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# Monofloral Honeys as Functional Interactomes: Navigating Bioavailability, Gut Microbiota Transformation, and Food Integrity Pressures

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## Abstract

While standard dietary frameworks and public health guidelines predominantly evaluate honey based on its high free-sugar content to regulate caloric intake, its comprehensive role as a complex and dense biological signaling matrix is often less characterized. This review integrates raw chemical profiling with physiological bioaccessibility to evaluate the effect of the floral source on the functional quality of monofloral honeys. Through a structured literature-based comparative synthesis, the specific enzymatic behaviors, gastrointestinal metabolic fates, and chemical biomarkers of four representative monofloral honeys—Manuka, Chestnut, Acacia, and Argan—were systematically mapped and contrasted. Honey bioactives undergo extensive metabolic transformation during gastrointestinal transit, actively communicating with the mucosal environment rather than acting as a static nutrient solution The synthesized literature highlights distinct botanical-specific mechanisms: Manuka honey matrices exhibit well-characterized topical antimicrobial kinetics, while data on Chestnut honey suggest a potential role in cellular redox homeostasis via the Nrf2 pathway and a highly specific, though preclinical, signaling axis through kynurenic acid–GPR35 interactions. Furthermore, in vitro profiles of Acacia honey support its potential to modulate digestive carbohydrate enzymes, providing a plausible framework for its lower postprandial glycemic responses. Conversely, Argan honey bioactivity remains restricted to preliminary baseline antioxidant screenings. We also note that sophisticated sugar adulteration and climate change create critical "phytochemical drift," endangering the therapeutic consistency of honey. In summary, the identification of honey as a "functional interactome" offers a strong scientific foundation for evidence-based dietetics. The targeted use of verified medical-grade monofloral honeys must be supported by advanced authenticity testing, metabolomics, and in vivo pharmacokinetics in future clinical dietetic interventions.

**Keywords:** *Apis mellifera*; bioavailability; monofloral honey; polyphenols; nutraceuticals



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## 1. Introduction

### *1.1. Beyond a Sugar Solution: Honey as a Complex Biological Matrix*

Honey is traditionally defined across global regulatory frameworks as a naturally sweet substance produced by Apis mellifera bees from the nectar of plants or secretions of

living parts of plants. However, major international statutory standards—including the European Union Council Directive 2001/110/EC, the FAO/WHO Codex Alimentarius Standard for Honey (Codex Stan 12-1981), and the United States Food and Drug Administration (FDA) industrial guidance—predominantly classify and evaluate this matrix based on rigid commercial and physicochemical thresholds, such as moisture content, fructose-to-glucose ratios, electrical conductivity, and diastase activity [1]. From a dietetic and clinical perspective, these criteria serve strictly as commercial quality baselines to ensure commodity compliance and detect macro-adulteration, while fundamentally overlooking the intricate nutraceutical fraction and secondary metabolites that drive its biological signaling capacity.

Moisture, acidity, and hydroxymethylfurfural (HMF) are physicochemical markers that are emphasized in current regulatory standards, such as the EU Directive 2001/110/EC, mainly for quality control and shelf-life assessment. Consequently, the true functional potential of honey cannot be attributed solely to an isolated class of trace molecules. Rather, its biological activity arises from a complex, synergistic network involving both major and minor constituents, where macro-components like specific sugars (including prebiotic oligosaccharides), organic acids, and bee-derived enzymes interact dynamically with micro-components such as polyphenols and volatile phytochemicals to dictate the overall bioactivity of the matrix.

The bioactive profile of honey in clinical nutrition is closely linked to its botanical source [2]. While the honeybee (*Apis mellifera*) contributes particular enzymatic and protein components during the ripening process, the secondary metabolism of foraging plants involves a wide variety of polyphenols. In terms of antioxidant density and enzymatic stability in particular, this synergy leads to notable intervariety variance. This operational distinction is further supported by recent systematic evidence mapping how processing dynamics affect native enzymatic structures without necessarily obliterating the baseline non-enzymatic antioxidant pool [3].

While in vitro profiling provides critical baseline screening and mechanistic insights, the primary barrier preventing the definitive classification and implementation of monofloral honeys as clinical functional foods is not the preclinical data itself. Rather, the translational bottleneck resides in the current lack of standardized human bioavailability studies and the scarce availability of robust, controlled human intervention trials [4]. The physiological gastric hurdle cannot be replicated by standard spectrophotometric assays (e.g., DPPH, FRAP), which measure radical scavenging capacity in buffered environments.

The stability of honey bioactives during gastrointestinal transit determines their metabolic fate [5]. When polyphenols, which are frequently found as complex glycosides, are consumed, their pH decreases sharply in the stomach (from 1.2 to 3.0) and then shifts to an alkaline state in the duodenum. As a result, biological accessibility depends on these compounds being released from the honey matrix and then hydrolyzed into absorbable aglycones by intestinal enzymes or the gut microbiota [6]. Moreover, the potential functional roles of bee-derived enzymes, such as glucose oxidase and diastase, warrant careful and conditional evaluation. Beyond their traditional industrial role as thermal damage markers, these proteins have been hypothesized to exert localized functions; however, their in situ catalytic stability under gastric conditions remains highly constrained. Because glucose oxidase kinetics are heavily dependent on severe luminal pH fluctuations, progressive bolus dilution, and proteolytic degradation by gastric pepsin, any local production of hydrogen peroxide ($H_2O_2$) cannot be considered an established physiological fact; rather, it must be framed strictly as a speculative, preclinical hypothesis that requires direct experimental validation within the complex environment of the mammalian intestinal lumen.

To bridge these gaps, this review aims to establish a systematic, multi-tiered framework that sequentially connects core dimensions of monofloral honey evaluation. Specifically, we integrate: chemical authenticity and botanical profiling, gastrointestinal transit dynamics and poly-phenolic bioaccessibility, and downstream target receptor and intracellular molecular mechanisms. By linking these components, this work provides a rigorous, hierarchy-graded appraisal of the realistic dietetic applications and metabolic implications of Manuka, Chestnut, Acacia, and Argan honeys, moving beyond raw chemical concentrations toward an evidence-based translational perspective.

