						
T1 (P+CPE)	9.80 \pm 0.02 ^{Aa}	9.63 \pm 0.07 ^{Ab}	9.39 \pm 0.04 ^{Ac}	8.90 \pm 0.01 ^{Ad}	8.59 \pm 0.03 ^{Ae}	8.41 \pm 0.05 ^{Af}
T2 (P)	9.59 \pm 0.03 ^{Ca}	9.15 \pm 0.04 ^{Cb}	8.36 \pm 0.05 ^{Cc}	8.25 \pm 0.06 ^{Cd}	7.99 \pm 0.01 ^{Be}	7.85 \pm 0.06 ^{Bf}
T3 (P+BHT)	9.69 \pm 0.06 ^{Ba}	9.24 \pm 0.02 ^{Bb}	9.06 \pm 0.01 ^{Bc}	8.97 \pm 0.08 ^{Ad}	8.54 \pm 0.02 ^{Ae}	8.47 \pm 0.03 ^{Af}
b^* (Yellowness)						
T1 (P+CPE)	15.54 \pm 0.02 ^{Ba}	15.34 \pm 0.01 ^{Bb}	15.39 \pm 0.01 ^{Bc}	15.42 \pm 0.02 ^{Bd}	14.44 \pm 0.05 ^{Be}	14.50 \pm 0.06 ^{Bf}
T2 (P)	15.83 \pm 0.04 ^{Aa}	14.72 \pm 0.03 ^{Ab}	14.63 \pm 0.02 ^{Ac}	14.24 \pm 0.02 ^{Ad}	14.06 \pm 0.05 ^{Ae}	13.45 \pm 0.07 ^{Af}
T3 (P+BHT)	15.60 \pm 0.01 ^{Ba}	15.40 \pm 0.01 ^{Bb}	15.31 \pm 0.03 ^{Bc}	15.20 \pm 0.01 ^{Bd}	15.10 \pm 0.01 ^{Be}	14.75 \pm 0.02 ^{Bf}

*Mean \pm S.E. Means bearing different superscripts within a row (lowercase) and within a column (uppercase) differ significantly ($P < 0.05$). $n = 6$. T1 (P + CPE) = pork sausages containing 0.30% cocktail phyto-extracts; T2 (P) = pork sausages without phyto-extracts; T3 (P + BHT) = pork sausages containing 100 ppm BHT.

Microbiological quality

The standard plate count of fibre-rich pork sausages stored under modified atmosphere packaging (MAP) is shown in Table 4, while the psychrophilic count is reported separately. Microbial numbers rose in every group as storage progressed; however, sausages containing BHT or the cocktail phyto-extract (CPE) maintained significantly ($P < 0.05$) lower SPC compared with the control under MAP, indicating reduced microbial proliferation. CPE-treated sausages showed the greatest inhibition of SPC during refrigerated storage. The findings of the present investigation demonstrate that the incorporation of a 0.30% phyto-extract blend in conjunction with modified atmosphere packaging (MAP) effectively suppressed the standard plate count in fibre-enriched pork sausages, and its inhibitory effect was higher than that of a control group of sausages with no phyto-extract added. This antibacterial

effect might be seen due to the bioactive compound present in the combination of extracts used in the study. However, the proanthocyanidins present in black currant extract in higher quantities can also inhibit the growth of certain bacteria. Recent studies indicate that plant extracts rich in phenolics and flavonoids can enhance microbial stability and extend shelf life in meat products by inhibiting spoilage organisms and delaying oxidative deterioration (Olvera-Aguirre *et al.*, 2023; Bai *et al.*, 2025; Tocai *et al.*, 2025) [3, 11, 14]. A significant ($P < 0.05$) rise in psychrophilic populations was observed as storage advanced, and the control exhibited the earliest growth. In contrast, BHT and CPE-treated sausages exhibited delayed psychrophilic growth under MAP. The reduced microbial growth in CPE-treated sausages suggests a synergistic antimicrobial effect of natural phyto-extracts and modified atmosphere packaging.

Table 4: Microbiological Profile of Fibre-Enriched Pork Sausages Treated with a Phyto-Extract Blend during Refrigerated Storage in MAP ($4 \pm 1 \text{ }^\circ\text{C}$) (Mean \pm S.E)*

