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Natural and biobased anti-melanosis strategies for penaeid shrimp: critically evaluating the link between enzymatic mechanisms and product-level preservation performance

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Abstract

Melanosis is a major postharvest quality defect in penaeid shrimp, substantially shortening product shelf life. To prevent black spot formation, the shrimp industry relies mainly on sulphites and 4-hexylresorcinol, two chemical benchmarks not aligned with clean-label and organic production goals. This scoping review critically evaluates natural and biobased interventions reported for shrimp melanosis control, with the explicit aim of assessing how consistently enzyme-level inhibition evidence translates to product-level preservation outcomes, a link that, as the available literature reveals, remains largely unresolved for most candidates. Literature and patent evidence published from 1950 to April 2026 were examined by separating enzyme-level evidence, such as inhibition of shrimp polyphenol oxidase/phenoloxidase (PPO/PO) activity, from product-level evidence, such as visible melanosis suppression during chilled or iced storage. Among the reviewed interventions, many act through overlapping mechanisms, including (partial) PPO/PO suppression, quinone interception, antioxidant effects, metal interactions, and microbial control. Overall, the available studies suggest that shrimp melanosis is rarely controlled by PPO/PO inhibition alone; in practice, delivery, redox effects, matrix interactions, and broader quality preservation often contribute substantially to the final outcome. Finally, we propose an author-defined Target Candidate Profile (TCP) and Target Product Profile (TPP) that integrate mechanism, cold-chain

efficacy, safety, sensory compatibility, residue burden, environmental profile, and industrial feasibility to support more rigorous prioritization of future biobased anti-melanosis candidates.

Keywords

Shrimp melanosis, polyphenol oxidase, natural inhibitors, postharvest preservation, translational prioritization

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Abstract

Melanosis is a major postharvest quality defect in *penaeid* shrimp, substantially shortening product shelf life. To prevent black spot formation, the shrimp industry relies mainly on sulphites and 4-hexylresorcinol, two chemical benchmarks not aligned with clean-label and organic production goals. This scoping review critically evaluates natural and biobased interventions reported for shrimp melanosis control, with the explicit aim of assessing how consistently enzyme-level inhibition evidence translates to product-level preservation outcomes, a link that, as the available literature reveals, remains largely unresolved for most candidates. Literature and patent evidence published from 1950 to April 2026 were examined by separating enzyme-level evidence, such as inhibition of shrimp polyphenol oxidase/phenoloxidase (PPO/PO) activity, from product-level evidence, such as visible melanosis suppression during chilled or iced storage. Among the reviewed interventions, many act through overlapping mechanisms, including (partial) PPO/PO suppression, quinone interception, antioxidant effects, metal interactions, and microbial control. Overall, the available studies suggest that shrimp melanosis is rarely controlled by PPO/PO inhibition alone; in practice, delivery, redox effects, matrix interactions, and broader quality preservation often contribute substantially to the final outcome. Finally, we propose an author-defined Target Candidate Profile (TCP) and Target Product Profile (TPP) that integrate mechanism, cold-chain efficacy, safety, sensory compatibility, residue burden, environmental profile, and industrial feasibility to support more rigorous prioritization of future biobased anti-melanosis candidates.

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Introduction

Shrimp is one of the most valuable aquaculture products worldwide (FAO, 2024), with global production reaching approximately six million tons in 2025 (FAO, 2025), including a growing niche sector for organic products. However, its commercialization is often limited by the rapid onset of melanosis (“blackening” or “black spot”), a *post-mortem* discoloration caused by phenoloxidase (PO) activity (often referred to as polyphenol oxidase, PPO) (Gonçalves & de Oliveira, 2016; Nirmal et al., 2015; Shao et al., 2019). In *penaeid* shrimp, this *post-mortem* PO activity arises from both the prophe-noloxidase (proPO) system and hemocyanin-derived PO (HdPO) activity, with relative contributions depending on species, tissue and handling (Adachi et al., 2003; García-Carreño et al., 2008; Martínez-Alvarez et al., 2008; Nirmal & Benjakul, 2012a).

These enzymes catalyze the oxidation of endogenous phenols into o-quinones, which subsequently polymerize into dark melanins (Nirmal et al., 2015; Yoruk & Marshall, 2003). As a result, a progressive pigmentation develops on the shell and cephalothoracic segments (Montero, Ávalos, et al., 2001; Shao et al., 2019), which, although harmless to human consumption (Laorenza et al., 2022; Y. F. Qian et al., 2019), significantly reduces the product commercial acceptability and shelf life (D. T. A. Phan et al., 2021; Sae-leaw & Benjakul, 2019a), generating substantial commercial losses and food waste (Khan & Lively, 2024; Lama-muñoz et al., 2021; Nirmal & Benjakul, 2012a).

In many shrimp-processing chains, melanosis control still relies predominantly on the use of sulphites (e.g., sodium/potassium metabisulphite); these reducing agents are capable of reconverting o-quinones into colorless phenols (Gómez-Guillén et al., 2005; Kuijpers et al., 2014; Otwell & Iyengar, 1992).

Despite their effectiveness, sulphites present important limitations, including the risk of hypersensitivity reactions in susceptible consumers (BfR - German Federal Institute for Risk Assessment, 2023; Vally & Misso, 2012; Younes et al., 2022)

and potential adverse effects on product color and flavor (Martínez-Alvarez et al., 2005; Rotllant et al., 2002). In the European Union, sulphur dioxide/sulphites are classified as allergens and must be declared when present at >10 mg/kg or >10 mg/L (expressed as total SO₂) in the food as consumed (European Parliament, 2011). In addition, the maximum sulphite levels allowed in crustaceans are category- and size-dependent under the EU additives framework: for fresh/frozen crustaceans and cephalopods, limits expressed as SO₂ are set at 150 mg/kg (up to 80 units/kg), 200 mg/kg (80-120 units/kg) and 300 mg/kg (>120 units/kg) for *penaeid*-type families (BfR - German Federal Institute for Risk Assessment, 2023; EU, 2008; European Parliament, 2011; Food Standards Agency, 2023). In the United States, a “detectable sulfite amount” triggering labelling is defined as ≥10 mg/kg total SO₂ in the finished food (21 CFR §101.100, 2020; U.S. Food and Drug Administration, 2019). While the EU Organic Regulation provides a specific derogation for sodium metabisulfite (SMBS; E223) application in the organic crustacean sector, the more restrictive USDA organic standards generally prohibit this use. Private organic labels such as Naturland and Demeter International set standards that exceed the EU baseline and restrict sulphite use during harvest and processing. SMBS is typically applied to fresh shrimp at relatively high concentrations through immersion in dipping solutions, although the exact solution-to-shrimp ratio and exposure time vary across protocols (Andrade et al., 2015). Instead, the second authorized anti-melanosis additive (the synthetic 4-hexylresorcinol (E586)) is applied at substantially lower quantities of 50 mg/kg on crustaceans with acceptable residue limits in edible tissues typically framed around 1-2 mg/kg. In contrast to sulphites, E586 is a highly potent PPO-directed melanosis inhibitor. While it is marketed as a non-allergenic alternative solution

for preventing melanosis, its synthetic nature prohibits its application in organic shrimp production.

Collectively, these constraints have accelerated the search for effective, biobased alternatives compatible with “clean-label” product positioning and with the specific needs of the organic shrimp sector (Hamdan et al., 2022; Moon et al., 2020; Sae-leaw & Benjakul, 2019a). Natural small molecules of microbial and plant origin offer clear promise in this regard, as exemplified by nisin (E234), natamycin (E235), carnosic acid/carnosol-rich extract (E392), and tocopherol-rich extract (E306), which are already used in food preservation, with E392 and E306 also permitted in organic farming. These examples are invoked here as regulatory precedents for natural food-preservation agents, not as shrimp-PPO-directed anti-melanosis inhibitors. Likewise, naturally derived compounds and complex mixtures (extracts/essential oils) have been investigated to inhibit PPO activity in shrimp and, consequently, delay melanosis development during product storage (Fang Tian et al., 2025; D. T. A. Phan et al., 2021; Sae-leaw & Benjakul, 2019a). Within this emerging space, structurally defined natural compounds such as flavonoids (e.g., catechin) (Nirmal & Benjakul, 2010, 2012b; Sae-leaw et al., 2017), phenolic acids (e.g., ferulic acid) (Nirmal & Benjakul, 2010, 2012b), non-protein amino acids (e.g., mimosine) (Nirmal & Benjakul, 2011b), and pyranones (e.g., kojic acid) (Chang, 2009; J. S. Chen et al., 1991) have been reported to interfere with melanosis-relevant chemistry through copper chelation, reduction of reactive intermediates, or substrate competition (Chang, 2009; Zolghadri et al., 2019). Likewise, various complex plant extracts (from leaves, seeds, fruit peels, and algae) (Basiri et al., 2015; Nirmal & Benjakul, 2011d; D. T. A. Phan et al., 2021; Sai-Ut et al., 2020; Sharifian et al., 2019) and essential oils are better understood as multitarget preservation systems combining PPO suppression with antioxidant and related quality-protective effects (Y. F. Qian et al., 2022; Tayel et al., 2025).

This review specifically addresses natural and biobased anti-melanosis interventions, including molecules of biological origin (plant, microbial, or biomass-derived), whether isolated or produced by fermentation, and complex mixtures such as extracts and essential oils, while excluding stand-alone physical/process interventions. Further, we focus on *penaeid* shrimp of greatest commercial relevance (Consulting, 2012; FAO, 2024), primarily *Litopenaeus vannamei* and *Penaeus monodon*, and, where data are available, also consider *Marsupenaeus japonicus*, *Parapenaeus longirostris*, *Aristeus antennatus*, *Pandalus borealis*, and other species to complement and expand beyond previous broad crustacean-centered reviews (Fang Tian et al., 2025; Gonçalves & de Oliveira, 2016; Sae-leaw & Benjakul, 2019a). Because both the PPO/proPO system and hemocyanin-derived PO contribute to shrimp melanosis, this mechanism is broadly conserved across crustaceans (Adachi et al., 2003; Cerenius & Söderhäll, 2004; García-Carreño et al., 2008; Martínez-Alvarez et al., 2008).

However, kinetic and biochemical parameters vary among species, and this variation is considered when comparing inhibitor efficacy (Nirmal & Benjakul, 2012a; Shao et al., 2019). Recognizing the complex nature of this system, we first summarize key developments in shrimp PPO biology and comparatively evaluate the biochemical properties reported across relevant species. This provides the basis for benchmarking and critically evaluating the reported natural solutions and their product-focused intervention performance against the practical industrial objective of near-complete melanosis prevention. In addition, we summarize evidence on mechanism of action, benchmark against common synthetic control agents and discuss patents and recent industrial applications toward scale-up.

Previous reviews have discussed crustacean melanosis and the inhibitory potential of phenolic compounds, including the CRFSFS review by Nirmal et al. (2015) and the later review by Sae-leaw and Benjakul (2019). The present review addresses a different and updated question. Rather than revisiting phenolic compounds alone, we focus specifically on *penaeid* shrimp and expand the evidence space to include structurally defined molecules, extracts, essential oils, coatings, delivery-enabled preservation systems, and relevant patent evidence. We further distinguish shrimp-relevant enzymology from product-level preservation evidence, identifying hemocyanin-derived phenoloxidase as a major unresolved mechanistic gap.

Finally, we propose a translational prioritization framework intended to separate academically active systems from candidates with realistic industrial potential. Therefore, translational prospects of the reported biobased approaches, based on their enzyme-inhibitory potency and modes of action, together with translational performance in storage trials are assessed. Building on this synthesis, we define a Target Product Profile (TPP) for a natural *penaeid* Head-On Shell-On (HOSO) shrimp anti-melanosis treatment concept and propose an author-defined Target Candidate Profile (TCP). This TCP extends beyond enzymatic potency alone by integrating multidimensional benchmarks, including robust storage performance in HOSO products (targeting near-complete melanosis suppression beyond 7 days), toxicological safety, environmental compatibility, and industrial feasibility. By doing so, we aim to support a more rigorous prioritization of natural product leads with genuine translational potential as next-generation clean-label processing aids, particularly for the organic shrimp sector.

