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Comparative Analysis of Butter spectra of FTIR and NMR in Combination with Tallow

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ABSTRACT: Butter is the product of milk fat, sometimes adulterations occurs with low-cost animal fat, which affects food safety and quality. The aim of this study was to evaluate the authenticity of butter in combination with bovine tallow (0-15% w/w) using FTIR and NMR methods. These studies have shown that by adding bovine tallow fat, there was a significant difference on peaks height in FTIR and value of NMR peaks. According to the statistical analysis obtained, L_Pseudo equations showed that in the FTIR spectra of 3473-3470, 723-720, 590-587 and 458-455 cm^{-1}, by increase in bovine tallow percentage, the height of these spectra was decreased. L_Pseudo equations for each ^{1}H NMR and ^{13}C NMR peak indicated that value (ppm) of these peaks decreased by increase percentage of tallow. Given value of R^2, it was found that none of the spectra were capable of estimating the presence of foreign fat.

Keywords: Bovine Tallow, FTIR, NMR, Pure Butter.

Introduction

One of the most important sources of nutrition in foods is milk fat (Hillbrick and Augustin, 2002). Typically, milk fat contains 66% saturated fatty acids, 30% monounsaturated fatty acids, and 4% polyunsaturated fatty acids (Aigster *et al*., 2000). Due to its taste, milk fat is accepted by the consumer because of its better quality than other fats. Butter is one of the products of milk fat that is economically important for the dairy industry and nutritionally and functionally for the customers (Nurrulhidayah *et al*., 2013b). One of the most common adulterations in butter is combination it with low-cost vegetable oil and animal fat, which affects food safety and quality (Javidnia *et al*., 2013). With increasing awareness of consumers about food safety and quality, they are always demanding a guarantee of

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food quality and content (Wilhelmsen, 2006).

Detection of adulteration and validation for butter usually require chromatographic techniques, which is a time consuming process (Chakrabarty *et al*., 1969). This method requires organic solvent to extract oil and fat, which can cause health problems for employees and it will also increase the environmental risk and, of course, the cost of analysis (Javidnia *et al*., 2013). Therefore, quality control laboratories strive to develop a fast, economical and reliable analytical method for determining authenticity of food and detecting any adulteration in product (Nurrulhidayah *et al*., 2013b). Due to the importance of detecting non-dairy fat in butter, a number of studies have been conducted by several research groups to develop analytical methods for this purpose (Lipp, 1995; Ulberth and Buchgraber, 2000).

Infrared spectroscopy (IR) methods are one of the effective alternatives that allow analytical methods to be carried out quickly without a need for sample preparation (Lee *et al*., 2009). Among the Infrared Spectroscopy (IR) methods, Fourier Transform Infrared Spectroscopy (FTIR) is a fast, inexpensive and high quality technology for material analysis that is easily used in foods to basic researches, quality control laboratories and industrial surveys (Karoui and De Baerdemaeker, 2007; Subramanian and Rodriguez-Saona, 2010). This powerful tool is also widely used for rapid quality control of olive oil (Maggio *et al*., 2009), honey (Bertellia *et al*., 2007), meat (Argyri *et al*., 2010) and dairy products (Lerma-Garcia *et al*., 2010). In fact, the FTIR spectroscopy for each sample separately shows information on the fundamental vibration and molecular stretching under infrared light in spectral range between 700 and 4000 cm^{-1}. As such, a spectral characteristic is obtained as a result of absorption by different chemicals (Karoui and De Baerdemaeker, 2007) and a fingerprint of each sample is presented.

NMR spectroscopy is other method which has been successfully used in recent years for analysis of various foods and their fats, chemical composition and authenticity of various products (e.g. cheese, oil, and milk) have been determined enough accurate (Brescia *et al*., 2004; Monakhova *et al*., 2012). NMR spectroscopy preserves the structure of foods and provides useful information from a complex and highly heterogeneous chemical system (Andreotti *et al*., 2000). NMR also supports food in industry and can help develop new ways to control food quality. This technology has been able to provide accurate spectral information on triglycerides, saturated and unsaturated fatty acids, and to detect structure of sample (Erich *et al*., 2015).

Based on the applications of FTIR and NMR and their benefits to determine food authenticity, our aim in this study was to evaluate the authenticity of butter in combination with animal fat of bovine tallow using FTIR and NMR methods.

Materials and Methods

- *Preparation of butter samples*

Milk sample from Holstein calf, collected at the end of the third month of spring season, was used to prevent from fluctuation of season changes in milk fat compositions level. Preparation of pure butter and its fat extraction were done (Nilchian *et al*., 2020). To prepare treatments, butter fat was mixed with different percentages of bovine tallow based on statistical analysis (0, 1.18, 2.63, 4.40, 6.06, 7.50, 9.94, 12.38, 13.69 and 15%).

- ***Collecting spectra based on FTIR spectroscopic system***

All spectra were obtained using Tensor 27TM FTIR spectrometer system. The system was equipped with an interferometer and a fully reflective DigiTect TM (ATR) detector. All analyzes were carried out at 35 ± 2 ° C. The spectra were obtained in the range of 700 to 4000 cm^{-1} with Fourier transform accuracy of 4 cm^{-1} and the spectra were recorded for each butter sample (Gori *et al*., 2012). 100 µl of each sample was used and the absorbance spectrum was collected against a background obtained from blank ATR cells. Before obtaining each spectrum, the ATR crystal was washed with hexane and then rinsed with acetone.

