

# Optimized Enzymatic Liquid Detergents: From Interfacial Stability and Colloidal Characterization to Enhanced Food Allergen Removal

*José M. Vicaria, Diego F. Salazar-Valdez, Juan F. Martínez-Gallegos, Ana I. García-López, Josefa Núñez-Olea, Irene Hurtado-París, Raquel Gómez-González, Alejandro Ávila-Sierra*

*Department of Chemical Engineering, Faculty of Sciences, University of Granada, Avda. Fuentenueva s/n, 18071 Granada, Spain.*

*VICARIA@UGR.ES*

## Introduction and Objective:

The development of sustainable detergent formulations increasingly relies on the incorporation of bioactive components (enzymes [1], essential oils [2], biosurfactants) to comply with environmental regulations while enhancing performance in allergen removal [3]. In this context, maintaining enzyme stability within complex colloidal systems remains a critical challenge. This study investigates the stability and compatibility of commercial protease (Blaze® Pro 100L) and  $\alpha$ -amylase (Achieve® Alpha 100L) in multicomponent detergent formulations containing anionic surfactants (LAS Linear Alkylbenzene Sulfonate, LES Sodium Lauryl Ether Sulfate), non-ionic surfactants (GLUCOPON 600® Alkyl Polyglucosides, FINDET 1214N23® Fatty Alcohol Ethoxylate, REWOFORM SL-ONE Sophorolipids), and essential oil-derived bioactives (e.g., bioflavonoids, polyphenols). The objective is to elucidate synergistic interactions between surfactants systems and bioactive additives that preserve enzymatic activity while maximizing the efficiency of food allergen removal under realistic cleaning conditions.

## Methods:

Accelerated aging tests were conducted at 40-50°C over 14 days to evaluate enzymatic stability under storage-relevant stress conditions. Enzymatic activity was quantified using modified Anson and DNS assays for protease and  $\alpha$ -amylase, respectively. Optimal formulations were characterized by Multi-Angle Dynamic Light Scattering (MADLS) using Zetasizer system to determine particle size distribution, zeta potential, and polydispersity index (PDI), providing insight into colloidal stability. Finally, cleaning efficiency was assessed on surfaces contaminated with fish, egg, and gluten allergens, complemented by mechanical soil removal analysis using a Millimanipulator.

## Results and Discussion:

Protease exhibited high thermal stability at 40°C, while amylase showed increased sensitivity to anionic surfactants. Among the systems evaluated, FOR1 (LAS/LES/ GLUCOPON 600®/FINDET 1214N23®) and FOR2 (LAS/LES/ REWOFORM SL-ONE/GLUCOPON 600® / FINDET 1214N23®) were identified as the most effective multicomponent formulations. FOR1 showed the highest overall stability, maintaining elevated relative enzymatic activity and a zeta potential  $>|30|$  mV, indicative of strong colloidal stability. FOR2 also proved to be a robust alternative, with a measured particle size of  $3.6 \pm 0.6$  nm and a zeta potential of  $-33.4 \pm 5.3$  mV, confirming its stability alongside high residual activity. Physicochemical characterization further suggested that enzyme incorporation promotes the formation of stable micellar aggregates in selected formulations. Cleaning assays demonstrated a significant reduction in allergen load (approx. 60-80%) for fish, egg, and gluten residues compared to water controls. In parallel, millimanipulator measurements revealed that enzymatic formulations effectively reduce the elastic modulus (E) of food soils, facilitating their mechanical detachment and overall removal efficiency.

## Conclusions:

The synergy between tailored surfactant systems and industrial enzymes not only preserves catalytic activity but also enhances the removal of persistent biological soils. In particular, formulations such as FOR1 and FOR2 demonstrate that the combination of anionic, non-ionic, and biosurfactants components can stabilize enzymes within complex colloidal environments while maintaining high cleaning performance. These systems represent a promising and sustainable approach for advanced industrial cleaning applications, contributing to improved allergen removal and enhanced food safety standards.

## Keywords:

Protease,  $\alpha$ -amylase, surfactant stability, allergen removal, colloidal characterization

## References:

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