Methods

Review design and reporting framework

This work was conducted as a scoping review (Fig. 1) with critical mechanistic synthesis to map and organize evidence on natural polyphenol oxidase (PPO)/phenoloxidase (PO)-targeted strategies for melanosis control in shrimp. Reporting was guided by the PRISMA extension for scoping reviews (PRISMA-ScR) (Tricco et al., 2018), whereas eligibility criteria, study selection, and data charting were structured following JBI guidance for scoping reviews under the Participants-Concept-Context (PCC) framework (Peters et al., 2020; Rethlefsen & Page, 2022; Tricco et al., 2018).

Eligibility criteria (PCC framework)

Participants (P) Commercially relevant *penaeid* shrimp, primarily *Litopenaeus vannamei* and *Penaeus monodon*, and, where informative data were available, other shrimp or prawn species such as *Marsupenaeus japonicus*, *Parapenaeus longirostris*, *Aristeus antennatus*, and *Pandalus borealis*. Studies on crustacean PPO or hemocyanin-derived PO were included when clearly relevant to shrimp melanosis biochemistry.

Concept (C) Eligible interventions included natural or biobased approaches intended to reduce melanosis through PPO/PO inhibition, quinone interception or reduction, or multifunctional preservation effects combining PPO suppression with antioxidant or related quality-protective mechanisms. For evidence mapping, interventions were classified into three definition tiers: 1) pure or structurally defined molecules, tested as single compounds; 2) plant extracts or agri-food byproducts, where composition was at least partially characterized and/or melanosis outcomes were reported; and 3) chemically profiled essential oils, in which major constituents were identified by chromatographic analysis, but isolated-compound causality was not demonstrated.

Context (C) Postharvest shrimp preservation under chilled, iced, refrigerated, frozen-thawed, or retail-relevant storage conditions; and *in vitro* assays using shrimp PPO/PO extracts, partially purified preparations, recombinant enzymes, or closely relevant crustacean PPO/PO systems.

Exclusion criteria

Physical/process-only interventions (e.g., high-pressure, irradiation, packaging atmospheres without chemical agents), purely synthetic inhibitors without a natural/biobased origin (except when used as

benchmark controls such as sulphites or 4-hexylresorcinol), and studies on non-crustacean tyrosinase/PPO (e.g., mushroom, plant) unless used strictly to support assay methodology rather than shrimp-specific conclusions. Process interventions were excluded when evaluated as stand-alone strategies but were retained when they functioned as delivery or co-intervention components of an otherwise eligible natural or biobased anti-melanosis system.

Information sources

Peer-reviewed literature was retrieved from Scopus, Web of Science Core Collection, and PubMed/MEDLINE. The search covered records from 1950 to April 2026 with no geographic restrictions. To capture intellectual-property and translational context, a targeted patent search was additionally performed in Espacenet (European Patent Office).

Search strategy

Search strategies combined controlled vocabulary (where supported) and free-text terms covering shrimp species, melanosis/black spot/browning, PPO/phenoloxidase/tyrosinase, and inhibitors/extracts/essential oils/coatings/formulations. Search strings were iteratively refined to balance sensitivity and specificity across databases and were adapted to the syntax requirements of each platform. A representative database query (adapted per platform) was: (Shrimp OR prawn OR "*Litopenaeus vannamei*" OR "*Penaeus monodon*") AND (melanosis OR "black spot" OR browning OR discoloration) AND (polyphenol oxidase OR PPO OR phenoloxidase OR prophenoloxidase OR proPO OR tyrosinase OR hemocyanin-derived phenoloxidase) AND (inhibit OR inhibitor OR anti-melanosis OR anti-browning OR chelat* OR antioxidant OR extract* OR "essential oil" OR coating OR film OR dipping OR soaking OR formulation) For patents, analogous keyword searches were combined with field-based queries (title/abstract) and restricted to 1950 to April 2026 (Rethlefsen & Page, 2022). Record screening was conducted sequentially at title/abstract and full-text level using the predefined eligibility criteria. Across the exported bibliographic corpus and targeted patent records, 195 records were identified, 56 duplicates were removed, and 139 records proceeded to title/abstract screening (Fig. 1). Of these, 67 records were excluded at title/abstract level, and 72 full-text records were assessed for eligibility. The 16 full-text exclusions were mainly due to: non-natural or purely synthetic intervention without relevance as a comparator, process-only intervention without a natural/biobased component, non-shrimp or non-crustacean PPO system without shrimp relevance, absence of melanosis/PPO-related endpoints, or insufficient methodological detail for evidence mapping. Fifty-six studies were ultimately included in the critical synthesis. Screening, data

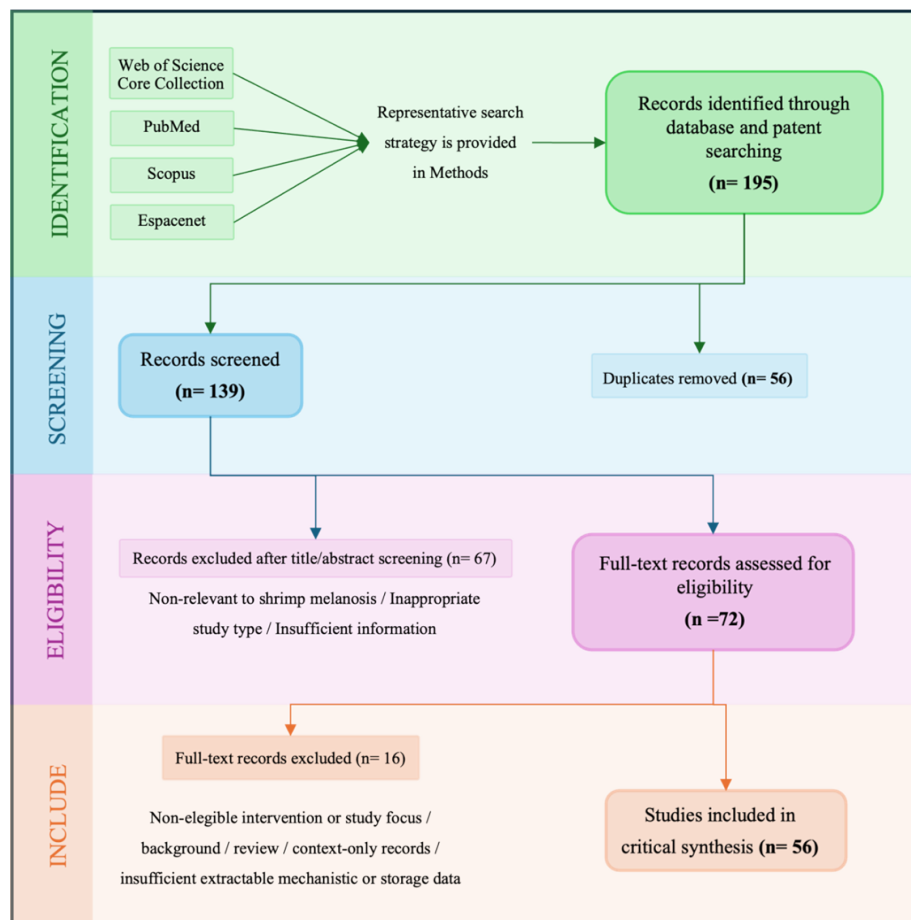


Figure 1: Figure 1 Study selection flowchart.

charting, and evidence mapping were conducted by one reviewer and subsequently discussed with senior authors to resolve eligibility uncertainties and interpretive decisions. To mitigate single-reviewer bias, all final inclusion/exclusion decisions at full-text level and all evidence-tier assignments were independently checked by a senior author. A random subset of extracted data items, including intervention dose, storage model, melanosis endpoint, comparator use, and mechanistic evidence, was cross-checked against the original articles. Disagreements were resolved by consensus. The final search update was completed in April 2026. Additional background, regulatory, comparator, and methodological references were incorporated through targeted hand-searching to contextualize shrimp PPO biology, assay interpretation, and current industrial benchmarks; these references were not necessarily part of the core evidence set contributing to the PRISMA screening flow and included-study count.

Data extraction

Data charting was performed by one reviewer using a standardized extraction form consistent with JBI scoping review practice. Eligibility uncertainties and interpretive decisions were discussed with senior authors during synthesis meetings, and extracted information was cross-checked during the development of the evidence maps. Extracted items included:

Study descriptors Year, country (if reported), study type (*in vitro* enzyme, whole shrimp storage, coating/film system, formulation), and shrimp species

Enzyme system (when applicable) Source tissue (cephalothorax, hemolymph, muscle), enzyme form (PPO/proPO, hemocyanin-derived PO), purification level, substrate (e.g., L-DOPA), pH/ionic strength, temperature, wavelength/readout, and controls/blanks

Intervention descriptors Compound/extract/oil identity; purity and compositional disclosure; extraction method (for extracts); formulation components (for defined formulations); dose window; application mode (immersion/dip/coating/film); and storage conditions (temperature, duration, handling history such as freeze-thaw)

Outcomes Enzyme-focused metrics (residual activity, % inhibition, IC_{50} , K_i , inhibition mode) and product-focused outcomes (melanosis index, CIELAB color metrics when reported, sensory acceptance, microbial/spoilage indices, and time-to-rejection under defined cold storage)

Mechanistic evidence Copper chelation/reduction assays, antioxidant assays, adduct formation evidence, oxygen-consumption or orthogonal kinetics, and reported limitations (e.g., spectral interference in dopachrome assays)

Evidence classification and critical synthesis

To prevent mixture of compositional association with causal inhibition, each entry was categorized as “tested as pure compound” versus “tested as extract/system” (including coatings/films and multicomponent formulations). For operational synthesis, studies were additionally mapped to internal evidence tiers: ET1, product-level evidence with an industrial comparator; ET2, product-level evidence without an industrial comparator; ET3, shrimp PPO/PO enzymology. The synthesis emphasizes studies where kinetic/mechanistic evidence is paired with storage outcomes under clearly specified conditions and explicitly flags assay-design dependencies and matrix constraints.

Critical evaluation

Consistent with scoping review methodology, studies were not excluded solely on the basis of reporting quality; instead, key reporting features relevant to interpretability, such as assay design details, controls/blanks, compositional disclosure, and storage conditions, were recorded and used to contextualize confidence in mechanistic and translational claims. Critical judgment was therefore embedded in the synthesis rather than applied as a formal exclusion criterion ^(Rethlefsen & Page, 2022; Tricco et al., 2018). A notable characteristic of the available evidence base is its concentration within a limited number of research groups. A substantial proportion of the high-resolution ET1 studies in *L. vannamei*, particularly those pairing shrimp PPO enzymology with iced or refrigerated storage validation and industrial comparators, originate from or in close collaboration with one research group (S. Benjakul and colleagues, Prince of Songkla University, Thailand). While this reflects the sustained and rigorous contribution of that group to the field, it introduces a limitation regarding independent cross-laboratory reproducibility. Candidate performance rankings derived predominantly from a single group's protocols, including specific melanosis scales, dipping conditions, and enzyme extraction procedures, should be interpreted with this caveat in mind. Independent replication of the most promising candidates (e.g., EGCG, ferulic acid, *Leucaena leucocephala* seed extract, guava leaf extract) in at least one additional laboratory, using distinct shrimp supply chains and storage facilities, would substantially strengthen translational confidence before industrial adoption is recommended.