- ***Measurement of 1H NMR and 13C NMR***

^{1}H NMR and ^{13}C NMR was carried out under automatic conditions on AVANCE III 300 MHz spectrometer (Monakhova *et al*., 2013). In order to prepare sample, 8 mg of the sample was mixed with 1 ml chloroform containing 0.1% tetra methyl silane (TMS) and transferred to NMR tube for direct measurement. To prevent the peroxide resonances increase, the samples were directly measured at δ 5.80 ppm and δ 6.30 ppm (^{1} H NMR) and δ 130 ppm (^{13} C NMR). Chemical changes were evaluated by TMS signal.

- ***Statistical analysis***

In this research, to examine authenticity percentage, experiments were designed by statistical method with optimum mixture response surface methodology and it made an attempt to predict authenticity optimal point, so statistical software of design expert, version 8 was used.

Results and Discussion

- ***Collecting spectra based on FTIR spectroscopic system***

All spectra were obtained using FTIR spectrometer system as compared to the pure butter and are shown in Figure 1.

In the infrared spectra obtained by FTIR spectroscopy, they indicated the absorption band of water, O–H groups, in the region of 3000-3700 cm^{-1} (Rodriguez-Saona *et al*., 2006; Karoui and De Baerdemaeker, 2007). In the FTIR spectra, the band of 3008 cm^{-1} was due to C–H stretching of the cis double bond (Javidnia *et al*., 2013) which in this study no peak was observed in this range. The strong adsorption in range of 2900 and 2800 cm^{-1} was related to C–H stretching vibration in CH2 and CH3, respectively (Koca *et al*., 2010). Only strong peak in 1745 cm^{-1} range was due to C=O stretching vibration of groups of carbonyl triglycerides or acids and esters (Javidnia *et al*., 2013; Lerma-García *et al*., 2010). The very weak band near 1654 cm^{-1} was related to the stretching vibration of C=C group of cis olefins, and peaks in 1400-1200 cm^{-1} range were mainly related to the bending vibration of the CH2 and CH3 aliphatic groups(Javidnia *et al*., 2013). In the range of 1000-1300 cm^{-1}, there were stretching vibrations of C–O band of ethers and bending vibration of methylene group (Lerma-García *et al*., 2010). Below 1000 cm^{-1}, at about 723 (Muik *et al*., 2007) and 966 cm^{-1}, it was related to vibration of HC=CH deformation which has been reported as a marker band for determination of Trans fatty acids in fats and oils (Nurrulhidayah *et al*., 2013a). Upon closer examination, slight differences in 1117 and 1097 cm^{-1} range were consistent with the C–H bending vibration and C–H deformation vibrations, respectively. The observed FTIR spectral changes have been used as a basis for selecting spectral ranges in measurement of bovine fat in butter samples, which was consistent with other investigations

(Nurrulhidayah *et al*., 2013a). These figures showed that in all samples, all spectra were the same, making it impossible to visualize the spectra. Identical FTIR spectra have also been observed by other researchers in the study of vegetable oils (Javidnia *et al*., 2013).

Based on the FTIR spectra, the results of height of each peak obtained from different butter treatments were detailed in Table1.

The height of each peak obtained from FTIR spectra in different treatments were analyzed statistically. Statistical results showed the effect of fat type on the height of each peak in different spectra in each treatment (Table2).

