

Optimization of passive modified atmosphere packaging to preserve physical and nutritional quality of ready-to-eat immature faba bean seeds

Amedeo Palma

amedeo.palma@cnr.it

National Research Council

Salvatore D'Aquino

National Research Council

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Abstract

A two-phase study identified optimal passive modified atmosphere packaging (MAP) conditions for ready-to-eat immature faba bean seeds (*Vicia faba* L. cv. 'Histal') stored at 5°C. A preliminary test compared seven treatments for in-package atmosphere, weight loss, texture and sensory quality: unpackaged seeds, a macro-perforated film (Film A, 1 × 5 mm hole), four laser micro-perforated films of decreasing perforation density (Film B, 28; Film C, 14; Film D, 9; Film E, 5 holes of 100 µm) and a continuous film (Film F). Films A, C and E were then examined for respiratory activity, biochemical composition, antioxidant systems and microbiological stability. Unpackaged seeds lost marketability within 6 days through severe weight loss and sensory degradation. Film E generated the most favourable atmosphere (CO₂ 11.5–16.8 kPa; O₂ 3.8–6.4 kPa), reducing respiration by 42% at day 1 relative to the near-atmospheric Film A. Suppression persisted until day 6, and the induction of superoxide dismutase and guaiacol peroxidase was limited, indicating lower oxidative stress. These conditions preserved chlorophylls, carotenoids, ascorbic acid, sucrose and starch, and maintained high sensory scores and low microbial loads over 9 days. The results support a framework in which perforation density regulates package permeability, cumulative in-package O₂ exposure, oxidative stress responses and ultimately quality retention. Because this cascade rests on general principles of oxidative biochemistry rather than legume-specific traits, perforation design offers a broadly applicable strategy for extending the shelf life of minimally processed, oxidation-sensitive foods, with immature faba bean as an effective model system.

1. Introduction

The faba bean (*Vicia faba* L.) is one of the oldest cultivated crops and among the most nutritionally significant grain legumes worldwide, with annual global production exceeding 5 million tonnes [1, 2]. It is an ecologically important rotational crop, contributing to biological nitrogen fixation and soil fertility. It is also a nutritionally dense food, rich in proteins, complex carbohydrates, dietary fibre, vitamins, phenolic compounds, flavonoids and L-DOPA, a dopamine precursor with therapeutic relevance for Parkinson's disease [3, 4, 5, 6]. On the other hand, it also contains antinutritional factors including condensed tannins, lectins and the pyrimidine glycosides vicine and convicine, which can cause favism-related haemolytic anaemia in glucose-6-phosphate dehydrogenase (G6PD)-deficient individuals [7, 8]. For this reason, the postharvest dynamics of these compounds is a relevant food-safety consideration. Immature faba beans, harvested before physiological maturity, differ markedly from dry seeds in composition and commercial value: they show high moisture (> 65%), elevated soluble sugars, vivid green coloration for the abundant chlorophylls and carotenoids, tender texture attributable to low starch and high turgor, and high concentrations of ascorbic acid and phenolic antioxidants [9, 10, 11, 12]. These attributes make them especially desirable for fresh consumption, particularly in Mediterranean countries where they are a traditional agri-food product [13, 14]. The natural morphology of the seed permits shelling without wounding the cotyledon surface, a fundamental advantage over conventional fresh-cut products: without the cellular disruption and exudate release associated with cutting, wound-induced respiratory bursts and prevented substrate-enzyme contact, quality evolution can be governed predominantly by the in-package atmosphere [15, 16]. The shelled immature seed is therefore an unusually clean model system in which the relationship between package permeability and atmosphere-driven quality loss can be clearly studied. Its main postharvest challenges are enzymatic browning of the seed integument through polyphenol oxidase (PPO) and peroxidase (POD) activity under aerobic conditions, dehydration-induced textural hardening, loss of vivid green colour through chlorophyll degradation, and oxidative decline of bioactive compounds [16, 17, 18]. Cold storage combined with modified atmosphere packaging (MAP) addresses all of these simultaneously: elevated CO₂ inhibits aerobic spoilage microorganisms, reduces respiration and senescence and attenuates PPO/POD activity, reduced O₂ limits oxidative degradation of pigments and phenolics, and near-saturated in-package humidity retards moisture loss and firmness changes [19, 20, 21, 22]. Despite this well-established framework, published research on MAP for minimally processed immature faba bean seeds is remarkably sparse and methodologically limited. Logegaray et al. [23] compared two packaging systems at 1 and 4°C, measuring firmness, colour and visual quality; Palma and D'Aquino [15] compared two films of contrasting oxygen transmission rate (OTR), adding TA, TSS and ascorbic acid; Collado et al. [24] used a single passive MAP condition (≈ 5 kPa CO₂/16 kPa O₂) while investigating the impact of sanitation agents on colour, firmness, vitamin C, protein and microbiology; and Collado et al. [16, 25] added UV-C pretreatment, measuring antioxidant capacity, phytic acid, condensed tannins and raffinose. All were limited to one or two packaging conditions, and none used laser micro-perforated films to control in-package CO₂ and O₂ systematically, measured antioxidant enzyme activities, or characterised the biochemical mechanisms linking MAP conditions to quality preservation. The present study addresses these gaps. Its objectives were: (a) to identify, in a preliminary test screening seven packaging solutions, the passive MAP atmosphere that best preserves the physical and sensory quality of ready-to-eat immature faba bean seeds; (b) to characterise, in a main trial on representative treatments, how the selected atmospheres influence respiratory activity, antioxidant enzyme systems and the nutritional and microbiological quality of the product over a 9-day cold storage period, through a full analytical profile - antioxidant enzyme activities (SOD and POD), respiratory rate, chlorophylls, carotenoids, individual carbohydrates, starch, vicine and convicine, TPC and TEAC - measured simultaneously for the first time in packaged immature faba bean seeds, together with the first Pearson and Spearman correlation analysis between TPC, TEAC and

ascorbic acid in this commodity; and (c) to test whether a single, design-controllable variable, film permeability, acting through the cumulative in-package O₂ exposure it imposes, can account for the observed oxidative quality loss and thereby provide a transferable design principle.

2. Materials and Methods

2.1. Plant material, processing, packaging, and storage

The study comprised two consecutive phases carried out on independent harvests: a screening phase (Preliminary Test, Section 2.1.1), in which seven packaging treatments were compared, and an in-depth characterisation of three selected films (Main Trial, Section 2.1.2), performed on a separate crop of the same cultivar, grown at the same site and harvested at the same physiological stage. Seven quality attributes were measured with identical procedures in both trials, so that the agreement between them provides an independent check of reproducibility across harvests (Section 3.2.3). Both trials followed the procedure described below. *Vicia faba* L. cv. 'Hista' pods were hand-harvested at an immature physiological stage in April from an open-field crop in Uri, Sardinia, Italy (40 °N, 8 °E, 128 m a.s.l.) and cold-transported to the laboratory within 2 h. Seeds showed vivid green coloration, dimensions of approximately 1.8 × 2.0 × 0.8 cm (width, length, thickness), mean weight 0.52 g and moisture content ≈ 85% (lyophilisation). Pods were held at 5°C and 85% RH overnight and manually shelled the following morning. Seeds were surface-sanitised in a cold (5°C) aqueous solution of 50 ppm peroxyacetic acid (Tsunami 100, Ecolab USA Inc.) to reduce the initial microbial load [26], then rinsed and packed dry. Approximately 150 g of sound, size-uniform seeds were placed in polypropylene trays (14 × 8.0 × 6.0 cm internal) and sealed with a 25 µm biaxially oriented polypropylene (BOPP) film (Coralene SWAF 400, Corapack, Brenna, Italy), with or without macro- and micro-perforations, using an Orved VGP heat sealer. The continuous film had a water-vapour transmission rate of 7 g m⁻² 24 h⁻¹ (37°C, 100% RH) and an O₂ permeance of 2150 cm³ m⁻² 24 h⁻¹ atm (23°C, 0% RH); laser micro-perforations of 100 µm diameter were produced in-line during film manufacturing. Package oxygen and carbon-dioxide transmission rates (OTR, CO₂TR) were estimated as the sum of the intrinsic film permeance and the diffusive transmission through the perforations, the latter modelled as steady-state Fickian diffusion in air [27, 28]. Each 100 µm perforation contributed ≈ 133 cm³ O₂ and ≈ 104 cm³ CO₂ d⁻¹ atm⁻¹ (per-pore β = CO₂TR/OTR ≈ 0.78), so package OTR increased approximately linearly with perforation number while β fell from ≈ 4 for the continuous Film F toward the near-unity value of pore diffusion; transmission rates and β for all six films are reported in Online Resource 1. The single 5 mm macro-perforation of Film A provided a far larger, edge-diffusion-dominated aperture that kept the headspace near-atmospheric. Packages were stored at 5°C and ≈ 85% RH for 3, 6 or 9 days, with temperature and humidity continuously monitored by three data loggers (LogTag Humidity and Temperature Recorder, Auckland, New Zealand) placed at different locations in the storage room.

2.1.1. Preliminary Test

Seven packaging treatments were compared against a un-packaged control: (1) unpackaged seeds in open polypropylene trays; (2) Film A: 1 macro-hole, 5 mm diameter; (3) Film B: 28 micro-holes (≈ 2 holes cm⁻², 100 µm diameter); (4) Film C: 14 micro-holes (1 hole cm⁻²); (5) Film D: 9 micro-holes (1 hole per 1.5 cm); (6) Film E: 5 micro-holes (1 hole per 2.5 cm); (7) Film F: continuous BOPP. The Preliminary Test assessed in-package CO₂ and O₂ (on days 1, 2, 3, 6, and 9 of storage), weight loss, instrumental texture, sensory quality, appearance, TA, and TSS (all on days 0, 3, 6, and 9).

2.1.2. Main Trial and justification of treatment selection

Based on the preliminary test outcomes (rationale detailed in Section 3.1.3), three treatments spanning the full atmospheric gradient were selected for comprehensive characterisation: Film A (near-atmospheric reference: CO₂ <2.3 kPa, O₂ >18.4 kPa), Film C (moderate MAP: CO₂ 6.6–7.9 kPa, O₂ 13.8–14.8 kPa; 14 micro-holes), whose atmosphere approximates the passive systems of Collado et al. [24], and Film E (optimal MAP: CO₂ 11.5–16.8 kPa, O₂ 3.8–6.4 kPa; 5 micro-holes), the best-performing treatment. The Main Trial included all parameters measured in the preliminary test plus: colour (L*, h°, C*); chlorophylls a and b; carotenoids; ascorbic acid; raffinose, sucrose, glucose, fructose, and starch; vicine and convicine; total phenolic compounds (TPC); TEAC (Trolox Equivalent Antioxidant Capacity); Superoxide Dismutase (SOD) and Peroxidase (POD) activities; respiratory rate; and complete microbiological profiling.

2.2. In-package gas composition and respiration rate

In-package CO₂ and O₂ concentrations were determined using an F-940 portable gas analyser (Felix Instruments, Camas, WA, USA). Air from the package headspace was sampled through two piercing needles connected to the analyser via inlet and outlet tubing and inserted through a self-sealing silicone septum previously attached to each package, thus maintaining a closed system. A built-in pump continuously circulated the headspace gas through the analyser and back into the package. Gas concentrations were recorded once CO₂

and O₂ readings had stabilized. Measurements were taken on days 1, 2, 3, 6, and 9 (Preliminary Test and Main Trial). The measurements were performed on separate dedicated packages (n = 6 per film per time point); other packages were used for quality analysis to avoid any compromise of headspace integrity in packages destined for chemical or sensory assessment. In-package temperature and RH were recorded every 4 h with a LogTag data logger placed inside three packages of each treatment.

