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Saponification Value of Fats and Oils as Determined from $^1\text{H-NMR}$ Data: The Case of Dairy Fats

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Abstract: The saponification value of fats and oils is one of the most common quality indices, reflecting the mean molecular weight of the constituting triacylglycerols. Proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra of fats and oils display specific resonances for the protons from the structural patterns of the triacylglycerols (i.e., the glycerol backbone), methylene ($-\text{CH}_2-$) groups, double bonds ($-\text{CH}=\text{CH}-$) and the terminal methyl ($-\text{CH}_3$) group from the three fatty acyl chains. Consequently, chemometric equations based on the integral values of the $^1\text{H-NMR}$ resonances allow for the calculation of the mean molecular weight of triacylglycerol species, leading to the determination of the number of moles of triacylglycerol species *per* 1 g of fat and eventually to the calculation of the saponification value (SV), expressed as mg KOH/g of fat. The algorithm was verified on a series of binary mixtures of tributyrin (TB) and vegetable oils (i.e., soybean and rapeseed oils) in various ratios, ensuring a wide range of SV. Compared to the conventional technique for SV determination (ISO 3657:2013) based on titration, the obtained $^1\text{H-NMR}$ -based saponification values differed by a mean percent deviation of 3%, suggesting the new method is a convenient and rapid alternate approach. Moreover, compared to other reported methods of determining the SV from spectroscopic data, this method is not based on regression equations and, consequently, does not require calibration from a database, as the SV is computed directly and independently from the $^1\text{H-NMR}$ spectrum of a given oil/fat sample.

Keywords: saponification value; $^1\text{H-NMR}$ spectroscopy; tributyrin; dairy fat; vegetable oils

1. Introduction

One of the most common oil quality indices is the saponification value (SV); it is defined as the amount of alkali (expressed as mg KOH/g sample) required to saponify a defined amount of sample. It is conventionally determined through saponification of a

known amount of oil/fat with excess KOH solution, followed by back titration of the excess base with acid solution in the presence of phenolphthalein as an indicator. The amount of base needed for saponification of the fatty acyl chains is then indirectly determined from the excess base that remains unreacted. Since the amount (moles) of base reacted is stoichiometrically equal to the amount (moles) of fatty acyl chains contained in 1 g of oil/fat, SV is then dependent on the length of the fatty acyl chains from triacylglycerols. Therefore, a small saponification value indicates long chain fatty acids on the glycerol backbone in a sample; on the contrary, a high SV indicates triacylglycerols with shorter fatty acyl chains. Consequently, SV becomes an easy approach to assess fatty acids' chain length of specific fats/oils.

For example, most of the common oils/fats of vegetable or animal origin (sunflower, soybean, rapeseed, pork lard, beef tallow, chicken fat, etc.) contain almost only long chain fatty acids (C18 and C16), having similar SV values (ranging from 168–196 mg KOH/g oil) [1]. Some vegetable oils, such as the coconut and palm kernel oils, contain large amounts of lauric (C12:0) and myristic (C14:0) acids; therefore, their saponification values are significantly higher (235–260 mg KOH/g oil) [2–5]. Milk fat differs substantially from other fats and oils in terms of the fatty acid profile (FAP), including relevant amounts of short chain (C4–C6) and medium chain (C8–C12) fatty acids, which is subsequently reflected in its high SV (213–227 mg KOH/g fat) [6,7]. Consequently, SV may be helpful in the detection of the adulteration of dairy products with cheaper fats and oils, because the addition of an oil/fat rich in C18 to a dairy product will result in a decrease in the SV.

Although easy and accurate, the reference method of SV determination requires specific glassware and harmful chemicals and is time consuming (according to the protocol, the saponification step takes one hour to complete, because it is critical that the saponification be complete prior to the final titration). In addition, several factors can cause errors in the titration step including misjudging the color of the indicator near the end point, misreading volumes or faulty technique. Therefore, a new, rapid and reliable method would be preferred.

In this respect, spectroscopic methods coupled with multivariate data analysis have attracted attention, being considerably faster and more practical from a procedural viewpoint. For example, SV has been determined through Fourier transform infrared spectroscopy (FTIR) coupled with multivariate analysis [8] with good accuracy, compared to the standard method; however, the main drawback of the methods based on spectroscopic data is that they require the existence of a large spectral base for the model calibration.

¹H-NMR spectroscopy is a fast (the recording of a ¹H-NMR spectrum takes approximately 2 min) and non-destructive technique that has widely been applied in the analysis of edible oils. ¹H-NMR spectra of fats and oils display signals assigned to both the unsaturated moiety and to various methylene groups of the fatty acyl chains. These signals may be used to calculate the average fatty acyl chain length of fat samples. The ¹H-NMR technique allows for full process automation, from the recording (due to the autosamplers) to data processing. Small amounts of samples are necessary, which—if needed—can further be recovered simply through solvent evaporation, after the spectra are recorded. Very importantly, the ¹H-NMR technique is also reliable, and several papers report the fatty acid profile of fats and oils computed from ¹H-NMR data in good agreement with chromatographic data [9–13]. Skiera et al. briefly reported a rapid method for the determination of the SV from NMR data based on the integral of the CH₂ protons adjacent to the ester groups (δ_{H} 2.2–2.4 ppm) and on the integral of the 1,2,4,5-tetrachloro-3-nitrobenzene (TCNB) signal at δ_{H} 7.7 ppm, used as an internal standard for quantitative NMR experiments. Five samples (with a single measurement *per* sample) were tested with the new method; the NMR results were in agreement with the values obtained through the ISO method, consequently pointing at the suitability of the NMR spectroscopy for the determination of the quality indices of fats and oils [14].

Based on our previous expertise on NMR chemometrics to edible oils [13], the present work reports a general algorithm for the calculation of the SV of fats and oils from the ¹H-

NMR data. The working model consists of a series of binary mixtures of tributyrin (TB) and vegetable oils in various ratios to obtain a wide range of SV. In addition, to ensure an even more variate composition also regarding the unsaturation, soybean and rapeseed oils—SO and RO, respectively—were used to prepare the model samples. The average length of the fatty acyl chains can be computed through chemometric equations from $^1\text{H-NMR}$ data, leading to the calculation of the average molecular weight of each sample and eventually to the SV. The new method was evaluated in comparison with the conventional method based on titration and was further applied to a series of edible fats and oils including butter and cheese extracted fats. Compared to other reported methods of determining the SV from spectroscopic data, the proposed method is not based on regression equations and, consequently, does not require calibration from a database. SV may be computed directly and independently from the $^1\text{H-NMR}$ spectrum of a given oil/fat sample.

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