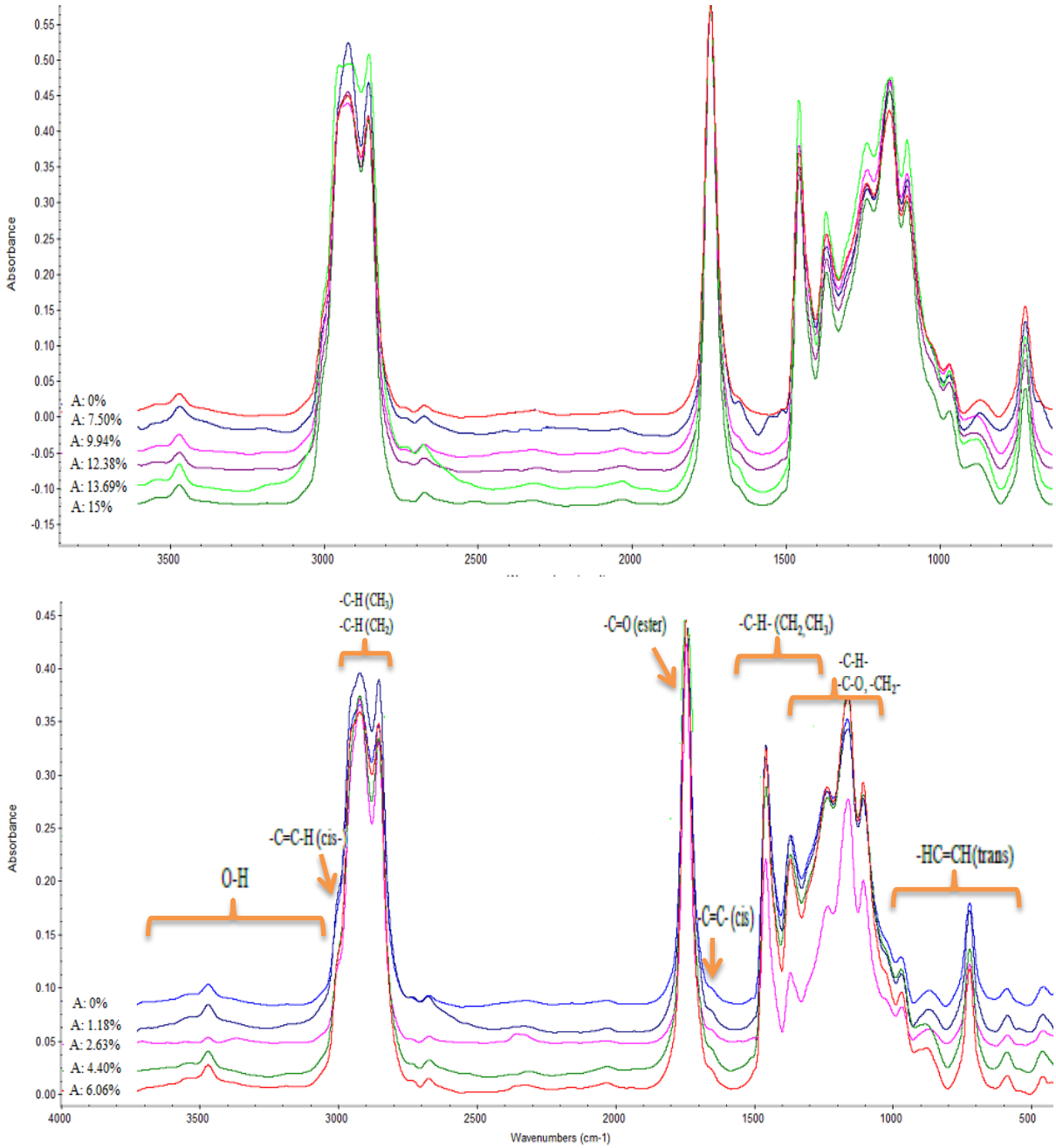

Fig. 1. Comparison of all FTIR spectra with pure butter

Table1. Peak Height of FTIR Spectra in Different Butter Treatments

The amount of tallow (%)	0	1.18	2.63	4.4	6.06	7.5	9.94	12.38	13.69	15
Wavelengths (cm^{-1})	The height of each peak									
3470-3473	0.031	0.03	0.006	0.022	0.026	0.009	0.028	0.022	0.035	0.024
2923-2926	0.449	0.408	0.337	0.381	0.358	0.143	0.503	0.49	0.561	0.482
2855-2858	0.419	0.399	0.295	0.337	0.347	0.129	0.48	0.46	0.572	0.454
2670-2673	0.014	0.038	0.007	0.013	0.013	0.005	0.014	0.015	0.06	0.015
2323-2329	0.007	0.006	0.009	0.001	0.008	0.011	0.007	0.002	0.011	0.006
2030-2036	0.007	0.003	0.003	0.007	0.009	0.001	0.008	0.005	0.013	0.007
1744-1747	0.574	0.467	0.388	0.456	0.443	0.157	0.642	0.601	0.638	0.586
1455-1458	0.367	0.325	0.18	0.29	0.323	0.106	0.442	0.393	0.513	0.389
1366-1369	0.254	0.222	0.069	0.22	0.22	0.068	0.315	0.273	0.367	0.274
1236-1240	0.325	0.273	0.133	0.278	0.287	0.089	0.407	0.369	0.457	0.36
1163-1166	0.427	0.343	0.235	0.385	0.369	0.128	0.531	0.505	0.541	0.485
1104-1107	0.308	0.265	0.159	0.281	0.292	0.093	0.403	0.369	0.461	0.357
969-972	0.072	0.064	0.035	0.105	0.094	0.02	0.127	0.113	0.157	0.111
867-870	0.022	0.024	0.014	0.048	0.041	0.007	0.053	0.046	0.067	0.048
720-723	0.153	0.137	0.077	0.125	0.117	0.04	0.156	0.143	0.202	0.137
587-590	0.024	0.017	0.007	0.021	0.016	0.006	0.017	0.015	0.027	0.016
455-458	0.027	0.019	0.012	0.022	0.015	0.018	0.022	0.019	0.036	0.02

All determinations were carried out in triplicate

Table 2. Statistical analysis of the effect of adding different amounts of bovine tallow in butter on the height of significant peaks obtained by FTIR

Range of spectra	Prob>F*	% C.V	L_Pseudo equation**
3470-3473	0.0345	33.45	3470-3473(height)=0.025A*** +0.031B*** -0.048AB+0.081AB(A-B) (R^2=0.3521)
2923-2926	0.0049	18.65	2923-2926(height)=0.48A+0.44B-0.81AB+0.64AB(A-B)+1.62AB(A-B)^2 (R^2=0.5812)
2855-2858	0.0048	19.93	2855-2858(height)=0.46A+0.41B-0.79AB+ 0.72AB(A-B)+1.56AB(A-B)^2 (R^2=0.5834)
1744-1747	0.0143	24.07	1744-1747(height)=0.65A+0.55B-0.93AB (R^2=0.3773)
1455-1458	0.0084	25.64	1455-1458(height)=0.41A+0.38B-0.58AB+0.93AB(A-B) (R^2=0.4849)
1366-1369	0.0087	30.63	1366-1369(height)=0.28A+0.26B-0.43AB+0.81AB(A-B) (R^2=0.4826)
1236-1240	0.0065	27.27	1236-1240(height)=0.38A+0.33B-0.53AB+0.92AB(A-B) (R^2=0.5054)
1163-1166	0.0071	24.34	1163-1166(height)=0.50A+0.43B-0.65AB+0.99AB(A-B) (R^2=0.4990)
1104-1107	0.0098	26.68	1104-1107(height)=0.38A+0.32B-0.47AB+0.79AB(A-B) (R^2=0.4725)
969-972	0.0092	38.73	969-972(height)=0.12A+0.053B (R^2=0.3315)
867-870	0.0024	40.68	867-870(height)=0.051A+0.018B (R^2=0.4338)
720-723	0.0187	26.35	720-723(height)=0.15A+0.16B-0.24AB+0.35AB(A-B) (R^2=0.4140)
587-590	0.0389	33.48	587-590(height)=0.017A+0.023B-0.036AB+0.050AB(A-B) (R^2=0.3394)
455-458	0.0482	23.71	455-458(height)=0.021A+0.026B-0.026AB+0.058AB(A-B) (R^2=0.3155)

All determinations were carried out in triplicate

* Values of "Prob> F" less than 0.05 indicate model terms are significant.