### *1.2. Review Methodology*

To ensure that the process of critical review was transparent and replicable, the current review has used structured narrative methodology. An extensive literature search was performed using international electronic databases, namely PubMed, Web of Science, Scopus, and ScienceDirect until May 2026. The literature search employed a combination of Boolean operators and specific keywords, including: ("monofloral honey" OR "Manuka" OR "Chestnut" OR "Acacia" OR "Argan honey") AND ("bioaccessibility" OR "bioavailability" OR "Nrf2" OR "GPR35" OR "NF-kappaB" OR "gut microbiota" OR "adulteration").

As for the inclusion criteria, peer-reviewed articles, analysis, and biochemistry, in vitro digestion studies, cell cultures, in vivo animal models concerning certain honey markers were chosen. Literature on general argan oil or any other botanical extracts, except when they are used for baseline comparisons, was not taken into account. No language limitations were imposed; however, all the selected papers were written in English.

## 2. Phytochemical Profiling and Botanical Markers

The identification of monofloral honey is based on the relationship between pollen analysis (melissopalynology) and the presence of secondary metabolites that act as chemical fingerprints (see also Table 1). These markers are not merely passive geographic or botanical indicators; they represent the signature phytochemical profile that establishes the baseline bioactive potential of the matrix. However, their ultimate physiological impact cannot be stated deterministically, as it remains strictly dependent on their quantitative concentration, gastrointestinal release kinetics, intestinal absorption rates, first-pass metabolism, and individual host factors.

### *2.1. The Dihydroxyacetone-Methylglyoxal Pathway (Manuka)*

The bioactive profile of *Leptospermum scoparium* (Manuka) honey is fundamentally characterized by the postharvest conversion of nectar-derived dihydroxyacetone (DHA) to methylglyoxal (MGO) [7].

The accumulation of DHA in L. scoparium nectar is not uniform; it is strictly governed by geographical and climatic clines across New Zealand, where specific regional soil dynamics and regional temperature variations directly dictate the baseline chemical potential of the postharvest matrix.

This non-peroxide activity (NPA) remains chemically stable across various storage temperatures, contrasting with the thermolabile peroxide-based activity typical of most blossom honeys. In terms of analytical quantification, while High-Performance Liquid Chromatography coupled with Diode Array Detection (HPLC-DAD) represents the historical routine method, current analytical standards utilize Ultra-High-Performance Liquid Chromatography–Tandem Mass Spectrometry (UHPLC-MS/MS) and quantitative Nuclear Magnetic Resonance (qNMR) to precisely map the DHA-to-MGO maturation kinetics without matrix interference.

According to official regulatory standards, commercial dietary Manuka honeys typically exhibit baseline MGO levels ranging from 100 to 500 mg/kg [8]. Concentrations exceeding 800 mg/kg do not represent typical commercial products; rather, they correspond to highly premium, certified Unique Manuka Factor (UMF) products (typically UMF 20+ and above) or specific, strictly standardized medical-grade honeys designed for clinical wound management.

### *2.2. Phenolic and Tryptophan Metabolites (Chestnut)*

Castanea sativa honey has high electrical conductivity (usually >0.8 mS/cm) and contains many phenolic acids, especially caffeic and gallic acids [9]. Kynurenic acid, on the other hand, is its most unique marker [10]. This metabolite derived from tryptophan is found in markedly greater concentrations than those in other monofloral honeys. Mechanistically, kynurenic acid functions as an endogenous N-methyl-D-aspartate (NMDA) receptor antagonist and a GPR35 agonist, establishing a clear biochemical foundation for its investigated anti-inflammatory and neuroprotective properties [11]. However, it must be explicitly clarified that these bioactivities have been demonstrated exclusively in experimental models utilizing kynurenic acid as an isolated, high-purity compound. Direct physiological evidence validating whether the dietary consumption of whole chestnut honey can achieve the systemic or central nervous system concentrations required to elicit these neuroprotective effects in humans is currently lacking, leaving this translational link strictly as a preclinical hypothesis. This translational caution is underscored by recent human dietary evaluations showing that while oral ingestion of tryptophan-derived metabolites shifts systemic plasma levels, the exact pharmacokinetics and central nervous system availability of honey-bound kynurenic acid in humans remain unvalidated due to rapid renal clearance and variable intestinal absorption [12].

The prominent mineral and phenolic profile of *Castanea sativa* honey is highly reflective of its European geographical footprint, where acidic and volcanic soil compositions typical of specific Mediterranean and temperate hilly woodlands significantly enrich the floral nectar in gallic acid and mineral escretes.

### *2.3. Specificity of Flavonoids in Acacia Honey*

Despite possessing a relatively low total phenolic content (TPC) compared to darker botanical varieties, honey derived from *Robinia pseudoacacia* (Acacia) exhibits a highly specialized and functionally distinct phytochemical signature. The main polyphenolic architecture of Acacia honey is characterized by a selective concentration of specific flavones and flavonols, most notably acacetin, quercetin, and kaempferol [13]. From a physiological perspective, these specific flavonoids drive distinct biological activities; for instance, acacetin and kaempferol have been shown to act as potent non-competitive inhibitors of key carbohydrate-digesting enzymes, such as alpha-glucosidase and alpha-amylase, thereby modulating glucose release kinetics [14].

Furthermore, from a clinical dietetic standpoint, Acacia honey is analytically identified by an exceptionally high fructose-to-glucose (F/G) ratio, typically exceeding 1.45. While this unique carbohydrate profile structurally prevents rapid crystallization—ensuring a permanently fluid matrix—it also carries profound metabolic implications. The marked predominance of fructose over glucose imparts a significantly lower glycemic index (GI) to Acacia honey relative to other monofloral and polyfloral variants [15].

The carbohydrate composition, particularly the fructose-to-glucose ratio, represents a major structural determinant of honey's glycemic response. However, it must be emphasized that reported Glycemic Index (GI) values are not static biochemical constants; they are significantly influenced by human metabolic variability—such as individual insulin sensitiv-

ity and gut microbiota architecture—as well as clinical study design parameters, including testing protocols, carbohydrate reference standards, and participant cohort selection.