Respiratory activity was determined in both unpackaged and packaged seeds by placing individual packages inside 10 L hermetically sealed jars and incubating them for 2 h at 5°C or 30 min at 20°C. CO₂ production was measured using a CM-100 CO₂ datalogger (CO₂Meter Inc., Ormond Beach, FL, USA). The jars were equipped with an internal fan to ensure air mixing, and the lids were fitted with two silicone septa connected to the analyser by inlet and outlet tubing. Each tube terminated in a needle inserted through a septum, thereby creating a closed-loop system. An integrated pump continuously circulated headspace air through the analyser via the inlet tube and returned the same air to the jar through the outlet tube. Measurements were recorded after approximately 60 s, once the CO₂ readings had stabilized. Values are expressed as µg CO₂ kg⁻¹ s⁻¹ (n = 6 replicates per temperature and sampling time for unpackaged seeds; n = 3 packages per film and sampling time for packaged seeds).

Ethylene was measured using a Varian 3300 gas chromatograph, equipped with a flame ionization detector (FID), Carbowax 20M 80/120 mesh Carbograph 1 AW 30 column (Alltech, Italy, Milan); the column temperature was 60°C, injector 110°C and detector 180°C. The injection volume was 0.5 mL, and helium was used as carrier gas. Ethylene was quantified against a certified standard [29].

2.3. Weight loss, texture, sensory evaluation, appearance, titratable acidity, and total soluble solids

Weight loss was calculated as the percentage mass reduction from the initial tray weight at each sampling time (n = 9). Instrumental texture was determined with a Zwick-Roell DO-FB 0.5 TS testing machine (Zwick-Roell, Ulm, Germany): a penetration test with a 1.2 mm flat-faced cylindrical probe at 3.3 mm s⁻¹ provided F Max (N) and L at F Max (mm), and a cut test with a 2 mm sharp blade provided F Max (N) (n = 10 seeds per treatment per period). Sensory quality (overall acceptability: aroma, taste, crispness) and appearance (seedcoat browning and external defects) were evaluated by an experienced panel of 10 assessors from the laboratory technical staff, familiarised with the product and calibrated on reference samples before each session, on a 9-point quality-rating scale (1 = extremely poor/completely browned; 5 = limit of marketability; 9 = excellent/no browning). At each sampling time ten seeds were withdrawn from each of six packages per treatment and pooled in a ceramic bowl, from which each assessor evaluated the sample independently, giving one score each (n = 10 per treatment per sampling time). Titratable acidity (TA) was determined by potentiometric titration of a 10 g seed homogenate diluted 1:5 in deionised water against 0.1 N NaOH to a pH 8.1 endpoint, expressed as g citric acid equivalents 100 g⁻¹ FW (n = 3); total soluble solids (TSS) were measured on clarified homogenate juice with a digital refractometer and expressed as °Brix (n = 3).

2.4. Chemical analyses (Main Trial)

All chemical analyses were performed at harvest and at 3, 6 and 9 d of storage (n = 3 independent packages per treatment per time point). Seeds were homogenised with an Ultra-Turrax® T25 homogeniser (IKA-Werke GmbH & Co. KG, Staufen, Germany) to a fine uniform powder and extracted in triplicate: 2.5 g aliquots were extracted with acetone:water (80:20, v/v) for chlorophylls and carotenoids and with methanol:water (80:20, v/v) for all other analytes, agitated 2 h in darkness at room temperature, centrifuged at 13,000 × g for 15 min at 4°C and filtered (0.45 µm cellulose acetate). Chlorophylls a and b and total carotenoids were determined spectrophotometrically at 663, 646 and 470 nm according to Albanese et al. [30] and ascorbic acid by HPLC-DAD according to Palma et al. [31], both expressed as mg 100 g⁻¹ FW. TPC was quantified by the Folin-Ciocalteu method at 760 nm, expressed as g kg⁻¹ gallic acid equivalents (GAE). Antioxidant activity (TEAC) was determined by the DPPH radical-scavenging assay according to Brand-Williams et al. [32]: 100 µL of extract added to 3.9 mL of 0.1 mM DPPH· in methanol, incubated at 25°C in the dark for 30 min, absorbance at 515 nm, expressed as mmol kg⁻¹ Trolox equivalents against a Trolox curve (0.05–1.0 mmol L⁻¹). Individual carbohydrates (raffinose, sucrose, glucose, fructose) were quantified by HPLC according to Palma et al. [29] and expressed as g kg⁻¹ FW, and starch by the amyloglucosidase/α-amylase method (AACC Method 76-13.01) [33]. Vicine and convicine were determined by HPLC at 280 nm on a C18 Prevail column at 30°C, with gradient elution of H₂O/0.1% TFA (A) and CH₃CN/0.1% TFA (B) – 2% B initial, 5% B at 15 min, 25% B at 30 min, 5% B at 45 min, 2% B at 50 min – at 1 mL min⁻¹; vicine was quantified against external standards and convicine expressed as vicine equivalents in g kg⁻¹ FW [34]. Calibration curves were linear over 10–100 µg mL⁻¹ (vicine: y = 0.6497x + 0.2397, R² = 0.999). Colour was measured with a Minolta CR-400 chromameter (Minolta Co., Osaka, Japan) on 30 randomly selected seeds per replicate, calculating L* (lightness), h° (hue angle) = arctan(b*/a*) and C* (chroma) = √(a*² + b*²).

2.4.1. Superoxide dismutase (SOD) and guaiacol peroxidase (POD) assays

Crude enzyme extracts were prepared from 1.5 g of powdered frozen tissue homogenised in 4.5 mL ice-cold 50 mM potassium phosphate buffer (pH 7.8) containing 1% (w/v) polyvinylpyrrolidone (PVP), 0.1 mM EDTA, and 0.5% (w/v) polyvinylpolypyrrolidone (PVPP) to minimise phenolic interference with enzyme assays [35]. Homogenates were centrifuged at $13,000 \times g$ for 20 min at 4°C , and the resulting supernatant was used immediately for enzyme assays. Total soluble protein was determined by the Bradford [36] assay with bovine serum albumin as standard. SOD activity (EC 1.15.1.1) was assayed by the riboflavin-mediated photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm [37], as adapted by Goffi et al. [38] and Wang et al. [35]. One unit of SOD was defined as the amount of enzyme required to inhibit NBT photoreduction by 50%; results expressed as U mg^{-1} protein. POD activity (EC 1.11.1.7) was determined by monitoring H_2O_2 -dependent guaiacol oxidation at 470 nm according to Song et al. [39] and Wang et al. [35]. One unit corresponded to $\Delta A_{470} = 0.001 \text{ min}^{-1}$; results expressed as $\Delta A_{470} \times 10^3 \text{ min}^{-1} \text{ g}^{-1} \text{ FW}$. Both enzymes were assayed at harvest and at 3, 6, and 9 d of storage ($n = 3$ independent packages per treatment per time point).

2.5. Microbiological analyses

Microbiological quality was evaluated by standard plate count methods on 10 g seed samples homogenised in 90 mL buffered peptone water (ISO 6887–1). Three independent packaging units per treatment per period were used as biological replicates; each replicate homogenate was plated in duplicate on each medium type, and the mean of the two technical replicates was used as the value for that biological replicate. Psychrotrophic bacteria: plate count agar (PCA), incubated at 5°C for 72 h. Mesophilic bacteria: PCA, 32°C , 48 h. Enterobacteriaceae: violet red bile glucose agar (VRBGA), 37°C , 48 h. Yeasts: yeast peptone dextrose agar (YPDA), 25°C , 48 h. Moulds: malt extract agar (MEA), 25°C , 4 d. *E. coli*: tryptone bile X-glucuronide agar (TBX), 37°C , 24 h. Staphylococci: Baird-Parker agar with egg yolk tellurite supplement, 37°C , 48 h. All results expressed as $\log_{10} \text{ CFU g}^{-1}$ ($n = 3$ replicates per treatment per time point). Values below the detection limit of the methods ($\log 0.5 \text{ CFU g}^{-1}$) are reported as < 1 .

2.6. Statistical analysis

Preliminary test: data were subjected to two-way factorial analysis of variance (ANOVA) with packaging treatment (7 levels, excluding harvest) and storage time (4 levels: harvest, 3, 6 and 9 d) as factors. Because the seeds were identical across treatments at harvest, day-0 values are common to all packaging treatments and are therefore reported as a single reference row, although they were included as the baseline level of the storage-time factor in the mean comparisons. Main Trial: a two-way factorial ANOVA (packaging treatment, 3 levels $\times$ storage time, 4 levels including harvest) was performed for each quality parameter, except weight loss, for which storage time comprised three levels (3, 6 and 9 d) since weight loss is zero at harvest by definition. In both trials homogeneous groups were identified by Tukey's HSD test ($P \leq 0.05$), applied separately among packaging treatments within each storage time and among storage times within each packaging treatment; a two-way factorial ANOVA summary is provided separately. Pearson (r) and Spearman (ρ) correlation coefficients between TPC, TEAC and ascorbic acid were computed for all Main Trial observations ($n = 36$: 3 films $\times$ 4 periods $\times$ 3 replicates), with partial correlations controlling for storage period to isolate the packaging effect from the temporal co-variation shared by all parameters, together with the coefficient of determination (R^2) and ordinary least-squares regression equations between TPC and TEAC. All analyses were performed using Statgraphics Centurion XV Professional (Herndon, VA, USA).

3. Results and Discussion

3.1. Preliminary Test

3.1.1. In-package atmosphere

The in-package CO_2 , O_2 and ethylene concentrations from day 1 to day 9 are reported in Table 1. The single 5 mm macro-hole of Film A offered negligible resistance to gas exchange, so its headspace underwent little changes (CO_2 1.4–2.1 kPa; O_2 19.0–19.4 kPa), matching the CO_2 accumulation reported for fresh faba beans in ventilated boxes [23]. The four micro-perforated films generated a CO_2 gradient inversely related to perforation number - Film B (28 holes) 4.9–5.3 kPa, Film C (14) 6.6–7.9 kPa, Film D (9) 9.2–11.2 kPa, Film E (5) 11.7–16.6 kPa - with equilibrium substantially established between days 2 and 3 and maintained through day 9, consistent with passive-MAP kinetics for high-respiration commodities [19, 20]. Because the equilibrium of a passive package is set by the balance between respiratory gas exchange and counter-diffusion through the film, fewer perforations lower package permeance and shift the steady state toward higher CO_2 and lower O_2 [40, 41]; the consistent ranking of equilibrium CO_2 with perforation number confirms that this respiration-permeation balance, rather than any uncontrolled variable, governed the in-package atmosphere. Since pore diffusion is nearly gas-independent ($\beta \approx 0.78$; Online Resource 1), fewer holes raised CO_2 while only moderately depressing O_2 , enabling CO_2 enrichment without the severe O_2 depletion of the continuous film, whose transmission depends on intrinsic film permeability rather than Fickian pore

diffusion [27, 42]. Film F never equilibrated: mean CO₂ rose from 13.0 kPa at day 1 to ≈ 31 kPa by day 6, while O₂ was already 1.6 kPa at day 1 and fell below 0.9 kPa from day 2, an atmosphere likely to favour fermentative metabolism and off flavours [43].