** The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

*** A: added tallow coefficient and B: butter coefficient.

These studies have shown that based on the cubic statistical model, by adding bovine tallow fat, there was a significant difference at 95% level on peak height in spectra of 3473-3470, 2926-2923, 2858-2855, 1758-1744, 1458-1455, 1369-1366, 1240-1236, 1166-1163, 1107-1104, 972-969, 870-867, 723-720, 590-587 and 458-455 cm^{-1} that this difference was not observed on other spectra. According to statistical results, the L_Pseudo equation was obtained, in which coefficients for fat and butter and their effects on each other were identified. Accordingly, equations for spectra of 3473-3470, 723-720, 590-587 and 458-455 cm^{-1} indicated that in general, both types of fat and butter had positive effects on these spectra, but tallow fat added was less effective than butter in increase of them, so that with increase in bovine tallow percentage, height of these spectra decreased. Equations for spectra of 2926-2923, 2858-2855, 1747-1744, 1458-1455, 1458-1455, 1369-1366, 1240-1236, 1166-1163, 1107-1104, 972-969 and 870-870 cm^{-1} showed both types of tallow and butter also had positive effects on these spectra, but the added tallow fat was more effective than butter in increasing the height of these spectra. In the L_Pseudo equation, height of the FTIR peaks in the sample could be determined using the coefficients of bovine tallow and pure butter and the relationship between these two fats. Given value of R^2, it was found that none of the spectra were capable of estimating the presence of foreign fat. According to the results of the effect of adding different amounts of bovine tallow in butter on the significant changes in height of each peak in different ranges, change in the amount of these spectra could indicate the adulteration in butter with different amount of bovine tallow in the sample.

- Measurement of 1H-NMR

The ^{1}H-NMR spectrum identified mixture of compounds such as triglycerides found in vegetable oils as well as animal fats and methyl esters with several prominent regions of fatty acid chains that contain specific proton signals (Nurrulhidayah *et al*., 2017). The ^{1}H-NMR spectra of pure butter samples and butter samples combined with bovine tallow in Figure 2 have been shown that presented similar profile.

According to the NMR spectra, these spectra mainly showed the signals of saturated unsaturated fatty acids, triglycerides, peroxide and glycerol. The chemical changes in the range of 0 to 4 ppm covered most of the free fatty acids and fatty acids attached to glycerol, while the chemical changes in the range of 4 to 6 ppm were specific to the proton signals in the glycerol column (Nurrulhidayah *et al*., 2017).

The ^{1}H-NMR spectra in the spectral range of 0.85-0.95, 1.90-2.10 and 2.70-2.90 ppm belonged to the CH3- ω1, the CH2- Δ2 and the base allyl methylene acid Alpha linolenic groups, respectively (Andreotti *et al*., 2000; Hu *et al*., 2007). Peaks in spectral region of 0.83-0.93 ppm were due to the overlap of the hydrogen atoms signals of the methyl group in the saturated acyl, oleic and linoleic chains and in spectral region of 0.93-1.03 ppm were due to the hydrogen atoms of methyl in the -3n acyl groups. Difference in chemical changes between both spectral regions was due to their proximity to the double bond. The spectral region of 1.22-1.42 ppm was due to the hydrogen atoms of the methylene groups in the β position of the olefinic groups, or in the γ position of the carbonic groups within the triacylglycerol molecule. The spectral region of 1.52-1.22 and 2.23-2.36 ppm

