CRITICAL REVIEW

Beyond High-Pressure Processing: Enzymatic Browning and Oxidative Instability in Refrigerated Guacamole

A critical review and multi-hurdle framework for clean-label control

By

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EDITORIAL POSITION — This manuscript treats browning as a multi-mechanistic stability problem rather than a single-enzyme defect. Product-specific commercial claims are not used as scientific evidence. The proposed "oxidative stability budget" is a conceptual framework intended to guide testable formulation and process hypotheses.

Abstract

High-pressure processing (HPP) has become a preferred preservation technology for refrigerated avocado products because it can deliver strong microbial control while retaining a fresh sensory profile. Yet HPP does not sterilize avocado biochemistry. Polyphenol oxidase (PPO) and lipoxygenase (LOX) are unusually pressure-resistant in avocado matrices, substantial residual activity can remain after commercially relevant treatments, and both enzymes may recover activity during refrigerated storage. Consequently, colour stability in guacamole cannot be understood as a simple function of initial PPO inactivation. This critical review integrates avocado-specific evidence on enzymatic browning, lipid oxidation, HPP-induced tissue disruption, antioxidant interventions, oxygen management and post-opening exposure. We argue that refrigerated guacamole behaves as a coupled aqueous–lipid oxidative system in which visual browning, loss of greenness, volatile oxidation products and sensory deterioration emerge from the balance between residual oxidative pressure and remaining defence capacity. This balance is formalized here as an "oxidative stability budget": a conceptual framework that considers residual or reactivated PPO/LOX, oxygen availability, phenolic and unsaturated-lipid substrates, reducing reserve, pH/chelating capacity, lipid-phase antioxidants, packaging barrier and temperature. The review also highlights an underappreciated paradox: a botanical extract may perform well in radical-scavenging assays yet worsen avocado browning if its phenolics serve as PPO substrates. Therefore, the term antioxidant should not be treated as synonymous with anti-browning. A translational multi-hurdle strategy is proposed, combining HPP with controlled acidification/chelation, a measurable reducing reserve, targeted PPO inhibition, lipid-phase protection and oxygen management. The most informative industrial endpoint may not be colour immediately after processing, but colour kinetics after opening late in shelf life, when oxygen is reintroduced and the reducing reserve may be depleted. A staged validation framework is provided to convert ingredient screening into mechanism-based formulation decisions for refrigerated guacamole and related avocado products.

Keywords: *avocado; guacamole; high-pressure processing; polyphenol oxidase; lipoxygenase; enzymatic browning; clean label; antioxidants; oxygen; shelf life*

Highlights

- HPP controls microorganisms more reliably than it controls oxidative enzymes in avocado matrices.
- Residual PPO and LOX can remain substantial after commercially relevant pressure treatments and may reactivate during cold storage.
- "Antioxidant" is not equivalent to "anti-browning": phenolic extracts can become PPO substrates and accelerate colour loss.
- Guacamole stability should be managed as a coupled aqueous-phase browning and lipid-phase oxidation problem.
- Post-opening colour kinetics at end of shelf life should become a primary formulation endpoint, not an afterthought.

1. Introduction: the unresolved quality problem behind commercially successful HPP

Guacamole is an unusually demanding refrigerated food matrix. Processing destroys the cellular compartmentation that normally separates oxidative enzymes, phenolic substrates, unsaturated lipids and oxygen. At the same time, consumers expect a colour, aroma and texture that remain recognizably "fresh", leaving little room for the flavour or colour changes associated with severe thermal preservation. High-pressure processing (HPP) fits this market requirement exceptionally well because pressure can markedly reduce vegetative microbial populations while preserving volatile and sensory attributes better than conventional heat processing. Commercial HPP guacamoles have therefore become an important model of successful nonthermal preservation (Palou et al., 2000; Alañón et al., 2022; Uzunlu, 2024).

However, the commercial success of HPP has occasionally encouraged an oversimplified interpretation: once microbial stability has been achieved, remaining shelf-life deterioration is treated as a secondary formulation issue. Avocado does not support that assumption. Its polyphenol oxidase (PPO) is notably resistant to several preservation stresses, and lipoxygenase (LOX) can contribute to oxidative deterioration of the lipid fraction and volatile profile. Under commercially realistic HPP conditions, neither enzyme should be assumed to be fully inactivated. Indeed, residual activities near one-half of the original level have been reported after 600 MPa for 3 min, followed by partial or substantial reactivation during cold storage (Jacobo-Velázquez & Hernández-Brenes, 2010).