Table 1

In-package CO₂ (kPa), O₂ (kPa), and ethylene C₂H₄ (ppm) concentrations at days 1, 2, 3, 6, and 9 during cold storage of minimally processed *Vicia faba* seeds under different packaging treatments at 5°C (Preliminary Test). Film A: 1 × 5 mm macro-hole; Films B-E: laser micro-perforated BOPP 25 µm with decreasing number of 100 µm holes; Film F: continuous non-perforated film.

Packaging treatment	Day 1	Day 2	Day 3	Day 6	Day 9
CO₂ (kPa)					
Film A	1.92 ± 0.17 <i>f AB</i>	2.13 ± 0.15 <i>f A</i>	1.98 ± 0.10 <i>f A</i>	1.68 ± 0.25 <i>e B</i>	1.37 ± 0.18 <i>d C</i>
Film B	5.10 ± 0.25 <i>e A</i>	5.30 ± 0.33 <i>e A</i>	5.17 ± 0.33 <i>e A</i>	5.17 ± 0.33 <i>de A</i>	4.88 ± 0.21 <i>cd A</i>
Film C	6.63 ± 0.41 <i>d B</i>	7.87 ± 0.72 <i>d A</i>	7.63 ± 0.77 <i>d AB</i>	7.30 ± 0.40 <i>cd AB</i>	6.90 ± 0.63 <i>cd AB</i>
Film D	9.18 ± 0.48 <i>c B</i>	11.20 ± 0.77 <i>c A</i>	11.23 ± 0.31 <i>c A</i>	10.95 ± 0.75 <i>c A</i>	9.85 ± 0.79 <i>c B</i>
Film E	11.68 ± 0.29 <i>b B</i>	15.72 ± 0.59 <i>b A</i>	16.15 ± 0.55 <i>b A</i>	16.63 ± 0.69 <i>b A</i>	16.20 ± 0.78 <i>b A</i>
Film F	13.00 ± 1.24 <i>a C</i>	20.98 ± 1.38 <i>a B</i>	24.24 ± 2.59 <i>a AB</i>	30.88 ± 6.16 <i>a A</i>	30.02 ± 7.85 <i>a A</i>
O₂ (kPa)					
Film A	18.98 ± 0.17 <i>a C</i>	19.00 ± 0.13 <i>a BC</i>	19.00 ± 0.09 <i>a BC</i>	19.40 ± 0.15 <i>a A</i>	19.30 ± 0.28 <i>a AB</i>
Film B	15.90 ± 0.32 <i>b B</i>	16.25 ± 0.27 <i>b AB</i>	16.47 ± 0.24 <i>b A</i>	16.47 ± 0.24 <i>b A</i>	16.52 ± 0.21 <i>b A</i>
Film C	13.85 ± 0.52 <i>c B</i>	13.77 ± 0.56 <i>c B</i>	13.85 ± 0.48 <i>c B</i>	14.28 ± 0.64 <i>c AB</i>	14.83 ± 0.51 <i>c A</i>
Film D	10.00 ± 0.74 <i>d C</i>	10.37 ± 0.91 <i>d BC</i>	10.37 ± 0.91 <i>d BC</i>	11.55 ± 0.64 <i>d AB</i>	12.57 ± 0.67 <i>d A</i>
Film E	5.35 ± 0.46 <i>e AB</i>	3.98 ± 0.75 <i>e C</i>	3.98 ± 0.75 <i>e C</i>	5.13 ± 0.87 <i>e BC</i>	6.50 ± 0.91 <i>e A</i>
Film F	1.55 ± 0.80 <i>f A</i>	0.32 ± 0.24 <i>f B</i>	0.38 ± 0.15 <i>f B</i>	0.42 ± 0.31 <i>f B</i>	0.87 ± 0.81 <i>f AB</i>
C₂H₄ (ppm)					
Film A	0.48 ± 0.16 <i>c B</i>	0.42 ± 0.15 <i>d B</i>	0.90 ± 0.17 <i>de A</i>	1.12 ± 0.18 <i>cd A</i>	1.01 ± 0.15 <i>cd A</i>
Film B	0.63 ± 0.21 <i>bc A</i>	0.55 ± 0.10 <i>d A</i>	0.73 ± 0.21 <i>e A</i>	0.81 ± 0.25 <i>d A</i>	0.74 ± 0.27 <i>d A</i>
Film C	0.90 ± 0.28 <i>b A</i>	1.00 ± 0.30 <i>c A</i>	1.21 ± 0.12 <i>cd A</i>	1.16 ± 0.20 <i>cd A</i>	1.24 ± 0.20 <i>bc A</i>
Film D	1.24 ± 0.12 <i>a A</i>	1.16 ± 0.23 <i>bc A</i>	1.36 ± 0.13 <i>bc A</i>	1.44 ± 0.17 <i>bc A</i>	1.32 ± 0.24 <i>bc A</i>
Film E	1.39 ± 0.16 <i>a B</i>	1.45 ± 0.14 <i>ab B</i>	1.59 ± 0.24 <i>ab AB</i>	1.76 ± 0.16 <i>ab A</i>	1.58 ± 0.16 <i>ab AB</i>
Film F	1.32 ± 0.11 <i>a B</i>	1.56 ± 0.24 <i>a AB</i>	1.89 ± 0.19 <i>a A</i>	1.94 ± 0.31 <i>a A</i>	1.99 ± 0.35 <i>a A</i>
Values are means ± SD (n = 6). For each storage period, different lowercase letters indicate significant differences among packaging treatments; for each treatment, different upper case letters indicate significant differences among storage periods (Tukey's HSD, P ≤ 0.05).					

The steady-state gas data provide an internal test of this interpretation that is independent of the absolute transmission rates. Since the apparent respiratory quotient of a passive package can be expressed as $RQ = (\Delta CO_2 / \Delta O_2) \times \beta$, errors in the absolute OTR and CO₂TR largely cancel because only their ratio enters the calculation. Using the equilibrium concentrations averaged over days 3–9 (Table 1), an ambient composition of 0.03 kPa CO₂ and 20.9 kPa O₂, and the per-pore β of 0.78 (Online Resource 1), the five perforated films yielded consistent apparent RQ values (0.77–0.89), well within the range expected for aerobic respiration on mixed substrates, whereas Film F, whose β is ≈ 4.0, yielded an apparent RQ of ≈ 5.6, indicating CO₂ production far more than O₂ consumption. Thus, the atmospheric data alone support the conclusion that fermentative metabolism developed in the continuous film but not in the perforated films.

The CO₂ range explored across the perforated films (1.4–16.6 kPa) greatly extends the single condition (≈ 5 kPa) previously used for this commodity [24] and, being achieved by film design alone, is a distinctive feature of laser micro-perforated BOPP films [44, 45] not available with the continuous or stretch films used in earlier faba bean studies.

In-package ethylene followed the same perforation-dependent pattern, broadly accumulating in inverse proportion to hole number but remaining low throughout (0.4–2.0 ppm): Films A and B were the lowest and near-atmospheric (Film A, day 1: 0.48 ppm, day 9: 1.01 ppm;

Film B, day 9: 0.74 ppm), Films C, D and E were intermediate and progressively higher, and Film F reached the highest values (day 9: 1.99 ppm, significantly above Film A, $P \leq 0.05$). The rise over storage, significant in Films A, E and F but not in the more permeable Films B, C and D, is consistent with the onset of senescence-associated ethylene biosynthesis in immature legume seeds [16]; the higher CO₂ of Films D, E and F may have further contributed to quality preservation by slowing senescence-related metabolism [19].

3.1.2. Weight loss, texture, sensory quality, appearance, titratable acidity, and total soluble solids

All measured attributes were significantly affected by packaging treatment and storage time, and by their interaction except penetration F Max (Table 2). Unpackaged seeds deteriorated most severely, weight loss reaching 8.7% at day 3, 14.6% at day 6 and 18.2% at day 9, against 1.4–1.6% of Film A and 0.6–0.9% of Films B-F, confirming that even minimally restrictive packaging sustains the near-saturated headspace (RH 93.8–98.5%, data not shown) that decisively limits transpiration [15, 23]. Instrumental texture was characterised for the first time in this commodity with three parameters (penetration F Max, deformation at F Max, cut-test F Max), a more complete description of seedcoat and flesh integrity than the single-probe penetrometer used previously [15, 23]; the three conveyed largely concordant information, penetration F Max discriminating most strongly. Penetration force rose with dehydration-driven seedcoat hardening in unpackaged seeds (0.57 N at harvest to 0.80 N at day 9) and, less markedly, in Film A (0.62 to 0.69 N), in both cases departing significantly from the harvest value already at day 3; cut force increased significantly only in unpackaged seeds, and only from day 6. Films D and E preserved all three parameters statistically unchanged from harvest throughout storage, whereas Films B, C and F departed from the harvest value only late in storage and by a small margin, penetration force differing significantly from harvest from day 6 in Film B and only at day 9 in Films C and F (0.53 to 0.59 N).

Table 2

Effects of packaging treatment and storage time on firmness (penetration; deformation; cut test), sensory quality, appearance, titratable acidity (TA), total soluble solids (TSS), and weight loss in minimally processed *Vicia faba* seeds stored at 5°C (Preliminary Test).

Packaging treatment	Penetration (F Max, N)	Deformation (L at F Max, mm)	Cut test (F Max, N)	Sensory quality†	Appearance†	TA (g cit. acid 100 g ⁻¹ FW)	TSS (°Brix)	Weight loss (%)
Harvest	0.57 ± 0.03	1.36 ± 0.09	0.88 ± 0.04	9.0 ± 0.00	9.00 ± 0.00	0.44 ± 0.01	9.17 ± 0.06	0.00
3 d at 5°C								
Un-packaged	0.69 ± 0.05 a B	1.62 ± 0.14 a B	0.96 ± 0.09 a B	6.3 ± 0.29 e B	5.1 ± 0.40 d B	0.44 ± 0.01 a A	9.07 ± 0.21 a A	8.65 ± 1.09 a C
Film A	0.62 ± 0.03 b B	1.45 ± 0.08 b BC	0.94 ± 0.08 a AB	6.9 ± 0.26 d B	6.2 ± 0.45 c B	0.43 ± 0.01 a AB	9.20 ± 0.10 a A	1.44 ± 0.19 b A
Film B	0.61 ± 0.04 b AB	1.46 ± 0.08 b AB	0.92 ± 0.05 a AB	6.7 ± 0.37 de B	6.6 ± 0.37 c B	0.42 ± 0.01 a B	9.10 ± 0.20 a A	0.80 ± 0.17 c A
Film C	0.60 ± 0.03 bc AB	1.42 ± 0.12 b AB	0.91 ± 0.05 a A	7.7 ± 0.39 c B	8.3 ± 0.32 b B	0.42 ± 0.01 a AB	9.07 ± 0.15 a A	0.77 ± 0.16 c A
Film D	0.57 ± 0.04 bc A	1.41 ± 0.13 b A	0.89 ± 0.04 a A	8.2 ± 0.37 b B	8.5 ± 0.25 b B	0.42 ± 0.01 a A	9.20 ± 0.10 a A	0.76 ± 0.15 c A
Film E	0.54 ± 0.08 c A	1.40 ± 0.13 b A	0.89 ± 0.04 a A	8.8 ± 0.21 a A	8.9 ± 0.13 a A	0.44 ± 0.01 a AB	9.07 ± 0.15 a A	0.68 ± 0.17 c B
Film F	0.53 ± 0.05 c B	1.40 ± 0.10 b AB	0.90 ± 0.05 a A	8.9 ± 0.19 a A	8.9 ± 0.11 a A	0.43 ± 0.01 a A	9.13 ± 0.06 a A	0.59 ± 0.12 c B
6 d at 5°C								
Un-packaged	0.73 ± 0.03 a B	1.72 ± 0.08 a B	1.09 ± 0.10 a A	4.1 ± 0.20 e C	4.5 ± 0.42 e C	0.40 ± 0.01 bc B	8.43 ± 0.15 c B	14.57 ± 0.47 a B
Film A	0.64 ± 0.03 b B	1.49 ± 0.08 b AB	0.98 ± 0.09 b A	5.3 ± 0.32 d C	5.2 ± 0.24 d C	0.42 ± 0.01 abc B	8.87 ± 0.06 b B	1.59 ± 0.28 b A
Film B	0.62 ± 0.04 b A	1.49 ± 0.10 b A	0.95 ± 0.06 bc A	5.9 ± 0.34 c C	6.8 ± 0.28 c B	0.42 ± 0.01 a AB	9.00 ± 0.10 ab A	0.85 ± 0.14 c A
Film C	0.62 ± 0.07 b AB	1.47 ± 0.08 b AB	0.92 ± 0.05 bc A	6.1 ± 0.16 c D	6.8 ± 0.23 c C	0.42 ± 0.00 ab B	9.13 ± 0.15 ab A	0.80 ± 0.12 c A
Film D	0.57 ± 0.05 b A	1.45 ± 0.08 b A	0.89 ± 0.03 bc A	7.6 ± 0.31 b C	7.6 ± 0.20 b C	0.41 ± 0.02 abc A	9.03 ± 0.15 ab A	0.86 ± 0.14 c A
Film E	0.58 ± 0.06 b A	1.42 ± 0.09 b A	0.89 ± 0.02 c A	8.2 ± 0.32 a B	8.4 ± 0.25 a B	0.42 ± 0.02 abc AB	9.20 ± 0.10 a A	0.86 ± 0.12 c A

