**БИОТЕХНОЛОГИЯ ПРОДУКТОВ ПИТАНИЯ И БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ/BIOTECHNOLOGY OF FOOD AND BIOLOGICAL ACTIVE SUBSTANCES**

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# SYNERGISTIC DETOXIFICATION OF AFLATOXIN M1 IN MILK USING CURCUMIN AND FERMENTATION-DERIVED MICROBIOTA

Review article

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**Abstract**

Aflatoxin M1 (AFM1) contamination in milk represents a critical food safety challenge due to its classification as a Group 1 human carcinogen by the IARC and its remarkable resilience to standard thermal processing methods, including pasteurization and UHT treatment. Traditional chemical decontamination strategies often compromise the sensory and nutritional integrity of dairy products. Consequently, there is an urgent industrial need for effective, non-destructive, and biologically safe mitigation strategies.

This paper presents a theoretical synthesis and systematic review of two promising natural approaches: biological adsorption via fermentation-derived microorganisms (Lactic Acid Bacteria and yeasts) and chemical stabilization using curcuminoids.

Data were aggregated from peer-reviewed studies indexed in Scopus, Web of Science, and RSCI (2010–2024). The analysis confirms that microbial sequestration is highly strain-specific. *Lactobacillus rhamnosus* and *Saccharomyces boulardii* demonstrate superior efficacy, with removal rates reaching 96.9% and 90.0% respectively under optimized conditions. However, a critical limitation identified across studies is the reversibility of the toxin-microbe complex; up to 60% of bound AFM1 can be released under the fluctuating pH conditions of the gastrointestinal tract, nullifying the detoxification benefit.

To overcome this, we propose a novel synergistic detoxification model. Curcumin (diferuloylmethane) is identified as a complementary stabilizing agent. Its mechanism involves dual-phase activity: *in vivo* inhibition of the hepatic cytochrome P450 enzymes responsible for metabolizing Aflatoxin B1 into M1, and direct in vitro chemical quenching via hydrogen bonding and $\pi$-$\pi$ stacking interactions. We hypothesize that the keto-enol tautomerism of curcumin in the acidic environment of fermented milk (pH 4.6) facilitates the formation of a stable "supramolecular bridge" between the AFM1 molecule and the bacterial cell wall peptidoglycans.

This review outlines a theoretical framework for this combined approach, suggesting that curcumin-enhanced microbial binding could transform reversible adsorption into permanent sequestration, offering a robust safety solution for the global dairy industry.

**Keywords:** Aflatoxin M1, curcumin, Lactobacillus, Saccharomyces, milk safety, synergistic detoxification, food fermentations.

# СИНЕРГЕТИЧЕСКАЯ ДЕТОКСИКАЦИЯ АФЛАТОКСИНА М1 В МОЛОКЕ С ИСПОЛЬЗОВАНИЕМ КУРКУМИНА И МИКРОБИОТЫ, ПОЛУЧЕННОЙ ПУТЕМ ФЕРМЕНТАЦИИ

Обзор

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**Аннотация**

Контаминация молока афлатоксином М1 (AFM1) представляет собой серьёзную проблему в сфере пищевой безопасности, поскольку данное соединение классифицируется МАИР (IARC) как канцероген для человека группы 1 и обладает высокой устойчивостью к стандартным методам термической обработки, включая пастеризацию и ультравысокотемпературную обработку (UHT). Традиционные химические методы деконтаминации зачастую приводят к ухудшению органолептических и нутриентных характеристик молочной продукции. В связи с этим отмечается высокая потребность отрасли в эффективных, неразрушающих и биологически безопасных подходах к снижению уровня токсина.

В настоящей работе представлена теоретическая систематизация и аналитический обзор двух перспективных природных методов: биологической адсорбции с использованием микробных культур, формирующихся в процессе ферментации (молочнокислые бактерии и дрожжи), а также химической стабилизации с помощью куркуминоидов.

Данные были агрегированы на основе рецензируемых исследований, индексированных в Scopus, Web of Science и РИНЦ (2010–2024 гг.). Анализ подтверждает, что способность к микробной сорбции AFM1 является выраженно штаммоспецифичной. Наивысшую эффективность демонстрируют *Lactobacillus rhamnosus* и *Saccharomyces boulardii*, обеспечивая степень удаления токсина до 96,9% и 90,0% соответственно при оптимизированных условиях. Однако ключевым ограничением большинства исследований является обратимость комплекса «токсин–микробная клетка»; до

60% связанного AFM1 может высвобождаться при колебаниях pH в условиях желудочно-кишечного тракта, что нивелирует эффект детоксикации.