The practical consequence is that browning in refrigerated guacamole is not a single-event defect occurring at processing. It is a time-dependent systems problem. Pressure treatment, acidification, oxygen availability, tissue disruption, enzyme

accessibility, reducing capacity, phenolic composition, lipid oxidation, package permeability and temperature all influence the rate at which quality is spent. The same formulation may look excellent immediately after HPP but deteriorate rapidly after opening near the end of shelf life because its reducing reserve has already been consumed. Conversely, a formulation that modestly reduces PPO activity at day 0 may perform exceptionally well if it also conserves reductive capacity and minimizes oxygen ingress.

This review therefore reframes guacamole browning as a multi-mechanistic oxidative stability problem. The objective is not merely to catalogue anti-browning agents, but to connect mechanism to industrial decision-making. Particular emphasis is placed on five questions: (i) why HPP does not reliably eliminate oxidative enzyme activity; (ii) how PPO-driven browning interacts with LOX and lipid oxidation; (iii) why natural antioxidant extracts may behave paradoxically in avocado; (iv) why post-opening testing late in shelf life is mechanistically informative; and (v) how ingredient developers and manufacturers can design staged experiments that identify the minimum effective multi-hurdle system rather than relying on trial-and-error formulation.

2. Avocado is a coupled aqueous–lipid oxidation system

The first conceptual error in many anti-browning programmes is to treat colour loss as if it were isolated from the rest of oxidative deterioration. Avocado pulp contains an aqueous phase in which PPO acts on phenolic substrates and a lipid-rich phase susceptible to enzymatic and non-enzymatic oxidation. These processes are analytically separable but biologically coupled through oxygen availability, cell disruption, redox status and storage conditions.

Failure axis	Primary drivers	Useful measurements	Industrial consequence
Enzymatic browning	PPO, phenolic substrates, O_2, pH, copper centre, tissue disruption	PPO activity; CIELAB L*, a*, b*; ΔE_{00}; browning index; image analysis	Loss of green colour, surface darkening, consumer rejection
Lipid oxidation	LOX, unsaturated lipids, O_2, metal catalysts, radical propagation	LOX activity; peroxide value; K232/K270; hexanal / volatile aldehydes	Rancid or "stale" notes, loss of fresh aroma, nutrient degradation
Pigment deterioration	Chlorophyll degradation, oxidation, acidification, processing stress	Chlorophyll a/b; hue angle; chroma	Loss of fresh green appearance independent of visible brown pigment
Reductant depletion	Oxidation of ascorbate and other reducing species over time	Ascorbic + dehydroascorbic acid; ORP; antioxidant capacity (supportive only)	Delayed but sudden post-opening browning when reserve becomes exhausted
Oxygen exposure	Headspace O_2, package OTR, dissolved O_2, repeated opening	Headspace O_2/CO_2; package OTR; optical O_2 sensor; open-pack challenge	Acceleration of both PPO and lipid oxidation, especially after opening

Table 1. Mechanistically distinct but interacting routes of oxidative quality loss in refrigerated avocado products.

2.1. PPO: a robust enzyme in a vulnerable matrix

PPO catalyses the oxidation of phenolic substrates to reactive o-quinones that subsequently participate in non-enzymatic reactions yielding dark polymers. The visual manifestation depends on more than enzyme concentration: substrate identity, oxygen availability, cellular disruption, pH and the presence of reductants or inhibitors all matter. Avocado PPO has been characterized as particularly difficult to control, and avocado-specific reviews emphasize the unusually persistent nature of the browning problem (Toledo & Aguirre, 2017).

Classic biochemical work demonstrated a pH optimum near neutrality for crude PPO extracts from avocado and showed that inhibitors differ substantially in potency; L-cysteine and ascorbic acid were among the more effective agents tested (Gómez-López, 2002). Industrial guacamole is normally acidified well below the optimum, which is beneficial but not equivalent to enzyme elimination. Acidification reduces activity and can influence copper coordination, yet residual PPO remains capable of driving colour change over extended storage—particularly after oxygen exposure increases.

2.2. LOX and the lipid phase: the part of the problem that colour trials can miss

Avocado is also a high-lipid fruit. Hass pulp commonly contains a substantial oil fraction rich in unsaturated fatty acids, creating a second oxidative substrate pool. Avocado LOX has been partially purified and characterized; it acts on polyunsaturated fatty-acid substrates and can contribute to formation of hydroperoxides and downstream volatile aldehydes (Jacobo-Velázquez et al., 2010). This matters because a formulation can preserve visible colour yet still lose sensory freshness through lipid oxidation.