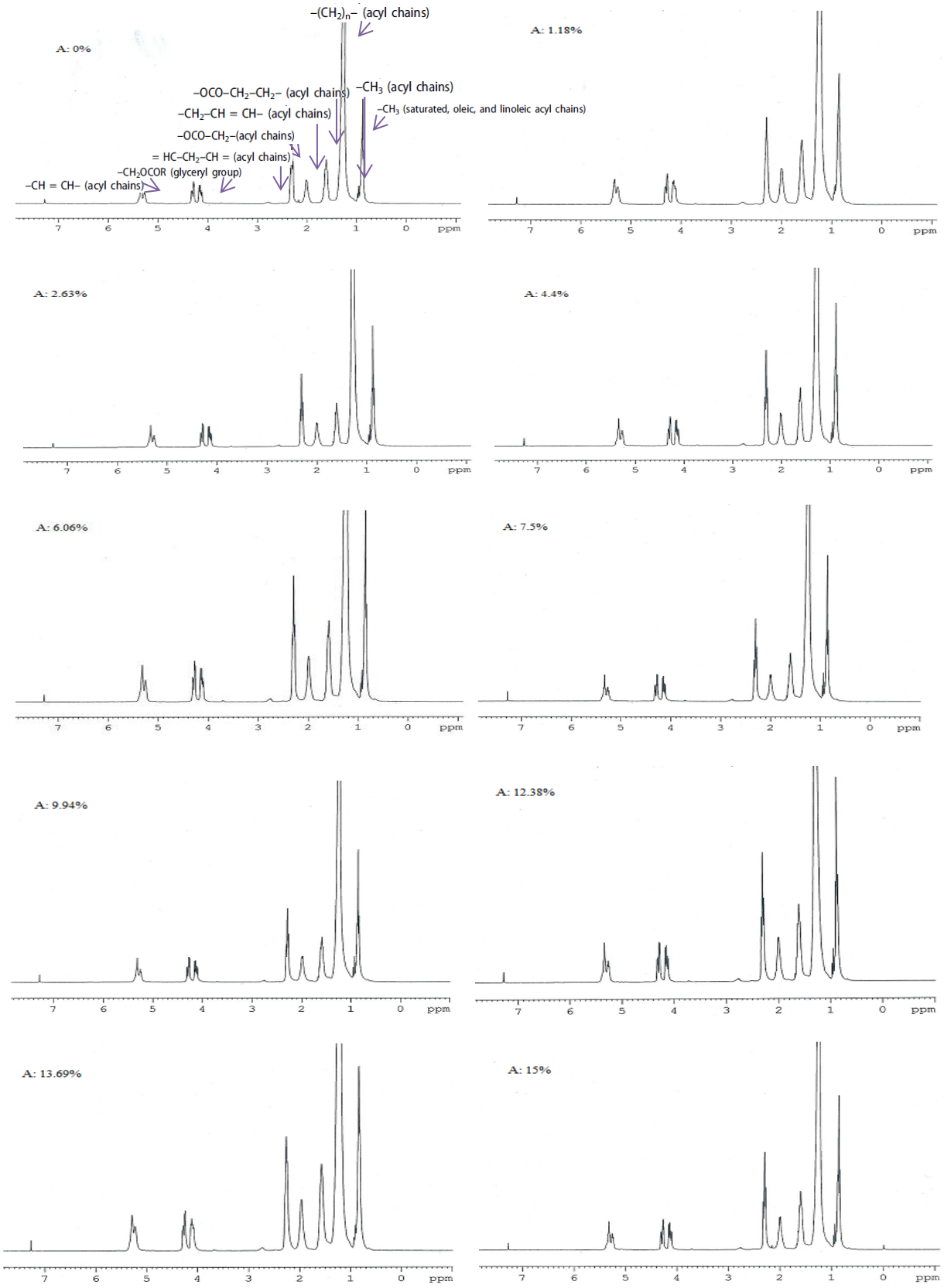

Fig. 2. H NMR spectra of each peak in different treatments of butter (A: percentage of bovine tallow).

was due to the methylene hydrogen atoms in β and α position of carbonyl groups, respectively. The spectral region of 1.94-2.14 ppm was resulted to α methylene hydrogen atom associated with a double bond. The spectral region of 4.32-4.10 ppm was due to the hydrogen atoms in 1 and 3 carbon atoms of glycerol groups and the spectral region of 5.20-5.26 ppm was resulted to the hydrogen atoms in 2 carbon atoms of glycerol groups. This region overlapped with the spectral region of 5.26-5.40 ppm, due to olefinic hydrogen atoms from different acyl groups (Nurrulhidayah *et al*., 2017). Spectrum of fat samples usually had similar profiles that appeared with differences in the amount of each peak. According to other researchers, careful observation in the spectra would indicate a significant difference between the samples. The general allocation of signals to these spectra depended on the different type of hydrogen atom (Guillén and Ruiz, 2001; Miyake *et al*., 1998). The exact position results of each peak obtained from the ^{1}H-NMR spectra in different butter treatments.

Table 3. Statistical analysis of the effect of adding different amounts of bovine tallow in butter on position (ppm) of ^{1}H NMR significant peaks

Range σ (ppm)	Prob>F*	% C.V	L_Pseudo equation**
0.823-0.866	0.0184	1.04	0.823-0.866 (ppm)=0.84A***+0.86B*** (R^2=0.2894)
0.867-0.889	0.0342	1.04	0.867-0.889 (ppm)=0.87A+0.88B (R^2=0.2167)
0.860-0.904	0.0407	0.66	0.860-0.904 (ppm)=0.89A+0.90B-0.028AB (R^2=0.3156)
0.913-0.956	0.0357	0.99	0.913-0.956 (ppm)=0.93A+0.95B (R^2=0.2127)
0.937-0.98	0.0405	0.96	0.937-0.98 (ppm)=0.96A+0.97B (R^2=0.2011)
1.227-1.266	0.0161	0.63	1.227-1.266 (ppm)=1.25A+1.26B (R^2=0.2839)
1.577-1.621	0.0213	0.56	1.577-1.621 (ppm)=1.60A+1.61B (R^2=0.2595)
1.599-1.644	0.0185	0.36	1.599-1.644 (ppm)=1.63A+1.64B-0.030AB (R^2=0.4002)
1.623-1.669	0.0210	0.36	1.623-1.669 (ppm)=1.66A+1.67B-0.033AB (R^2=0.3874)
1.649-1.694	0.0265	0.36	1.649-1.694 (ppm)=1.68A+1.69B- 0.031AB (R^2=0.3628)
1.969-2.011	0.0004	0.39	1.969-2.011 (ppm)=1.99A+2.01B (R^2=0.5494)
1.984-2.117	0.0024	0.98	1.984-2.117 (ppm)=2.01A+2.03B-0.081AB-0.32AB(A-B)+ 0.85AB $(A-B)^2$ (R^2=0.6947)
2.252-2.298	0.0238	0.43	2.252-2.298 (ppm)=2.27A+2.29B (R^2=0.2656)
2.273-2.321	0.0168	0.42	2.273-2.321 (ppm)=2.30A+2.31B (R^2=0.2802)
2.298-2.344	0.0150	0.26	2.298-2.344 (ppm)=2.33A+2.34B- 0.035AB (R^2=0.4204)
4.08-4.125	0.0158	0.22	4.08-4.125 (ppm)=4.10A+4.12B (R^2=0.2855)
4.100-4.144	0.0206	0.22	4.100-4.144 (ppm)=4.12A+4.14B (R^2=0.2624)
4.119-4.164	0.0230	0.23	4.119-4.164 (ppm)=4.14A+4.16B (R^2=0.2526)
4.138-4.183	0.0237	0.14	4.138-4.183 (ppm)=4.17A+4.18B- 0.029AB+0.058AB(A-B) (R^2=0.4155)
4.247-4.283	0.0115	0.17	4.247-4.283 (ppm)=4.26A+4.28B (R^2=0.3125)
4.259-4.296	0.0268	0.18	4.259-4.296 (ppm)=4.28A+4.29B (R^2=0.2391)
4.286-4.322	0.0106	0.16	4.286-4.322 (ppm)=4.30A+4.32B (R^2=0.3197)
4.300-4.335	0.0238	0.17	4.300-4.335 (ppm)=4.32A+4.33B (R^2=0.2498)
5.221-5.264	0.0028	0.086	5.221-5.264 (ppm)=5.25A+5.26B- 0.029AB+0.050AB(A-B) (R^2=0.6290)
5.237-5.279	0.0149	0.16	5.237-5.279 (ppm)=5.26A+5.27B (R^2=0.2910)
5.251-5.294	0.0058	0.093	5.251-5.294 (ppm)=5.28A+5.29B- 0.033AB (R^2=0.5049)
5.300-5.349	0.0433	0.20	5.300-5.349 (ppm)=5.33A+5.34B (R^2=0.1949)