This lower GI restricts postprandial glycemic spikes and reduces immediate insulinemic demands, rendering authenticated Acaciaan intriguing model for targeted carbohydrate modulation in metabolic research, even in setups where raw native enzymatic activity (such as glucose oxidase) is baseline lower than in darker, colloid-rich honeys.

Although *R. pseudoacacia* is a cosmopolitan species, its premium monofloral honey production is highly localized within specific geographical corridors of Central-Eastern Europe and East Asia, where stable spring microclimates prevent early crystallization by optimizing the nectar's fructose-to-glucose ratio.

### *2.4. Argan Honey: Secondary Metabolites in Xerophytes*

Argan honey, derived from *Argania spinosa* (L.) Skeels (synonym *Sideroxylon spinosum*), is an exceptionally rare and geographically restricted monofloral matrix produced exclusively within the endemic Argan forests of southwestern Morocco. The phytochemical uniqueness of Argan honey is inherently tied to its strict geographical confinement. Produced exclusively within the UNESCO biosphere reserve of southwestern Morocco, the extreme arid climate, coupled with localized soil traits, triggers a distinct secondary metabolic response in the plant, driving the synthesis of its signature marker sterols.

It is critical to establish a strict analytical boundary between this matrix and the widely studied Argan oil; while the oil is characterized by a specific lipidic profile rich in unique phytosterols, Argan honey constitutes a distinct, water-soluble floral secretion whose bioactivity depends primarily on its hydrophilic fraction. Currently, comprehensive metabolomic characterization of this honey remains limited compared to other established monofloral varieties [16].

Standard physicochemical screenings confirm its strict compliance with international regulatory quality benchmarks. Analytical datasets demonstrate that Argan honey is characterized by dark amber coloration, a high total phenolic content (TPC), and robust baseline radical scavenging capacity in vitro, suggesting that non-esterified phenolic acids are major contributors to its antioxidant profile [17].

Regarding its biological properties, significant growth inhibition has been documented in laboratory assays against key pathogenic strains, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus subtilis* [18].

However, while traditional Moroccan medicine and preliminary in vitro scratching assays suggest a potential supportive role in wound-healing tissue dynamics, these properties remain strictly suggestive at a preclinical level. In the absence of documented human intervention trials, these laboratory outcomes must not be over-translated into clinical or therapeutic recommendations, and further bioaccessibility studies are required to isolate its definitive floral markers.

**Table 1.** Comparative analysis of monofloral honeys: chemical markers, bioaccessibility, and molecular targets.

| *Honey Variety* | *Primary Botanical Marker(s)* | *Key Bioactive Mechanism* | *Digestive Stability (Bioaccessibility)* | *Main Molecular Target(s)* | *Level of Evidence/ Validation Stage* |
|---|---|---|---|---|---|
| Manuka (*L. scoparium*) | Methylglyoxal (MGO), DHA, Leptosperin | Nonperoxide antibacterial (NPA) activity via dicarbonyl stress. | High; MGO is relatively stable at gastric pH but sensitive to high protein diets. | Bacterial cell wall/DNA cross-linking (demonstrated for pure MGO); validated in whole medical-grade matrices for wound care [7,8,19]. | *Topical/Antimicrobial:* High (Clinical Evidence/Certified Medical-Grade) *Oral/Systemic:* Low to Moderate (In vitro & Animal Models) |

**Table 1.** *Cont.*

| *Honey Variety* | *Primary Botanical Marker(s)* | *Key Bioactive Mechanism* | *Digestive Stability (Bioaccessibility)* | *Main Molecular Target(s)* | *Level of Evidence/ Validation Stage* |
|---|---|---|---|---|---|
| Chestnut (*C. sativa*) | Kynurenic acid, Gallic acid, 1-O-caffeoylquinic acid | NMDA receptor antagonism; High antioxidant scavenging. | Moderate; Phenolic acids absorbed via passive diffusion in upper GI tract. | GPR35 receptor (validated for isolated kynurenic acid); Nrf2 activation (suggested for whole matrix fractions); ROS neutralization [9]. | Biomarker Authentication: High (Analytical Validation) Neuroprotective/Anti-inflammatory claims: Low (Preclinical/Isolated Compound Models Only) |
| Acacia (*R. pseudoacacia*) | Acacetin, Kaempferol, High F/G ratio (>1.45) | Low glycemic index (GI); Specific flavonoid-mediated anti-inflammatory flux. | Moderate; Flavonoid glycosides require colonic microbial hydrolysis. | α-glucosidase (allosteric inhibition demonstrated for isolated fractions); IL-6/TNF-α downregulation (inferred from extracts); confirmed via low-GI human profiles for whole honey [13]. | Glycemic Index Profiling: Moderate to High (Human Screening, subject to cohort variability) |
| Argan (*A. spinosa*) | Vanillic acid, Schottenol, Spinasterol | Lipid-associated antioxidant pathway; Xerophytic adaptogens. | Low/Complex; Sterols require bile salt-mediated micellar solubilization. | Macrophage modulation & PPAR-gamma (hypothesized for isolated sterols); Redox homeostasis (validated via whole-matrix in vitro screenings) [18]. | Botanical Authenticity: High (Pollen/Analytical Marker Validation) Micellar/Lipid Uptake Claims: Low (Theoretical/In vitro Preclinical Hypothesis) |

## 3. The Digestive Stability and Bioavailability of Honey Bioactives

The therapeutic efficacy of monofloral honey is contingent upon the biological accessibility of its metabolites, which is defined as the proportion of compounds released from the food matrix during digestion and rendered available for absorption. This process is not linear; it is controlled by the interaction between the high osmotic pressure of honey, its colloidal structure, and the environment in the gastrointestinal tract.