The separation between "colour stability" and "oxidative stability" is therefore artificial from a product-quality standpoint. Commercial HPP guacamoles retain a desirable fresh, green sensory character relative to thermally processed products, but their volatile profiles include aliphatic compounds such as hexanal and trans-2-hexanal that are relevant to oxidative state and sensory discrimination (Alañón et al., 2022). A rigorous formulation programme should therefore pair colour metrics with at least one lipid-oxidation endpoint whenever a lipid-phase antioxidant is being evaluated.

3. The HPP paradox: microbiologically stable, biochemically active

HPP is often described as "cold pasteurization", a useful commercial shorthand that can obscure a critical mechanistic distinction. Microbial lethality and enzyme inactivation do not follow the same pressure–time relationships. Enzymes are compact proteins and can exhibit marked pressure resistance; in some systems pressure perturbs quaternary structure or cell compartmentation without irreversibly destroying the catalytic site. For avocado, the practical result is clear: a pressure schedule sufficient for strong microbial control may leave enough PPO and LOX activity to determine colour and aroma during subsequent storage.

López-Malo et al. (1998) demonstrated that increasing pressure and decreasing initial pH reduced residual PPO in avocado puree. In their system, puree with residual PPO below approximately 45% and storage at 5 °C maintained acceptable colour for at least 60 days. Palou et al. (2000), working directly with guacamole, found that increasing treatment time and pressure cycling reduced PPO and LOX. LOX could be inactivated under stronger conditions, whereas the lowest PPO residual reported was approximately 15% after four cycles at 689 MPa with 5-min holding periods. These studies established two durable principles: pressure can contribute significantly to colour protection, but PPO is the harder target; and pressure, pH, time and cycling interact.

Commercial feasibility changes the equation. Jacobo-Velázquez and Hernández-Brenes (2010) examined avocado paste treated at 600 MPa for 3 min, a far more realistic industrial schedule than extended multi-cycle treatments. Residual PPO and LOX activities were 50.72% and 55.16%, respectively. More importantly, both enzymes recovered activity during storage, reaching values close to those of fresh paste around days 10–15 before declining. This finding shifts the problem from "how much enzyme survives HPP?" to "how does residual enzyme behave over the full refrigerated life?"

Recent work confirms that 600 MPa for 3 min can markedly improve refrigerated stability, especially when puree is acidified. Uzunlu (2024) reported extended stability from roughly one week in untreated puree to 28 days in HHP-treated puree at 4 °C, with better control of colour and microbiological quality. These results support HPP as a central hurdle, but they should not be interpreted as evidence that ingredient-level protection is unnecessary. Rather, they illustrate that HPP creates a lower-risk biochemical starting point on which formulation and packaging can act.

Study	Matrix / treatment	Key observation	Industrial inference
López-Malo et al., 1998	Avocado puree; 345–689 MPa; pH 3.9–4.3	Residual PPO decreased with pressure and lower pH; colour related strongly to a*	Pressure and acidification are synergistic; residual activity must be interpreted with storage temperature
Palou et al., 2000	Guacamole; continuous / oscillatory HPP up to 689 MPa	LOX easier to inactivate; PPO residual as low as ~15% only under intensive cycling	Commercially feasible HPP may not deliver near-complete PPO inactivation
Jacobo-Velázquez & Hernández-Brenes, 2010	Avocado paste; 600 MPa, 3 min, 4 °C	~51% PPO and ~55% LOX residual; both reactivated during storage	Day-0 enzyme data can underestimate late-shelf-life oxidative risk
Woolf et al., 2013	Avocado slices; HPP	PPO/POD largely not inactivated; structural and physiological effects observed	HPP response depends on tissue structure and matrix state
Jacobo-Velázquez et al., 2013	Avocado puree + natural extracts; 600 MPa, 3 min	Botanical extracts showed divergent PPO/LOX effects; rosemary could increase apparent PPO activity	Screen botanicals in the real matrix; antioxidant assays are insufficient
Alañón et al., 2022	Commercial HPP vs thermal guacamole	HPP better preserved fresh/green sensory identity; volatile profiles differed	Quality protection must include aroma and volatile oxidation, not colour alone
Uzunlu, 2024	Acidified avocado puree; 600 MPa, 3 min	HHP improved colour and microbiological stability through 28 d at 4 °C	HPP is an effective core hurdle but still requires matrix-specific validation

Table 2. Selected avocado-specific HPP evidence and its formulation implications.

