Enzymatic Extraction and Sulphur Dioxide Stabilisation Strategies for Preserving Phenolics in Olive Mill Waste

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1. Introduction

Olive mill waste (OMW) is a major by-product of olive oil production, representing both an environmental challenge and a valuable source of bioactive phenolic compounds with antioxidant, antimicrobial, and anti-inflammatory properties (Obied *et al* 2007). Due to their polar nature, approximately 98% of olive phenolics are lost to the aqueous and solid waste streams during oil extraction (Rodis *et al* 2002). Post-production degradation of OMW is largely driven by polyphenol oxidase-mediated oxidation, resulting in rapid losses of ortho-diphenolic compounds during storage and drying (Gutiérrez-Rosales *et al* 2003). Sulphur dioxide (SO_2), released in situ from sodium metabisulfite, is a known inhibitor of oxidative enzymes and microbial activity in food systems (Bokulich *et al* 2015); however, its targeted application for enzymatic stabilisation of olive pomace prior to drying and extraction remains underexplored. This study therefore investigates SO_2-mediated enzymatic inhibition as a strategy to preserve phenolic integrity and enhance extraction efficiency from olive pomace.

2. Materials and Methods

Two complementary experimental designs were conducted to investigate enzymatic, chemical, and processing factors affecting the extraction and preservation of bioactive compounds from OMW. Fresh olive pomace was collected from a single local olive oil producer to ensure homogeneity and processed within 24 hours of production. Experiment 1 employed a full factorial design to optimise pectinase-assisted extraction, evaluating the effects of temperature (25, 40 and 60°C), pH (3–5), and enzyme concentration (0, 1, 5 and 10%) over a fixed extraction time of 3 h. Experiment 2 assessed the impact of sulphur dioxide stabilisation and drying conditions, where fresh olive pomace was treated with sodium metabisulfite (0, 10, 15 and 20% w/w), combined with enzyme concentrations (0%, 1%, 5%, and 10%), and subjected to controlled convective drying (40, 60 and 80 °C). Total phenolic content (TPC) (Singleton *et al* 1999), total flavonoid content (TFC) (Mabry *et al* 1970), total ortho-diphenolic compounds (TdOPC) (Mateos *et al* 2001), cupric reducing antioxidant capacity and ferric reducing antioxidant power assays (Apak *et al* 2016 and Benzie *et al* 1996) were determined spectrophotometrically for all experiments.

3. Results and Discussion

Factorial analysis for experiment 1 demonstrated that temperature, pH, and pectinase concentration significantly influenced phenolic recovery and antioxidant capacity. Total phenolic content (TPC) and total flavonoid content (TFC) increased with temperature and enzyme loading, confirming that pectinase-enhanced cell wall degradation facilitates the release of bound phenolics. Temperature was the dominant factor governing extraction efficiency, while significant temperature–enzyme interactions indicated that enzymatic activity is maximised under moderate thermal conditions, consistent with enzyme-assisted extraction behaviour reported in plant matrices (Carciochi *et al* 2015; Che Sulaiman *et al* 2017). Antioxidant-related responses (TdOPC, CUPRAC, FRAP) were primarily influenced by pH and its interaction with temperature, rather than enzyme concentration alone. Mildly acidic conditions promoted higher ortho-diphenolic stability and antioxidant capacity, reflecting reduced oxidative degradation and improved phenolic stability under acidic environments (Ruenroengklin *et al* 2008).

Sulphur dioxide pretreatment in experiment 2 significantly improved phenolic preservation during drying. Metabisulfite-treated samples consistently exhibited higher TPC and TFC values and lower TdOPC formation than untreated controls across all drying temperatures. Intermediate SO_2 levels (≈15% w/w) combined with controlled drying at 60 °C provided the most favourable balance between preservation and processing efficiency. These results indicate that enzymatic inhibition, rather than extreme drying conditions, is the primary mechanism governing phenolic retention. Sulphur dioxide suppresses polyphenol oxidase activity by reducing quinone intermediates, thereby limiting oxidative degradation (Cape, 1984; Obied *et al* 2007). Similar dose-dependent stabilisation effects of SO_2 have been reported in wine and other food systems (Bokulich *et al* 2015; Fišera *et al* 2022). Importantly, industrial drying preceded by metabisulfite treatment yielded acceptable preservation outcomes, demonstrating process scalability.

4. Conclusions

The results show that phenolic recovery and stability in OMW are strongly influenced by enzymatic activity, processing conditions, and chemical environment. Enzyme-assisted extraction improved phenolic release, while sulphur dioxide generated from sodium metabisulfite effectively inhibited polyphenol oxidase activity during drying, leading to higher phenolic retention and reduced oxidative degradation. Moderate SO_2 levels combined with controlled drying provided optimal preservation. Overall, this work demonstrates a scalable strategy for the sustainable valorisation of OMW into high-value bioactive extracts.

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6. References

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