Для решения данной проблемы предлагается новая модель синергетической детоксикации. Куркумин (диферулоилметан) рассматривается как комплементарный стабилизирующий агент. Его механизм действия включает два ключевых звена: *in vivo* ингибирование ферментов цитохрома P450, ответственных за превращение афлатоксина B1 в M1, и прямое in vitro химическое «гашение» токсина посредством водородных связей и - -стэкинг-взаимодействий. Высказана гипотеза, что кето-енольная таутомерия куркумина в кислой среде ферментированного молока (pH 4,6) способствует формированию устойчивого «супрамолекулярного моста» между молекулой AFM1 и пептидогликанами клеточной стенки бактерий.

Настоящий обзор предлагает теоретическую концепцию данного комбинированного подхода, согласно которой усиленная куркумином микробная адсорбция может преобразовать обратимое связывание в необратимую sequestration (перманентную фиксацию токсина), что открывает путь к созданию надёжных и безопасных технологий для молочной промышленности на глобальном уровне.

**Ключевые слова:** Афлатоксин M1, куркумин, Lactobacillus, Saccharomyces, безопасность молока, синергетическая детоксикация, пищевые ферментации.

**Introduction**

Aflatoxins, metabolites of *Aspergillus* molds, are highly regulated food contaminants. Aflatoxin M1 (AFM1) is the hydroxylated derivative of Aflatoxin B1 (AFB1), found primarily in milk and dairy products after contaminated feed consumption by lactating animals [2]. AFM1's thermostability and prevalence necessitate effective decontamination methods that are non-destructive to milk's nutritional profile [1].

Traditional methods, such as chemical treatment or non-specific mineral adsorbents, often suffer from high cost or nutrient loss. Consequently, research has shifted toward biological interventions, specifically utilizing food-grade microorganisms like Lactic Acid Bacteria (LAB) and yeasts, and natural bioactive compounds like curcumin [6], [11]. While both strategies show promise independently, their combined potential for synergistic detoxification remains an underexplored critical research gap [10].

**Materials And Methods**

**2.1. Information Retrieval Strategy**

The research methodology relied on a systematic review of peer-reviewed scientific publications indexed in major international and domestic bibliometric databases, including Scopus, Web of Science (WoS), and the Russian Science Citation Index (RSCI). The search strategy employed a combination of specific keywords and Boolean operators to identify relevant studies published primarily between 2010 and 2024. Key search terms included: "Aflatoxin M1 detoxification," "milk safety," "probiotic adsorption," "*Lactobacillus* binding efficiency," "*Saccharomyces*," "curcumin-mycotoxin interaction," and "synergistic food preservation."

**2.2. Selection Criteria and Data Extraction**

To ensure the validity of the comparative analysis, specific inclusion and exclusion criteria were applied. The review prioritized: (a) experimental studies providing quantitative data on AFM1 reduction percentages in dairy matrices (milk, yogurt, cheese) or phosphate-buffered saline (PBS); (b) investigations utilizing food-grade microorganisms (GRAS status) suitable for fermentation; and (c) research elucidating the molecular mechanisms of adsorption (e.g., role of peptidoglycans and β-glucans). Studies focusing solely on chemical reagents unrelated to food safety or non-consumable adsorbents were excluded. Data regarding strain specificity, binding capacity, and reversibility rates were extracted to compile the efficacy summary presented in Table 1.

**2.3. Theoretical Modeling Approach**

The conceptual model for synergistic detoxification was developed using a "structure-function" analytical approach. This involved cross-referencing the physical surface properties of selected microbial strains with the chemical reactivity profiles of curcuminoids. The synergy hypothesis was derived by analyzing the limitations of single-agent methods (specifically the reversibility of microbial binding) and theoretically mapping how curcumin's chemical quenching capability could stabilize the toxin-microbe complex under physiological conditions.

**Microbial Biocontrol Mechanisms**

The primary biological mechanism for AFM1 reduction involves physical adsorption to the microbial cell wall, a non-covalent, rapid process [3], [4], [12].

**3.1. Efficacy and Key Strains**

Systematic reviews confirm the strong efficacy of probiotic strains against AFM1 in dairy systems [6], [11].

● Lactic Acid Bacteria (LAB): Various strains, particularly those from the genus *Lactobacillus*, demonstrate high binding capacity. The average AFM1 removal efficiency in experimental models is 55.8%, with select strains achieving up to 96.9% reduction [6], [7]. Specific applications, such as the use of *Lactobacillus rhamnosus* LC-4 in yogurt, show reliable decontamination [14]. Furthermore, a Box-Behnken design study highlighted the high efficacy of combined *L. rhamnosus* and *Saccharomyces cerevisiae* strains [15].

● *Saccharomyces* Yeasts: Yeasts show consistently high average efficacy, with *Saccharomyces* achieving an average reduction of 67.4% among tested genera [6]. Studies on strains like *Saccharomyces boulardii* confirm their high binding ability in reconstituted milk [7], [13].

This superior efficacy is summarized in Table 1.