All determinations were carried out in triplicate

* Values of "Prob> F" less than 0.05 indicate model terms are significant.

** The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

*** A: added tallow coefficient and B: butter coefficient.

Position of each peak (ppm) obtained from ^{1}H NMR in different treatments were statistically analyzed. The statistical results of the effect of fat type on position of each peak in each treatment were shown in (Table 3). These studies have shown that addition of bovine tallow fat indicated a significant difference on ^{1}H NMR peaks at positions of 0.823-0.866 ،0.867-0.889 ، 0.860-0.904 ،0.913-0.956 ،0.937-0.980 ، 1.227-1.266 ،1.577-1.621 ،1.599-1.644 ، 1.623-1.669 ،1.649-1.694 ،1.969-2.011 ، 1.984-2.117 ،2.252-2.298 ،2.273-2.321 ، 2.298-2.344 ،4.080-4.125 ،4.100-4.144 ، 4.119-4.164 ،4.138-4.183 ،4.247-4.283 ، 4.259-4.296 ،4.286-4.322 ،4.300-4.335 ، 5.221-5.264 ،5.237-5.279 ،5.251-5.294 ، 5.300-5.349 ppm. According to statistical results for each factor, L_Pseudo equation was obtained, in which coefficients for fat of tallow and butter and their effects on each other were specified. Accordingly, equations for all ^{1}H NMR peaks indicated that both fat of bovine tallow and butter had a positive effect on these peaks but added tallow fat had less effective than butter in their increase so that value (ppm) of ^{1}H NMR peaks decreased with increasing percentage of bovine tallow. In L_Pseudo equation, position of each ^{1}H NMR peak in the sample could be determined using coefficients of bovine tallow and pure butter and relationship between these two fats. Given the R^2 value for each ^{1}H NMR peak, it was determined that none of the peaks position (ppm) could predict the presence of foreign fat added. According to the results of the effect of adding different amounts of bovine tallow in butter on the significant changes in peaks position (ppm) in different ranges, change in the amount of these positions could indicate the adulteration in butter with different amount of bovine tallow in the sample.

- ^{13}C NMR value

Examination of ^{13}C NMR spectra of pure butter samples and butter samples combined with bovine tallow also yielded similar profiles (Figure 3), but position of each peak differed therefore the spectra of fat samples usually had similar profiles and appeared with differences in value of each peak.

The high resolution of ^{13}C NMR spectrometer allows many positional isomers of long chain fatty acids or esters to be distinguished (Nurrulhidayah *et al*., 2017). Accordingly, in the range of 60 to 70 ppm, carbons are located on the glycerol column. Carbonyl ester is approximately visible at 173 ppm, i.e. ^{13}C NMR in the range of 172.5- 174.5 ppm belongs to the carboxyl group of butyric acid and long-chain fatty acids (Andreotti *et al*., 2000; Hu *et al*., 2007). Saturated carbons of methyl group appear at 14 ppm and methylenes at 20-40 ppm. At 130 ppm, unsaturated ones only appear in the spectrum of olive oil and do not exist for animal oils (Molly, 2008).

The accurate positioning values of each peak obtained from ^{13}C NMR spectra in different butter treatments. Position of each peak (ppm) obtained from ^{13}C NMR in different treatments was analyzed statistically. Statistical results of the effect of fat type on position of each peak (ppm) in each treatment were shown in (Table 4). These studies have specified that addition of bovine tallow fat showed a significant difference in ^{13}C NMR on position of peaks of 13.962-14.028 ،18.261-18.308 ، 22.599-22.639 ،24.801-24.842 ،27.099-27.148 ،27.15-27.195 ،29.025-29.088 ، 29.206-29.236 ،29.288-29.317 ،29.411-29.442 ،29.629-29.660 ،31.862-31.892 ، 33.931-34.018 ،34.103-34.182 ،61.996-62.068 ،129.57-129.657 ،129.869-129.960 ،172.931-173.139 ppm.