Polyphenol Oxidase Biology in Shrimp

In living shrimp, polyphenol oxidase (PPO), often stored/present in eukaryotic cells as inactive prophenoloxidase (proPO), is a type-3 copper metalloprotein that catalyzes the hydroxylation of monophenols and the oxidation of o-diphenols to reactive quinones, two rate-limiting steps in melanin synthesis (Deri et al., 2016). PPO is also an immune effector expressed largely in hemocytes and plays protective roles in shrimp immunity, where it drives melanin synthesis for pathogen encapsulation and cuticle hardening (Dinçer et al., 2015; Masuda, 2021). However, the same enzymatic activity, when triggered after harvest, leads to undesirable blackening of shrimp tissues, causing quality deterioration and economic loss (Masuda, 2021). Accordingly, the distribution, activation, and properties of shrimp PPO are of prime interest in devising strategies to control melanosis. Melanosis in post-harvest shrimp typically begins at the cephalothorax (head and carapace) and in cuticular “joint” regions (e.g., where shell segments meet), then progresses to the abdomen and appendages (Dinçer et al., 2015; Y. F. Qian et al., 2022). This spatial pattern reflects the broad, though not uniform distribution of PPO (as its zymogen proPO) across shrimp tissues, with particularly high abundance in hemocytes (circulating immune cells in the hemolymph) and in peripheral cuticular tissues that are prone to injury (H. N. T. Phan et al., 2023). In black tiger shrimp (*Penaeus monodon*), for example, two proPO isoforms show tissue-specific expression: proPO₁ predominates in hemolymph and in external structures like the uropod (tail fan), whereas proPO₂ is most abundant in the hepatopancreas (digestive gland) (H. N. T. Phan et al., 2023). Upon experimental activation of shrimp proPO, the highest phenoloxidase activities are detected in the hemolymph and appendages (uropods, pleopods, etc.), with much lower activity in muscle (H. N. T. Phan et al., 2023). These observations align with the fact that black spots form first in the shell (especially the head region where hemolymph and pigment precursors are concentrated) before spreading to muscle tissue. The cephalothorax therefore appears functionally enriched in PPO-related activity, consistent with its role in rapid wound sealing and microbial defense (Nirmal & Benjakul, 2012a; Y. F. Qian et al., 2022). The main external regions associated with early melanosis development and higher PPO/proPO-related activity are summarized in Fig. 2.

Figure 2: Figure 2 External anatomy and regions commonly reported as early melanosis sites and higher PPO activity in tissue assays.

Hemocyanin as a Latent Phenoloxidase

In addition to PPO enzymes, shrimp hemolymph contains hemocyanin (Hc), a copper-based oxygen transport protein that can act as a phenoloxidase. Arthropod hemocyanin and PPOs are evolutionarily related type-III copper proteins with similar active sites (Deri et al., 2016). Each Hc subunit carries a binuclear Cu center coordinated by six histidines, broadly comparable to PPO active sites (Cong et al., 2009; Deri et al., 2016). In native Hc, this catalytic pocket is sterically shielded by protein domains, as Hc's physiological role is O₂ transport rather than substrate oxidation (Coates & Talbot, 2018; Cong et al., 2009). However, under certain conditions Hc can be converted into an enzyme with PPO-like catalytic behavior (often named hemocyanin-derived phenoloxidase, HdPO) (Cubillos et al., 2021; Mei et al., 2024). Partial denaturation or proteolysis can expose the active site; for example, SDS treatment induces a conformational change that displaces a blocking phenylalanine-containing loop and allows phenolic substrates access to the dicopper center (Cong et al., 2009). Similarly, limited protease digestion or endogenous activation factors from hemocytes can trigger phenoloxidase activity in Hc (Adachi et al., 2003; Cong et al., 2009). Empirically, purified Hc from *L. vannamei* can slowly oxidize L-DOPA, and adding trypsin modestly increases this activity (Nirmal & Benjakul, 2012a). The contribution of Hc to *post-mortem* melanosis is likely significant; for example, Hc has been reported to be more abundant than PPO in *M. japonicus* hemolymph (Adachi et al., 2005). Indeed, Hc's latent monophenol oxidase activity has been implicated as a major driver of black spot in stored shrimp (García-Carreño et al., 2008; Martínez-Alvarez et al., 2008). *In vitro* evidence indicates that part of *post-mortem* phenoloxidase activity can be attributed to Hc; for example, treatments that denature Hc eliminate a substantial fraction of phenoloxidase activity in shrimp extracts (Manheem et al., 2012). More recently, isolated *L. vannamei* HdPO and PPO were comparatively evaluated, with HdPO showing roughly 20% of the specific activity of the native PPO enzyme (Mei et al., 2024). This lower specific activity is partly offset by the high abundance of Hc in *penaeid* shrimp tissues (Manheem et al., 2012). Hc in *penaeid* shrimp exists as large oligomers (hexamers or dodecamers of ~75 kDa subunits, total mass ~450-800 kDa) (Martínez-Alvarez et al., 2008). When activated to PO, these complexes may dissociate, e.g., deep-water shrimp Hc/PPO shows active forms near 500 kDa (intact oligomer) and ~200 kDa (partial subunits) (Martínez-Alvarez et al., 2008; Zamorano et al., 2009). In summary, shrimp Hc serves as a latent phenoloxidase reservoir, adaptable under stress to reinforce the proPO system. Its type-III copper active site and reaction mechanism are fundamentally the same as PPO, highlighting the functional overlap between oxygen transport and latent phenoloxidase activity in shrimp hemolymph. However, despite its likely relevance for real-world efficacy in storage trials, HdPO remains essentially absent from the natural-products inhibition literature. We

found no HdPO-focused enzymology (kinetics, substrate scope) or inhibitor profiling embedded in natural products anti-melanosis studies. Accordingly, this review centers on PPO/proPO-focused evidence and treats HdPO inhibition as a clear mechanistic gap for future work.

Biochemical Properties of Shrimp PPO Across Species

Purified or partially purified PPO enzymes from various shrimp species reveal both conserved traits and notable differences. Most shrimp PPOs are binuclear enzymes that function as either monomers or oligomers in solution. The apparent molecular weight of active PPO often suggests an oligomeric state. For example, Pacific white shrimp (*L. vannamei*) PPO appears as ~200-220 kDa on activity-stained gels (Manheem et al., 2012; Shao et al., 2019), implying a trimer of ~70 kDa subunits (consistent with a ~75 kDa monomer that retains activity in a trimeric form). By contrast, the *M. japonicus* PPO was reported at ~160 kDa (Benjakul et al., 2005), potentially a dimer. Black tiger prawn (*Penaeus monodon*) PPO has been observed in a similar ~150-170 kDa range upon partial purification (H. N. T. Phan et al., 2023; ROLLE et al., 1991). These differences could reflect species-specific propensity to form stable homo-oligomers or isoforms with different glycosylation. All shrimp PPOs require a binuclear copper center for activity, but in some species the enzyme is isolated pre-activated (likely with copper bound and the pro-peptide removed). For instance, *L. vannamei* cephalothorax PPO is isolated in an active form: adding SDS, trypsin, or Cu^{2+} does not further increase activity (Manheem et al., 2012; Nirmal & Benjakul, 2012a). Similarly, *M. japonicus* PPO was not activatable by trypsin or SDS, indicating it was already in the active state upon extraction (Benjakul et al., 2005). This suggests that handling, or endogenous proteases may activate proPO during extraction for those species. In contrast, older studies on other shrimp species (e.g., *Penaeus setiferus*) noted that adding copper could enhance PPO activity (Simpson et al., 1987), hinting that some preparations had lost copper and could be reconstituted, or contained inactive proPO that could be partially activated by copper loading (Adachi et al., 1999; Simpson et al., 1987). Despite such variations, shrimp PPOs show broadly comparable assay optima, but with clear species- and preparation-dependent shifts. Across published L-DOPA assays, pH optima often fall within mildly acidic to near-neutral conditions, for example around pH 6.0 in *L. vannamei* (Shao et al., 2019) and within a broader range of approximately pH 6.0–8.0 in *P. setiferus* (J. S. Chen et al., 1991; Nirmal & Benjakul, 2012a; SIMPSON et al., 1988). Notable exceptions exist: *Parapenaeus longirostris* (Zamorano et al., 2009) has been reported with markedly acidic optima (pH ~4.0–4.5), whereas *Marsupenaeus japonicus* (Benjakul et al., 2005) can extend into more alkaline conditions depending on preparation and assay format. Temperature optima are likewise heterogeneous. In our compilation (Table 1), apparent

activity maxima span ~30-55 °C, with *P. longirostris* clustering at ~30-35 °C, *M. japonicus* at ~35-37 °C (Benjakul et al., 2005), and multiple coastal *penaeids* showing higher optima (e.g., *P. monodon* and *L. setiferus* ~45 °C; *L. vannamei* ~50-55 °C) (Benjakul et al., 2005; ROLLE et al., 1991; Shao et al., 2019). Importantly, “optimum temperature” is not a fixed enzyme constant and can shift with assay duration and enzyme concentration; it should therefore be interpreted as an operational assay optimum rather than a direct proxy for environmental temperature or physiological set-points^(Almeida & Marana, 2019). Although *penaeid* species differ in habitat and life-history context, these ecological differences should not be overinterpreted as direct determinants of reported PPO assay optima, which are strongly influenced by enzyme source, extraction/activation state, substrate, and assay configuration (W. Dall et al., 1991). Table 1 therefore compiles reported kinetic parameters and assay conditions across shrimp PPO preparations, but these values should be interpreted primarily as assay- and preparation-dependent operational parameters rather than as species-level physiological constants. Comparative profiling of reported shrimp PPO preparations reveals substantial functional heterogeneity, with marked differences in apparent affinity, reported catalytic capacity, and operational pH and temperature optima. Apparent K_m values span more than an order of magnitude, from high-affinity preparations such as *Marsupenaeus japonicus* PPO ($K_m \approx 0.26$ mM) to markedly lower-affinity preparations reported for *Penaeus monodon* and *Penaeus setiferus* ($K_m > 4$ mM). Reported V_{max} values likewise vary widely, but because they were obtained under non-equivalent assay and normalization conditions, they are best interpreted as study-specific operational values rather than directly comparable catalytic metrics. Taken together, the literature supports clear species- and preparation-dependent variability in shrimp PPO behavior, while also underscoring that cross-study comparisons remain limited by differences in enzyme source, activation state, substrate system, assay duration, and activity normalization.

Mechanistic basis of natural PPO inhibitors

Building on the tissue distribution and activation routes described above (proPO activation and Hc-derived phenoloxidase activity), natural anti-melanosis agents can be framed along the PPO-quinone-melanin cascade. In *penaeid* shrimp, the apparent contribution of PPO is shaped by enzyme form and activation state (latent/pro-forms versus activated enzyme), which helps explain why potencies derived from purified enzyme preparations or crude extracts do not translate linearly to intact tissues (Amparyup et al., 2013; Jearaphunt et al., 2015). Inhibition therefore arises either from direct interference with catalysis (Cu coordination and active-site effects, like 4-hexylresorcinol), downstream interception of

reactive intermediates (as exemplified by sulphites), or system-level constraints that modulate reaction rates *in situ* (Sae-leaw & Benjakul, 2019a). Across studies, inhibitor potency is typically reported using enzyme-focused readouts (residual activity, IC_{50}/K_i , inhibition mode) and product-focused outcomes (melanosis index, CIELAB lightness (L^*), total color difference (CIE 1976) (ΔE), sensory scores, and days of acceptable quality during cold storage) (Y. F. Qian et al., 2019; Sae-leaw & Benjakul, 2019a). Because these endpoints are highly assay-dependent (enzyme source, substrate choice such as L-DOPA, pH/ionic strength, extraction method, and matrix composition), mechanistic interpretation is strongest when enzyme kinetics and storage trials are reported together (Sae-leaw & Benjakul, 2019a). In dopachrome-based spectrophotometric assays (typically monitored at 475 nm), colored extracts and polyphenols can bias apparent inhibition because intrinsic sample absorbance contributes to the measured signal and some “inhibitors” are also tyrosinase substrates that generate oxidation products with distinct absorption maxima, potentially distorting calculated inhibition constants; rigorous analyses therefore require sample blanks/controls to correct background and, where possible, orthogonal readouts (e.g., oxygen-consumption-based kinetics) to validate inhibition mode (Mayr et al., 2019a, 2019b). Although individual compounds may display overlapping behavior, shrimp-related literature supports four dominant mechanistic archetypes:

Copper coordination and chelation at the active site The catalytic copper ions are indispensable for PPO turnover; consequently, molecules that coordinate copper can suppress quinone generation. Kojic acid is a canonical example reported to inhibit crustacean (including shrimp) PPO through Cu-associated interactions and mixed/competitive features depending on substrate and enzyme source (J. S. Chen et al., 1991).

Substrate competition and active-site occupancy Many phenolics and polyphenols can behave as alternative substrates or active-site ligands, reducing access of endogenous shrimp phenols to PPO. In *L. vannamei* PPO, catechin and ferulic acid have been mechanistically characterized: catechin shows mixed-type reversible inhibition, whereas ferulic acid exhibits non-competitive reversible inhibition, and both can interfere with dopachrome formation through more than one route (Nirmal & Benjakul, 2012b).

