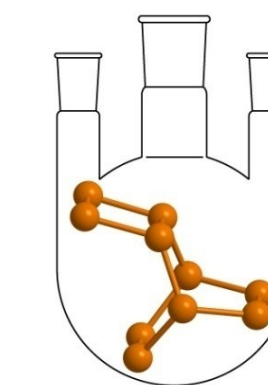


NMR Based Descriptors for the Detection
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1. INTRODUCTION

The substitution or replacement of milk fat with lower-cost oils or fats represents one of the most common fraudulent practices in the dairy industry, primarily due to the economic value of milk fat in the production of dairy derivatives. The main adulterants include vegetable oils (such as soybean, sunflower, coconut, and palm oils) and animal fats (such as beef tallow and pork lard) [1–3]. All structural configurations of triacylglycerols produce characteristic signals in the ¹H-NMR spectra of fats and oils, which depend on the number of contributing protons (Figure 1).

In this work, we present an original approach, based on three ¹H-NMR descriptors related to chain length, butyric and/or linolenic moieties and unsaturation to characterize the fat samples. 3D representation of the descriptors leads to samples clustering according to fat type. Further method development (data processing and clusters geometrical characterization) allows for the detection of non-dairy fats in cheese.

2. EXPERIMENTAL

Cheese samples.

Cheese (n=62) samples of bovine origin were obtained from ISO certified dairy companies. Cheese fat was extracted according to ISO 1735|IDF 5:2004 protocol. Adulterated cheese samples were prepared from cow milk with controlled addition of non-dairy fats.

Oil and fat samples.

Soybean, rapeseed and sunflower seeds were obtained from the NARDI Fundulea, Romania. Oil was extracted from seeds according to standard Soxhlet protocol [29]. Beef and sheep tallow were extracted with CH₂Cl₂ from subcutaneous adipose tissue, dried on anhydrous MgSO₄, followed by evaporation of the solvent. Coconut oil was purchased from Trio Verde S.R.L., Romania (distributor), palm stearin and palm kernel oil from Scintilla Silk, Romania (distributor).