Treatments/Days	Day 1.	Day 7.	Day 14.	Day 21.	Day 28.	Day 35.
Standard Plate Count (Log₁₀ cfu/g)						
T1 (P+CPE)	1.04 \pm 0.06 ^f	1.11 \pm 0.09 ^{Be}	2.14 \pm 0.11 ^{Bd}	2.85 \pm 0.08 ^{Cc}	4.20 \pm 0.10 ^{Cb}	4.50 \pm 0.10 ^{Ca}
T2 (P)	1.05 \pm 0.04 ^f	1.52 \pm 0.06 ^{Ae}	2.76 \pm 0.06 ^{Ad}	3.45 \pm 0.14 ^{Ac}	4.95 \pm 0.19 ^{Ab}	6.56 \pm 0.15 ^{Aa}
T3 (P+BHT)	1.01 \pm 0.03 ^f	1.23 \pm 0.08 ^{Be}	2.16 \pm 0.12 ^{Bd}	3.02 \pm 0.06 ^{Bc}	4.45 \pm 0.11 ^{Bb}	5.42 \pm 0.13 ^{Ba}
Coliforms Count (Log₁₀ cfu/g)						
T1 (P+CPE)	ND.	ND.	ND.	ND.	ND	ND
T2 (P)	ND	ND	ND	ND	ND	ND
T3 (P+BHT)	ND	ND	ND	ND.	ND	ND
Psychrophillic Count (Log₁₀ cfu/g)						
T1 (P+CPE)	ND.	ND	ND.	ND	ND	1.10 \pm 0.04 ^C
T2 (P)	ND.	ND	ND.	1.00 \pm 0.02 ^c	1.40 \pm 0.02 ^b	2.26 \pm 0.02 ^{Aa}
T3 (P+BHT)	ND.	ND.	ND.	ND.	ND	1.15 \pm 0.03 ^B

*Mean \pm S.E. Means bearing different superscripts within a row (lowercase) and within a column (uppercase) differ significantly ($P < 0.05$). $n = 6$. T1 (P + CPE) = pork sausages containing 0.30% cocktail phyto-extracts; T2 (P) = pork sausages without phyto-extracts; T3 (P + BHT) = pork sausages containing 100 ppm BHT.

Sensory evaluation

The radar plots presented in Figure 1 illustrate the evolution of sensory attributes of fibre-enriched pork sausages during refrigerated storage under MAP. At the beginning of storage (day 1), all treatments exhibited relatively high scores, although samples containing the cocktail phyto-extract blend (CPE) showed marginally better ratings for most parameters compared with the control and BHT groups.

With progression of storage to day 35, a reduction in the sensory polygon area was evident for all treatments, confirming a general decline in organoleptic quality over time. Nevertheless, the contraction was more pronounced in the control, whereas CPE-treated sausages maintained comparatively greater extensions along the flavour axis. The better preservation of flavour in CPE-treated sausages may be attributed to the antioxidant activity of plant bioactives, which likely suppressed the generation of secondary oxidation compounds responsible for sensory deterioration, as also reported by Ahmad *et al.* (2023) [2].

Texture scores followed a similar tendency. Although a gradual decrease was observed, the higher ratings in CPE-treated sausages suggest better preservation of structural integrity and mouthfeel during storage. Comparable observations have been reported, where phenolic-rich extracts contributed to the maintenance of textural attributes by restricting protein and moisture degradation (Bergamaschi *et al.*, 2023) [4]. Juiciness values were nearly similar among treatments on day 1; however, antioxidant incorporation appeared beneficial at later stages, as reflected by relatively higher scores than the control. Overall acceptability mirrored the behaviour of individual attributes. From day 1 through day 35, sausages with CPE consistently received superior panel ratings, indicating that mitigation of oxidative changes along with improved retention of desirable characteristics contributed to sustained consumer appeal.

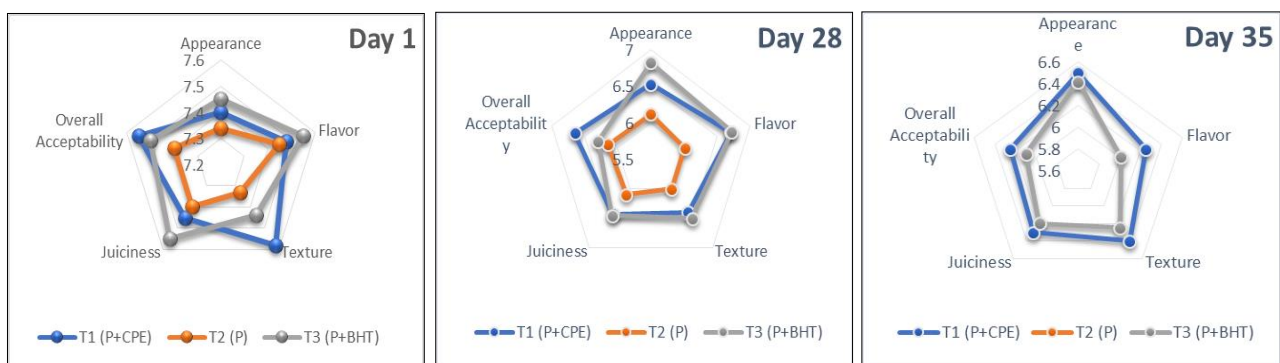


Fig 1: Radar plots illustrating sensory attributes (appearance, flavour, texture, juiciness and overall acceptability) of fibre-enriched pork sausages treated with cocktail phyto-extracts (P+CPE), BHT (P+BHT) and control (P) under modified atmosphere packaging on (a) Day 1, (b) Day 21 and (c) Day 28 of refrigerated storage

Conclusion

The present study demonstrates that the combined application of a polyphenol-rich phyto-extract cocktail (0.30%) and modified atmosphere packaging (MAP) effectively enhanced the storage stability of fibre-enriched pork sausages during refrigerated storage. The integrated approach significantly retarded lipid oxidation, maintained colour stability, suppressed microbial growth, and preserved sensory quality compared to untreated control samples, with performance comparable to or better than the synthetic

antioxidant BHT. The observed improvements are attributable to the synergistic effects of reduced oxygen availability under MAP and the antioxidative and antimicrobial activities of plant-derived bioactive compounds. Importantly, sausages treated with the phyto-extract blend remained microbiologically safe and sensorially acceptable for up to 35 days of refrigerated storage. These findings support the potential of combining natural antioxidant blends with MAP as an effective clean-label strategy for extending the shelf life and quality of

functional pork products, offering a viable alternative to synthetic antioxidants in meat processing.