Reductive quenching of quinones and interception of reactive intermediates A distinct (and often fast-acting) strategy is to short-circuit pigment formation downstream of catalysis, either by reducing quinones back to phenols or by preventing colored intermediates from accumulating. Thiol-containing compounds are central here: cysteine and

glutathione inhibit prawn/shrimp PPO activity and, crucially, can also prevent color development by trapping intermediates and/or reducing o-quinones to colorless products (Benjakul et al., 2006).

System-level and multifunctional preservation effects Some natural anti-melanosis interventions do not act primarily as tight PPO ligands but rather suppress melanosis through system-level constraints and multifunctional preservation effects. These may include altered oxygen availability, local redox balance, diffusion and penetration barriers, changes in the physicochemical microenvironment, and coupled reductions in spoilage processes that accelerate discoloration *in situ* (Bono et al., 2012; Fang Tian et al., 2025; Kimbuathong et al., 2020). In this context, many of the best-performing natural interventions in shrimp are better understood as multitarget preservation systems that combine partial PPO suppression with antioxidant, antimicrobial, barrier, or quality-protective effects. For example, tea catechin and its derivatives have been evaluated in *L. vannamei* with parallel readouts of PPO inhibition and melanosis during refrigerated storage, illustrating how enzyme-related effects can be integrated with broader product-level preservation outcomes (Nirmal & Benjakul, 2012b). Collectively, these mechanisms illustrate how PPO inhibition in shrimp can be approached from multiple molecular perspectives. They also suggest that combining compounds with complementary modes of action therefore represents a particularly promising strategy to extend shrimp shelf life.

Pure natural compounds

Structurally defined natural compounds provide the most informative starting point for benchmarking biobased anti-melanosis leads beyond sulphites and 4-hexylresorcinol. Testing pure natural compounds enables the clearest mechanistic evaluations, allowing hypotheses on copper coordination, redox interception, or active-site effects to be examined alongside defined enzyme kinetics and, where available, challenged in storage trials. That said, apparent potency is highly context-dependent: many phenolics are themselves PPO substrates and/or interfere with dopachrome-based assays (Gasowska-Bajger & Wojtasek, 2016), while dose requirements in whole-shrimp dipping can be driven as much by diffusion and matrix constraints as by intrinsic PPO binding affinity. For clarity, the compounds below are discussed by chemical class, emphasizing first the assay system (enzyme preparation vs whole shrimp), second the practical dose window, and third the reported inhibition mode.

Flavonoids

Flavonoids are the most extensively studied phenolic scaffold class in shrimp anti-melanosis research. However, only a limited subset has been evaluated as structurally defined molecules, whereas many other flavonoids are inferred as dominant constituents of active extracts. To avoid overinterpretation, the literature is best synthesized by separating compound-tested flavonoids, applied as purified standards or as compositionally disclosed formulations, from compound-identified flavonoids annotated as major extract constituents in studies that report PPO inhibition and/or storage outcomes (see below under plant extracts and agri-food byproducts as multi-mode anti-melanosis systems).

Flavan-3-ols: the “core” shrimp flavonoid evidence Among plant polyphenols tested in *penaeid* shrimp, tea catechins (flavan-3-ols) provide the strongest defined-compound flavonoid evidence because they are chemically well characterized and supported by both PPO-focused assays and storage validation (Sae-leaw & Benjakul, 2019a). In *L. vannamei*, immersion (dipping) in (+)-catechin solutions (0.05-0.1%, w/v) markedly retarded melanosis during ~10-12 days of iced storage, while also reducing lipid oxidation versus untreated controls and a SMBS comparator (Nirmal & Benjakul, 2009b). Mechanistically, catechin-family compounds do not behave as a single inhibition class: depending on the derivative and assay conditions, inhibition has been reported as uncompetitive or mixed-type, underscoring the structure sensitivity of this scaffold (Nirmal & Benjakul, 2009b, 2010, 2012b). Beyond (+)-catechin itself, several major tea flavan-3-ol derivatives like (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) have been systematically compared in a shrimp-relevant context, revealing structure-dependent differences in both mechanism and practical anti-melanosis performance. All compounds inhibited *L. vannamei* PPO in a dose-dependent manner (0.10-2.00 mM; reported as % inhibition rather than IC_{50} values), but kinetic behavior was distributed as follows: catechin and EC showed uncompetitive inhibition, whereas ECG, EGC, and EGCG exhibited mixed-type inhibition, leading to the authors’ interpretation that a 5'-hydroxyl group (EGC) and a gallate (ECG/EGCG) may be the differentiating moieties (Sae-leaw et al., 2017). In whole-shrimp trials, EGCG dipping at 0.5-0.75% (w/v) produced the lowest melanosis scores during refrigerated storage, with the clearest advantage visible by the end of the 10-day study period relative to both untreated shrimp and the 1.25% SMBS comparator (Sae-leaw et al., 2017). More broadly, this dataset highlights both the promise and the reporting gap in shrimp-relevant PPO inhibition: clear structure-dependent shifts in inhibition mode are observed, yet the lack of harmonized potency metrics (IC_{50}/K_i under matched assay conditions) still limits

cross-study ranking. Outside flavan-3-ols, purified-compound evidence is much thinner. Quercetin, although frequently mentioned, appears in shrimp largely through a compositionally disclosed preservative formulation containing quercetin, cinnamic acid, and 4-hexylresorcinol, with or without modified-atmosphere packaging. These studies support formulation-level preservation effects, but because the system includes 4-HR, they should not be interpreted as clean evidence for quercetin as an independently validated biobased PPO-directed lead (Y. Qian et al., 2018; Y. F. Qian et al., 2015). Across flavonoid subclasses, a conservative synthesis restricted to purified standards and compositionally disclosed formulations is that: catechin-family flavan-3-ols remain the most consistently examined as defined molecules, with enzyme-kinetic characterization paired to direct storage validation in *L. vannamei*; outside flavan-3-ols, the purified-compound evidence base is narrower and, in practice, quercetin enters the literature mainly through a multi-component preservative formulation (rather than as a repeatedly validated single-compound dip); and for most other flavonoid scaffolds, robust shrimp storage datasets using isolated molecules remain comparatively scarce, motivating their separate treatment of plant-extract-driven datasets later in the review.

Phenolic acids

Phenolic acids recure across shrimp anti-melanosis studies because they combine redox activity, potential copper interactions, and, in some cases, direct PPO engagement ^(Nirmal et al., 2015; Sae-leaw & Benjakul, 2019a). Their apparent “PPO inhibition” can therefore arise from multiple, partially overlapping processes, including true enzyme inhibition (competitive/non-competitive/mixed), copper-associated effects at the binuclear site, and downstream interception of reactive quinones ^(Nirmal & Benjakul, 2009a, 2010, 2012b). A key caveat for this class is that several phenolic acids can also perturb dopachrome-based readouts, so inhibition claims based solely on absorbance should be interpreted with appropriate blanks and, where possible, orthogonal confirmation.

Hydroxycinnamic acids Ferulic acid (FA) is the most robustly supported pure-compound example in *penaeid* shrimp that is supported by enzyme-focused and product-focused outcomes. In *L. vannamei*, ferulic acid (FA) inhibits PPO in a concentration-dependent manner and translates into reduced melanosis and improved quality attributes during iced storage. In the same study framework, FA outperformed oxygenated ferulic acid (OFA) at matched concentrations, supporting structure-dependent differences within this scaffold. A harsher freeze-thaw/refrigerated-storage model also supported FA as a dose-dependent anti-melanosis treatment ^{(Nirmal & Benjakul, 2012b) (Nirmal & Benjakul, 2010)}. Mech-

anistic inhibition kinetics against cephalothorax PPO classify FA as a reversible non-competitive inhibitor with a reported $K_i \approx 37$ mM, i.e., weak binding in enzymology terms rather than high-affinity target engagement. Accordingly, its preservation efficacy is best interpreted within a broader multi-mode framework that includes downstream redox and quinone-interception effects rather than PPO binding alone (Nirmal & Benjakul, 2012b). By contrast, shrimp-specific evidence for caffeic acid is much less mature and is better treated as fixed-dose inhibitor-panel evidence than as a validated translational lead. In *P. longirostris*, it appears mainly in comparative inhibitor testing using crude PPO preparations, and the available product-level confirmation is limited and insufficiently quantified for robust cross-study benchmarking. Caffeic acid is therefore better cited as assay-conditional evidence of possible activity than as a compound with well-established shrimp anti-melanosis potency (Barbagallo et al., 2012).

Hydroxybenzoic acids Among hydroxybenzoic acids, gallic acid provides useful mechanistic support rather than a strong translational lead. In PPO from deepwater pink shrimp (*P. longirostris*), gallic acid was reported as a reversible mixed-type inhibitor against L-DOPA oxidation with a K_i of approximately 0.96 mM, supporting direct interaction with the crustacean PPO system under the reported assay conditions (Serap Dogan et al., 2016). Beyond dosing phenolic acids as free molecules, a substantial shrimp-facing literature deploys them embedded in biopolymer matrices, where performance reflects a coupled set of effects (barrier properties, antioxidant action, and quinone interception) rather than a clean readout of direct PPO binding. Accordingly, these studies are best framed as phenolic-acid-enabled preservation systems and should not be overinterpreted as evidence that the acid is *per se*, a superior PPO ligand. This system-level pattern is further illustrated by the phenolic-acid chitosan film studies of Liu et al. (2021) and Huang et al. (2022). Although these authors prepared a matched series of composite films containing p-coumaric, ferulic, gallic, vanillic, and salicylic acids at equivalent phenolic loading, only the ferulic- and p-coumaric-based films were advanced to shrimp preservation testing, while the remaining acids were characterized mainly at the level of film properties. Chlorogenic acid (CGA) offers a comparable example. In *L. vannamei* stored under partial-freezing conditions, a CGA-gelatin coating outperformed free CGA across spoilage- and oxidation-related indices, including TVB-N and TBA. Similarly, in *L. vannamei*, covalent grafting of CGA onto chitosan films yielded stronger preservation outcomes than simple physical incorporation, as evidenced by differences in weight loss, pH, TVB-N, total bacterial counts, and sensory quality. Collectively, these cases show that the observed preservation benefits are more convincingly interpreted as emergent properties of the formulated system than as direct evidence

of superior intrinsic PPO affinity of the free phenolic itself (Hu et al., 2020; C. Huang et al., 2022; W. Liu et al., 2021). Finally, a shrimp study in *Fenneropenaeus chinensis* screened “phenolic acid + water-soluble chitosan” formulations and selected water-soluble chitosan + gallic acid as the optimal coating; under 4 °C storage, the treatment is reported to slow pH increase, reduce TVB-N formation and lower TBARS versus control (M. X. Huang et al., 2025). A final methodological caution is warranted for this class. Because several phenolic acids are redox-active and can intercept o-quinones or related intermediates, dopachrome- or absorbance-based PPO assays may confound true catalytic inhibition with altered product chemistry unless appropriate blanks and orthogonal endpoints are implemented (García-molina et al., 2021; Ortiz-Ruiz et al., 2015; Rescigno et al., 2002). Fixed-dose screening datasets should therefore be interpreted as assay- and matrix-conditional rankings rather than as universal potency hierarchies transferable across shrimp species, enzyme preparations, substrates, and storage contexts (Fan et al., 2021; Nirmal et al., 2015; Zolghadri et al., 2019).

Amino acids and derivatives

In contrast to phenolic acids, whose apparent PPO inhibition often merges target engagement with redox and downstream quinone chemistry, nitrogen-containing small molecules introduce a more chemically explicit anti-melanosis framework. Their effects may still combine direct enzyme inhibition with nucleophilic interception or reduction of reactive browning intermediates; meaning visible melanosis suppression can occur even when binding affinity is only moderate.

Thiols In *M. japonicus* PO assays (L-DOPA oxidation), both L-cysteine and glutathione inhibit PPO with competitive kinetics and K_i values ~0.45-0.46 mM under the reported conditions. Partial recovery of inactivated enzyme upon copper acetate addition supports a copper-linked component in this system, consistent with thiols impacting PPO function beyond simple active-site competition. Importantly for interpretation, the same study emphasizes that these thiols can also reduce o-quinones and/or form colorless adducts with intermediates, meaning spectrophotometric “dopachrome” readouts can reflect a coupled enzyme + product-chemistry effect rather than binding alone (Benjakul et al., 2006).

