

Comparative analysis of butter thermal behavior in combination with bovine tallow

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Abstract

Assessment of butter authenticity in combination with animal fat is a major challenge. Changing the butter composition can affect crystallization and polymorphism properties. The purpose of this study was to evaluate the thermal behavior of butter combined with (0-15% w/w) bovine tallow using Digital Calorimetric Scanning, and to determine fatty acid profile and isothermal microstructure in order to complete DSC data. According to the statistical analysis, obtained L_Pseudo equations show that in the crystallization curve, with increase of bovine tallow percentage in the butter, the onset temperature of peak 1 and amount of heat released in peak 2 increased and the onset temperature of peak 2 decreased and peak 1 became wider. In the melting curves, with increase of bovine tallow percentage, the onset temperature of peaks 1 and 2 increased. Regarding the value of R^2 , the onset temperature of peak 2 in the melting curve had capability of estimation 78%. The crystal morphology of butter samples was different and with increased temperature, larger crystals were observed. In general, scans of calorimetric results revealed that DSC is a valuable method for studying butter in combination with animal fat and also can be utilized for distinguishing pure fat from adulterated one.

Keywords: butter; bovine tallow; DSC; fatty acids; isothermal microstructure.

Practical Application: Determination of butter authenticity in combination with bovine tallow.

1 Introduction

Vegetable oils and animal fats contain lipid compounds that are important in terms of nutrition and processing in food products (Akoh, 1998). Today, there are various types of vegetable oils and animal fats like milk fat whose chemical properties have been obtained through scientific research and development (O'Brien, 1998). Due to the relatively high price of milk fat compared to other fats and oils, milk fat is subjected to adulteration by replacement of cheaper animal fats or vegetable oils (Ntakatsane et al., 2013) and butter is not excluded in this regard and its detection methods have been studied (Deelstra et al., 2014). Vegetable oils and animal fats are complex compounds that contain a mixture of fatty acids and triacylglycerols (TAGs) (Tsimidou et al., 1987) which affected physical and chemical properties of oils and fats. The methods to check the authenticity of butter, even today, are internationally accepted as standard tests in spite of their low repeatability (Nogala-Kańska et al., 2008). Recently, significant works have been performed on the authenticity of butter. One of the first methods is the analysis of fatty acid compounds using gas chromatography (GC). Given high variation in mass approximate percentage of butyric acid, it is difficult to provide qualitative and quantitative information on foreign fat in pure milk fat up to 20% (Molkentin & Precht, 1987; Glaeser, 2002). Therefore, due to high variability of fatty acids of milk, use of fatty acid composition is not sufficient in order for evaluation and fraud recognition (Derewiaka et al., 2011; Anklam & Lipp, 1997).

Differential Scanning Calorimetric (DSC) has been widely used in the study of oils and fats (Cebula & Smith, 1992). The DSC provides a detailed analysis of melting behavior of oils and fats (O'Brien, 1998). Differential Scanning Calorimetric has been tested for use in the assessment of authenticity of fats, in which crystallization curves have been initially recommended for this evaluation (Lambelet & Ganguli, 1983; Aktas & Kaya, 2001). The measurements based on crystallization curves have advantages such as avoiding problems associated with polymorphism of fats (Lambelet & Ganguli, 1983). By studying milk fat (Tomaszewska-Gras, 2013) it was found that the melting curve could also be better than crystallization curves to study this sample. Each curve of DSC is composed of three parts; the beginning of peak that shows melting of the first crystals, the top of peak that shows melting of all crystals and the end of peak that shows completion of a process (Farah et al., 2018). In milk fat, fatty acids and triacylglycerols make a lot of changes in the tissue properties. Each type of triacylglycerol scan can be converted to different forms of crystalline α , β and β' from a less stable phase to a more stable phase. These phases are characterized by melting temperature and enthalpy (Heyen et al., 1999). There are not many researches in comparison with the feasibility of DSC crystallization and melting curves to monitor the presence of non-milk fats (Tomaszewska-Gras, 2016) but the results indicated the potential of DSC method because it is a sensitive, rapid and repeatable method for identifying oils and fats. In DSC, any change in the shape of the melting curve, identified the adulteration (Farah et al., 2018). Therefore, the evaluation

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of butter thermal behavior in combination with bovine tallow is still very limited and in the present research, the behavior of melting and crystallization of butter in combination with bovine tallow and their relationship with fatty acids were studied.

2 Materials and methods

2.1 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%).