To evaluate the nutritional physiology of honey, three distinct pharmacological boundaries must be defined: (i) Bioaccessibility, which refers to the precise fraction of bioactive compounds released from the honey matrix within the gastrointestinal lumen, becoming available for absorption; (ii) Bioavailability, indicating the rate and extent to which these free compounds or their metabolites cross the epithelial barrier, enter systemic circulation, and reach their target sites; and (iii) Bioactivity, which represents the specific, quantified intracellular signaling cascades or physiological endpoints triggered by the bioavailable compounds within the host cells.

### *3.1. Gastric Barriers–pH, Viscosity, and Protein Degradation*

The digestive cascade initiates as the naturally acidic honey matrix (pH 3.4–6.1) encounters the highly acidic environment of the gastric lumen, where the ambient pH drops sharply to 1.2–2.5. With respect to enzymes, especially glucose oxidase and diastase, such an environment is rather denaturing. Nevertheless, because of the high content of sugars (fructose and glucose) and complicated oligosaccharides, some protection of proteins against pepsin-induced degradation is likely, which is referred to as “protein crowding” [5,20].

During the gastric phase, the structural fate of honey bioactives is strictly governed by the rheological and chemical divergence of the specific floral sources. Manuka and Chestnut honeys, characterized by higher colloidal protein content and higher viscosities, exert a more pronounced delay on gastric emptying compared to the Newtonian, high-fructose matrices of Acacia honey. This dense carbohydrate environment triggers a ‘preferential

hydration' or 'protein crowding' mechanism [21,22]; the high osmolarity excludes water from the protein core, thereby thermodynamically stabilizing bee-derived enzymes like glucose oxidase against peptic cleavage and low-pH denaturation.

Regarding polyphenols, their gastric stability depends on specific pKa thresholds. For instance, the main flavones in Acacia (acacetin, pKa 6.25) and molecules like ferulic acid (pKa 4.6) remain predominantly un-ionized in the highly acidic gastric lumen (pH 1.5–2.0). This un-ionized state limits chemical degradation, resulting in high quantitative recovery rates (ranging from 85% to 92% recovery for simple phenolic acids and glycosylated flavonoids) after simulated gastric digestion, ensuring their structurally intact transit into the duodenum [23].

### *3.2. Duodenal Transition and Micellar Solubilization*

An alkaline challenge (pH 6.8–7.5) along with the effects of bile salts and pancreatic lipases characterize the duodenal stage of digestion. While this phase represents the primary site for the passive uptake of the hydrophilic matrix, micellar solubilization is by no means a general feature of honey digestion; rather, it remains strictly restricted to the micro-niche of lipophilic phytochemicals present in specific botanical varieties. For instance, the presence of rare phytosterols such as schottenol and spinasterol has been documented as a highly specific authenticity marker for Argan honey. Mechanistically, the absorption of these lipophilic structures by enterocytes relies on their incorporation into mixed micelles, a process that represents the rate-determining factor for lipid bioaccessibility [5].

However, it must be verified that these sterols occur in Argan honey strictly in trace amounts—primarily associated with suspended pollen grains—contrasting sharply with their high concentration in Argan oil. Therefore, while their theoretical metabolic fate requires micellar integration, their dietary relevance within the whole honey matrix remains limited and strictly preliminary.

In contrast, the baseline duodenal behavior of standard honey components is governed by polarity and molecular weight: small phenolic acids (gallic, caffeic, and vanillic acids) demonstrate rapid bioaccessibility in the upper gastrointestinal tract via passive diffusion. Conversely, larger, less soluble flavonoid glycosides—such as acacetin derivatives from Acacia or quercetin complexes in Chestnut honey—resist immediate duodenal absorption and transit largely unaltered toward the distal regions of the small intestine and the colon [24].

### *3.3. Microbial Biotransformation and Colonic Absorption*

The relationship between honey phytochemicals and the gut microbiome could be termed the "second wave" of the absorption process [24]. The activity of β-glucosidase and α-rhamnosidase enzymes secreted by the microbiota leads to the hydrolysis of flavone and flavonol glycosides to free aglycones that are metabolized to simple phenols such as protocatechuic acid and several hydroxybenzoic acids. These metabolic products often exhibit distinct systemic bioavailability profiles and diverse biological functions compared to their precursor molecules. Moreover, the prebiotic fraction of honey—which includes specialized oligosaccharides such as melezitose and erlose—is well-documented to modulate gut microbiota composition by favoring the proliferation of beneficial taxa. From a mechanistic standpoint, it represents an interesting, yet currently unproven, hypothesis that such oligosaccharide-driven shifts in microbial architecture might indirectly influence or favor the metabolic transformation of specific honey-derived metabolites, such as methylglyoxal or kynurenic acid. Because direct experimental evidence validating this specific biotransformation cascade within the mammalian colon is currently lacking, this pathway must be interpreted strictly as a theoretical, predictive framework. Nonetheless, during

colonic fermentation, the delayed biological potential of honey continues to be evaluated via the general microbial transformation of non-absorbable polyphenolic precursors into low-molecular-weight active catabolites [25] (Figure 1).