Non-protein amino acid Mimosine is a non-protein amino acid for which shrimp PPO inhibition is explicitly parameterized in a *penaeid* model. Using *L. vannamei* cephalothorax PPO, mimosine was reported as a reversible mixed-type inhibitor of L-DOPA oxidation with $K_i = 3.7$ mM. The same work documents dose-dependent Cu^{2+} reduction and chelating

capacity and reports that it can react with intermediate browning products to yield lower red-brown color formation, supporting a multi-mode framework (enzyme inhibition + microchemical interception) for its anti-melanosis explanation ^(Nirmal & Benjakul, 2011b).

Sulfinic amino acid derivative Hypotaurine (2-aminoethanesulfinic acid) occupies an unusual translational position because patent and peer-reviewed evidence are not directly dose-comparable. The issued patent frames hypotaurine as an in-food anti-browning intervention at low ppm levels, whereas the shrimp papers apply much higher concentrations in dipping solution or coatings. In *L. vannamei*, immersion in hypotaurine solutions (1, 10, and 20 g/L) improved quality relative to the untreated control, with 20 g/L performing best and sensory analysis indicating roughly a 3-4-day shelf-life extension. In a separate coating model, 2% hypotaurine combined with 1% chitosan gave the best preservation performance over 10 days at 4 °C. Because edible tissue residues are not quantified in these studies, the practical comparability of patent and peer-reviewed dose frameworks remains unresolved (M. Chen et al., 2022; Zhou et al., 2022). Because thiols/sulfinates are strong reductants and nucleophiles, they can quench or distract quinone/dopachrome chemistry in standard PPO assays; therefore, “inhibition” datasets are most defensibly discussed with appropriate blanks and orthogonal endpoints and compared across papers only with explicit acknowledgment of assay dependence.

Resorcinols

The benchmark synthetic anti-melanosis agent 4-hexylresorcinol (4-HR; E586) is a high-potency alkyl-resorcinol used as a color-retention/antioxidant additive in foods (European Commission, 2015). Beyond the practical benchmark role of 4-HR, patent literature also suggests that naturally sourced resorcinol-derived leads may have relevance for shrimp melanosis control. However, these observations remain largely patent-based, are not embedded in the peer-reviewed natural products literature and still lack independent validation under standardized shrimp PPO/HdPO assays and modern storage models. Accordingly, they are noted here only as an underexplored translational signal rather than as validated natural anti-melanosis leads (Arthur J. McEvily et al., 1991).

Other natural metabolites

Beyond flavonoids, phenolic acids, resorcinols, amino-acids and their derivatives, shrimp anti-melanosis studies repeatedly invoke organic acids. Importantly, these molecules often act through process chemistry

(quinone reduction/adduction, pH and metal-ion effects) as much as through direct PPO binding, so they should be discussed with explicit attention to readout-dependence and storage context.

Organic acids Organic acids are better treated as co-actives than as stand-alone shrimp PPO inhibitors. In *P. japonicus*, ascorbic acid and citric acid were evaluated alongside other anti-melanosis agents under both non-pressure and high-pressure conditions; under those study conditions, neither acid alone ranked among the most effective melanosis inhibitors. Instead, they appear more consistently as supporting redox, pH, or chelation components in combination systems. Complementary evidence from refrigerated *P. japonicus* also suggests that citric acid performs better than lactic acid, whereas acetic acid has little effect, supporting only modest stand-alone utility for organic-acid dipping (Gökoğlu, 2004; Montero, Lopez-Caballero, et al., 2001).

Kojic acid Kojic acid has one of the more developed shrimp-relevant evidence bases among non-flavonoid natural metabolites, because it is supported by both crustacean PPO inhibition data and whole-product melanosis outcomes. Classical work on plant and crustacean PPOs showed that kojic acid can display mixed-type inhibition in crustacean systems depending on the substrate used. This mechanistic relevance is supported by product-level evidence: in *P. japonicus*, dipping in 0.5% (w/v) kojic acid markedly delayed visible black spot development during chilled storage. When delivered within coatings, however, reported benefits should be interpreted as preservation-system performance rather than as direct evidence of free kojic acid affinity alone; for example, a fully deacetylated chitosan coating containing kojic acid and clove essential oil improved the preservation of *L. vannamei* during cold storage (J. S. Chen et al., 1991; X. Liu et al., 2020; Montero, Lopez-Caballero, et al., 2001). Across structurally defined natural inhibitors in shrimp, the most credible compound-level cases are those in which a named molecule is evaluated both in shrimp-relevant enzymology and in direct storage trials. At present, these criteria are met most consistently by tea catechin-family flavan-3-ols and selected phenolic-acid examples such as ferulic acid, whereas thiols and other reductants often act strongly through quinone-interception chemistry and therefore demand careful assay design and interpretation. In parallel, a large fraction of the “natural compound” literature is better understood as compound-enabled preservation systems, in which product performance reflects barrier, antioxidant, antimicrobial, and browning-microchemistry effects rather than a single inhibitory constant. Fixed-dose screens and dopachrome-based readouts should therefore be treated as assay-conditional rankings, and cross-paper potency comparisons should be made only when enzyme preparation, substrate system, and endpoints are explicitly aligned.

Plant extracts and agri-food byproducts as multi-mode anti-melanosis systems

Across extract-based studies, claims of “SMBS-equivalent” or “4-HR-superior” performance should only be interpreted within matched protocols, because melanosis scales, storage models, dipping ratios, and comparator designs vary substantially across the literature. Beyond purified PPO inhibitors, a large fraction of the shrimp anti-melanosis literature is built around complex extracts from leaves, peels, seeds, and marine biomass. These systems rarely operate as “single-target PPO inhibitors” in the strict enzymology sense. Instead, their storage performance typically reflects stacked mechanisms: Cu chelation and PPO suppression, redox buffering/quinone interception, antioxidant control of lipid/protein oxidation, and antimicrobial action that slows the broader spoilage path that covaries with blackspot development. Consequently, extract studies are most defensible when they couple a chemical fingerprint (at minimum LC–MS/UV profiling) with PPO-relevant readouts and/or direct cold-chain validation against industrial comparators.

Leaf extracts

A recurring practical lesson is that extract “activity” is often limited not only by composition but also by matrix interference and delivery (Xie & Schaich, 2014; Yeo & Shahidi, 2019). In leaf-derived extracts, dechlorophyllization can both improve interpretability of absorbance-based assays and enrich phenolic fractions that show stronger copper-chelating/PPO-inhibitory signatures, translating into clearer storage effects.

Composition-controlled leaf phenolics Among leaf extracts, cashew leaf extract provides one of the more chemically and functionally anchored benchmarks. In a comparative evaluation of several leaf extracts, cashew leaf extract was among the most effective at delaying melanosis relative to the untreated control, reducing black spot progression during chilled storage (up to ~12 days at 4 °C). Under the same study conditions, its anti-melanosis performance was discussed against SMBS (~1.25%) as the conventional benchmark. Mechanistically, the reported protection was associated with PPO inhibition and copper-chelating capacity, alongside broader quality benefits across microbiological, chemical (TVB-N/TBARS), and sensory endpoints (Saeleaw & Benjakul, 2019b). Guava (*Psidium guajava*) leaf extract is one of the better-supported leaf systems because the same study combines dechlorophyllization, PPO-related assays, and refrigerated storage validation against SMBS. Olatunde et al. (2021) prepared ethanolic guava

leaf extracts using different chlorophyll-removal strategies and reported that the sedimentation-dechlorophyllized fraction showed the strongest PPO-inhibitory and Cu-chelating activities. Crucially, in refrigerated *L. vannamei* storage trials (0.5-1% extract; 12 days at 4 °C), guava leaf treatment also directly delayed visible melanosis development relative to the untreated control, while concurrently improving oxidative and spoilage-related indices. Under the same conditions, SMBS (1.25%) served as the conventional benchmark, allowing the anti-melanosis performance of the guava system to be interpreted against an industry-standard reference (Olatunde et al., 2021). A further precedent reinforcing the same principle, namely that phenolic enrichment can be advantageous, comes from the tea-leaf extract literature. Nirmal & Benjakul (2011) explicitly assessed ethanolic green tea extract with prior chlorophyll removal, reporting stronger antioxidant and copper-chelating properties, clear inhibition of shrimp PPO, and improved storage performance in *L. vannamei* during iced storage; under some conditions, melanosis control was reported to be comparable to that obtained with SMBS (12.5 g/L) (Nirmal & Benjakul, 2011d). A related but distinct green-tea study used green tea extract (GTE) in combination with modified-atmosphere packaging (MAP). Because this study used a different storage model from the iced-storage work, its results should be interpreted separately: under refrigerated MAP, shrimp were treated with GTE at 1 g/L with or without ascorbic acid (AA) at 0.05 g/L, whereas in the iced-storage model, GTE at 0.1% (1 g/L) was combined with AA at 0.005 or 0.01% (0.05 or 0.1 g/L). Thus, claims about enhanced PPO inhibition and melanosis retardation should be assigned to the corresponding storage model rather than generalized across both studies. Accordingly, the latter study should underpin claims regarding enhanced PPO inhibition and melanosis retardation (Nirmal & Benjakul, 2011c). A more informative evidence tier comes from head-to-head screening within a shared cold-storage protocol, where dose and comparator are held constant across candidates. In a refrigerated *L. vannamei* study, Ahmad et al. (2023) evaluated dechlorophyllized ethanolic extracts from Thai indigenous leaves, including soursop (*Annona muricata*), noni (*Morinda citrifolia*), and Jik (*Barringtonia acutangula*) and reported efficacy across 0.25-1%, with 1% providing the most consistent protection during 12 days at 4 °C relative to SMBS (1.25%). Such within-study ranking helps distinguish between the general claim that “extracts can work” and the more useful question of which extract, at which dose range appears most credible, although mechanistic support still relies largely on activity-based assays such as PPO inhibition and copper chelation rather than on full kinetic characterization against shrimp PPO (Ahmad et al., 2023). *Lonicera japonica* Thunb. extract (LJT) is one of the stronger leaf-extracts because it explicitly couples PPO-relevant readouts with a direct commercial comparator. In the underlying study, *L. vannamei* were dipped in 16 mg/mL LJT or 20 mg/mL 4-HR for 15 min at 4 °C (shrimp:solution 1:2,

w/v) and then stored at 4 °C. LJT inhibited shrimp PPO and chelated Cu²⁺ in a dose-dependent manner, with the inhibitory response increasing up to the tested range and approaching a plateau beyond ~16 mg/mL. For melanosis, the key outcome was comparative rather than absolute: no visible melanosis was detected at day 0 (score = 0), both treated groups remained significantly below the untreated control throughout storage, and no significant melanosis was observed in the LJT and 4-HR groups during the first 3 days. By day 6, however, the LJT group showed significantly less melanosis than the 4-HR group, and by day 9 only slight blackening was described in the LJT-treated shrimp, supporting a stronger anti-melanosis effect than 4-HR under that matched protocol. The same study also reported lower TVC, TVB-N, and TBARS values for LJT than for 4-HR. Importantly, however, this remains extract-level evidence: although the study provides PPO-relevant inhibition and cold-storage validation, it does not resolve purified active constituents, compound-level IC₅₀/K_i values, or substrate-resolved shrimp-PPO/HdPO kinetics, so it should be interpreted as strong performance-first evidence rather than definitive mechanistic proof (Zheng et al., 2025).