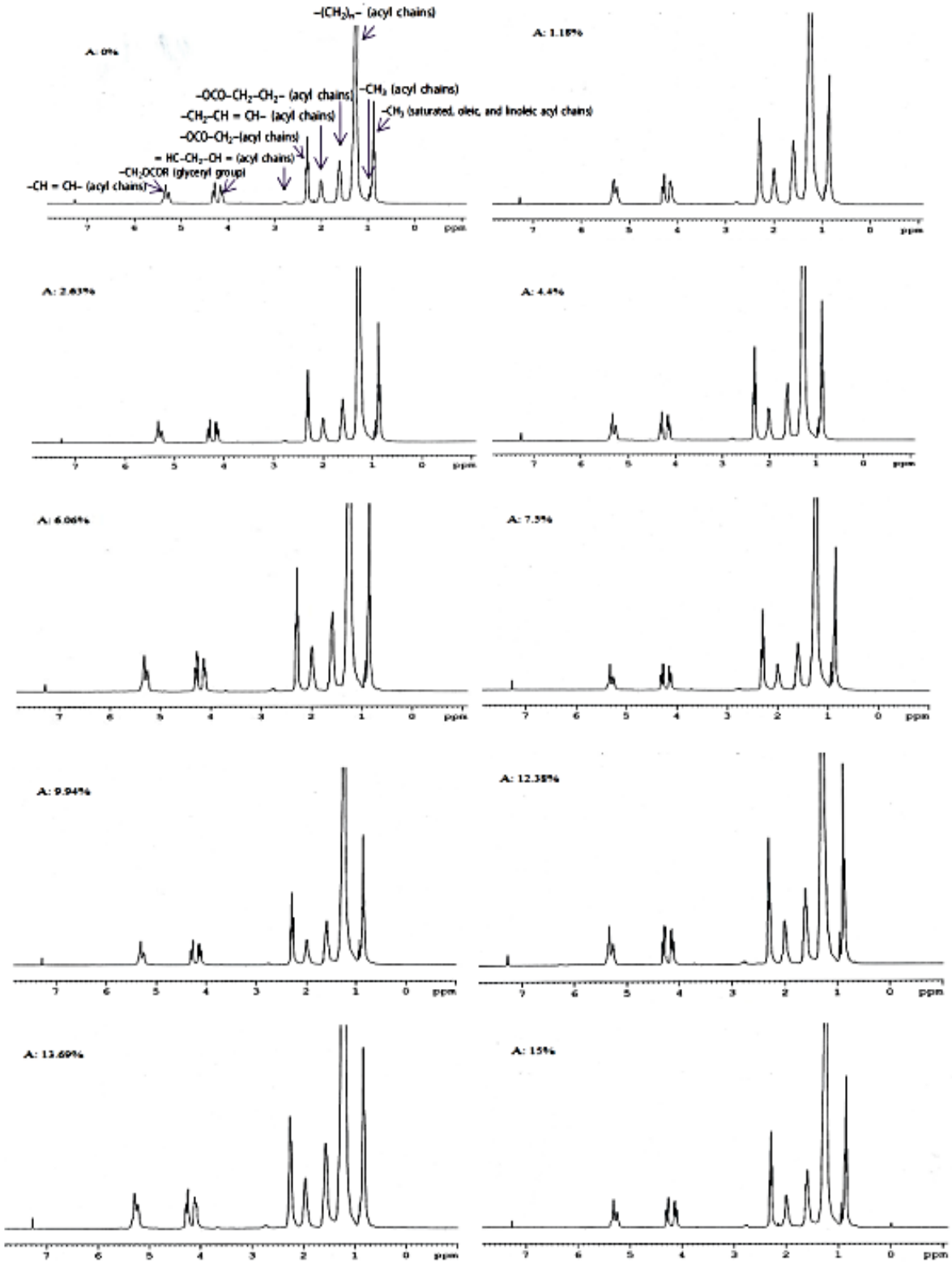

Fig. 3. ^{13}C NMR spectra of each peak in different butter treatments (A: bovine tallow percent).

Table 4. Statistical analysis of the effect of adding different amounts of bovine tallow in butter on position (ppm) of significant peaks obtained from ^{13}C NMR

Range σ (ppm)	Prob > F*	% C.V	L_Pseudo equation**
13.962-14.028	0.0051	0.072	13.962-14.028 (ppm)=14.00A***+14.03B***- 0.037AB-0.095AB(A-B)-0.41AB(A-B)^2 (R^2=0.6428)
18.261-18.308	0.0061	0.041	18.261-18.308 (ppm)=18.29A+18.31B- 2.812E-003AB-0.093AB(A-B)-0.33AB(A-B)^2 (R^2=0.6290)
22.599-22.639	0.0086	0.035	22.599-22.639 (ppm)=22.62A+22.63B (R^2=0.3372)
24.801-24.842	0.0192	0.034	24.801-24.842 (ppm)=24.82A+24.84B (R^2=0.2687)
27.099-27.148	0.0043	0.034	27.099-27.148 (ppm)=27.12A+27.14B (R^2=0.3921)
27.15-27.195	0.0118	0.034	27.15-27.195 (ppm)=27.17A+27.19B (R^2=0.3291)
29.025-29.088	0.0492	0.50	29.025-29.088 (ppm)=29.08A+29.11B+ 1.19AB-0.22AB(A-B)-3.48AB(A-B)^2 (R^2=0.3629)
29.206-29.236	0.0008	0.020	29.206-29.236 (ppm)=29.22A+29.23B (R^2=0.5062)
29.288-29.317	0.0056	0.020	29.288-29.317 (ppm)=29.30A+29.31B (R^2=0.3714)
29.411-29.442	0.0021	0.020	29.411-29.442 (ppm)=29.42A+29.44B (R^2=0.4449)
29.629-29.660	0.0043	0.020	29.629-29.660 (ppm)=29.64A+29.66B (R^2=0.3905)
31.862-31.892	0.0051	0.018	31.862-31.892 (ppm)=31.88A+31.89B (R^2=0.3789)
33.931-34.018	0.0269	0.052	33.931-34.018 (ppm)=33.98A+34.01B (R^2=0.2388)
34.103-34.182	0.0179	0.047	34.103-34.182 (ppm)=34.15A+34.17B (R^2=0.2747)
61.996-62.068	0.0001	6.29	61.996-62.068 (ppm)=27.41A+61.47B+ 67.31AB+ 123.14AB(A-B)+155.65AB(A-B)^2 (R^2=0.9386)
129.57-129.657	0.0220	0.014	129.57-129.657 (ppm)=129.62A+129.65B (R^2=0.2565)
129.869-129.960	0.0106	0.014	129.869-129.960 (ppm)=129.92A+129.95B (R^2=0.3199)
172.931-173.139	0.0204	0.025	172.931-173.139 (ppm)=173.04A+173.11B (R^2=0.2632)

All determinations were carried out in triplicate

* Values of "Prob> F" less than 0.05 indicate model terms are significant.