4. Why "antioxidant" is not synonymous with "anti-browning"

The food industry often screens natural extracts using chemical antioxidant assays and then assumes that high radical-scavenging capacity will translate into superior colour preservation. In avocado, this logic can fail. PPO requires phenolic

substrates. A botanical extract rich in reducible phenolics may scavenge radicals in an in-vitro assay yet simultaneously provide additional substrate for PPO in the food matrix. The direction of the net effect depends on extract composition, concentration, solubility, phase distribution, pH and the activity of the endogenous enzyme system.

Avocado-specific evidence makes this paradox unusually concrete. Jacobo-Velázquez et al. (2013) evaluated natural antioxidant extracts in HHP avocado puree. Although the extracts were selected for antioxidant potential, their biochemical effects diverged. Rosemary-containing systems could increase apparent PPO activity, consistent with the possibility that some phenolic constituents act as enzyme substrates, whereas thyme performed better on LOX/hexanal-related stability. The lesson is not that rosemary is intrinsically unsuitable, but that "natural antioxidant" is a chemical descriptor too broad to predict anti-browning behaviour in avocado.

This distinction should change how extracts of green tea, olive, rosemary, grape, berries or other polyphenol-rich sources are developed for guacamole. The first-stage screen should include PPO kinetics or at least matrix browning under controlled oxygen and matched pH. Extracts that darken the matrix, increase a* or accelerate ΔE_{00} despite strong radical-scavenging capacity should be eliminated or redirected toward lipid-phase applications. Where a polyphenolic system is retained, its sensory contribution—bitterness, astringency, herbal notes and intrinsic colour—must be assessed at the same concentrations used for efficacy.

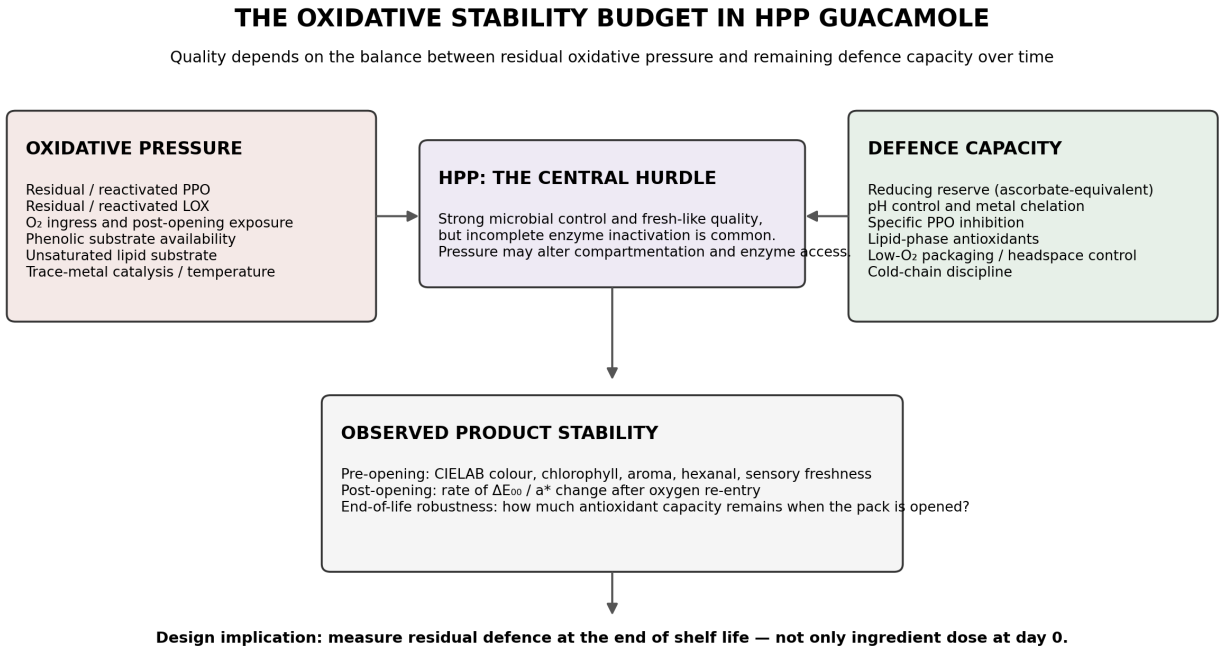

Figure 1. The "oxidative stability budget" proposed in this review. The framework is conceptual rather than a validated mathematical model. Stability is treated as the time-dependent balance between oxidative pressure and remaining defence capacity; HPP lowers several risks but does not eliminate the biochemical system.