Leaf extracts as a barrier component Some leaf-extract systems are better understood not as intrinsically stronger inhibitors, but as preservation platforms whose apparent efficacy depends strongly on delivery, penetration, and retention of active phenolic fractions within the shrimp matrix. The chamuang literature illustrates this especially well: the extract alone slows melanosis, but performance improves further when transport and persistence are engineered through PEF, vacuum impregnation, MAP, or plasma-based process combinations (Shiekh et al., 2019). Notably, several delivery-enabled chamuang studies originate from the same research group, which strengthens internal coherence but limits independent cross-laboratory validation. Follow-up work makes the transport constraint explicit by progressively shifting the intervention from “extract-only” toward engineered exposure and persistence. In *L. vannamei*, pulsed electric field (PEF) pretreatment followed by soaking in 1% chamuang leaf extract and storage under CO₂-modified atmosphere packaging reduced melanosis more effectively than extract alone across refrigerated storage, consistent with enhanced mass transfer and altered surface redox/microbial dynamics rather than a simple change in inhibitor strength (Shiekh et al., 2020). The same logic is reinforced when the extract is driven into tissue by vacuum impregnation (reported at 5 kPa absolute) using 10-20 g/L extract solutions under MAP, extending preservation in a longer cold-chain window (Shiekh, Benjakul, et al., 2021). A parallel “in-package” axis appears with high-voltage cold atmospheric plasma, where shrimp pre-soaked with 0.5-1% chamuang leaf extract and treated for 10 min under Ar/air (80:20) maintained lower melanosis throughout 15 days at 4 °C; when

combined with PEF (15 kV/cm; 800 pulses; 697 kJ/kg) and 2% extract prior to plasma, melanosis control was further strengthened over 18 days at 4 °C (Shiekh & Benjakul, 2020). Finally, when PEF + 2% extract precedes plasma treatment, the reported shelf-life and microbial control improve further, reinforcing that the phenotype is governed by process-extract-atmosphere, not necessarily extract “intrinsic potency” (Shiekh, Zhou, et al., 2021). The soursop leaf extract literature provides a useful counterpart to the chamuang series because it also progresses from extract-only treatment toward delivery-enabled preservation designs (Ahmad et al., 2023). Subsequent studies then shift the lever from “what’s in the extract” to “how much reaches the reactive sites”: PEF pretreatment (reported to create pores on shell/membrane surfaces that facilitate passage of solutes) is paired with SLE and vacuum impregnation to enhance uptake without altering extract chemistry, reaching a lowest alteration on the quality after 15 days with the SLE1-VI (Ahmad et al., 2024). And, in a separate barrier design, PEF + 1% SLE ($\pm$ VI) followed by MAP extends acceptability to 18 days at 4 °C while keeping TVC at $\sim$ 5.9 log CFU/g (Ahmad et al., 2025). Together, these studies suggest that the strongest outcomes in this literature are often barrier-engineered preservation phenotypes rather than simple reflections of extract intrinsic potency.

Additional leaf matrices Beyond the chemically anchored, comparator-benchmarked leaf systems, more studies support a more pragmatic claim: leaf phenolic preparations can shift cold-chain quality trajectories, even when they cannot be interpreted as well-resolved shrimp-PPO inhibitor systems. In *Litopenaeus vannamei*, curry (*Murraya koenigii*) and moringa (*Moringa oleifera*) leaf extracts applied as simple dips (1% each, or a 1:1 blend; 15 min) reduced melanosis progression over an extended chilled-storage window while simultaneously improving spoilage/oxidation and microbial indices, useful storage-led validation, but notably without industrial benchmarking (SMBS/4-HR) or extract characterization that would enable mechanistic attribution (Soni et al., 2021). In a more application-ready format, Myrtaceae leaf extract powders (*Cleistocalyx operculatus*, *Psidium guajava*, *Rhodomyrtus tomentosa*) bring stronger process-facing detail (TPC/antioxidant capacity; antibacterial zones against spoilage isolates) and a direct comparator: as 0.25-0.5% dips during cold storage, they decelerated melanosis alongside TBARS/TVB-N and microbial growth, yet the study’s own head-to-head data show 1.25% SMBS still outperforms the powders on melanosis at the end of storage, reinforcing that the benefit is best read as multi-mode quality retardation (Ha et al., 2022). Regional herb extracts extend this “quality-panel-driven” tier: *Houttuynia cordata* extract at 0.025% (w/v) slowed microbial growth and reduced pH/grey-value/TBARS changes during 7-day cold storage, with several endpoints reported as not significantly different from 1.25% SMBS (D. T. A. Phan et al., 2019). Similarly, *Persicaria odorata*

ethanolic leaf extract was optimized against SMBS for frozen storage (-21 °C, 5 days) and improved microbiological and chemical freshness indices versus untreated controls, again positioning the extract as a preservative system rather than a clean enzymology claim (T. D. Nguyen et al., 2023). Rosemary extract (E392) should be treated primarily as a translationally relevant antioxidant system with regulatory familiarity, whereas mechanistic work on rosmarinic-acid-containing systems may provide only indirect support for hemocyanin/phenoloxidase-linked pathways. Within this context, rosemary extract is relevant because it is already authorized in the EU as an antioxidant for processed fish and fishery products including molluscs and crustaceans, with maximum permitted levels set at 15 mg/kg for products with fat content ≤10% (and 150 mg/kg on fat basis when fat content >10%), expressed as the sum of carnosic acid and carnosol (European Commission, 2013). In EU organic processing, E392 is also listed among permitted additives only when derived from organic production, which further underscores its “real-world” relevance as a biobased preservative option. Shrimp studies that incorporate rosemary extract typically position it as a multi-mode quality stabilizer (redox buffering/oxidation control, often alongside acidification) rather than as a well-parameterized shrimp-PPO inhibitor. For example, a chilled *L. vannamei* study reported that combining citric acid with rosemary extract improved microbial and oxidative indices and preserved quality, including melanosis scoring, over 8 days at 4 °C (Zeng et al., 2026; X. Zhang et al., 2021). In a sulphite-reduction framing, *A. antennatus* was treated with rosemary or green-tea extracts (2 g/100 mL) and SMBS (1.25 g/100 mL), where extract-sulphite combinations outperformed extracts alone, supporting the use of plant extracts as co-adjuvants to lower sulphite reliance (Yatmaz & Gokoglu, 2016). Overall, rosemary-based interventions are best interpreted as process-relevant antioxidant systems with possible hemocyanin/phenoloxidase-linked effects, but with limited shrimp-specific enzymology (IC_{50}/K_i vs shrimp PPO/HdPO) available for rigorous cross-study ranking. More recent mechanistic work on rosmarinic-acid-containing natural preservative systems suggests that hemocyanin-associated pathways may also be relevant during shrimp quality deterioration (Zeng et al., 2026). Altogether, the leaf-extract literature supports a performance-first conclusion: when extracts are composition-controlled (especially after dechlorophyllization) and tested under matched cold-storage protocols, effective dose windows commonly fall around ~0.5-1.0% (w/v) for crude/dechlorophyllized leaf extracts or ~5 g/L for selected tea extracts, and these treatments can markedly suppress melanosis relative to untreated controls and, in the strongest within-study comparisons, approach the performance of 1.25% SMBS; direct superiority over 4-HR has been shown only in isolated head-to-head cases, rather than as a generalizable rule. However, this should not be interpreted as proof of interchangeable efficacy across the literature: melanosis scales, storage formats (ice vs 4 °C), shrimp form,

dipping protocols, and comparator designs vary substantially among studies.

Seed, peel, and byproduct extracts

Agro-industrial byproducts are attractive because they are abundant and often rich in tannins, ellagitannins, and related polyphenols with chemical properties relevant to melanosis control, including copper binding, redox buffering, and antimicrobial activity. An example is a pomegranate peel extract (PPE). In *L. vannamei*, Basiri et al. (2015) tested PPE at 10-20 g/L and explicitly connected shrimp PPO activity to storage outcomes during ~10 days at 4 °C, reporting lower melanosis alongside improvements in oxidative/spoilage indices (e.g., TBARS and volatile base metrics); Although the mechanistic enzymology is limited, the study remains informative because it directly links PPO-related measurements to cold-storage performance (Basiri et al., 2015). Grape seed extract (GSE) is the clearest early reference point for condensed-tannin (proanthocyanidin-rich (Unusan, 2020)) anti-melanosis strategies in shrimp, precisely because the intervention is simple, and the dose-response is explicit. In deep-water pink shrimp (*P. longirostris*), Gokoglu & Yerlikaya (2008) prepared aqueous dipping solutions containing 7.5 and 15 g/L GSE and applied a standardized 1-min dip at 15 °C (shrimp:solution 1:4) before refrigerated storage at 4 °C for 3 days; melanosis was tracked by a sensory black spot scale alongside instrumental color and the 15 g/L treatment was the only condition that remained acceptable through day 3, while controls progressed to heavy melanosis by the end of storage (Gokoglu & Yerlikaya, 2008). A later iced-storage study in Pacific white shrimp (*L. vannamei*) tested the same practical concentration window (7.5, 15 g/L) over 9 days on ice, again reporting concentration-dependent suppression of melanosis with parallel improvements in freshness indices (retarded rises in pH, TVB-N, and total bacterial counts), with 15 g/L consistently outperforming 7.5 g/L on melanosis score and quality endpoints (Sun et al., 2014). Lead (*Leucaena leucocephala*) seed extract is one of the few seed-derived cases where shrimp PPO inhibition is quantified and linked to iced-storage performance. Nirmal & Benjakul quantified both phenolics and mimosine in the extract powder and showed dose-dependent PPO inhibition across 0.05-1% (w/v). In *L. vannamei*, 0.5% (w/v) gave the best melanosis control across 12 days, outperforming 1.25% SMBS in their scoring at day 12 (Nirmal & Benjakul, 2011a). Mango seed kernel extract (MSKE) is a waste-to-value example with explicit shrimp-PPO anchoring. In *L. vannamei*, Sai-Ut et al. (2020) prepared an ethanolic MSKE and demonstrated dose-dependent inhibition of partially purified shrimp PPO, reaching ~90% inhibition at 5 g/kg in a L-DOPA assay. In parallel cold-chain validation, shrimp were dipped in 0.1-0.5% (w/v) MSKE solutions (10 min, 4 °C) and stored on ice for 12

days; MSKE reduced melanosis scores and decelerated coupled spoilage path, including lower TVB-N and TBARS relative to untreated controls (Sai-Ut et al., 2020). A particularly strong agri-food byproduct fix is the avocado by-product study because it combines a low treatment dose with an explicit industrial comparator. *L. vannamei* shrimp were immersed for 15 min at 4 °C in 0.025% (w/v) ethanolic extracts (shrimp:solution 1:2, w/v) prepared from nine avocado seed/peel extracts, then stored for 8 days at 4 °C against 1.25% (w/v) SMBS and water controls. Six of the nine extracts significantly reduced both melanosis and lipid oxidation relative to SMBS. After 8 days, the best-performing treatments retained mean gray values of 47.0 ± 0.7 to $57.3 \pm 0.4\%$, compared with $39.8 \pm 0.4\%$ for SMBS, with the Nuoc peel extract performing best ($57.3 \pm 0.4\%$). All extracts also lowered TBARS relative to SMBS and control, and Nuoc peel gave the lowest final TBARS (3.3 ± 0.1 mg MDA/kg). Mechanistically, the study is useful because it links performance to chemistry: total phenolic content ranged from 44.5 ± 1.1 to 144.7 ± 1.9 mg GAE/g DW, and melanosis inhibition correlated most strongly with TPC and DPPH activity ($r > 0.80$), followed by tyrosinase inhibition and FRAP ($r > 0.50$). The strongest extracts also exceeded 50% tyrosinase inhibition at 100 µg/mL, with reported IC₅₀ values of 48.0-94.6 µg/mL for the most active fractions. This makes avocado by-products a strong “multi-mode” example, although the enzymology here is based on a tyrosinase proxy assay rather than shrimp PPO kinetics (D. T. A. Phan et al., 2021). Similarly, *Perilla frutescens* hydro distillation residue is one of the better recent “waste-stream valorization” examples because the authors move from compositional profiling to storage validation in the same study. They identified 14 bio compounds by HPLC-MS and selected the PF-E-2 fraction based on its superior antioxidant and tyrosinase-inhibition profile. In the application step, 0.25% (w/v) PF-E-2 outperformed 0.1% and performed equivalently to 0.5% (w/v) and 1.25% (w/v) SMBS during an initial 12-h refrigerated preservation screen and was therefore chosen for the long-term test. Over 8 days at 1-3 °C, shrimp treated with 0.25% PF-E-2 showed no statistical difference from SMBS for melanosis development, pH, lipid peroxidation, microbial counts, nitrogen-based spoilage indices, or texture/stiffness. Importantly, this study merges compositional characterization of a valorized residue stream with direct cold-storage benchmarking, supporting SMBS-comparable performance under the tested conditions. As with the avocado work, however, the mechanistic enzymology is built around tyrosinase inhibition plus storage endpoints, not full shrimp-PPO kinetic characterization (Q. Van Nguyen & Phan, 2026).