** The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

*** A: added tallow coefficient and B: butter coefficient.

According to the statistical results for each factor, the L_Pseudo equation was obtained, in which coefficients for fat and butter and their effects on each other were specified. Accordingly, equations of ^{13}C NMR peaks indicated that both fat of bovine tallow and butter had a positive effect on these peaks, but that added tallow fat had less effective than butter in increasing them so that they have shown that the position of the ^{13}C NMR peaks decreases with increasing percentage of bovine tallow. In the L_Pseudo equation, the position of each ^{13}C NMR peak in the sample can be determined using the coefficients of pure butter and bovine tallow and the relationship between these two fats. Given the value of R^2 for each peak in ^{13}C NMR, it was determined that none of positions of the peaks could predict the presence of added foreign fat.

Changes of FTIR spectra in all samples indicated that the wavelengths of 1744, 1458, 1366, 1160, 1107, 969, 870, 720, 590 and 455 cm^{-1} along with the NMR spectra, could be used to determine authenticity of butter in combination with bovine tallow. In a review by other researchers, the FTIR spectrum of a butter sample combined with different concentrations of palm oil was obtained, which butter spectra were assigned to fatty acid or ester functional groups, and with increasing palm oil concentration, absorption of wavelength in butter samples increased (Cuibus *et al*., 2015). Using the FTIR method, accurate validity of a set of butter was obtained, which the most

important IR absorption bands was identified at 3000, 1740 and 1460 cm^{-1}, which were related to fat and protein (Rodriguez-Saona *et al*., 2006). Many types of functional groups (hydroxyl groups, acids, esters, amide I and amide II, aliphatic fatty acid chains and amino acid acids) provided the entire spectrum of butter, which was very complex and not be easy to understand and made it difficult to identify small changes resulted to less adulterations in spectrum. During a study of researchers on FTIR spectra of butter and fat of lamb, it was found that they had peaks with characteristics related to the spectra of edible oils and fats, as described by Guillen and Cabo (1997) because glycerides were the main component of fats. The FTIR spectra in butter and lamb fat also had slight differences in terms of band intensities and precise frequencies that showed the maximum absorption in the wavelength range of 2923, 2852, 1236 and 1098 cm^{-1} that these wavelengths were selected for better analysis of lamb fat in butter (Nurrulhidayah *et al*., 2013b). In our study on butter in combination with bovine tallow, it was also found that the maximum absorption was observed in the wavelength range of 2923, 2855 and 1240 cm^{-1} which their equations could be used to determine the authenticity of butter in combination with bovine tallow.

The NMR spectra showed that amplitude of the spectra variations did not differ between pure butter and butter combined with bovine tallow, but differences in the samples combined with bovine tallow could be observed by statistical analysis of peak intensities in the amplitude range of 0–4 ppm and 4–6 ppm. According to other researchers, careful observation in the broad spectrum would show a significant difference between the samples that the general allocation of signals to these spectra has depended on the different type of hydrogen atom (Guillén and Ruiz, 2001; Miyake *et al*., 1998). In the study of butter samples in combination with bovine tallow, the intensity of ^{1}H-NMR peaks in the range of 4 ppm related to hydrogen atoms in the carbon atoms 1 and 3 of the glycerol group decreased with increase of tallow amount in butter, which showed the decrease of short and average chains fatty acids. In addition, increasing bovine tallow caused to raise peak intensities in the range of 0.9, 1 and 5 ppm, which strongly correlated with the hydrogen atoms of the methyl group in the saturated acyl, oleic and linoleic chains as well as the hydrogen atom in the carbon atom 2 of glycerol group. Based on other researches, the NMR spectra mainly showed signals of saturated and unsaturated fatty acids, triglycerides, peroxide and glycerol, which covered range of peak chemical changes in 0 to 4 ppm of most free fatty acids and glycerol-bound fatty acids, while chemical changes in range of 4 to 6 ppm were assigned to proton signals in the glycerol column (Nurrulhidayah *et al*., 2017). Based on the statistical model obtained, use of these factors has been able to assist in authenticity of butter in combination with bovine tallow.

Examination of ^{13}C NMR spectra of pure butter samples and butter samples combined with bovine tallow also showed a similar profile. Increase of bovine tallow in butter has shown decreasing changes in the position of the peaks in ^{13}C NMR system of the samples. In other researches on the pig fat spectrum, changes in the carbonyl region in 173.29 ppm signal have correlated with different fatty acid carbonyl carbons that generally chemical changes in this signal depend on triglycerides, i.e. fatty acid position in sn-1,3α or sn-2β in acyl glycerol and distance (Δ) of the closest double bond to carbonyl

carbon. Positional distribution of fatty acids in triglycerides is unique for each species (Aursand *et al*., 2007) Comparison between ^{13}C spectra of butter and bovine tallow triglycerides has indicated that they are very similar in view of quality but with statistical models, discrepancy is clear. Therefore, based on the obtained statistical model, use of these spectra along with the FTIR spectra can help to authenticate butter in combination with bovine tallow.

Conclusion

The results of this study showed that the peaks resulting from FTIR and NMR of the samples are mainly related to the distribution of fatty acid and TAG. Comparison between FTIR and NMR spectra of butter and bovine tallow triglycerides has indicated that they are similar in quality but with statistical models, discrepancy is clear. Therefore, based on the obtained statistical model, use of these spectra can help in the authentication of butter in combination with bovine tallow and can be used simply and quickly before TAG analysis.

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