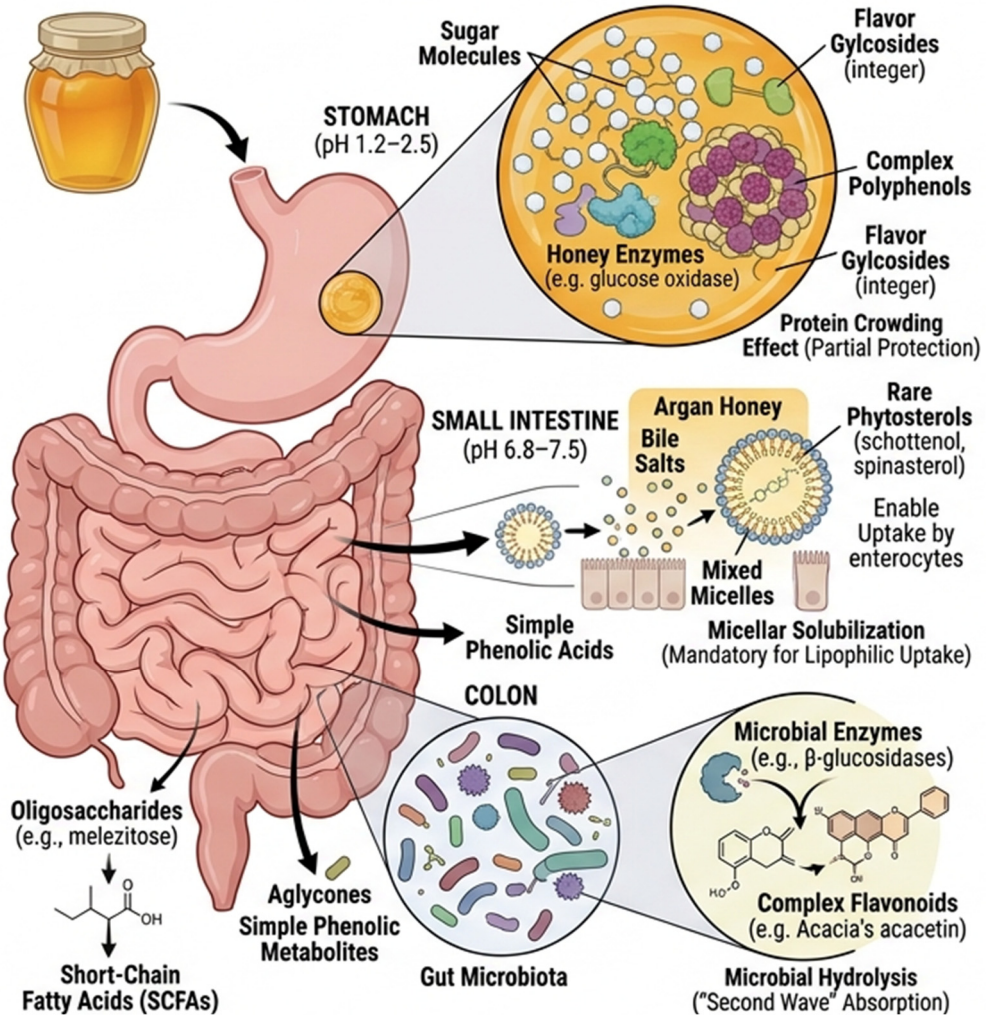


**Figure 1.** Conceptual schematic of the potential gastrointestinal processing and phase-dependent bioaccessibility of honey-derived bioactives. Gastric phase: potential modulation of gastric emptying driven by matrix viscosity, evaluating hypothetical macronutrient matrix protection of native enzymes against rapid peptic degradation as a preliminary model. Duodenal-jejunal phase: localized passive diffusion of simple hydrophilic phenolic fractions under neutral-alkaline conditions, alongside the localized micellar incorporation required exclusively for trace lipophilic structures, such as Argan honey phytosterols. Colonic phase: microbial deglycosylation of complex flavonoids, where bacterial hydrolases liberate absorbable aglycones to generate a systemic secondary wave of metabolites, while structural oligosaccharides undergo fermentation into short-chain fatty acids (SCFAs). Note: This is a conceptual framework based on synthesized preclinical and in vitro models; direct human translational evidence for specific metabolic sub-pathways remains under evaluation. Schematic layout initially drafted and refined using Gemini (Google) and fully verified for scientific accuracy and calibrated by the authors.

## 4. Biological Functions and Target Sites

The systemic physiological responses elicited by monofloral honey are not produced by a single "active ingredient" but by an interactome targeting several sites.

Here, we rigorously define the term functional interactome in the context of honey biology as the multi-tiered network of reciprocal, dynamic interactions occurring between: (i) the intrinsic components of the honey matrix (sugars, bee-derived enzymes, and botan-

ical phytochemicals), (ii) the host gastrointestinal physiology and symbiotic microbiota-encoded enzymes, and (iii) the downstream intracellular mammalian signaling pathways.

Rather than viewing honey as a collection of isolated compounds, this framework treats it as an integrated biological signaling system whose final physiological efficacy is determined by the synergistic crosstalk of its components during gastrointestinal transit.

The interaction between the phenolic metabolites absorbed and the enzymes present in honey regulates crucial signal transduction pathways associated with chronic inflammation and oxidative stress [26].

The therapeutic and signaling pathways triggered by monofloral honey matrices operate via distinct molecular networks. A comprehensive hierarchical analysis of the evidence supporting each proposed biological function—distinguishing between in vitro models, animal studies, and human clinical trials—is detailed in Table 2.

**Table 2.** Hierarchical classification of evidence levels for proposed monofloral honey bioactivities.

| Honey Type | Proposed Bioactivity/ Mechanism | Compositional Profiling | In Vitro Digestion | Cell Culture Assays | In Vivo Animal Studies | Human Clinical Trials | Current Evidence |
|---|---|---|---|---|---|---|---|
| Manuka | Topical Wound Healing & Antimicrobial | ✓ | ✓ | ✓ | ✓ | ✓ | Clinically Validated (for topical medical-grade formulations) |
| | Oral Gut Microbiota Modulation | ✓ | ✓ | ✓ | ✓ | x | Preclinical/ Suggestive (lacks clinical trial confirmation) |
| Chestnut | GPR35 Agonism & Gut Anti-inflammation | ✓ | ✓ | ✓ | ✓ | x | Mechanistically Suggestive (validated for pure kynurenic acid) |
| | Nrf2/ARE Redox Activation | ✓ | ✓ | ✓ | x | x | In Vitro Evidence (restricted to extract fractions) |
| Acacia | alpha-Glucosidase Inhibition & Low GI | ✓ | ✓ | ✓ | ✓ | ✓ | Clinically Sup-ported (validated via postprandial glycemic trials) |
| | NF-\kappaB Suppres-sion/Cytokine Reduction | ✓ | x | ✓ | ✓ | x | Preclinical/ Hypothesis (tested via extracts/ animal models) |
| Argan | Radical Scavenging & Redox Homeostasis | ✓ | x | ✓ | x | x | Preliminary In Vitro (restricted to crude antioxidant profiles) |
| | Macrophage/ PPAR-\gamma Modulation | ✓ | x | ✓ | x | x | Speculative Hypothesis (requires direct characterization) |

### *4.1. Activation of Nrf2 and Redox Buffer Systems*

The antioxidant activity of honey goes beyond the mere scavenging of radicals. While individual polyphenolic constituents isolated from honey (such as quercetin or ferulic acid) are well-documented Nrf2/ARE activators in cellular models, evidence regarding whole honey matrices remains predominantly indirect. In vitro assays utilizing whole dark honey fractions (such as vanillic, gallic, and caffeic acids from Chestnut and Argan honeys)

indicate a potential cascade induction and eliminate ROS, showing high activity in in vitro endothelial models to mitigate early atherogenic steps [27,28].