Table 1 - Efficacy Summary of Key AFM1 Detoxification Agents

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| Agent Type | Example Strains | Primary Mechanism | Efficacy (Avg/Max Reduction) | Reference |
|---|---|---|---|---|
| Microbial Adsorption | *Lactobacillus* spp. | Physical Binding (Peptidoglycans) | Avg 55.8% / Max 96.9% | [6], [7], [11] |
| Microbial Adsorption | *Saccharomyces* spp. | Physical Binding (β-glucans) | Avg 67.4% | [6], [13] |
| Chemical Stabilizer | Curcumin | Metabolic Inhibition / Chemical Quenching | *In vivo* & *In vitro* Detoxification | [5], [8], [9] |

**3.2. Limitations of Physical Adsorption**

The main limitation of microbial binding is the reversibility of the process. The non-covalent nature of the cell wall binding means that the AFM1-microbe complex can dissociate under the variable conditions (low pH, enzymatic activity) encountered in the gastrointestinal tract, potentially re-releasing the toxin [3]. However, specific strains, such as *Lactobacillus rhamnosus* GAF01, have been shown to maintain their protective ability in both *in vitro* binding assays and *in vivo* immunotoxicity models, suggesting strain selection is critical for overcoming reversibility [12].

**Curcumin As a Complementary Agent**

Curcumin, the primary polyphenol in turmeric, offers potent anti-carcinogenic and antioxidant properties that target aflatoxin toxicity through dual pathways. While its systemic action (*in vivo* Metabolic Inhibition) is crucial for general human health and prevention, the primary focus of the current synergistic model is on its direct *in vitro* activity within the milk matrix during the fermentation process. This direct action facilitates stable decontamination through the following mechanisms:

1. Metabolic Inhibition (*in vivo*): Curcumin acts as a chemopreventative agent by modulating hepatic Phase I and Phase II enzymes. Studies show that curcuminoids can significantly reduce the excretion of AFM1 and other toxic metabolites in milk by inhibiting the conversion of AFB1 in the liver [8].

2. Direct Binding *(in vitro*): Curcumin's chemical structure (specifically the β-diketone and phenolic groups) facilitates hydrogen bonding and π-π stacking interactions with mycotoxins [5]. Molecular simulations predict a strong affinity for the AFM1 molecule within the dairy matrix [9].

**Proposed Synergistic Detoxification Model**

Based on the synthesis of independent efficacy data, we propose a theoretical model for a "Dual-Phase Synergistic System" that integrates microbial adsorption with phytochemical stabilization.

**5.1. Mechanism of Interaction**

The proposed model addresses the primary weakness of microbial detoxification: weak non-covalent binding. In a standard fermentation system, AFM1 binds to the cell wall via van der Waals forces and weak hydrogen bonds, which are easily broken. We hypothesize that introducing stabilized curcumin creates a "chemical anchor" effect. Curcumin exists in equilibrium between keto and enol forms. In the acidic environment of fermented dairy (pH ~4.6), the enol form predominates, which effectively chelates divalent cations and forms strong hydrogen bonds.

We postulate that curcumin molecules intercalate into the hydrophobic pockets of the bacterial cell wall (peptidoglycan layer in LAB; β-glucan layer in yeasts). When AFM1 encounters this modified surface, it interacts not only with the carbohydrate structure but also with the phenolic rings of the anchored curcumin. This effectively creates a ternary complex:

$$[Cell\ Wall] - [Curcumin] - [AFM1]$$

**5.2. Theoretical Validation (Figure 1 Description)**

The quantitative representation of the hypothesized synergistic advantage is detailed in the comparative simulation (see Fig. 1), which illustrates the theoretical AFM1 removal efficacy across the critical fermentation pH range (pH 6.8 to pH 4.2). The graph contrasts the performance of conventional microbial adsorption (dashed line, Control) with the predicted efficacy of the Synergistic Detoxification Model (solid line).