Algal extracts and formulation-style deployments

Among algal-derived interventions, formulation-based systems provide a stronger translational evidence tier than extract-only reports. A particularly informative example is the phlorotannin-alginate system developed from the brown seaweed *Nizimuddinia zanardinii*. After solvent screening, the methanolic extract yielded the highest phlorotannin content (19.14 ± 0.65 mg phloroglucinol/g), together with strong antioxidant activity ($98.95 \pm 0.74\%$, DPPH inhibition) and $73.44 \pm 1.64\%$ copper-chelating activity. In direct *L. vannamei* shrimp-PPO assays, the combination of 2% phlorotannins (PT) + 1% alginate (AG) achieved $84.51 \pm 2.15\%$ inhibition, exceeding 1% phlorotannins alone ($68.37 \pm 0.65\%$), 1% alginate ($27.39 \pm 1.05\%$), and 1% ascorbic acid ($72.43 \pm 1.42\%$). For storage validation, whole shrimp was dipped for 10 min at 4 °C (shrimp:solution 1:2, w/v) in 1% PT, 1% PT + 0.5% AG, or 2% PT + 1% AG, using 1.25% SMBS as a positive control. Over 16 days of ice storage, the best treatment (2% PT + 1% AG) reduced the melanosis score to 2.67, compared with 6.93 in the untreated control, while also improving pH, lipid oxidation, and microbial quality indices (Sharifian & Bitar, 2025). A simpler extract-only precedent is provided by *Sargassum horneri*, where a 0.2% extract fraction (sample III; 49.88% total phenolics) produced the lowest melanosis scores during 10 days at 4 °C, outperformed both 0.1% extract and 0.1% kojic acid, and extended shelf life to 8 days under the reported conditions (Fang et al., 2021). Importantly, the algal literature remains dominated by extract-, fraction-, or formulation-level evidence. Thus, although these studies demonstrate PPO-relevant inhibition and clear cold-storage benefits, they do not yet provide the compound-level enzymology required for rigorous mechanistic ranking against defined shrimp PPO inhibitors.

Protease-based pretreatment benchmark: ficin

Ficin appears in the shrimp melanosis literature primarily as a pretreatment additive rather than as a defined PPO inhibitor. As a plant-derived protease, its relevance here is not as a classical active-site-directed anti-melanosis agent, but as an example that measurable black spot suppression can also arise from non-classical pretreatments whose efficacy is evaluated at the product level rather than through compound-level PPO kinetics. In a quantitative melanosis-kinetics study on *Pleoticus muelleri* stored at 4 °C for 96 h, pretreatments were compared using percentage darkened area (PDA) under defined dose ranges: SMBS (0.6-1.25% w/v), 4-HR (0.0025-0.01% w/v), and ficin (0.5-2% w/v). Ficin reduced darkening relative to the untreated control, with PDA decreasing from 30.32% (control) to 12.93% at 24 h and from 44.05% to 18.09% at 96 h in the 2% ficin treatment; the corresponding apparent zero-order rate constant decreased from 0.18 1/h (control) to 0.068 1/h. However, the

best performance in that dataset was still obtained with 4-HR, not ficin, indicating that ficin is better treated here as a practical anti-melanosis pretreatment benchmark than as a potent reference inhibitor (Loubes et al., 2012). A second, patent-based mechanistic thread links ficin preparations to separable small-molecule PPO-inhibitory activity. In the patent, crude ficin was ultrafiltered to generate a ficin-free YM5 eluate, which was then assayed in a model system (mushroom PPO + L-DOPA, pH 6.5). The patent reports that crude ficin, the 0.45 μ m filtrate, and the ficin-free YM5 eluate showed comparable PPO inhibition and therefore argues that the inhibitory activity is not attributable to ficin protease itself. This same patent stream then moves from ficin-derived preparations to defined resorcinol-type inhibitors, including Formula IV (2,4-dihydroxyphenylpropionic acid) and 4-hexylresorcinol, thereby providing a conceptual bridge from extract/preparation-level observations to the pure-compound anti-melanosis scaffold (Compositions and methods for inhibiting browning in foods using resorcinol derivatives) (Arthur J. McEvily et al., 1991). Accordingly, Table 3 compiles the key product-level melanosis outcomes as reported in each study, alongside dose/application, storage format, and industrial comparators where included. Overall, the extract and by-product literature support a pragmatic, application-first demonstration conclusion: many polyphenol-rich systems can measurably delay black spot development under cold-chain conditions, often while simultaneously improving broader quality trajectories (oxidation, spoilage indices, and microbial growth). However, because studies differ widely in melanosis scales, shrimp form, storage format (ice vs 4 °C), dipping ratios/exposure, and whether an industrial benchmark is included, cross-study “ranking” is rarely defensible. Notably, at least one citrus peel extract report (lemon/pomelo peel extracts in Indian white prawn) has been retracted, with the retraction notice stating that an unattributed reused/edited figure rendered the article’s conclusions unreliable; we therefore exclude it from the evidentiary core and treat similar single-study claims cautiously unless supported by head-to-head benchmarking and transparent reporting. Mechanistic assays (PPO inhibition, copper chelation, antioxidant/antimicrobial read-outs) should thus be treated as supportive evidence that helps rationalize product outcomes rather than as a substitute for cold-storage validation.

Essential oils and volatile compounds

Moving from non-volatile plant extracts to volatile-rich botanical systems, essential oils provide a closely related but mechanistically distinct evidence block, in which phenolic enrichment may still be advantageous, yet anti-melanosis performance is additionally shaped by volatility, antimicrobial potency, and delivery format. Essential oils and volatile-rich systems represent a distinct evidence class within the

shrimp anti-melanosis literature. Unlike purified phenolics or other defined low-molecular-weight inhibitors, these interventions are usually tested as dips, coatings, films, or nanoemulsions; therefore, their efficacy should be interpreted primarily as a multi-hurdle preservation effect, in which melanosis retardation may arise from concurrent attenuation of PPO-associated browning, antioxidant activity, antimicrobial action, and barrier-mediated slowing of *post-mortem* deterioration. This distinction is important for evidence of grading, because storage performance is often convincing even where direct shrimp-PPO mechanistic resolution remains limited. Within this group, cinnamon-derived systems provide the clearest bridge between product preservation and PPO-linked mechanism. In *L. vannamei*, cinnamaldehyde was evaluated at 1 and 5 g/kg during chilled storage at 4 °C; residual PPO activity decreased with increasing cinnamaldehyde concentration, kinetic analysis identified cinnamaldehyde as a non-competitive inhibitor for L-DOPA oxidation by shrimp PPO, and the treatment extended shelf life to 8 days (Mu et al., 2012). A later study reinforced this PPO-linked signal at the formulation level: cinnamon essential oil incorporated into an electrospun polyvinyl alcohol/chitosan film directly inhibited PPO and spoilage bacteria from Pacific white shrimp, with a reported PPO inhibition of $57.52 \pm 3.33\%$ after 7 h at 4 °C (M. Zhang et al., 2024). Taken together, cinnamon is one of the few volatile-rich systems in literature that can be discussed not only through quality preservation, but also through explicit shrimp-PPO-related measurements. Oregano and clove are also supported by quantitatively useful shrimp studies, although their evidence remains more strongly anchored at the *in situ*/formulation level than at the level of purified target kinetics. In a comparative evaluation of four commercial essential oils, oregano essential oil (OEO) and clove-leaf essential oil (CLEO) were selected for cold-storage testing in *L. vannamei*. On day 10, treatment with 2% OEO and 2% CLEO reduced total viable counts from 9.05 log CFU/g in the control to 8.18 and 8.34 log CFU/g, respectively, while melanosis ratio decreased from 38.16% in the control to 28.98% with OEO and 26.35% with CLEO. The same study also reported inhibition of PPO increase during storage, and the combined treatment (1% OEO + 1% CLEO) gave the best overall preservation outcome. These numbers make oregano/clove one of the strongest quantitative product-level precedents in the essential-oil block, even though the data still do not amount to full kinetic characterization against purified shrimp PPO (Y. F. Qian et al., 2022). The clove literature is further strengthened by coating studies. In *L. vannamei* shrimp, carboxymethyl cellulose (CMC) coatings containing clove oil nanoemulsion were tested at 10, 20, and 30 mg/mL during 10 days at 4 ± 0.5 °C, and the treatment improved melanosis, texture, and sensory acceptability while suppressing increases in volatile nitrogen indices and lipid oxidation markers (Rezaei et al., 2021). In another study, fully deacetylated chitosan coating containing clove essential oil together with kojic acid significantly retarded melanosis and color

deterioration during 15 days of cold storage. These studies support clove as a quantitatively credible anti-melanosis system, but again mainly as a preservation platform rather than as a mechanistically resolved single-agent PPO inhibitor. For citrus-derived oils, the most persuasive data come from shelf-life extension studies in *P. longirostris* rather than from direct PPO assays. In *P. longirostris*, chitosan film combined with orange peel essential oil prolonged shelf life to 15 days, compared with 10 days for chitosan alone and 7 days for the untreated control (Alparslan & Baygar, 2017). A related gelatin-based coating enriched with orange peel essential oil similarly extended shelf life by almost 8 additional days relative to the uncoated control, reaching approximately 15 days versus 7 days in controls and 12 days for gelatin alone (Alparslan et al., 2019). Orange leaf essential oil incorporated into gelatin coating was also reported to improve melanosis control and overall quality during a 14-day refrigerated-storage trial (Alparslan et al., 2016). Collectively, these data justify inclusion of citrus oils in the subsection, but they should be framed as citrus-based volatile preservation systems rather than examples of direct shrimp-PPO inhibition. Thyme-based systems also deserve inclusion, although the evidence is predominantly formulation-driven. A sweet-potato-starch coating containing thyme essential oil was developed with 0, 2, 4, and 6 g per 100 g thyme oil, and the coated shrimp showed reduced lipid oxidation, freshness loss, and melanosis during refrigerated storage (Alotaibi & Tahergorabi, 2018). In a separate study, CMC coating containing Shirazi thyme (*Zataria multiflora*) nanoemulsion at 10, 20, and 30 mg/mL was tested for 10 days at 4 ± 0.5 °C; the treatment improved microbial and oxidative quality, melanosis, and sensory acceptability, although 30 mg/mL adversely affected color coordinates and acceptability, making the lower concentrations more practical (Rezaei et al., 2023). Thus, thyme is supported by clear dose ranges and storage times, but the current evidence still supports its discussion more strongly as a volatile-rich preservation system than as a direct PPO inhibitor. In a 2024 study using African basil (*Ocimum gratissimum*), shrimp were stored for 16 days at 4 ± 0.5 °C with formulations based on this essential oil, either with or without cashew gum. The nanoemulsified system containing cashew gum produced particles approximately threefold smaller than the corresponding formulation without gum and showed more pronounced inhibition of melanosis: by day 16, melanosis was still classified as mild in the nanoemulsion-treated shrimp, compared with moderate in the non-optimized emulsion and severe in untreated controls. Chemically, the oil was dominated by eugenol (47.31%) and 1,8-cineole (30.74%), which provides a plausible volatile basis for its preservation performance (Araujo et al., 2024). At the level of signature volatile compounds, the literature also contains a smaller but useful mechanistic bridge. Besides cinnamaldehyde, biodegradable active films containing carvacrol, citral, or α -terpineol were evaluated in *L. vannamei*, and all essential-oil-compounded films prevented melanosis while also

reducing quality deterioration. Within that study, films containing 6% citral showed particularly favorable film characteristics, whereas citral and carvacrol also stabilized muscle protein conformation and delayed textural deterioration ^(Laorenza & Harnkarnsujarit, 2021). Likewise, a chitosan-carvacrol coating was reported to delay melanosis formation, reduce ΔE change, and improve shrimp texture and sensory properties. These studies strengthen the “volatile compounds” component of the subsection by showing that some dominant constituents of oregano-, thyme-, citrus-, or clove-type systems are associated with measurable anti-melanosis benefits in shrimp models. A narrower secondary tier can also be recognized, but it should be framed cautiously rather than blended with the higher-confidence core. A recent example is dill seed essential oil, which has been evaluated in refrigerated *L. vannamei* as an emulsion system (100-2000 ppm; 12 days) with readouts centered on physicochemical, microbiological, sensory, and color/browning-related quality changes. This makes dill a useful preservation-first addition to the volatile-rich evidence block, but not yet a strong mechanistic anchor for direct shrimp-PPO inhibition. Likewise, not every oil screened *in vitro* warrants equal weight in a translational anti-melanosis review: in the four-oil comparative study that included tea tree and wild orange essential oils, oregano and clove were the systems advanced to *in situ* shrimp validation, whereas tea tree and wild orange remained secondary screening-level evidence. Essential oils should not be discussed as a single mechanistic category. Cinnamon provides the clearest PPO-linked evidence, whereas oregano and clove are currently stronger as product-level preservation systems and selected volatile constituents such as citral and carvacrol emerging as mechanistically plausible leads. This distinction matters because some systems inform inhibitor discovery, whereas others are mainly useful as applied preservation tools.