Under normal physiological conditions, this factor is bound by the Keap1 protein in the cytosol of cells. As soon as cells encounter these electrophilic fractions, Nrf2 dissociates from Keap1 and moves into the nucleus, where it activates ARE binding and triggers the synthesis of phase II enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [29,30].

This priming effect increases the efficiency of the redox buffer system of cells, thereby protecting vascular endothelial cells from lipid peroxidation in vitro and in preclinical model [31]. Rather than representing a direct preventative cure for atherosclerosis, this pathway should be interpreted strictly as a potential, localized cytoprotective mechanism that warrants rigorous, long-term clinical validation in human cardiovascular trials.

### *4.2. NF-κB Suppression and Proinflammatory Cytokines*

The mechanism underlying the anti-inflammatory activity of honey includes the suppression of the NF-κB signaling pathway. However, a clear distinction must be made regarding the nature of the tested matrices: the suppression of the IKK complex phosphorylation and the subsequent inhibition of the nuclear translocation of the p65 subunit have been observed primarily in cell culture setups treating macrophages with either total honey dry extracts or highly purified flavonoid fractions (e.g., acacetin and kaempferol characteristic of Acacia honey), rather than raw, whole crude honey interventions [32].

Studies using quantitative PCR and ELISA have shown that these specific extract fractions successfully suppress the downstream expression of IL-6, IL-1β, and TNF-α [1,33].

Beyond traditional polyphenolic fractions, recent investigations have identified kynurenic acid (KA) as a highly specific, low-molecular-weight biomarker characteristic of Chestnut (*Castanea sativa*) honey. From a mechanistic standpoint, KA represents a promising molecular candidate due to its role as an endogenous agonist for the G protein-coupled receptor 35 (GPR35), which is highly expressed on human epithelial cells and macrophages [31,34]. In vitro and preliminary animal models suggest that the activation of the KA–GPR35 pathway downregulates the downstream release of high mobility group box 1 (HMGB1), a critical late-phase pro-inflammatory alarmin. Consequently, this axis is hypothesized to mitigate localized intestinal inflammation and mucosal damage [35]. While these preclinical findings offer a compelling suggestive framework for the targeted management of inflammatory bowel dynamics, rigorous human clinical trials remain mandatory to definitively validate the therapeutic efficacy and translational bioavailability of honey-derived KA in clinical dietetics [36].

### *4.3. Kinetic Models of Antimicrobial Activity: Peroxides or MGO*

In some types of honey, antimicrobial activity can be described by two different kinetic mechanisms. The first is nonperoxide activity (NPA), which occurs in Manuka (*Leptospermum scoparium*) [37]. Methylglyoxal (MGO) causes rapid stress to bacteria because of the formation of a dicarbonyl reaction. MGO forms covalent bonds with the amino acid residues of proteins and DNA. As a result, the activity of key enzymes, such as RNA polymerase, is inhibited. Nonperoxide activity does not depend on the concentration of oxygen. It does not degrade under the effect of catalase [38].

On the other hand, Acacia and Argan honeys rely predominantly on a peroxide-based mechanism governed by the glucose oxidase system [39]. Unlike the dilution-independent kinetics of MGO in Manuka, this enzymatic system requires matrix dilution with water or physiological fluids to reduce osmotic inhibition and trigger catalyst activation. Once

activated, glucose oxidase converts glucose into gluconic acid and hydrogen peroxide ($H_2O_2$) [40].

This antimicrobial pathway operates on a localized 'slow-release' kinetic pattern. Rather than accumulating in sustained toxic millimolar quantities, $H_2O_2$ is continuously generated at low-dose, steady-state concentrations (typically maintaining narrow micromolar fluxes or transient localized ranges) [41]. This specific kinetic profile is high enough to inflict oxidative stress on bacterial pathogens—which often lack robust protective enzymes—but does not overwhelm healthy mammalian tissues. Human cells can rapidly clear these low, continuous peroxide influxes via endogenous catalase and glutathione peroxidase systems, thereby promoting tissue cleansing and structural regeneration without suffering the severe cytotoxicity associated with high, unbuffered pharmaceutical doses of hydrogen peroxide [2,33].

### *4.4. Glucose Response and Enzymatic Inhibition*

In evidence-based dietetics, the metabolic effect of honey consumption on postprandial glycemia is crucial. Even though honey contains many sugars, its dietary classification is frequently oversimplified. The particular botanical source and the presence of nonsugar bioactive components influence the metabolic fate of its sugars.

The glycemic index and fructose/glucose (F/G) ratio of Acacia honey are much lower than those of sucrose, despite the exceptionally high F/G ratio (usually >1.45) [42]. This ratio decreases the immediate insulinogenic load on pancreatic β-cells by preventing rapid crystallization and ensuring a more gradual absorption of glucose.

In vitro enzymatic inhibition assays confirm that total phenolic extracts from specific honeys, as well as their isolated flavonols like kaempferol and acacetin, can directly bind to allosteric sites of alpha-glucosidase and alpha-amylase, thereby flattening the theoretical glucose curve by delaying the breakdown of complex carbohydrates. This compound-level behavior provides a plausible mechanistic explanation for the lower postprandial glycemic index observed during whole-matrix clinical screenings of Acacia honey. Furthermore, regarding systemic low-grade inflammation, experimental rodent models show that these specific flavonoid fractions can reduce the phosphorylation of the IKK complex [43].