References

1. Abdullah FAA, Dordevic D, Kabourkova E. Oxidation status and antioxidant activity of analogue meat products in modified atmosphere packaging. *Applied Sciences*,2024;14(15):6713.
2. Ahmad A, Mahmood N, Hussain M, Aiman U, Al-Mijalli SH, Raza MA, *et al.* Improvement in oxidative stability and quality characteristics of functional chicken meat product supplemented with aqueous coriander extract. *International Journal of Food Properties*,2023;26(1):855-865.
3. Bai T, Wang X, Du W, Cheng J, Zhang J, Zhang Y, *et al.* Recent Advances, Challenges, and Functional Applications of Natural Phenolic Compounds in the Meat Products Industry. *Antioxidants*,2025;14(2):138.
4. Bergamaschi M, Simoncini N, Spezzano VM, Ferri M, Tassoni A. Antioxidant and sensory properties of raw and cooked pork meat burgers formulated with extract from non-compliant green coffee beans. *Foods*,2023;12(6):1264.
5. Fernandez-Lopez J, Zhi N, Aleson-Carbonell L, Pérez-Alvarez JA, Kuri V. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. *Meat Science*,2005;69(3):371-380.
6. Jandyal M, Malav OP, Mehta N, Wagh RV. Quality characteristics of functional pork sausages incorporated with oat bran powder. *Meat Science*,2022;17:1-7.
7. Koniecko ES. Handbook for meat chemists, 1979.
8. Kumar D, Chatli MK, Mehta N, Verma AK, Kumar P. Quality evaluation of chevon patties fortified with dietary fibre. *Indian Journal of Small Ruminants (The)*,2015;21(1):85-91.
9. Lorenzo JM, Sineiro J, Amado IR, Franco D. Influence of natural extracts on the shelf life of modified atmosphere-packaged pork patties. *Meat Science*,2014;96(1):526-534.
10. Moawad RK, Mohamed OSS, Abdelmaguid NM. Shelf-life evaluation of raw chicken sausage incorporated with green tea and clove powder extracts at refrigerated storage, 2020, 8821-8830.
11. Olvera-Aguirre G, Piñeiro-Vázquez ÁT, Sanginés-García JR, Zárate AS, Ochoa-Flores AA, Segura-Campos MR, *et al.* Using plant-based compounds as preservatives for meat products: A review. *Heliyon*, 2023, 9(6).
12. Rahman MH, Alam MS, Monir MM, Ahmed K. Comprehensive effects of black cumin (*Nigella sativa*) and synthetic antioxidant on sensory and physicochemical quality of beef patties during refrigerant storage. *Journal of Agriculture and Food Research*,2021;4:100145.
13. Salejda AM, Tril U, Krasnowska G. The effect of sea buckthorn (*Hippophae rhamnoides* L.) berries on some quality characteristics of cooked pork sausages. *International Journal of Food Engineering*,2014;8:604-607.
14. Tocai AC, Rosan CA, Teodorescu AG, Venter AC, Vicas SI. Multifunctional Roles of Medicinal Plants in the Meat Industry: Antioxidant, Antimicrobial, and Color Preservation Perspectives. *Plants*,2025;14(17):2737.
15. Wang FS. Effects of three preservative agents on the shelf life of vacuum packaged Chinese-style sausage stored at 20°C. *Meat Science*,2000;56(1):67-71.
16. Wang Q, Chen Q, Xu J, Sun F, Liu H, Kong B, *et al.* Effects of modified atmosphere packaging with various CO₂ concentrations on the bacterial community and shelf-life of smoked chicken legs. *Foods*,2022;11(4):559.
17. Witte VC, Krause GF, Bailey ME. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*,1970;35(5):582-585.
18. Yan Y, Zhang Y, Fang Z, Wang ZC, Nan Y, Shi H, *et al.* Modified atmosphere packaging and plant extracts synergistically enhance the preservation of meat: A review. *Food Control*,2024;164:110622.
19. Zhai Y, Huang J, Khan IA, Guo Y, Huang M, Zhou G, *et al.* Shelf-Life of boiled salted duck meat stored under normal and modified atmosphere. *Journal of Food Science*,2018;83(1):147-152.
20. Zhou T, Wu J, Zhang M, Ke W, Shan K, Zhao D, *et al.* Effect of natural plant extracts on the quality of meat products: A meta-analysis. *Food Materials Research*, 2023, 3(1).