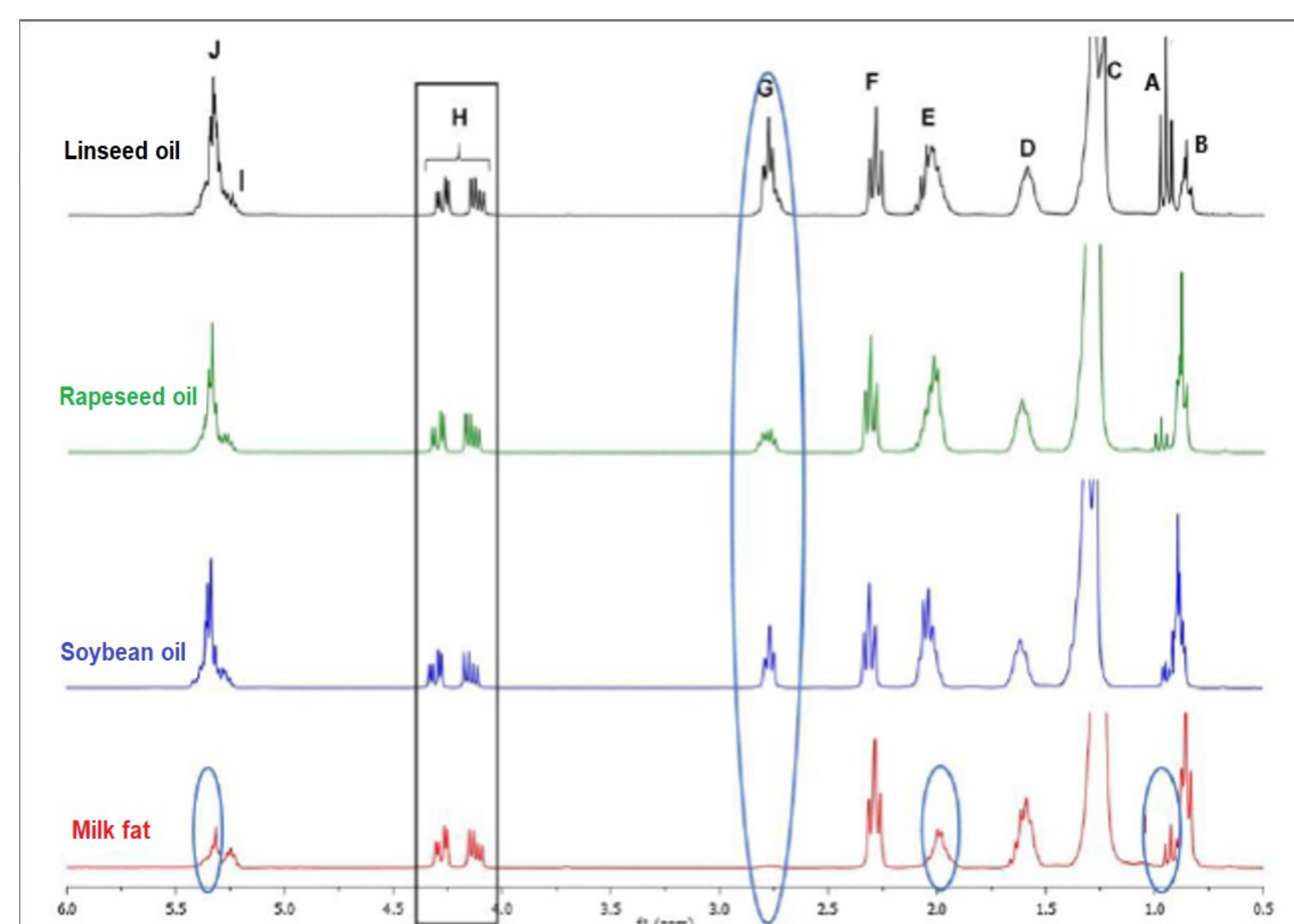
3. ¹H-NMR CHARACTERIZATION OF MILK FAT AND ADULTERANTS

Figure 1: ¹H-NMR spectra of milk fat and non-dairy fats and oils

4. RESULTS

Each fat sample, denoted as F, is characterized by two numerical values corresponding to the three conformity descriptors, expressed as F(D₁, D₂). When represented in a two-dimensional Cartesian coordinate system, the samples form distinct clusters according to their origin (Figure 2) [1].