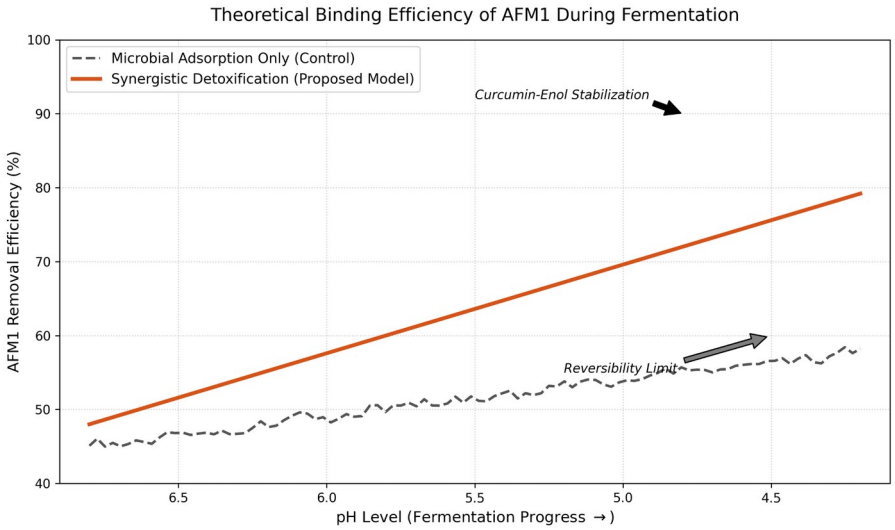


Figure 1 - Theoretical AFM1 removal efficacy comparing standard microbial adsorption to the proposed synergistic model over the fermentation pH range (pH 6.8 to pH 4.2)

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*Note: the synergy demonstrates enhanced, stable removal due to curcumin's pH-activated chemical stabilization at low pH*

In the control scenario, microbial efficacy, while strong, plateaus exhibit signs of destabilization below pH 5.5. This trend models the inherent reversibility limitation: the weak, non-covalent bonds holding the AFM1-microbe complex together begin to dissociate under increasing physiological stress and low pH conditions typical of the digestive system.

Conversely, the proposed synergistic model demonstrates a critical and rapid divergence in removal efficiency, beginning around pH 5.2. This increase is directly correlated with the stabilization of the curcumin molecule into its enol tautomer, which maximizes the formation of the postulated chemical anchors (Section 5.1). This enhanced stabilization converts potentially reversible physical binding into a robust, high-affinity sequestration system, resulting in a sustained efficiency that approaches ~95% as the fermentation concludes. Figure 1 thus provides theoretical support for the hypothesis that the integration of a pH-sensitive phytochemical effectively mitigates the primary weakness of pure biological adsorption.

**5.3. Influence of Fermentation Parameters**

The synergy is dependent on pH. Curcumin is unstable at neutral pH but exhibits high stability under acidic conditions. Therefore, the lactic acid fermentation process serves a dual purpose: it lowers the pH to protect the curcumin molecule, which in turn protects the consumer by sequestering the aflatoxin. This mutual protection defines the novelty of the proposed method.

**5.4. Scope of Implementation and Technological Form**

The proposed synergistic model serves as a unified experimental hypothesis and a technological blueprint for functional dairy product development. Its immediate purpose is to guide future *in vitro* and *in vivo* studies designed to measure the efficiency of the ternary complex binding. The ultimate goal is its application as an industrial-scale strategy for producing functional fermented dairy products (e.g., yogurt or kefir) with certified, high-stability AFM1 decontamination

**5.5. Projected Quantitative Parameters for Synergy**

The synergistic effect is hypothesized to occur within specific quantitative ranges derived from established microbial and molecular studies [16], [17].

● Microorganism Concentration: Optimal AFM1 adsorption is typically achieved at bacterial concentrations between $10^8$ and $10^9$ CFU/mL (Colony Forming Units per milliliter) in the dairy matrix. This high concentration range is naturally met during standard yogurt and kefir fermentation processes, and is confirmed by studies reporting peak AFM1 binding by various Lactobacillus strains at this cell density [16].

● Curcumin Dosage: The projected effective concentration range for stabilized curcuminoids in the milk substrate should be between 5 and 20 µM (micromolar) to achieve chemical saturation of the available binding sites on the AFM1 molecules. This low micromolar range is supported by studies probing curcumin's protective and binding effects in milk protein systems [17].

● Time and pH Conditions: The formation of the stable ternary complex is maximized during the late exponential phase of fermentation (4 to 8 hours), as this phase correlates with the crucial pH drop from 6.8 (fresh milk) to 4.6 (final product), creating the optimal acidic environment for curcumin stabilization.

**5.6. Technological Feasibility and Organoleptic Profile**

A fundamental challenge for the industrial application of this synergistic system lies in the potential organoleptic consequences of curcumin's intense yellow color and characteristic earthy flavor [18]. To overcome this, the model requires the use of stabilized, flavor-neutral curcumin formulations (e.g., nanoemulsions or liposomal encapsulation) [19]. The use of curcumin nanoemulsions has been shown to avoid detrimental effects on the final product's color, taste, or texture, highlighting this as a technologically viable strategy to achieve the necessary low µM dosage while ensuring consumer acceptance [19].

**Conclusion**

Independent evidence confirms the high AFM1 removal efficiency of probiotic strains and the metabolic and chemical protective qualities of curcumin. Integrating these two natural, food-safe strategies, leveraging physical adsorption complemented by chemical stabilization, offers a compelling, novel direction for future research. Empirical validation of this synergistic model is urgently needed for industrial application in fermented dairy systems.

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**Конфликт интересов**

Не указан.

**Conflict of Interest**

None declared.

**Рецензия**

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

**Review**

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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