Values are means ± SD (n = 10 for texture, sensory quality, and appearance; n = 3 for TA and TSS; n = 9 for weight loss). Different lowercase letters indicate significant differences among packaging treatments within each storage period. Within each packaging treatment, different uppercase letters indicate significant differences among the three storage periods (3, 6, and 9 d; Tukey's HSD, P ≤ 0.05).

† Quality-rating scale: 1 = extremely poor/completely browned; 3 = poor/heavily browned; 5 = acceptable/moderately browned (marketability limit); 7 = good/lightly browned; 9 = excellent/no browning.

Packaging treatment	Penetration (F Max, N)	Deformation (L at F Max, mm)	Cut test (F Max, N)	Sensory quality†	Appearance†	TA (g cit. acid 100 g ⁻¹ FW)	TSS (°Brix)	Weight loss (%)
Film F	0.59 ± 0.03 b AB	1.42 ± 0.06 b AB	0.88 ± 0.04 c A	7.8 ± 0.24 ab B	8.3 ± 0.20 a B	0.39 ± 0.01 c B	9.13 ± 0.06 ab A	0.75 ± 0.19 c AB
9 d at 5°C								
Un-packaged	0.80 ± 0.03 a A	1.94 ± 0.13 a A	1.11 ± 0.09 a A	2.3 ± 0.25 d D	2.4 ± 0.21 f D	0.36 ± 0.01 b C	8.30 ± 0.10 b B	18.20 ± 1.22 a A
Film A	0.69 ± 0.04 b A	1.57 ± 0.12 b A	0.97 ± 0.05 b A	4.3 ± 0.28 c D	4.3 ± 0.34 e D	0.38 ± 0.01 ab C	8.77 ± 0.15 a B	1.58 ± 0.19 b A
Film B	0.65 ± 0.03 b A	1.49 ± 0.12 b A	0.96 ± 0.04 b A	4.7 ± 0.52 c D	6.1 ± 0.12 c C	0.39 ± 0.01 ab C	8.83 ± 0.12 a A	0.88 ± 0.08 c A
Film C	0.65 ± 0.05 b A	1.49 ± 0.08 b A	0.92 ± 0.06 bc A	6.9 ± 0.26 b C	5.4 ± 0.27 d D	0.39 ± 0.01 ab C	9.00 ± 0.10 a A	0.86 ± 0.12 c A
Film D	0.58 ± 0.03 c A	1.48 ± 0.11 b A	0.89 ± 0.04 c A	7.5 ± 0.22 a C	7.5 ± 0.31 b C	0.41 ± 0.02 a A	9.03 ± 0.12 a A	0.86 ± 0.11 c A
Film E	0.57 ± 0.03 c A	1.45 ± 0.09 b A	0.88 ± 0.02 c A	7.9 ± 0.16 a C	8.3 ± 0.38 a B	0.41 ± 0.01 a B	9.10 ± 0.17 a A	0.81 ± 0.11 c AB
Film F	0.59 ± 0.04 c A	1.46 ± 0.05 b A	0.88 ± 0.03 c A	7.7 ± 0.23 a B	7.6 ± 0.13 b C	0.39 ± 0.01 ab B	9.10 ± 0.10 a A	0.79 ± 0.11 c A
Values are means ± SD (n = 10 for texture, sensory quality, and appearance; n = 3 for TA and TSS; n = 9 for weight loss). Different lowercase letters indicate significant differences among packaging treatments within each storage period. Within each packaging treatment, different uppercase letters indicate significant differences among the three storage periods (3, 6, and 9 d; Tukey's HSD, P ≤ 0.05).								
† Quality-rating scale: 1 = extremely poor/completely browned; 3 = poor/heavily browned; 5 = acceptable/moderately browned (marketability limit); 7 = good/lightly browned; 9 = excellent/no browning.								

Sensory quality declined in inverse relation to in-package CO₂. Unpackaged seeds fell below the marketability threshold (score 5) by day 6 and Film A by day 9, consistent with the ≈ 7-day acceptable shelf life reported at 4°C [23, 24], while Films D, E and F kept sensory and appearance scores between 7.5 and 8.9 throughout, the decline from the harvest value becoming significant already at day 3 in Film D but only from day 6 in Films E and F. The intermediate films revealed a diagnostic decoupling between flavour and appearance: Film C retained acceptable eating quality (6.9) but a declining appearance (5.4) at day 9, whereas Film B showed the opposite pattern (sensory 4.7 vs. appearance 6.1), indicating that moderate CO₂ (≈ 4–8 kPa) suppresses surface browning. Titratable acidity (0.44 g citric acid 100 g⁻¹ FW at harvest) declined significantly in all treatments except the high-CO₂ Film D, which remained statistically unchanged (0.41 g at day 9); in Film E the decrease reached significance only at day 9 (0.41 g), whereas it was greatest and earliest in unpackaged seeds (0.36 g, – 18%), showing that elevated CO₂ attenuates rather than prevents the respiratory catabolism of organic acids [19, 20]. Total soluble solids fell significantly only in unpackaged seeds (9.07 to 8.30 °Brix) and Film A (9.20 to 8.77 °Brix), remaining unchanged in Films B–F, consistent with MAP-mediated suppression of respiratory sugar consumption.

3.1.3. Selection of treatments for the Main Trial

Three films spanning the full atmospheric gradient were retained for the Main Trial, so as to characterise the physiological and quality responses across progressively different MAP conditions rather than only to identify the best system. Film A provided the near-atmospheric aerobic baseline against which metabolic suppression could be quantified; Film C represented the moderate MAP range comparable to published passive systems for this commodity (≈ 5–7 kPa CO₂ [24]) and the transition between inadequate and optimal protection; Film E was the best-performing treatment across all Preliminary-Test parameters. Film D was excluded as largely redundant with the more restrictive Film E, being statistically indistinguishable from it for weight loss, texture, TA and TSS at every sampling time and differing only by slightly lower sensory and appearance scores. Film F was excluded because its uncontrolled CO₂ accumulation (>

23 kPa) and associated off-odours placed it outside the safe atmospheric window, and Film B because its sensory quality was indistinguishable from Film A at days 3 and 9 and only marginally higher at day 6, adding little beyond the near-atmospheric reference. Unpackaged seeds were omitted from the factorial design because their extreme deterioration - weight loss > 14% by day 6 and sensory scores below 2.5 at day 9 - would have precluded a meaningful two-factor analysis and distorted treatment means, although they were retained as an open reference in the respiration measurements (Section 3.2.2).

3.2. Main Trial

3.2.1. In-package atmosphere

The three selected films maintained clearly differentiated and temporally stable atmospheres throughout the Main Trial (Table 3), reproducing the preliminary gradient: Film A had a modest impact on gas changes compared to the atmosphere (CO₂ 1.4–2.2 kPa; O₂ 18.8–19.5 kPa), Film C established a stable moderate MAP (CO₂ 6.6–7.7 kPa; O₂ 13.4–14.8 kPa) and Film E the most restrictive environment (CO₂ 11.5–16.8 kPa; O₂ 3.8–6.4 kPa), reaching equilibrium at day 2. CO₂ and O₂ differed significantly among films at every sampling time, and within each film the atmosphere was essentially established within two to three days: the only significant within-film changes were the initial CO₂ rise in Film E from 11.5 kPa at day 1 to ≈ 16 kPa from day 2, a modest late CO₂ decline in Film A, and a partial O₂ recovery in Films C and E after the day-2 to day-3 minimum. As in the Preliminary Test, this reflects a passive equilibrium system in which lower permeance displaces the steady state toward higher CO₂ and lower O₂, the constancy within each film indicating a stable respiration-permeation balance [40, 41]. The absence of atmospheric convergence allows the quality differences described below to be attributed directly to the gaseous environment of each package. Cumulative oxygen exposure, computed by trapezoidal integration of the O₂ concentration–time curve between day 1 and day 9 (AUC-O₂), was 153.4, 115.2 and 39.8 kPa·d for Films A, C and E, a 3.9-fold reduction in Film E relative to Film A. This gradient paralleled the ranking of respiratory activity, antioxidant-enzyme induction and pigment and phenolic retention. The limited number of treatments precluded formal correlation analysis, but the concordance is consistent with the established role of oxygen availability in regulating respiration, senescence and oxidative metabolism [19, 41], and indicates that cumulative O₂ exposure, particularly over the first six days, is an important determinant of the quality trajectories that diverged from day 3 and widened through day 9. Notably, the equilibrium of Film E is more restrictive than the ≈ 6 kPa CO₂/9 kPa O₂ recently obtained with a 0.03 mm polyethylene film for whole fresh faba beans combined with electron-beam irradiation [46]; here the same protective window was attained passively, through perforation alone.

Table 3

In-package CO₂ and O₂ (kPa) and ethylene (ppm) changes during cold storage at 5°C of minimally processed *Vicia faba* seeds as affected by packaging treatments(Main Trial). Film A: 1 × 5 mm macro-hole; Film C: 14 × 100 µm micro-holes; Film E: 5 × 100 µm micro-holes.