5. Intervention classes: what each hurdle can and cannot do

5.1. Reducing agents and the concept of a residual reducing reserve

Ascorbic acid is one of the most familiar anti-browning agents because it can reduce o-quinones back toward their phenolic precursors, interrupting visible pigment formation. In practical terms, however, ascorbate is sacrificial: it is consumed as it performs its function. A formulation can therefore show excellent early colour and fail abruptly once the reducing pool is depleted. For long-life refrigerated guacamole, the relevant variable is not merely the ascorbate-equivalent dose at manufacture but the residual reducing reserve at the moment oxygen exposure occurs.

This is especially important after opening. If an unopened product has consumed most of its reducing reserve during distribution, the first major influx of oxygen may expose active PPO with little remaining chemical defence. This provides a plausible mechanism for products that appear stable in-package but brown rapidly after consumer or foodservice opening. Analytical monitoring of ascorbic acid and, where feasible, dehydroascorbic acid during shelf life is therefore more informative than formulation dose alone.

Natural vitamin-C sources such as acerola can be useful where label strategy favours food-derived ingredients, but they should be standardized by active ascorbate-equivalent content rather than dosed solely as grams of botanical powder. Carrier composition, intrinsic colour, accompanying polyphenols and batch-to-batch variability can otherwise confound interpretation. The same principle applies to citrus-derived systems and fermented ingredients: the active chemical contribution must be quantified if mechanistic conclusions are expected.

5.2. Acidification and chelation: necessary context, not a standalone answer

Lowering pH generally suppresses PPO activity and can improve the performance of complementary hurdles. Citric acid also provides metal-chelating functionality. Historic HPP studies in avocado clearly show that pressure and low initial pH act synergistically (López-Malo et al., 1998). Yet indiscriminate acidification has a sensory ceiling. Guacamole is expected to taste fresh and avocado-forward; excessive acid can move the product toward a lime-dominant or pickled profile and can alter texture.

A properly designed ingredient screen should therefore separate pH effects from ingredient-specific effects. During mechanistic screening, candidate systems should be compared at matched pH wherever possible. A second arm can deliberately vary pH to quantify the additional benefit of acidification. Without this separation, a botanical or proprietary blend may appear superior simply because it drives the matrix 0.2–0.3 pH units lower than the control.

5.3. Thiol chemistry: a high-efficacy benchmark with formulation trade-offs

L-cysteine is a powerful anti-browning benchmark because thiol chemistry can intercept quinones and inhibit colour formation. In fresh-cut avocado, a dip containing 2% L-cysteine with 1% citric acid and 1% calcium lactate was highly effective in preserving colour and texture when combined with intense light pulses (Ramos-Villarroel et al., 2011). For guacamole, however, cysteine may be less attractive commercially because sulphur notes, labelling perception and formulation interactions can become limiting. Its value in development is therefore often as a positive-control benchmark: if a natural system approaches the performance of a cysteine-based reference without its sensory liabilities, that is meaningful evidence of efficacy.

5.4. Allium-derived strategies

Allium extracts are particularly interesting because their sensory identity can be compatible with guacamole itself. Bustos et al. (2015) found that scallion, onion and selected Brassica extracts reduced colour changes in refrigerated avocado pulp and that browning retardation paralleled PPO inhibition. Ascorbic acid and scallion were among the strongest treatments for preserving green/yellow colour over extended refrigerated storage. This does not justify arbitrary use of crude onion or garlic extracts; rather, it supports a targeted programme using standardized, low-colour, low-aroma food-grade preparations whose effect can be separated from the recipe contribution of onion or garlic already present in many guacamoles.

5.5. Lipid-phase antioxidants: protecting quality that colour measurements cannot see

Alpha-tocopherol provides a useful demonstration of why phase-specific protection matters. In minimally processed avocado puree, 100 ppm alpha-tocopherol provided stronger protection of the lipid fraction than 200 ppm ascorbic acid or 200 ppm EDTA, with minimal peroxide formation and a prolonged induction period (Elez-Martínez et al., 2005). The experiment was not an HPP guacamole trial, but it establishes that lipid oxidation is independently modifiable.