However, characterizing whole honey as a fully validated immunoregulatory intervention for human metabolic syndrome requires caution; tentative extrapolation from high-dose isolated molecules or animal models is necessary, and whole-matrix human intervention studies remain essential to map the real-world impact on clinical metabolic endpoints [44].

Melezitose and erlose are two examples of special trisaccharides and oligosaccharides that have major prebiotic effects [2]. By being fermented in the colon, these substances encourage the gut microbiota to produce short-chain fatty acids (SCFAs). SCFAs alter systemic metabolic pathways and improve peripheral insulin sensitivity [44].

Secondary metabolic protection may be offered by certain flavonoids, such as kaempferol and acacetin, which are present in acacia. These substances lessen systemic low-grade inflammation, which is frequently linked to metabolic syndrome and type 2 diabetes, by inhibiting the phosphorylation of the IKK complex.

From a dietetic standpoint, botanical purity is paramount to maintaining the integrity of these metabolic properties. Patients who depend on honey as a functional sweetener may be at risk if foreign syrups (such as high-fructose corn syrup or rice syrup) are added because they can negate the natural hypoglycemic benefits of monofloral honeys such as chestnut or acacia.

Combining sugar kinetics, enzymatic inhibition, and prebiotic modulation, this multifactorial approach implies that if their chemical fingerprint is verified, particular monoflo-

ral honeys can be deliberately incorporated into dietetic plans for metabolic management (Figure 2).

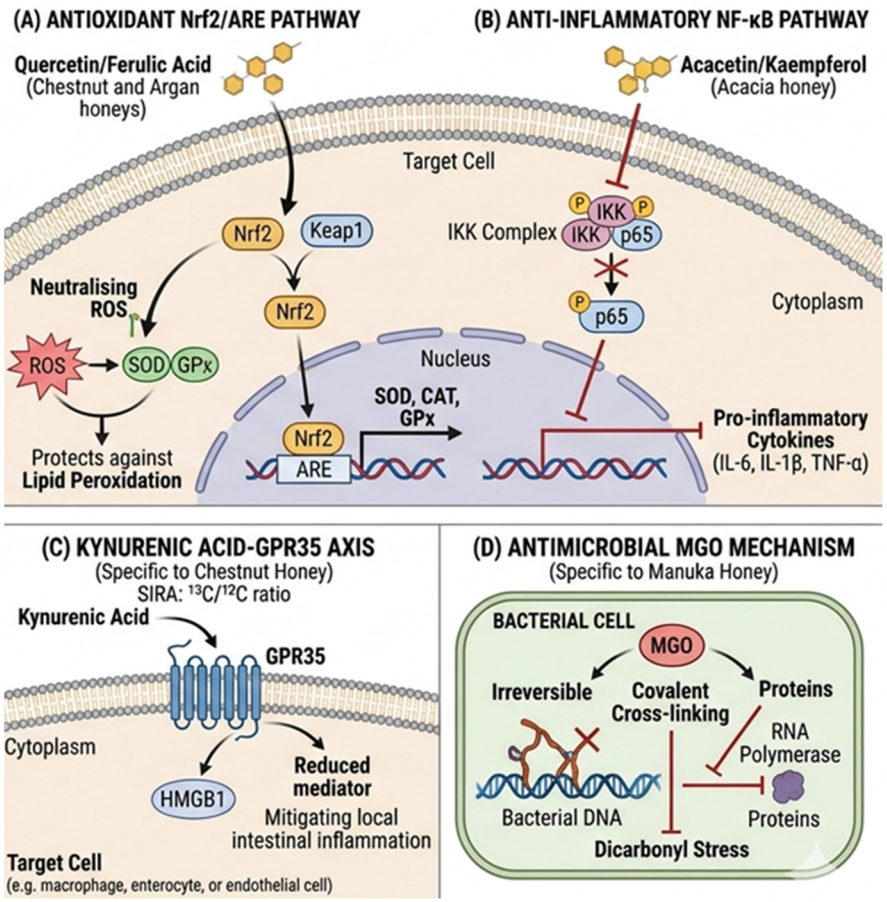


**Figure 2.** Conceptual model of the potential pleiotropic cellular effects and signaling pathways of targeted monofloral honey fractions. (**A**) Endothelial and epithelial cytoprotection: theoretical upregulation of Phase II antioxidant enzymes (SOD, CAT, GPx) via Nrf2 dissociation from Keap1, hypothesized to be stimulated by Chestnut and Argan polyphenolic fractions. (**B**) Macrophage-mediated anti-inflammatory cascade: attenuation of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) via the potential inhibition of IKK phosphorylation, modeled from in vitro Acacia honey data. (**C**) Monocyte immunomodulation: inhibition of late-stage HMGB1 exocytosis via GPR35 activation, demonstrated strictly for isolated kynurenic acid (highly enriched in Chestnut honey) in preclinical models. (**D**) Pathogen elimination: direct antimicrobial kinetics driven by Manuka MGO via dicarbonyl cross-linking of bacterial DNA and structural inactivation of RNA polymerase within the bacterial cell. Note: This diagram represents a non-generalized, conceptual framework synthesizing distinct experimental models. Individual pathways take place in specific cell types (macrophages, endothelial cells, or bacteria) rather than a generalized target cell. Schematic layout initially drafted and refined using Gemini (Google) and fully verified for scientific precision by the authors.

## 5. Environmental Pressures and Food Integrity: Climate Change and Frauds

There are currently two interlinked issues that threaten the stability and nutritional value of monofloral honeys: climate change and increasingly elaborate cases of food fraud. Both of these factors not only represent economic factors for dietary specialists but also directly affect the dosage and toxicity of the results [45].

### *5.1. Climate Change and Phytochemical Shifts*

Climate change is a leading force in changing the phenology of flowering plants. Changes in precipitation and temperature levels in vulnerable areas, such as the Argan tree environments in Morocco or the Manuka honey environment in Oceania, lead to changes in the yield of nectar and its phytochemicals [46]. Heat may influence the DHA-to-MGO process in the case of Manuka honey or change the concentration of certain flavonoids in the case of Acacia because of premature wilting of the Robinia flowers [47,48].