Film	Day 1	Day 2	Day 3	Day 6	Day 9
CO₂ (kPa)					
Film A	1.81 ± 0.45 c AB	2.18 ± 0.48 c A	1.98 ± 0.60 c AB	1.96 ± 0.29 c AB	1.37 ± 0.16 c B
Film C	6.79 ± 0.75 b A	7.68 ± 0.73 b A	7.65 ± 0.82 b A	7.37 ± 0.73 b A	6.56 ± 0.46 b A
Film E	11.47 ± 0.46 a B	16.00 ± 0.63 a A	16.02 ± 0.52 a A	16.76 ± 1.00 a A	15.99 ± 0.93 a A
O₂ (kPa)					
Film A	18.93 ± 0.49 a A	19.18 ± 0.62 a A	18.77 ± 0.48 a A	19.45 ± 0.49 a A	19.24 ± 0.63 a A
Film C	13.87 ± 0.47 b AB	13.42 ± 0.78 b B	14.38 ± 0.75 b AB	14.64 ± 0.52 b A	14.79 ± 0.62 b A
Film E	5.50 ± 0.29 c A	3.89 ± 0.68 c BC	3.80 ± 1.05 c C	5.30 ± 0.88 c AB	6.41 ± 1.13 c A
C₂H₄ (ppm)					
Film A	0.58 ± 0.53 a AB	0.45 ± 0.48 b B	0.55 ± 0.45 b AB	1.18 ± 0.47 a AB	1.21 ± 0.26 a A
Film C	0.77 ± 0.53 a A	1.09 ± 0.65 ab A	1.55 ± 0.42 a A	1.23 ± 0.48 a A	1.24 ± 0.38 a A
Film E	1.12 ± 0.36 a A	1.55 ± 0.51 a A	1.72 ± 0.47 a A	1.66 ± 0.30 a A	1.49 ± 0.66 a A
Values are means ± SD (n = 6); different lowercase letters indicate significant differences among films within each measurement day. Different uppercase letters indicate significant differences within each film across measurement days (Tukey's HSD, P ≤ 0.05).					

Ethylene remained low in all packages (0.45–1.72 ppm) and, unlike CO₂ and O₂, was only weakly modulated by perforation density. Concentrations did not differ among films on day 1; Film A showed a transient decline on day 2 (0.45 ppm) and Films C and E increased slightly to day 3 before stabilising, but at day 6 ethylene had converged and remained statistically similar to the end of storage despite the markedly different atmospheres. Once senescence-associated ethylene biosynthesis is initiated in immature seeds [16], its intensity therefore appears only partially governed by the storage atmosphere, in contrast to the film-dependent response seen over the wider perforation range of the Preliminary Test.

3.2.2. Respiratory activity

Respiratory activity provided the physiological link between the in-package atmosphere and the quality responses. Unpackaged seeds showed high respiration and strong temperature dependence (Table 4), with rates of 155.2, 65.8 and 46.9 µg CO₂ kg⁻¹ s⁻¹ at 20, 10 and 5°C. This corresponds to a Q₁₀ of 2.22 (95% CI 2.05–2.40) between 5 and 20°C, a 3.3-fold increase over that interval. The Arrhenius plot was strictly linear at harvest (r² = 0.987; Online Resource 2a), indicating no metabolic transition or chilling-related discontinuity within 5–20°C. These values classify immature faba bean seeds as a high-respiration commodity and underscore the need for an unbroken cold chain, since a 15°C abuse would more than triple the deteriorative rate [40, 43]. Temperature sensitivity was not constant: Q₁₀ declined from 2.22 at harvest to 1.87 at day 9 (regression on storage time, P = 0.028) (Table 4; Online Resource 2b), visible as a progressive flattening of the fitted lines (Online Resource 2a). Such a loss of temperature sensitivity is expected when respiratory flux ceases to be governed mainly by enzyme kinetics and becomes constrained by substrate availability or residual respiratory capacity; it is the quantitative counterpart of the senescence-driven decline discussed below and implies that the benefit of a strict cold chain is greatest in the first days after processing. Over storage the respiration of unpackaged seeds fell steeply (46.9 to 16.9 µg CO₂ kg⁻¹ s⁻¹ at 5°C by day 9), driven by desiccation stress superimposed on senescence. In packaged seeds at 5°C (Table 5), day-1 respiration was significantly stratified by atmosphere: Film E (18.9 µg CO₂ kg⁻¹ s⁻¹) was 42% lower than Film A (32.7), which was statistically indistinguishable from the unpackaged control (31.7), while Film C was intermediate (25.6). The equivalence of Film A and unpackaged seeds confirms that the single macro-hole conferred no metabolic suppression, whereas the 42% suppression by Film E is consistent with the respiratory response of other leguminous vegetables to comparable atmospheres of 10–16 kPa CO₂ and 3–7 kPa O₂ [16, 24] and with the effect of MAP on whole fresh faba beans [46].

Table 4

Respiratory activity (µg CO₂ kg⁻¹ s⁻¹) of unpackaged minimally processed *Vicia faba* seeds at three storage temperatures (5, 10, and 20°C) over a 9-day storage time and Q₁₀ coefficients calculated between 5°C and 20°C as (R₂₀/R₅)^{10/ΔT}.

Treatment	Harvest	Day 1	Day 2	Day 3	Day 6	Day 9
Unpackaged seeds (µg CO₂ kg⁻¹ s⁻¹)						
5°C	46.87 ± 2.62 c A	32.65 ± 1.34 c B	26.82 ± 1.27 c C	22.62 ± 0.66 c D	16.72 ± 0.99 c E	16.92 ± 2.36 c E
10°C	65.81 ± 3.88 b A	45.11 ± 3.15 b B	36.55 ± 1.51 b C	29.15 ± 0.89 b D	24.61 ± 0.52 b E	20.36 ± 0.62 b F
20°C	155.19 ± 6.09 a A	116.83 ± 5.88 a B	80.81 ± 4.65 a C	66.13 ± 4.10 a D	49.29 ± 4.18 a E	43.22 ± 3.11 a E
Q ₁₀ (5–20°C)	2.22	2.34	2.09	2.04	2.06	1.87
Film E	1.12 ± 0.36 a A	1.55 ± 0.51 a A	1.72 ± 0.47 a A	1.66 ± 0.30 a A	1.49 ± 0.66 a A	
Values are means ± SD (n = 6); different lowercase letters indicate significant differences among storage temperatures within each measurement day. Different uppercase letters indicate significant differences within each temperature among measurement days (Tukey's HSD, P ≤ 0.05).						

Table 5

Respiratory activity ($\mu\text{g CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$) of unpackaged and packaged minimally processed *Vicia faba* seeds (Films A, C, E) stored at 5°C.

Treatment	Day 1	Day 2	Day 3	Day 6	Day 9
Packaged seeds at 5°C ($\mu\text{g CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$)					
Unpackaged	31.74 ± 0.59 a A	29.81 ± 1.01 a AB	27.70 ± 1.19 a B	19.69 ± 0.92 a C	17.07 ± 0.63 b D
Film A	32.65 ± 0.67 a A	28.65 ± 0.48 a B	27.01 ± 0.86 a C	20.05 ± 0.32 a D	18.25 ± 0.54 ab E
Film C	25.56 ± 0.87 b A	24.22 ± 0.14 b AB	23.11 ± 0.82 b B	20.61 ± 0.60 a C	19.28 ± 0.26 a C
Film E	18.85 ± 1.10 c A	19.27 ± 0.13 c A	18.97 ± 0.61 c A	18.68 ± 1.04 a A	18.15 ± 1.10 ab A
Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within Storage time; for each treatment, different upper case letters indicate significant differences among measurement days (Tukey's HSD, $P \leq 0.05$).					

The comparison between Films E and A illustrates the self-regulating nature of a passive MAP system: by day 1 the Film E atmosphere (11.5 kPa CO_2 , 5.5 kPa O_2) was already associated with 42% lower respiration than Film A, a protective effect operating before the maximum CO_2/O_2 divergence was reached. Respiratory suppression was thus proportional to the diffusive resistance of the film, and the day-1 ranking (Film A $\approx$ unpackaged > Film C > Film E) mirrored that of permeance and equilibrium O_2 tension, confirming that the quality gradient originates in a permeability-controlled modulation of seed metabolism [19, 41].

All treatments declined progressively over storage, converging toward similar values by day 9 (Film A 18.3; Film C 19.3; Film E 18.2 $\mu\text{g CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$, close to the 17.1 of unpackaged seeds), but through distinct routes. In Film E the rate remained low and statistically unchanged across all five sampling points (18.9 to 18.2 $\mu\text{g CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$), reflecting MAP-mediated metabolic stabilisation, whereas the continuous fall in Film A from 32.7 to 18.3 reflects a senescence-driven collapse of respiratory capacity rather than any protective effect [47, 48]. This convergence does not diminish the efficacy of MAP: the critical period for metabolic suppression fell within the first six days, when differences in cumulative O_2 exposure were greatest (AUC- O_2 153.4 vs. 39.8 kPa·d for Films A and E), and it was then that the divergence in colour, texture, pigment, phenolic and microbial parameters became established. These results support targeted MAP with CO_2 above 10 kPa as a practical approach to metabolic suppression in this commodity. More generally, expressing the atmosphere effect as cumulative O_2 exposure rather than an instantaneous concentration provides an integrative descriptor linking film permeability to physiological response, transferable in principle to any commodity whose respiration and senescence are O_2 -limited.

3.2.3. Firmness (penetration, deformation, cut test), sensory quality, appearance, titratable acidity (TA), total soluble solids (TSS), and weight loss

The impact of the three films on physical, organoleptic and physicochemical attributes is summarised in Table 6, with the corresponding two-way factorial ANOVA in Online Resource 3. Weight loss was governed by both factors and their interaction (S, P and $S \times P$ all $P \leq 0.001$), reflecting the divergent water-vapour barrier of the packages. Film A lost most moisture, rising from 1.44% at day 3 to 2.39% at day 9, against 0.68–0.98% in Film E and 0.76–1.32% in Film C, so that all three differed significantly at day 9 (2.39% > 1.32% > 0.98%), Film A losing $\approx$ 2.4-fold more water than Film E. This reflects the higher effective water-vapour permeability of the macro-perforated package and its lower in-package humidity [20, 23]. Even Film A, however, maintained a humid environment and its maximum weight loss (2.39%) remained well below the $\approx$ 5% threshold for visible shrivelling, so turgor stayed marketable in all packages. Instrumental texture followed a coherent pattern in which the near-atmospheric package promoted toughening while the restrictive MAP preserved harvest texture. Penetration F Max increased significantly over storage (S and P, $P \leq 0.001$) with a non-significant $S \times P$ interaction, indicating similar temporal trends of differing magnitude; the increase was greatest in Films A (0.62 to 0.71 N) and C, whereas Film E remained statistically indistinguishable from harvest (0.57–0.61 N) and significantly softer than Film A at days 3 and 9. Deformation at F Max followed the same trend with less pronounced treatment differences (no packaging or interaction effect), and cut-test firmness showed significant packaging and storage effects but no interaction, Film A remaining slightly tougher than Film E at days 6 and 9. This reproduces the response previously described for minimally processed faba bean seeds, in which puncture resistance and specific deformation rose faster under the more permeable film while the cut test was barely affected [15]. Toughening under near-atmospheric conditions is generally ascribed to partial dehydration and to cell-wall reinforcement accompanying senescence [18]; its suppression in Film E indicates that the low- O_2 /high- CO_2 atmosphere ($\approx$ 5.5 kPa O_2 , 11 kPa CO_2) retarded the changes underlying the loss of tenderness, in agreement with the delayed softening reported for faba beans under MAP with electron-beam irradiation [46].

Table 6

Effects of packaging treatment and storage time on firmness (penetration, deformation, cut test), sensory quality, appearance, titratable acidity (TA), total soluble solids (TSS), and weight loss in ready-to-eat minimally processed *Vicia faba* seeds stored at 5°C (Main Trial).