For industrial formulations, tocopherols or other oil-soluble antioxidant systems should therefore be evaluated with lipid endpoints—peroxide value, K232/K270, hexanal or a validated volatile marker—rather than dismissed if their effect on L*, a* or ΔE_{00} is modest. A dual-phase system may combine an aqueous reducing/anti-PPO hurdle with a lipid-phase antioxidant. The optimum solution is not necessarily the ingredient that gives the greenest sample on day 1; it is the formulation that preserves colour, aroma and consumer acceptability together across the intended life.

5.6. Circular-economy candidates: avocado-derived anti-browning systems

The avocado itself may provide future anti-browning resources. Yepes-Betancur et al. (2025) reported that a fermented Hass avocado seed extract competitively inhibited PPO in vitro and delayed browning in avocado pulp at relatively low application levels. The concept is scientifically attractive because it combines activity with by-product valorisation and could support circular-economy development.

However, the translational gap is substantial. Seed-derived extracts require compositional standardization, toxicological and contaminant assessment, sensory evaluation, stability data, regulatory classification and freedom-to-operate review before they can be considered routine food ingredients. The appropriate industrial response is therefore not immediate adoption but a controlled research programme. Circularity does not remove the need for food-safety and regulatory evidence.

Intervention class	Primary mechanism	Strengths	Main risks / limitations
Ascorbate / vitamin-C sources	Reduces quinones; supports reducing reserve	Direct mechanistic fit; familiar; easy to quantify	Sacrificial depletion; acid/sensory effects; botanical variability
Citric acid / citrate systems	pH suppression; metal chelation	Synergistic with HPP and reductants	Sensory ceiling; may mask rather than identify ingredient-specific effects
L-cysteine / thiols	Quinone trapping; PPO inhibition	Very strong benchmark efficacy	Sulphur notes; label perception; formulation interactions
Allium extracts	PPO inhibition / redox effects depending composition	Sensory compatibility with guacamole; promising avocado-specific evidence	Variability; aroma intensity; needs standardization
Polyphenol-rich botanicals	Radical scavenging; possible metal chelation	Natural positioning; broad oxidative activity	May become PPO substrates; colour, bitterness, astringency
Tocopherols / lipid antioxidants	Chain-breaking lipid antioxidant action	Targets avocado oil fraction and aroma stability	Limited direct PPO effect; dispersion and phase distribution matter
Avocado-seed extracts	Potential direct PPO inhibition; circularity	Avocado-derived narrative; emerging efficacy evidence	Regulatory, toxicology, standardization and sensory gaps
Oxygen management	Lowers co-substrate for PPO/LOX and radical propagation	Acts across multiple oxidation pathways	Packaging cost; ineffective after opening unless residual defence remains

Table 3. Mechanistic interpretation of candidate intervention classes.

6. Oxygen and packaging: the missing co-formulant

Oxygen is not a passive environmental variable in guacamole; it is a reactant. PPO requires molecular oxygen, LOX chemistry is oxygen-dependent, and non-enzymatic lipid oxidation accelerates as oxygen becomes available. Consequently, package headspace, dissolved oxygen, oxygen-transmission rate (OTR), fill geometry and consumer opening behaviour all belong in an anti-browning strategy.

Vacuum or reduced-oxygen conditions have long been associated with improved oxidative stability in avocado systems. Elez-Martínez et al. (2005) observed better preservation of lipid-quality indices under vacuum. In HPP products, package selection has an additional role because the product is commonly processed in its final flexible pack. A low initial headspace oxygen concentration is helpful, but it should not be confused with resilience after opening.

The post-opening state is mechanistically different. The surface-to-volume ratio changes, oxygen can diffuse rapidly into disrupted tissue, mixing by the consumer can repeatedly renew oxygen contact, and the formulation may be late in its chemical shelf life. This is why a robust product-development protocol should challenge samples after opening not only at day 0, but also at the middle and end of intended refrigerated storage. The end-of-life post-opening challenge is particularly informative because it combines the highest realistic history of antioxidant depletion with a sudden increase in oxygen availability.

7. The oxidative stability budget: a framework for formulation decisions

The evidence reviewed above can be organized as a time-dependent “oxidative stability budget”. This is deliberately a conceptual model rather than a validated equation. The central idea is that every package begins with a finite defence capacity and a particular oxidative pressure. HPP changes both sides of that balance: it lowers microbial risk and can reduce enzyme activity, but it also disrupts cell structures and may increase contact among enzymes, substrates and

intracellular components. Ingredient systems add reducing, inhibitory, chelating or lipid-protective capacity. Packaging limits oxygen input. Storage progressively spends these defences.