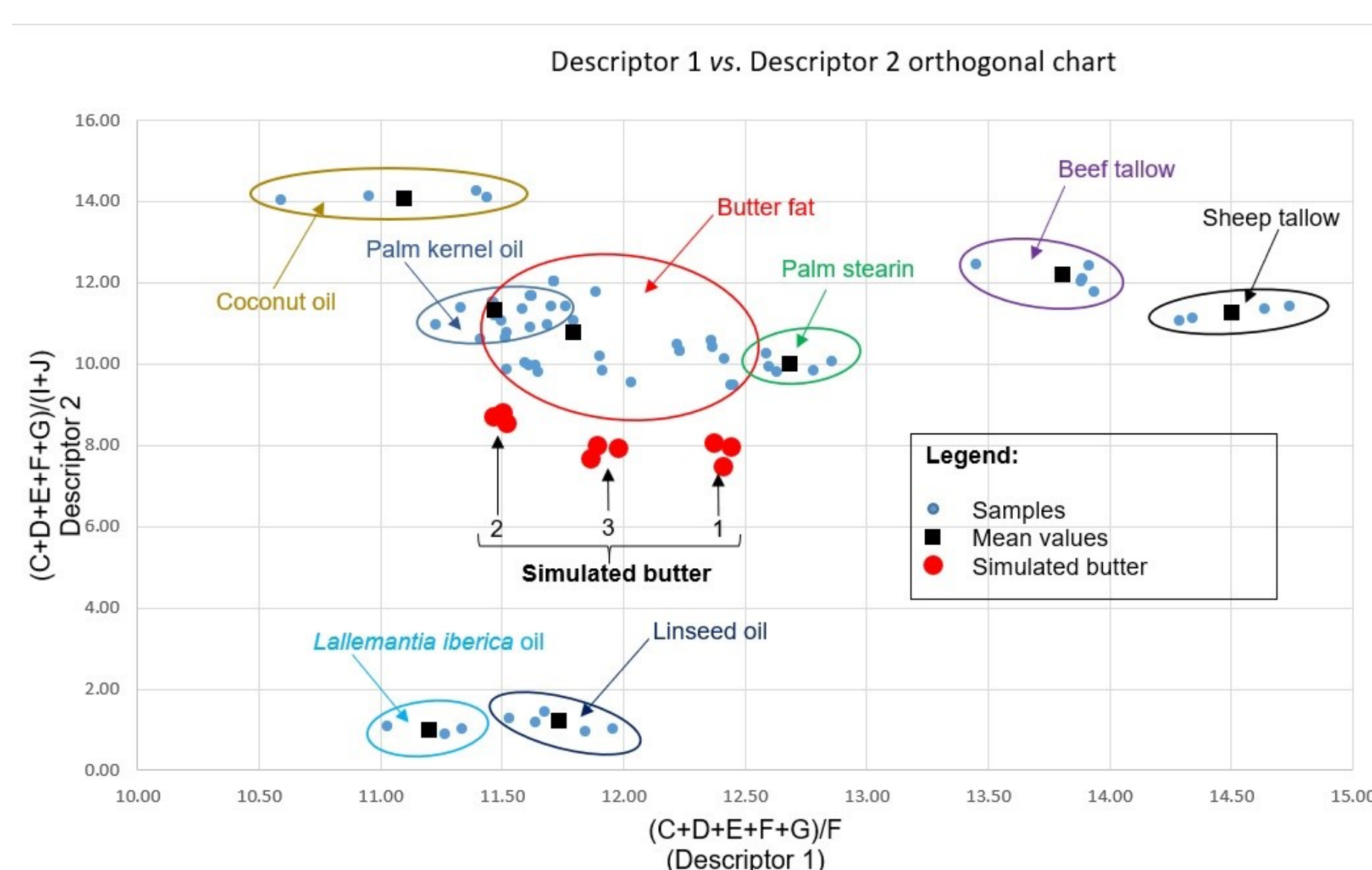


Figure 2: Orthogonal 2D representation of butter fat and non-dairy fats and oils

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Table 1: Peak assignment of ¹H-NMR spectra of fats and oils [4,5]

Signal	δ (ppm)	Proton	Compound
A	0.85	-CH ₂ -CH ₂ -CH ₂ -CH ₃	all acids except butyric acid and linolenic acid
B	0.96	-OOC-CH ₂ -CH ₂ -CH ₃	butyric acid
B'	0.96	-CH=CH-CH ₂ -CH ₃	linolenic acid
C	1.24	-(CH ₂) _n	all fatty acids
D	1.64	-CH ₂ -CH ₂ -COO-	all fatty acids
E	2.02	-CH ₂ -CH=CH-	all unsaturated fatty acids
F	2.26	-CH ₂ -COO-	all fatty acids
G	2.76	-CH=CH-CH ₂ -CH=CH-	n-6 (linoleic) acid and n-3 (linolenic) acid
H	4.19	-CH ₂ OCOR	H in the sn-1/3 position of the glycerol backbone
I	5.15	-CHOCOR	H in the sn-2 position of the glycerol backbone
J	5.29	-CH=CH-	all unsaturated fatty acids

Descriptors of conformity
computed from the ¹H-NMR data [1]:

$$D_1 = \frac{I_C + I_D + I_E + I_F + I_G}{I_F}$$

$$D_2 = \frac{I_C + I_D + I_E + I_F + I_G}{I_I + I_J}$$

Calculating the distance of each sample from the center of the soybean (SO) group enables the discrimination of samples according to their respective categories (Figure 3). Samples belonging to the same group exhibit similar distances relative to the SO group, while these distances vary significantly between different groups. Cheese samples display distances to the SO group that are markedly distinct from those of other fat and oil clusters, confirming the effectiveness of the proposed approach in differentiating genuine cheese from potential adulterants. For adulterated cheese samples, the distance to the SO group deviates substantially from that of authentic samples, thereby allowing their classification as non-genuine (fraudulent) products.