Treatment	Penetration (F Max, N)	Deformation (L at F Max, mm)	Cut test (F Max, N)	Sensory quality†	Appearance†	TA (g cit. acid 100 g ⁻¹ FW)	TSS (°Brix)	Weight loss (%)
Harvest	0.62 ± 0.05	1.40 ± 0.10	0.85 ± 0.06	9.0 ± 0.00	9.0 ± 0.00	0.43 ± 0.02	9.33 ± 0.08	0.00
3 d at 5°C								
Film A	0.64 ± 0.03 a B	1.42 ± 0.07 a B	0.88 ± 0.04 a AB	6.8 ± 0.34 c B	6.2 ± 0.50 c B	0.42 ± 0.02 a A	9.51 ± 0.12 a A	1.44 ± 0.19 a B
Film C	0.63 ± 0.04 ab AB	1.39 ± 0.13 a A	0.87 ± 0.06 a A	7.6 ± 0.37 b B	8.1 ± 0.37 b B	0.41 ± 0.01 a A	9.48 ± 0.11 a A	0.76 ± 0.15 b B
Film E	0.57 ± 0.08 b A	1.38 ± 0.14 a A	0.86 ± 0.04 a A	8.9 ± 0.26 a A	8.8 ± 0.30 a A	0.42 ± 0.01 a A	9.49 ± 0.13 a A	0.68 ± 0.17 b B
6 d at 5°C								
Film A	0.67 ± 0.03 a AB	1.45 ± 0.09 a AB	0.95 ± 0.09 a A	5.2 ± 0.37 c C	5.2 ± 0.26 c C	0.41 ± 0.01 a A	9.02 ± 0.21 a B	1.59 ± 0.28 a B
Film C	0.65 ± 0.08 a A	1.43 ± 0.09 a A	0.89 ± 0.05 ab A	6.0 ± 0.24 b D	6.8 ± 0.24 b C	0.42 ± 0.00 a A	9.31 ± 0.15 a AB	0.89 ± 0.10 b B
Film E	0.61 ± 0.07 a A	1.40 ± 0.12 a A	0.86 ± 0.03 b A	8.1 ± 0.40 a B	8.3 ± 0.39 a B	0.40 ± 0.02 a A	9.37 ± 0.04 a A	0.83 ± 0.15 b AB
9 d at 5°C								
Film A	0.71 ± 0.05 a A	1.54 ± 0.13 a A	0.94 ± 0.05 a A	4.2 ± 0.41 c D	4.3 ± 0.52 c D	0.40 ± 0.01 a A	8.95 ± 0.15 a B	2.39 ± 0.29 a A
Film C	0.67 ± 0.05 a A	1.45 ± 0.09 ab A	0.88 ± 0.05 b A	6.8 ± 0.31 b C	5.4 ± 0.34 b D	0.41 ± 0.01 a A	9.06 ± 0.12 a B	1.32 ± 0.12 b A
Film E	0.61 ± 0.03 b A	1.42 ± 0.09 b A	0.84 ± 0.03 b A	7.9 ± 0.28 a B	8.2 ± 0.39 a B	0.40 ± 0.01 a A	9.07 ± 0.15 a B	0.98 ± 0.23 c A
Values are means ± SD (n = 3 for TA and TSS; n = 9 for weight loss; n = 10 for texture, sensory quality, and appearance). Lowercase letters show significant differences among packaging treatments within each storage period. Different uppercase letters show significant differences for each packaging treatment among measurement days (Tukey's HSD, P ≤ 0.05).								

Sensory quality and appearance were the most discriminating attributes, with significant effects of storage time, packaging and their interaction (Online Resource 3), quality ranking Film E > Film C > Film A at all sampling times. Film E maintained excellent sensory quality throughout (8.94–7.90), whereas Film A reached the marketability limit at day 6 (5.19) and fell below it at day 9 (4.22); Film C remained acceptable (6.04–7.58) although appearance declined faster than flavour, approaching the acceptability threshold by day 9. This earlier loss of visual than eating quality reflects the seedcoat browning that drives consumer purchase decisions [15]. The retention of sensory and visual acceptability in Film E over 9 days, against loss of marketability in Film A by day 6, confirms that a sufficiently restrictive MAP extends the commercial life of fresh faba bean seeds [16, 24, 25]. Importantly, the Film E atmosphere (≈ 11 kPa CO₂, 5.5 kPa O₂) remained near the lower bound of the CO₂ range associated with fermentative off-flavours (above ≈ 15–20 kPa CO₂, or below ≈ 0.5 kPa O₂ [49]) and its O₂ tension never entered the fermentative range, so that metabolic suppression improved rather than impaired sensory quality. Titratable acidity remained stable (0.40–0.43 g citric acid 100 g⁻¹ FW) with a small but significant storage effect and no packaging or interaction effect; the only appreciable trend was a slight decline in Film A by day 9 (0.40), consistent with marginally greater consumption of organic acids under its higher O₂ tension, although within-film comparisons did not reach significance in any

package. Total soluble solids showed a significant storage effect only: TSS rose marginally at day 3 and then declined, significantly from day 6 in Film A (9.51 to 9.02 °Brix) and only at day 9 in Films C and E ($\approx$ 9.06–9.07 °Brix), a pattern consistent with initial mobilisation of reserves through starch hydrolysis followed by respiratory consumption of soluble sugars (Section 3.2.5). Harvest values of TA and TSS (0.43 g 100 g⁻¹ FW; 9.33 °Brix) fall within the range reported for Mediterranean faba bean landraces and cultivars [50], confirming the representativeness of the material. Taken together, the significant S $\times$ P interactions for weight loss, sensory quality and appearance (Online Resource 3) show that Film E did not merely offset these attributes but progressively slowed their rate of decline, the signature of an atmosphere that alters the kinetics of senescence.

3.2.4. Colour, chlorophylls, carotenoids, and ascorbic acid

Colour parameters and pigment concentrations were significantly affected by packaging (Table 7). Film E kept hue angle (h°) statistically unchanged over the 9 days and limited the decline in lightness (L*) and chroma (C*) to a small late reduction, whereas Film A declined significantly in all three (L* 68.9 to 61.5; h° 121.3 to 113.4; C* 48.1 to 40.6). The decrease in h° within the yellow-green region is particularly important, since the shift toward lower hue angles reflects the green-to-yellow transition associated with chlorophyll degradation. Its close correspondence with the measured loss of chlorophyll a indicates that hue angle is a reliable non-destructive indicator of pigment status in faba bean seeds, as reported for asparagus and other green vegetables [30, 51]. At day 9 chlorophyll a was 32.2 mg 100 g⁻¹ FW in Film E versus 28.1 in Film A, and carotenoids and ascorbic acid behaved analogously (Film E retaining 41.5 and 50.4 mg 100 g⁻¹ FW against 37.4 and 45.8 in Film A, i.e. -14.6% and - 16.8% relative to harvest). The better retention of these oxidation-sensitive constituents under the low-O₂/high-CO₂ atmosphere is consistent with the reported link between reduced ROS accumulation and preservation of chloroplast pigments and ascorbate [35, 52] and with the protective effect of restrictive atmospheres in other commodities [53, 54]; the enzyme responses in Section 3.2.6 are compatible with this interpretation, although ROS were not measured directly.

Table 7

Effects of packaging treatment and storage time on colour (L^* , h° , C^*), chlorophyll a and b, carotenoids, and ascorbic acid in ready-to-eat minimally processed *Vicia faba* seeds stored at 5°C (Main Trial).

Treatment	L^*	h°	C^*	Chlorophyll a (mg 100 g ⁻¹ FW)	Chlorophyll b (mg 100 g ⁻¹ FW)	Carotenoids (mg 100 g ⁻¹ FW)	Ascorbic acid (mg 100 g ⁻¹ FW)
Harvest	68.87 ± 2.52	121.31 ± 1.09	48.06 ± 1.76	33.72 ± 0.10	10.09 ± 0.20	43.81 ± 0.30	55.06 ± 1.22
3 d at 5°C							
Film A	66.55 ± 3.32 b A	120.37 ± 0.98 b A	45.05 ± 2.28 a B	32.59 ± 0.09 c B	8.81 ± 0.86 a AB	41.40 ± 0.84 b B	48.91 ± 0.30 a B
Film C	66.21 ± 3.49 b B	121.02 ± 1.42 ab A	46.66 ± 3.20 a A	33.09 ± 0.12 b B	8.78 ± 0.77 a B	41.87 ± 0.66 b B	49.15 ± 1.16 a B
Film E	68.94 ± 3.17 a A	121.51 ± 1.62 a A	45.17 ± 2.59 a B	33.66 ± 0.16 a A	10.21 ± 0.27 a A	43.87 ± 0.26 a A	49.37 ± 0.73 a B
6 d at 5°C							
Film A	61.59 ± 4.75 b B	114.15 ± 4.97 b B	41.44 ± 4.79 b C	31.76 ± 0.07 c C	8.41 ± 0.09 b B	40.18 ± 0.16 c B	47.45 ± 0.53 b BC
Film C	63.38 ± 3.31 b C	120.64 ± 1.40 a A	46.17 ± 3.15 a A	32.08 ± 0.06 b C	8.65 ± 0.27 b B	40.73 ± 0.22 b B	48.00 ± 0.62 b B
Film E	66.96 ± 3.17 a B	121.39 ± 1.26 a A	47.71 ± 1.85 a A	33.05 ± 0.11 a B	9.80 ± 0.12 a AB	42.85 ± 0.08 a B	49.77 ± 0.77 a B
9 d at 5°C							
Film A	61.52 ± 5.07 b B	113.37 ± 5.78 c B	40.56 ± 5.43 b C	28.07 ± 0.14 c D	9.03 ± 0.57 ab AB	37.43 ± 0.67 b C	45.81 ± 0.86 b C
Film C	63.40 ± 3.42 b C	117.51 ± 3.01 b B	42.95 ± 3.76 ab B	29.76 ± 0.30 b D	8.42 ± 0.22 b B	38.47 ± 0.98 b C	46.68 ± 0.71 b B
Film E	67.37 ± 2.56 a AB	121.31 ± 1.05 a A	44.79 ± 1.95 a B	32.16 ± 0.09 a C	9.33 ± 0.14 a B	41.48 ± 0.20 a C	50.43 ± 0.83 a B
Values are means ± SD (n = 30 for colour parameters; n = 3 for chlorophylls, carotenoids, and ascorbic acid). Different lowercase letters indicate significant differences among packaging treatments for each storage period. Different uppercase letters indicate significant differences for packaging treatment among measurement days (Tukey's HSD, P ≤ 0.05).							

3.2.5. Carbohydrates, starch, and antinutritional factors

Sucrose, the primary determinant of the sweetness that distinguishes immature from mature faba beans [11], was the most atmosphere-sensitive carbohydrate (Table 8): it fell by ≈ 50% in Film A at day 9 (6.90 to 3.45 g kg⁻¹ FW) but remained at harvest level in Film E (6.87–6.89 g kg⁻¹), so its preservation under the restrictive atmosphere has direct consumer and commercial relevance. Glucose and starch behaved similarly, starch declining by 22% in Film A (to 33.6 g kg⁻¹) while remaining stable in Film E. This coupling is physiologically coherent: under the near-atmospheric O₂ of Film A, rapid respiratory consumption of sucrose likely depleted the primary substrate pool and promoted compensatory mobilisation of starch reserves during senescence, whereas the metabolic suppression in Film E was associated with greater sucrose retention and a reduced requirement for starch hydrolysis [55]. Fructose was unaffected by packaging at any time, remaining at 3.0–3.2 g kg⁻¹ FW. Raffinose (8.73 g kg⁻¹ FW at harvest) was stable until day 6 and declined at day 9, significantly in Film A (– 15%) and Film E (– 11%) but not in Film C (– 4%), indicating a general senescence-associated catabolism only marginally modulated by MAP.