On the pressure side of the budget are residual and reactivated PPO, residual and reactivated LOX, oxygen ingress, phenolic substrate accessibility, unsaturated lipid substrate, trace-metal catalysis and temperature excursions. On the defence side are pH suppression, chelation, reducing reserve, specific enzyme inhibition, lipid-phase antioxidants, low oxygen permeability and cold-chain control. Quality remains acceptable while the combined defence capacity is sufficient to keep the rate of visible and sensory deterioration below the rejection threshold.

The framework explains several otherwise confusing observations. A high initial dose of ascorbate may create a large day-0 defence but be rapidly consumed. A polyphenolic botanical may increase chemical antioxidant capacity yet simultaneously raise PPO substrate load. A tocopherol system may appear ineffective if only colour is measured while it is substantially delaying lipid oxidation. HPP may produce a low initial enzyme activity followed by storage reactivation. Each of these cases represents a redistribution of the budget rather than a contradiction.

For industrial research, the most useful consequence is experimental: the programme should measure both the forces that drive deterioration and the defences expected to oppose them. This reduces the risk of choosing ingredients on the basis of correlated but non-causal endpoints.

8. A staged multi-hurdle development strategy for HPP guacamole

An industrial programme should be broad enough to exploit all plausible technological levers but disciplined enough to avoid an uninterpretable "cocktail" of ingredients. The recommended sequence is screening → mechanism confirmation → HPP validation → post-opening challenge → industrial confirmation. A manufacturer with access to several anti-browning, antioxidant, acidifying and bioprotective technologies should initially evaluate them as mechanism classes rather than as a single pre-mixed solution.

8.1. Phase I — Controlled micro-screening

- Use one avocado lot and standardize maturity, dry matter/oil content, mixing energy, temperature and time from comminution to measurement.
- Include the current commercial formulation as the true industrial control, not a laboratory "blank" that the factory never produces.
- Include a conventional ascorbate/citrate benchmark to define the performance ceiling of a familiar reducing/acidifying system.
- Test natural vitamin-C systems on an ascorbate-equivalent basis; test anti-PPO fresh-keeping systems separately; test lipid antioxidants with lipid endpoints.
- Match pH across candidate formulations in the mechanistic arm so that ingredient-specific effects are not confused with acidification.
- Screen polyphenol-rich botanicals at low doses with immediate colour and sensory checks before committing them to long HPP studies.
- Run small post-opening exposures (e.g., controlled surface area and fixed oxygen contact) to identify formulations that collapse once exposed to air.

8.2. Phase II — HPP interaction study

The leading candidates should then be manufactured under an identical industrially relevant HPP schedule. Measurements immediately before and after HPP should include pH, CIELAB colour, PPO, LOX if available, reducing reserve and a lipid-oxidation baseline. The purpose is to identify pressure–ingredient interactions. A formulation that looks strong before HPP but loses its protection after pressure should not advance on the basis of bench data.

8.3. Phase III — Refrigerated shelf life and end-of-life post-opening challenge

Closed-pack storage should be sampled at time zero, early life, mid-life and the declared end of shelf life, with at least one exploratory over-life point where food-safety rules permit. Independent packs should be used for destructive measurements. At selected ages—especially end of life—new packs should be opened and followed over 0, 2, 6, 12, 24

and 48 h at controlled refrigeration. A separate temperature-abuse arm may be informative, but it should not replace the primary validated cold-chain condition.

8.4. Analytical hierarchy

Not every development laboratory needs every technique. The hierarchy below distinguishes core decision metrics from mechanistic or advanced measurements.

Tier	Measurements	Decision value
Core	L*, a*, b*, ΔE_{00}, hue/chroma; pH; standardized photography; trained sensory	Directly determines visual/sensory success and is feasible for routine development
Mechanistic	PPO activity; ascorbic/dehydroascorbic acid; ORP; headspace O_2/CO_2	Explains why a formulation succeeds or fails and predicts post-opening robustness
Lipid protection	LOX activity; peroxide value; K232/K270; hexanal or selected volatiles	Required to judge oil-phase antioxidant strategies fairly
Advanced	Chlorophyll/carotenoids; dissolved O_2; microscopy; untargeted volatile/metabolomic profiling	Useful for mechanism resolution and publication-quality validation
Safety / shelf life	Aerobic counts, yeast/mould, relevant pathogen/challenge testing according to product risk	Confirms that quality reformulation does not compromise microbiological validation

Table 4. Recommended analytical hierarchy for industrial development.

