F.13. ADAPTATION OF ACID HYDROLYSIS-SOXHLET EXTRACTION METHOD FOR TOTAL FAT DETERMINATION IN GLUTEN-FREE MUFFINS FORMULATED WITH WAX-BASED OLEOGELS AS BUTTER REPLACERS

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Abstract. The growing use of wax-structured oleogels as solid fat replacers in bakery products creates a methodological gap in routine fat analysis: standard solvent extraction protocols, originally developed for conventional triglyceride-based fats, may yield systematically biased results when applied to wax-containing food matrices. This study evaluated and adapted the acid hydrolysis–Soxhlet extraction method (ISO 11085/AOAC 954.02) for accurate total fat determination in gluten-free muffins in which butter was replaced 100% by wax-based oleogels. Two gluten-free muffin series were investigated: sorghum flour-based muffins formulated with corn oil/sunflower wax oleogels (2, 4, 6, 8, and 10% wax), and buckwheat flour-based muffins with soybean oil/beeswax oleogel (2, 4, 6, 8, and 10% wax), alongside a butter-based control. Fat content was determined using three protocols: direct Soxhlet extraction with petroleum ether (b.p. 40-60 °C); acid hydrolysis (4 N HCl) followed by Soxhlet extraction with petroleum ether (40-60 °C), and acid hydrolysis followed by Soxhlet extraction with n-hexane (b.p. 68-70 °C) on a VELP SER 148 apparatus.

Results demonstrated that petroleum ether (40-60 °C) produced significantly underestimated fat values (up to 3.1-4.8% lower on a dry weight basis) compared to n-hexane extraction, particularly at higher wax concentrations (≥ 6%). This discrepancy is attributed to the incomplete dissolution of wax crystalline networks at the solvent boiling point, which falls below the melting temperature of both sunflower wax (74-77 °C) and beeswax (62-65 °C). Furthermore, sorghum flour matrices required an extended hydrolysis time of 80-90 min due to the resistance of kafirin protein bodies to acid cleavage, whereas standard 60 min hydrolysis was sufficient for buckwheat and control formulations.

The adapted protocol – acid hydrolysis with matrix-specific duration followed by n-hexane extraction – yielded reproducible and complete fat recovery (RSD < 1.8%, recovery 97.2-99.6%). A correction for the wax ester contribution to the crude fat value is proposed for nutritional labelling purposes. These findings establish a validated analytical approach for fat analysis in the emerging category of wax-oleogel bakery products and highlight the critical importance of solvent selection in Soxhlet-based lipid extraction from non-conventional food fat systems.

Keywords: bakery products, buckwheat flour, lipid analysis, kafirin protein bodies, n-hexane, sorghum flour.

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