Table 8

Effects of packaging treatment and storage time on individual carbohydrates, starch, vicine, convicine, total phenolic content (TPC), antioxidant activity (TEAC), superoxide dismutase (SOD), and guaiacol peroxidase (POD) activities in ready-to-eat minimally processed *Vicia faba* seeds stored at 5°C (Main Trial).

Treatment	Sucrose (g kg ⁻¹ FW)	Glucose (g kg ⁻¹ FW)	Fructose (g kg ⁻¹ FW)	Raffinose (g kg ⁻¹ FW)	Starch (g kg ⁻¹ FW)	Vicine (g kg ⁻¹ FW)	Convicine (g kg ⁻¹ FW)	TPC (g kg ⁻¹ FW)	TEAC (mmol kg ⁻¹)	SOD (U mg ⁻¹ protein)	POD (ΔA470 ×10 ³)
Harvest	6.90 ± 0.09	2.59 ± 0.04	3.12 ± 0.02	8.73 ± 0.21	43.16 ± 2.98	4.42 ± 0.19	1.70 ± 0.07	2.74 ± 0.07	0.83 ± 0.02	28.30 ± 2.80	1.18 ± 0.07
3 d at 5°C											
Film A	5.72 ± 0.12 c B	2.39 ± 0.02 b B	3.12 ± 0.01 a A	8.64 ± 0.17 a A	42.60 ± 0.51 a A	4.52 ± 0.25 a A	1.67 ± 0.05 a A	2.66 ± 0.03 a A	0.74 ± 0.02 b B	35.10 ± 1.93 a B	1.52 ± 0.33 a AB
Film C	6.39 ± 0.16 b B	2.45 ± 0.03 b B	3.14 ± 0.03 a A	8.65 ± 0.18 a A	42.54 ± 1.70 a A	4.48 ± 0.21 a A	1.72 ± 0.06 a A	2.73 ± 0.02 a A	0.76 ± 0.01 b B	30.40 ± 2.36 ab BC	1.28 ± 0.42 a A
Film E	6.87 ± 0.04 a A	2.53 ± 0.02 a A	3.13 ± 0.02 a A	8.67 ± 0.25 a A	44.34 ± 1.78 a A	4.14 ± 0.25 a B	1.67 ± 0.10 a A	2.71 ± 0.04 a A	0.83 ± 0.01 a A	28.90 ± 2.19 b A	1.22 ± 0.39 a A
6 d at 5°C											
Film A	3.62 ± 0.07 c C	2.20 ± 0.01 c C	3.10 ± 0.05 a AB	8.73 ± 0.15 a A	33.91 ± 0.87 b B	4.58 ± 0.27 a A	1.73 ± 0.03 a A	2.30 ± 0.00 c B	0.70 ± 0.02 b B	44.80 ± 3.04 a A	2.12 ± 0.42 a A
Film C	5.95 ± 0.04 b B	2.31 ± 0.02 b C	3.04 ± 0.03 a A	8.95 ± 0.25 a A	36.20 ± 1.74 b B	4.41 ± 0.12 a A	1.67 ± 0.03 a A	2.55 ± 0.05 b B	0.74 ± 0.01 b B	37.20 ± 1.25 b AB	1.58 ± 0.28 ab A
Film E	6.83 ± 0.08 a A	2.50 ± 0.01 a A	3.11 ± 0.03 a A	8.68 ± 0.23 a A	43.21 ± 0.74 a A	4.60 ± 0.10 a A	1.66 ± 0.06 a A	2.68 ± 0.03 a A	0.82 ± 0.01 a A	30.60 ± 1.93 c A	1.28 ± 0.20 b A
9 d at 5°C											
Film A	3.45 ± 0.18 c C	2.12 ± 0.02 c D	3.02 ± 0.05 a B	7.43 ± 0.30 b B	33.56 ± 1.46 b B	4.32 ± 0.06 b A	1.75 ± 0.04 a A	2.13 ± 0.08 c C	0.63 ± 0.01 c C	42.50 ± 2.69 a A	1.98 ± 0.39 a AB
Film C	5.30 ± 0.28 b C	2.27 ± 0.02 b C	3.11 ± 0.07 a A	8.40 ± 0.28 a A	34.52 ± 1.65 b B	4.52 ± 0.06 a A	1.71 ± 0.07 ab A	2.35 ± 0.05 b C	0.73 ± 0.02 b B	38.40 ± 3.56 ab A	1.72 ± 0.16 ab A
Film E	6.89 ± 0.08 a A	2.50 ± 0.06 a A	3.13 ± 0.02 a A	7.77 ± 0.27 ab B	43.55 ± 2.09 a A	4.47 ± 0.10 ab AB	1.59 ± 0.05 b A	2.55 ± 0.06 a B	0.80 ± 0.02 a A	32.60 ± 2.61 b A	1.34 ± 0.13 b A
Values are means ± SD (n = 3). Different lower case letters indicate significant differences among packaging treatments for each storage period. Different uppercase letters indicate significant differences within each packaging treatment among measurement days (Tukey's HSD, P ≤ 0.05).											

Vicine and convicine, characterised here under MAP for the first time in this commodity, proved to be remarkably stable (Table 8): neither was significantly affected by packaging, storage time or their interaction (Online Resource 3), their concentrations remaining close to the harvest values of 4.42 and 1.70 g kg⁻¹ FW in all three films over the 9 days. The glycosidic bonds of these pyrimidine glycosides are therefore considerably less sensitive to oxidative and respiratory activity than the pigments, sugars and phenolics that changed markedly over the same period. From a food-safety standpoint, the vicine and convicine content relevant to glucose-6-phosphate dehydrogenase

(G6PD)-deficient, favism-susceptible consumers thus remains close to harvest levels irrespective of packaging over the declared shelf life [7].

3.2.6. Total phenolic content, antioxidant activity, and enzyme activities

TPC (2.74 g kg^{-1} GAE at harvest) and antioxidant capacity (TEAC $0.83 \text{ mmol kg}^{-1}$) declined during storage in proportion to in-package O_2 exposure (Table 8), following the gradient observed for pigments and vitamin C. In Film A, TPC fell steadily to 2.13 g kg^{-1} by day 9 (-22%) and TEAC to $0.63 \text{ mmol kg}^{-1}$, whereas in Film E TPC was maintained through day 6 and declined only modestly at day 9 (2.55 g kg^{-1} , -7%) and TEAC remained statistically unchanged ($0.83\text{--}0.80 \text{ mmol kg}^{-1}$); Film C was intermediate for both. The declining gradient (Film A > Film C > Film E) closely mirrored that of chlorophyll a, carotenoids and ascorbic acid, suggesting a common O_2 -dependent oxidative process, and its attenuation under restrictive atmospheres agrees with reports of reduced oxidative deterioration and browning under elevated CO_2 and low O_2 [17, 56, 57].

The antioxidant enzymes provided direct evidence for this interpretation. At harvest, SOD and POD activities were 28.3 U mg^{-1} protein and $1.18 \Delta A_{470} \times 10^3$, consistent with values reported for legume cotyledons [38, 39, 58, 59], and during storage both increased in proportion to in-package O_2 . In Film A, SOD rose from 28.3 to 35.1 U mg^{-1} at day 3 ($+24\%$, not yet significant), peaked at 44.8 at day 6 ($+58\%$) and then declined slightly and non-significantly to 42.5 at day 9 - an induction-plateau pattern typical of senescent tissues under sustained oxidative stress [52, 59] and of a magnitude consistent with plant tissues subjected to oxidative challenge [60, 61]. In Film E, SOD increased only modestly ($+15\%$ over 9 days) and never significantly, indicating that the ROS burden under $11.5\text{--}16.8 \text{ kPa CO}_2$ and $3.8\text{--}6.4 \text{ kPa O}_2$ was insufficient to trigger a substantial antioxidant response; Film C was intermediate, with significant induction only from day 6. POD followed a broadly similar but more variable pattern: in Film A it peaked at $2.12 \Delta A_{470} \times 10^3$ on day 6, the only value significantly above harvest, and remained elevated at day 9, whereas in Film E it stayed stable ($1.22\text{--}1.34$) with no significant change. This day-6 divergence reflects the greater oxidative pressure under the more O_2 -permeable atmosphere [62, 63], and the later induction of POD relative to SOD reflects the sequential activation of antioxidant defences reported for kiwifruit during cold storage [60, 61, 64]. The positive association between POD activity, phenolic depletion and seed-coat browning agrees with observations in stored *Vicia faba* seeds and other horticultural products [59, 65]. In Film E the near absence of enzyme induction coincided with preservation of TPC, TEAC, pigments and ascorbic acid: the lower O_2 reduced the oxidative burden while the elevated CO_2 may have further delayed senescence-related changes, as reported for fresh-cut produce under restrictive atmospheres [66]. Taken together, the parallel, permeability-ranked behaviour of respiration, antioxidant-enzyme induction and the depletion of pigments, phenolics and ascorbic acid indicates successive steps of a single cascade rather than independent responses: package permeability sets the cumulative O_2 exposure, which sets the reactive-oxygen load and the antioxidant-enzyme response, which in turn governs the consumption of oxidisable quality constituents. As each step reflects generic oxidative biochemistry rather than a legume-specific trait, the same permeability-based control may operate in other minimally processed, oxidation-sensitive plant foods.

3.2.7. Microbiological quality

All microbial counts remained within commonly accepted quality limits and regulated criteria complied with EU requirements [67] throughout storage in the three films (Table 9). At harvest, psychrotrophs and moulds were below the detection limit - moulds becoming detectable from day 3 only in Film A ($1.42 \text{ log CFU g}^{-1}$) - while mesophilic bacteria ($1.86 \text{ log CFU g}^{-1}$), Enterobacteriaceae (1.97) and yeasts (1.48) reflected normal post-sanitisation levels after peroxyacetic acid treatment; staphylococci stayed below detection throughout. Film E consistently maintained the lowest spoilage loads: at day 9 its mesophilic count ($3.48 \text{ log CFU g}^{-1}$) was significantly below Film A (4.12), moulds differed by $\approx 2.1 \text{ log}$ (1.13 vs. 3.20) and *E. coli* remained below detection in Film E while reaching $1.87 \text{ log CFU g}^{-1}$ in Film A. Yeasts, being CO_2 -tolerant and facultatively fermentative, were not differentiated up to day 6, and only at day 9 did Film A reach a significantly higher count (2.07) than Films C and E (1.53 and 1.51) [68]. The *E. coli* value in Film A at day 9 approached the 2 log CFU g^{-1} process-hygiene criterion for ready-to-eat products: although compliant at 5°C , this suggests, without demonstrating, that under a sub-optimal cold chain ($8\text{--}10^\circ\text{C}$) near-atmospheric packaging could exceed the limit within the declared shelf life. These results quantitatively extend earlier findings: under the single passive MAP condition of Collado et al. [16, 24] ($\approx 5 \text{ kPa CO}_2$, comparable to Film C), mesophilic counts reached $\approx 5.0 \text{ log CFU g}^{-1}$ by day 9 against 3.61 here, an improvement likely attributable to the lower storage temperature and to peroxyacetic acid rather than hypochlorite. Film E provided a further reduction of $0.1\text{--}1.1 \text{ log CFU g}^{-1}$ across the aerobic groups, an additional benefit associated with CO_2 above 10 kPa . The elevated- CO_2 atmosphere thus achieved microbial suppression similar in direction to that reported by Wang et al. [46] after electron-beam irradiation, but without any additional decontamination step [69].