8.5. Statistical design: move beyond p-values alone

A minimum of three independent production batches is preferable for confirmation. A mixed-effects model with formulation, storage time and their interaction as fixed effects and batch as a random effect is more informative than separate one-way ANOVAs at each time point. Where opening is repeated within an experimental unit, the correlation structure should be modelled explicitly. Effect sizes and confidence intervals should accompany p-values.

The formulation target is inherently multi-objective. Colour retention, post-opening stability, sensory neutrality, lipid protection, label acceptability, regulatory feasibility and cost may pull in different directions. A desirability function or pre-specified weighted decision matrix can make this trade-off transparent. Crucially, the acceptance threshold for ΔE_{00} should be calibrated against visual/sensory rejection in the actual guacamole matrix rather than borrowed as a universal number from unrelated foods.

9. Clean label and regulatory reality

Clean label is a powerful commercial objective but not a universal scientific category. Consumer research has repeatedly shown that the term lacks a single objective definition and is interpreted through ideas such as “natural”, short ingredient lists and absence of unfamiliar additives (Asioli et al., 2017). Product developers should therefore avoid using “clean label” as a mechanism of action. It is a communication constraint layered on top of chemistry, safety and regulation.

In the European Union, technological function remains central to additive classification. Regulation (EC) No 1333/2008 defines antioxidants as substances that prolong shelf life by protecting foods against oxidation, including rancidity and colour changes. A plant origin does not automatically convert a technologically active extract into a conventional food ingredient; extraction selectivity, composition, intended function and conditions of use can affect regulatory treatment. For botanicals used in guacamole, a legal classification review should therefore precede commercial claims, especially where highly standardized extracts are employed.

This regulatory reality strengthens rather than weakens the case for mechanism-based development. A system that can achieve adequate stability using lower concentrations, familiar food sources or a more favourable declaration route has commercial value—but only after efficacy has been demonstrated under the real product process. Conversely, a “natural” ingredient that worsens browning or compromises sensory quality is not a cleaner technical solution simply because its source is botanical.

10. Research priorities

- Quantify the relationship between residual reducing reserve at end of shelf life and post-opening browning kinetics.
- Determine how HPP modifies partitioning and accessibility of PPO, LOX, phenolics and oil-phase substrates over time.
- Develop standardized matrix-based screens that identify when botanical polyphenols are PPO substrates rather than inhibitors.
- Compare dual-phase antioxidant systems (aqueous anti-PPO/reductant + lipid-phase antioxidant) against single-hurdle formulations.
- Integrate headspace oxygen and package OTR into shelf-life models instead of treating packaging as a fixed background variable.
- Validate consumer-relevant post-opening protocols for retail and foodservice formats, including repeated opening and mixing.
- Establish toxicological, compositional and regulatory dossiers for avocado-by-product extracts before circular-economy concepts are commercialized.
- Use multi-omics selectively to identify biochemical signatures that predict colour collapse before it becomes visually obvious.

11. Conclusions

The modern guacamole problem is not simply “PPO browning”, and HPP is not a complete biochemical solution. Avocado products remain dynamic oxidative systems after pressure treatment. PPO and LOX can survive commercially realistic HPP schedules, residual activity can change during storage, and the final quality response depends on oxygen, substrate accessibility, reducing reserve, lipid protection, pH, packaging and temperature.

The most important formulation principle is therefore specificity. Reducing agents should be judged by how long their reserve persists; botanical antioxidants should be screened for PPO-substrate behaviour; lipid antioxidants should be judged with lipid endpoints; acidification should be separated analytically from ingredient-specific effects; and packaging should be treated as an active hurdle. A formulation that is stable in a closed pack at day 7 is not necessarily robust after opening at day 35.

The “oxidative stability budget” provides a practical language for integrating these variables. Its central question is not how much antioxidant is added at manufacture, but whether sufficient defence capacity remains when oxidative pressure peaks. For refrigerated HPP guacamole, that peak may occur not during processing but at the moment an aged pack is opened. Designing products around that reality offers a more rational route to longer visual life, better aroma retention, lower waste and credible clean-label innovation.

Declarations and publication notes

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Review type. Critical narrative review. The article prioritizes avocado-specific experimental evidence and recent broader literature on HPP, enzymatic browning and natural inhibitors. It is not presented as a systematic review or meta-analysis.

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