Conclusion and future trends

Translational outlook: TCP and TPP for natural shrimp anti-melanosis agents

To move the field from descriptive screening toward application-relevant prioritization, we propose an author-defined translational framework composed of a Target Candidate Profile (TCP) and a Target Product Profile (TPP) for next-generation natural shrimp anti-melanosis agents. In this review, the TCP is used to describe desirable attributes of the active candidate, whereas the TPP is used to describe desirable attributes of the final treatment concept under shrimp-processing conditions. Importantly, the numerical values and criteria proposed below should be interpreted as author-defined target ranges and prioritization aids, not as formal regulatory thresholds or universally validated cut-offs. Rather, they are intended to help distinguish candidates that are merely

bioactive from those that appear more plausible for translation into industrial anti-melanosis use.

Within that precision, the central translational question is not whether a natural treatment reduces melanosis relative to an untreated control, but whether it can approach benchmark-level performance at a dose, residue, sensory burden, and cost compatible with commercial shrimp processing. This distinction matters because current industrial comparators are not merely academic controls; they define highly efficient translational use-window. In comparative shrimp work, 4-HR was reported as highly effective at around 100 mg/kg shrimp, whereas SMBS required 1 g/kg under the same experimental logic; internationally, 4-HR is also framed as a low-residue melanosis inhibitor, with Codex listing a treatment level of 50 mg/kg for crustaceans and expected residues <1 mg/kg, while EFSA considered 4-HR toxicologically acceptable for crustaceans provided residues in edible tissue do not exceed 2 mg/kg (Guandalini et al., 1998).

Against that benchmark, the current evidence base is still insufficient to identify a natural candidate that matches benchmark performance, dose efficiency, and practical usability at the same time. Many natural extracts, essential-oil systems, and formulation-based approaches do reduce melanosis relative to untreated controls, but very few have demonstrated the combination of near-complete visual protection, low applied dose, low residue burden, and acceptable sensory performance that would be required for realistic adoption in raw or cooked/chilled Head-On Shell-On (HOSO) products. This is particularly important because even limited residual blackening may remain commercially unacceptable, especially in product categories where visual cleanliness is a primary purchase criterion. Thus, the practical challenge is not simply to identify “active” natural treatments, but to find candidates that compete with the translational efficiency of 4-HR (Guandalini et al., 1998) and, where relevant, SMBS.

A useful distinction therefore emerges between complex extracts and defined natural compounds. Complex extracts may offer an immediate clean-label narrative and can perform well as multi-component preservation systems, but they are also more vulnerable to high-dose dependency, batch variability, flavor carry-over, bitterness, staining, and poorly resolved exposure profiles. A useful illustrative analogy is rosemary extract (E392): although it represents an established natural antioxidant system with regulatory familiarity, it usefully illustrates some translational limitations of complex extracts, namely compositional complexity, higher dose burden, and greater risk of sensory, or color interference compared with a defined high-potency compound. By contrast, defined natural compounds are more attractive when the goal is to achieve PPO-directed efficacy at a substantially lower chemical load, thereby minimizing sensory, color, and toxicological burdens. In

this context, the most valuable natural leads are unlikely to be those that merely produce a statistically significant effect versus control, but rather those that can move into a low-dose application window while preserving efficacy under realistic storage conditions. In practice, the key distinction is whether a treatment remains effective at low applied dose under realistic storage conditions, rather than merely showing statistical activity against an untreated control.

To move the field from descriptive screening toward application-relevant experimental settings and lead selection, we propose a Target Candidate Profile (TCP) for next-generation natural anti-melanosis leads. In addition, a Target Product Profile (TPP) is defined because successful translation will ultimately be determined not only by the intrinsic properties of a candidate molecule, but by the performance of the final treatment concept under commercial shrimp-processing conditions. Accordingly, the TCP captures the desired attributes of the active candidate at the molecular level, whereas the TPP defines the functional requirements of the end-use solution, including (near-)complete melanosis prevention, low-dose applicability, minimal sensory and visual interference, acceptable residue levels, operational compatibility with dipping or coating workflows, and economic feasibility. These provisional benchmarks are proposed as author-defined translational screening gates intended to prioritize candidates with realistic application potential, rather than formal regulatory cut-offs.

Target Candidate Profile (TCP) for natural shrimp anti-melanosis leads

- **Precision inhibition:**

- o **Minimum:** measurable inhibition of shrimp PPO/PO activity using a defined enzyme preparation and appropriate blanks/controls and $IC_{50} < 1$ mM. **Ideal:** IC_{50} in the 10-50 μ M range and, where feasible, K_i/K_r values in the sub-mM to low- μ M range against shrimp PPO and/or HdPO.

- **Supportive functional traits:**

- o **Minimum:** assessment of at least one complementary mechanism relevant to melanosis control, such as copper chelation, copper reduction, quinone interception, or antioxidant capacity. **Ideal:** supportive copper-active and antioxidant effects comparable to reference systems such as ascorbic acid, Trolox, EDTA, or kojic acid (e.g., >50% effect in aligned assays).

- **Physicochemical fitness:**

- o **Minimum:** sufficient aqueous/process compatibility for dipping or coating applications. **Ideal:** moderate lipophilicity, for example logP 1–4,

supporting cuticle interaction, formulation compatibility, and limited migration into edible tissues.

- **Residue and safety positioning:**

- o **Minimum:** no obvious structural alerts for genotoxicity or unacceptable food-safety risk, supported by early *in silico* and *in vitro* de-risking. **Ideal:** residue potential compatible with edible-tissue levels below 2 mg/kg, with <20 mg/kg used as an early triage boundary, and a toxicological profile compatible with food-additive assessment, optimally providing an ADI (Acceptable Daily Intake) >0.5 mg/kg body weight/day.

- **Environmental profile:**

- o **Minimum:** no obvious persistence, bioaccumulation, aquatic-toxicity, or endocrine-disruption alerts. **Ideal:** favorable biodegradability and low aquatic toxicity (e.g., EC₅₀ >50 mg/L) in standard screening assays, for example Daphnia, algae, or fish-embryo models.

- **Sensory liability:**

- o **Minimum:** no strong visible color, odor, or taste interference at the intended working concentration. **Ideal:** minimal absorbance in the 400-700 nm range and no detectable adverse effects on shrimp odor, taste, or appearance in sensory testing.

- **Scalability and economic feasibility:**

- o **Minimum:** plausible access through extraction, fermentation, synthesis from natural precursors, or byproduct valorization, with < 1500 €/kg representing a preliminary upper feasibility boundary depending on dose efficiency.

Ideal: compatibility with industrial production at approximately 10-20 t/year and cost of goods in the low hundreds of €/kg.

Target Product Profile (TPP) for natural shrimp anti-melanosis leads

- **Storage efficacy:**

- o **Minimum:** visible melanosis suppression relative to untreated control during □7 days refrigerated storage. **Ideal:** >90% suppression of visible melanosis in HOSO shrimp during □10 days, benchmarked against 4-HR and/or SMBS.

- **Applied dose window:**

- o **Minimum:** efficacy at a practically applicable dipping concentration, with □ 500 mg/L representing a preliminary upper feasibility boundary.

Ideal: efficacy at □250 mg/L active ingredient in dipping solution under defined 1:2 shrimp:solution ratios.

- **Species relevance:**
 - o **Minimum:** demonstrated functionality in *Litopenaeus vannamei* and *Penaeus monodon*. **Ideal:** confirmed performance in both *L. vannamei* and *Penaeus monodon*, with additional validation in other commercially relevant *penaeids*.
- **Operational compatibility:**
 - o **Minimum:** compatible with dipping or coating workflows. **Ideal:** no need for specialized equipment, long contact times, extreme pH, or conditions incompatible with industrial shrimp processing.

Together, these two profiles provide a practical basis for distinguishing between nominal activity and realistic application potential in shrimp processing. They are intended as heuristic tools for candidate triage and translational prioritization, rather than formal regulatory or universal cut-offs. For clarity, the proposed translational framework is summarized in Table 5.

Values are proposed as heuristic translational aids intended to support candidate ranking, not as formal regulatory thresholds or universally validated cut-offs.

Table 1:

Framework	Criteria	Minimum requirement	Preferred target
TCP	PPO/HdPO potency	Measurable: shrimp PPO/PO inhibition with adequate controls, $IC_{50} < 1 \text{ mM}$	IC_{50} : 10-50 μM ; K_i/K_i' sub-mM to low- μM
TCP	Supportive functional traits	At least one complementary mechanism as-sessed	Copper-active/antioxidant effects comparable to reference systems
TCP	Physicochemical fitness	Compatible with dipping/coating use	Chemical log P 1-4 preferred
TCP	Residue/Safety	Safe for tissue residue <20 mg/kg as triage boundary. No obvious safety alerts.	Safety profile supporting an ADI of >0.5 mg/kg, Edible tissue residue ideally <2 mg/kg.
TCP	Environmental profile	Non-toxic to various environments	Favorable biodegradability

Based on this framework, simply adding more extract screens is unlikely to solve the translational problem. What the field lacks is no more nominal activity, but candidates that hold up under dose, residue, sensory, and process constraints. Hence, the field should shift toward targeted discovery of defined natural molecules that combine PPO-directed potency with low-dose functionality and minimal sensory penalty. Within that search space, plant-derived phenolics appear particularly attractive as a hypothesis-driven direction, because they most plausibly approximate the low-dose, PPO-directed logic that makes 4-HR such a strong benchmark. This does not mean that complex extracts are irrelevant; rather, their value may lie more in immediate clean-label preservation strategies, whereas the most promising path toward a truly competitive next-generation anti-melanosis additive likely lies in defined natural leads that can match benchmark efficacy with lower formulation burden.

Outlook and translational perspective

Despite substantial academic progress, the field has not yet delivered a natural product or complex biobased preparation that consistently satisfies the efficacy, dose, residue, sensory, safety, scalability and cost-structure requirements needed for realistic translation into shrimp processing. This gap is especially consequential for organic shrimp production, where the anti-melanosis toolbox remains limited, and the demand for an effective biobased solution is particularly acute. Overall, natural products have clearly demonstrated the capacity to modulate shrimp melanosis, but no candidate has yet convincingly matched the combined standard required for industrial adoption. The proposed TCP and TPP should therefore be viewed not merely as organizational tools, but as a corrective translational framework that redirects the field from descriptive positivity toward candidates and treatment concepts with realistic industrial potential. Applied rigorously, this framework could help narrow the gap between mechanistic promise and commercial feasibility and thereby accelerate the identification of truly translatable clean-label anti-melanosis solutions suitable for development as a next-generation processing aid or food additive.

Author contributions

Nicolas Reyes Castillo: Investigation, Methodology, Writing - original draft, Visualization, Validation, Formal Analysis, Data Curation. **Stefan Holler, Eric Bernard and Florian Birk:** Review & Editing, Validation, Data Curation. **Till F. Schäberle:** Writing - Review & Editing, Resources, Project administration, Funding acquisition. **Marius Spohn:** Supervision, Investigation. Writing - review & editing, Resources, Project administration, Funding acquisition.

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Conflict of interest

M.S. and T.F.S. declare a pending patent application (EP26168492.2) related to the content of this review. The remaining authors declare no competing interests.

Data availability

No new experimental data were generated. Data underlying this review consist of the published literature cited herein.

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