Table 9

Effects of packaging treatment and storage time on microbiological quality (log CFU g⁻¹) of ready-to-eat minimally processed *Vicia faba* seeds stored at 5°C (Main Trial). Values < 1 indicate below detection limit (log 0.5 CFU g⁻¹).

Treatment	Psychrotrophic bacteria	Mesophilic aerobic bacteria	Enterobacteriaceae	Yeasts	Moulds	E. coli	Staphylococci
Harvest	< 1.0 ± 0.00	1.86 ± 0.03	1.97 ± 0.06	1.48 ± 0.17	< 1.0 ± 0.00	< 1.0 ± 0.00	< 1.0 ± 0.00
3 d at 5°C							
Film A	< 1.0 ± 0.00 a C	2.43 ± 0.08 a C	2.45 ± 0.09 a B	1.53 ± 0.12 a B	1.42 ± 0.08 a C	< 1.0 ± 0.00 a C	< 1.0 ± 0.00 a A
Film C	< 1.0 ± 0.00 a C	2.21 ± 0.08 b C	1.86 ± 0.14 b B	1.43 ± 0.10 a A	< 1.0 ± 0.00 b C	< 1.0 ± 0.00 a B	< 1.0 ± 0.00 a A
Film E	< 1.0 ± 0.00 a B	1.85 ± 0.05 c B	1.83 ± 0.18 b A	1.56 ± 0.10 a A	< 1.0 ± 0.00 b B	< 1.0 ± 0.00 a A	< 1.0 ± 0.00 a A
6 d at 5°C							
Film A	1.89 ± 0.06 a A	3.75 ± 0.01 a B	3.21 ± 0.06 a A	1.56 ± 0.17 a B	2.05 ± 0.05 a B	1.39 ± 0.06 a B	< 1.0 ± 0.00 a A
Film C	1.13 ± 0.05 b B	3.40 ± 0.02 b B	2.52 ± 0.05 b A	1.54 ± 0.08 a A	1.37 ± 0.30 b B	1.11 ± 0.07 b A	< 1.0 ± 0.00 a A
Film E	< 1.0 ± 0.00 c B	3.17 ± 0.10 c A	1.88 ± 0.29 c A	1.53 ± 0.15 a A	< 1.0 ± 0.00 c B	< 1.0 ± 0.00 c A	< 1.0 ± 0.00 a A
9 d at 5°C							
Film A	1.78 ± 0.03 a B	4.12 ± 0.02 a A	3.20 ± 0.15 a A	2.07 ± 0.13 a A	3.20 ± 0.12 a A	1.87 ± 0.05 a A	< 1.0 ± 0.00 a A
Film C	1.60 ± 0.04 a A	3.61 ± 0.03 b A	2.80 ± 0.29 a A	1.53 ± 0.14 b A	2.21 ± 0.13 b A	1.23 ± 0.13 b A	< 1.0 ± 0.00 a A
Film E	1.37 ± 0.12 b A	3.48 ± 0.23 b A	2.04 ± 0.15 b A	1.51 ± 0.16 b A	1.13 ± 0.06 c A	< 1.0 ± 0.00 c A	< 1.0 ± 0.00 a A
Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among packaging treatments for each each storage period. Different uppercase letters indicate significant differences foreach packaging treatment among measurement days (Tukey's HSD, P ≤ 0.05).							

3.3. Correlation between TPC, TEAC, and ascorbic acid

The relative contributions of phenolics and ascorbic acid to antioxidant capacity were assessed by correlating each with TEAC (Table 10). Across the full dataset TPC was strongly and robustly related to TEAC (Pearson $r = 0.854$, Spearman $\rho = 0.741$, $R^2 = 0.730$; $P < 0.001$), the agreement between parametric and non-parametric coefficients indicating that the relationship was not driven by outliers or departures from normality. Phenolic content alone thus explained 73% of the variance in antioxidant capacity, establishing TPC as its primary biochemical determinant in immature faba bean seeds under MAP. The coupling was largely time-independent (partial $r = 0.750$ after controlling for storage period) and strengthened as oxidative divergence among films accumulated, being weak at harvest and day 3 ($r = 0.300$ and 0.307) but strong at days 6 and 9 ($r = 0.882$ and 0.932). Consistently, it was highest in Film A ($r = 0.902$), whose wide range of phenolic values maximised the detectable covariance, and weakest in the compositionally stable Film E ($r = 0.664$), where the restricted dynamic range limited statistical power. Ascorbic acid was also positively correlated with TEAC ($r = 0.786$, $R^2 = 0.618$), confirming a genuine contribution to the antioxidant pool, but this association was markedly attenuated after controlling for storage period (partial $r = 0.612$), indicating that much of it reflected the shared temporal decline of the two variables rather than a direct, packaging-driven coupling. Mechanistically, phenolics are oxidised directly by peroxidase in proportion to O₂ tension, whereas ascorbic

acid degrades through multiple routes less tightly modulated by the atmosphere [24, 54]. TPC therefore provides a more responsive, packaging-sensitive index than ascorbic acid for validating MAP strategies in this commodity, a phenolic dominance consistent with the strong phenolic-antioxidant relationships reported for faba bean genotypes [70] and other leguminous vegetables under MAP [49, 56].

Table 10

Pearson (r) and Spearman (ρ) correlation analyses between TPC (total phenolic content), TEAC (antioxidant capacity), and AA (ascorbic acid) in minimally processed *Vicia faba* seeds under passive MAP, stratified by storage period and packaging treatment. Partial correlations control for storage period. R²: coefficient of determination from simple linear regression. *** P ≤ 0.001; ** P ≤ 0.01; * P ≤ 0.05; ns: not significant.

Stratification	Pearson r	P (r)	Spearman ρ	P (ρ)	R ²	Regression equation	Sig.
TPC vs TEAC							
All data (n = 36)	0.854	< 0.001	0.741	< 0.001	0.730	TEAC = 0.267·TPC + 0.082	***
Harvest (n = 3)	0.300	0.806	-0.500	0.667	0.090	TEAC = 0.105·TPC + 0.540	ns
3 d at 5°C (n = 9)	0.307	0.422	0.533	0.139	0.094	TEAC = 0.292·TPC - 0.012	ns
6 d at 5°C (n = 9)	0.882	0.002	0.879	0.002	0.777	TEAC = 0.288·TPC + 0.033	**
9 d at 5°C (n = 9)	0.932	< 0.001	0.917	0.001	0.869	TEAC = 0.375·TPC - 0.160	***
Film A (n = 12)	0.902	< 0.001	0.888	< 0.001	0.813	TEAC = 0.264·TPC + 0.077	***
Film C (n = 12)	0.703	0.011	0.741	0.006	0.494	TEAC = 0.174·TPC + 0.314	*
Film E (n = 12)	0.664	0.019	0.516	0.086	0.441	TEAC = 0.137·TPC + 0.452	*
Partial r (controlled for storage period)	0.750	< 0.001	n.a.	n.a.	0.562	n.a.	***
TEAC vs Ascorbic acid							
All data (n = 36)	0.786	< 0.001	0.804	< 0.001	0.618	AA = 40.27·TEAC + 19.06	***
Partial r (controlled for storage period)	0.612	< 0.001	n.a.	n.a.	0.375	n.a.	***
<i>n = total observations per analysis group. OLS = ordinary least squares regression. Partial r: storage period included as a quantitative (numeric) covariate. Note: at harvest the three films share a single harvest batch, so only n = 3 independent replicates are available; the harvest correlation is therefore descriptive and not a statistically powerful estimate, and its non-significant coefficients should be interpreted with caution. In the pooled analysis (n = 36) these three replicates constitute the day-0 baseline. For the individual storage periods (n = 9) the estimates are statistically robust. n.a.: partial Spearman correlations (ρ) and their corresponding regression equations were not computed, as robust partial rank-correlation procedures were not implemented in the software used for this analysis; only partial Pearson r, controlling for storage period as a quantitative (numeric) covariate, is reported for the partial-correlation rows.</i>							

4. Conclusions

This two-phase study provides one of the most comprehensive postharvest characterisations of ready-to-eat immature faba bean seeds under passive MAP to date, integrating atmospheric, physical, biochemical and microbiological responses in a single framework, and shows that an optimal in-package atmosphere can be established for this commodity by film perforation design alone, without active gas flushing or any supplementary treatment.

The five-micro-hole film maintained overall quality closest to harvest condition throughout storage, simultaneously restraining respiration and the oxidative-enzyme response, preserving pigments, sugars, ascorbic acid and antioxidant capacity, and retaining the highest sensory, visual and microbiological quality, thereby remaining marketable for at least the 9 days examined at 5°C. Correlation analysis established phenolics as the principal determinant of antioxidant capacity, with ascorbic acid as a secondary contributor, and the first characterisation of vicine and convicine under modified atmosphere showed that packaging did not significantly modify their

concentrations. These conclusions derive from a single cultivar grown in one season, a limited number of biological replicates and a 9-day window; their generality would benefit from confirmation across further cultivars, seasons and longer storage.

The principal advance of this work is mechanistic and, we argue, transferable beyond the model commodity. By varying only perforation density, the study isolates film permeability as a single design lever that sets cumulative in-package O₂ exposure and, through it, the intensity of oxidative stress and the rate of quality loss - a permeability→cumulative-O₂→oxidative-stress→quality chain consistently supported by the parallel respiratory, enzymatic, pigment, phenolic and vitamin responses. Immature faba bean is a particularly clean system in which to demonstrate it, because shelling exposes no cut surface and therefore removes the wound-response confounders that obscure the atmosphere-quality relationship in most fresh-cut products. As the individual steps rest on generic oxidative biochemistry rather than legume-specific traits, the same design logic may be relevant to other minimally processed, oxidation-sensitive foods, although this requires experimental validation in each commodity. In practical terms, this atmosphere-only optimisation matched, and in gas-composition terms exceeded, the protection previously attainable for fresh faba beans only by coupling MAP with electron-beam irradiation, offering a simple, low-cost and gas-flush-free route to passive-MAP optimisation across the ready-to-eat sector.

Declarations

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Competing interests: The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval and Consent to Participate: This study did not involve any clinical trial, medical procedure, or invasive intervention on human participants or animals. The sensory evaluation consisted solely of the tasting of a commonly consumed food product (immature faba bean seeds) and was performed by ten trained staff members of the Institute of Sciences of Food Production (ISPA), National Research Council (CNR), as part of routine food quality assessment activities. Participation was voluntary, participants were informed about the purpose and procedures of the evaluation, and no personal, medical, or sensitive data were collected.

The requirement for ethical approval for this study was formally waived by the Commission for Ethics and Research Integrity (Commissione per l'Etica e l'Integrità nella Ricerca) of the National Research Council of Italy (CNR), the relevant named ethics committee for CNR-affiliated research. In its official written communication (Prot. CNR No. 0390524, 15 September 2026), it was confirmed that, under Italian regulations, no ethical opinion is required for studies of this nature, namely sensory evaluations aimed at assessing the organoleptic acceptability of food products.

The study was conducted in accordance with the principles of research integrity and good scientific practice adopted by the CNR and with the principles of the Declaration of Helsinki.

Consent to participate: Written informed consent was obtained from all panellists who took part in the sensory evaluation.

Accordance Statement: This study was conducted in accordance with the relevant institutional guidelines and regulations of Institute of Sciences of Food Production (ISPA), National Research Council (CNR), and in accordance with the ethical principles of the Declaration of Helsinki.

Consent to Publish: Not applicable

Data availability: The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Author contributions: A. Palma: conceptualization, methodology, investigation, formal analysis, writing – original draft. S. D'Aquino: conceptualization, supervision, writing – review and editing. Both authors read and approved the final manuscript.

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