Moreover, the "dilution effect" due to elevated atmospheric levels of carbon dioxide has been found to reduce the amount of protein and minerals present in the pollen grains of flowers, which could impair the enzymatic secretion of the honeybee [49]. This leads to what is termed "phytochemical drift," wherein honey that passes the stipulated monofloral test might not have adequate concentrations of bioactive compounds [3,50].

### *5.2. Challenges in the Detection of Complex Frauds*

Owing to the globalization of the honey trade, complex means of adulteration have been devised that cannot be controlled through conventional means [51]. Mixing of foreign syrups (such as C4 sugars such as corn/cane syrup or C3 sugars such as rice/beet syrup) can now be hidden through the processing of highly refined syrups that have the same fructose/glucose ratio as specific types of honey such as acacia [52].

Conventional tests such as HMF/diastase tests are easily fooled. For this reason, modern science is shifting toward a more complex approach:

- Stable Isotope Ratio Analysis (SIRA): Useful for testing the carbon isotope ratio ($^{13}C/^{12}C$) and identifying the botanical source of sugars [53].
- NMR Metabolomics: Allows for the comprehensive detection of the composition of honey, including nontargeted adulteration that cannot be detected using simple HPLC techniques [54].

To secure the botanical traceability of these high-value matrices, melissopalynolog, i.e., the microscopic identification and quantification of pollen grains, remains the accepted legal reference method under Codex Alimentarius Stan 12-1981 and EU Directive 2001/110/EC.

In analytical practice, pollen assemblages serve as a geographic and botanical fingerprint. Laboratories subject the honey matrix to acetolysis, mounting the sediment to count a statistically robust sample of 500–1000 grains to determine percentage frequencies. Under these regulatory frameworks, strict species-specific thresholds dictate monofloral compliance: a honey is certified as monofloral only if the dominant pollen exceeds targets such as $\geq$45% for Acacia or $\geq$70% for Chestnut.

Conversely, due to the low pollen-shedding nature of Leptospermum species, Manuka honey requires a threshold of just $\geq$18%, making over-reliance on raw counts susceptible to false-negative fraud assessments.

This legal framework directly targets sophisticated physical adulteration, such as industrial ultrafiltration (membranes <0.2 μM). While removing pollen is structurally straightforward for fraudulent packers, hiding the intervention is difficult: 'pollen-free' honey immediately fails compliance tests and cannot legally be labeled as 'honey'.

However, melissopalynology presents inherent operational bottlenecks: it is heavily labor-intensive, requires elite taxonomic expertise, and suffers from natural biases due to under- or over-represented pollen caused by specific bee foraging behaviors. To overcome these limitations, DNA barcoding is rapidly gaining traction as a high-resolution complement. DNA barcoding successfully extracts and amplifies trace plant plastid DNA even from highly filtered matrices or when pollen morphology is completely ambiguous.

Although not yet fully standardized for international trade litigation, integrating DNA barcoding alongside classic melissopalynology provides the multi-tiered analytical grid required to safeguard the integrity of medical grade monofloral markets [55].

### *5.3. Conclusions About Evidence-Based Dietetics and Honey Adulteration*

From a dietetic and clinical perspective, honey adulteration extends beyond mere commercial fraud; it compromises the matrix authenticity and poses a metabolic risk by altering the expected glycemic response and depriving the consumer of bioactive phytochemicals. Given that the gastrointestinal and prebiotic effects of honey are strictly dictated by its chemical composition, the introduction of an undefined syrup could negate the specific hypoglycemic properties of Chestnut and Acacia honeys [56].

## 6. Conclusions and Future Directions

The comparative analysis of Acacia, Chestnut, Manuka, and Argan honeys highlights an evolving paradigm in food science: the transition from evaluating honey as a uniform commodity toward exploring it as a complex, multi-component biological signaling matrix.

While current data strongly support the role of botanical origin in dictating raw chemical composition, establishing whether these specific phytochemical profiles can predictably translate into distinct biological kinetics within the human body remains a major research priority rather than an empirically proven fact. In this context, conventional global parameters like 'Total Phenolic Content' (TPC) remain highly valuable and cost-effective screening indices for initial matrix characterization; however, they are inherently insufficient on their own for modern evidence-based dietetics, necessitating the parallel development of rigorous metabolic fingerprinting based on targeted biomarkers [2].

Several critical translational bottlenecks must be systematically addressed by future investigations. First, the low systemic bioavailability of honey-derived polyphenols poses a structural challenge. Because physiological blood concentrations after digestion are often insufficient for direct systemic radical scavenging, it is hypothesized that the health outcomes observed in preclinical models may derive from a localized or systemic hormetic effect, potentially stimulating endogenous antioxidant defenses via the Nrf2/ARE pathway—a mechanistic model that requires rigorous in vivo pharmacokinetic validation using isotopic labeling or advanced LC-MS/MS methods. Similarly, the structural stability of bee-derived enzymes during gastric transit warrants further exploration. The hypothesized 'macromolecular crowding effect,' wherein the dense sugar matrix is proposed to temporarily shield enzymes like glucose oxidase from rapid peptic degradation and allow a transient, localized release of $H_2O_2$ in the stomach, must be treated strictly as a research hypothesis until directly demonstrated in clinical settings.

Furthermore, the precise role of the human gut microbiota in converting complex non-absorbable flavonoid glycosides into highly bioavailable phenolic acids remains a nascent field. Evaluating how individual microbiome variations impact the release of unique metabolites—such as Chestnut kynurenic acid or Argan phytosterols—is a mandatory prerequisite before any therapeutic or standardized dietary applications can be considered. Finally, while Manuka honey represents an established gold standard for topical antimicrobial applications within validated medical-grade wound care frameworks, current evidence does not support its oral or systemic clinical superiority over other varieties. Future research efforts should leverage multi-omics platforms—including metabolomics, proteomics, and metagenomics—to systematically map the path from specific floral sources to human metabolic fates, aiming at the long-term characterization of biomarker-standardized honeys tailored for targeted dietetic interventions [57].

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