2.2 Evaluation of the percentage of fatty acids

Fat extraction from different treatments and then direct fatty acids methylation were performed. Fatty acids were analyzed according to gas chromatography (Nilchian et al., 2020).

2.3 Isothermal behavior of various butter treatments

To study the isothermal behavior of crystallization of various butter treatments, DSC was used at temperature of -60 °C to + 80 °C where the crystallization and melting kinetics varied by changing the triacylglycerols (American Oil Chemists' Society, 2000).

2.4 Shape and crystalline structure

The crystal structure of the samples was examined in isothermal conditions using a polarized optical microscope equipped with a digital camera. For this, the samples were heated at 80 °C in an oven and kept at this temperature for 30 minutes to ensure that the fats were totally melted. A drop of melted fat was placed on a pre-heated microscope slide and covered with a pre-heated slipper coating. Slides were stored at -20, 10 and 20 °C for 5 hours. Then images were taken at 200x magnification, and analyzed (Zhang et al., 2013).

2.5 Statistical analysis

To examine authenticity percentage, experiments were designed by response surface methodology with statistical software of Design Expert 8. ANOVA in this methodology was used to evaluate significant differences, Adj R-Squared, CV% and L-Pseudo equations (significance levels at $p < 0.05$). For each treatment, experiments were repeated three times. This statistical method was used to predict authenticity optimal point.

3 Results and discussion

According to statistical analysis, the addition of bovine tallow fat revealed a significant difference at 95% level on the percentage of short and long chains saturated and mono-unsaturated fatty acids. Based on the results for each fatty acid in different butter treatments, $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{10:1}$, $C_{11:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$ and CLA_{c9:11} fatty acids decreased 0.52%, 0.62%, 0.47%, 0.79%, 0.12%, 0.03%, 0.92%, 7.05%, 0.86%, 28.77%, 0.43%, irrespectively, and

$C_{17:0}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1C}$ fatty acids raised 1.09%, 0.62%, 15.08%, 32.56%, irrespectively, with increase of the percentage of bovine tallow (up to 15%) in butter. Based on analysis carried out in this study, the composition of butter and bovine tallow fatty acids is similar, but their percentage is different. The increase of bovine tallow in butter has affected the composition of butter fatty acids in a way that the percentage of long-chain saturated and mono-unsaturated fatty acids in butter has increased. In this study, bovine tallow had a higher percentage of $C_{17:0}$, $C_{17:1}$, $C_{18:0}$ and $C_{18:1C}$ fatty acids, which also affects the thermal behavior of its triacylglycerols. Therefore, by adding bovine tallow to butter, isothermal behavior of adulterated butter changes compared to that of pure butter.

The results for isothermal behavior of butter crystallization in combination with different percentages of bovine tallow are shown in Table 1. According to the statistical method, the effect of fat type on different parts of the crystallization curves was evaluated.

Review results indicated that addition of tallow fat had been a significant difference at 95% level in the onset temperature of peak 1 and 2, the temperature of peak 2 top and enthalpy changes of peak 2 in the crystallization curves. According to the statistical result for each of the four factors, the L_Pseudo equation was obtained (Table 2), showing that both fat types of tallow and butter have influenced the crystallization curve factors. Regarding the onset temperature of peak 1, it has been shown that added tallow fat had a greater effect on the rise of this temperature than butter did, and that with increase of bovine tallow in butter, the onset temperature of peak 1 has increased. In relation to the onset temperature of peak 2 and the temperature of peak 2 top, it has been shown that added tallow fat has a lesser effect on the rise of the amount of these two points than butter does, and that with increase of bovine tallow in the butter, the onset temperature of peak 2 and the temperature of peak 2 top decreased. Equation for the enthalpy changes of peak 2 revealed that added tallow fat has a greater effect on the rise of peak 2 enthalpy than butter does, and that with increase of percentage of bovine tallow in butter, amount of heat released in the peak 2 of crystallization has increased. Regarding the value of R^2 , it is determined that the onset temperature of peak 1, the onset temperature of peak 2, the temperature of peak 2 top and enthalpy changes of peak 2 in the crystallization curve cannot be estimated for bovine tallow added in butter. According to studies by other researchers, there is a difference between thermal properties of different vegetable oils, mainly due to difference in the distribution of TAG molecules (Breitschuh & Windhab, 1996). In other research, Jahurul et al. (2019) investigated the thermal properties of bambangan kernel fat and palm stearin in cocoa butter alternatives that the melting onset temperatures decreased and the crystallization onset temperatures increased (Jahurul et al., 2019).

According to the results from Table 1, difference between the onset temperature of peak 1 and the temperature of peak 1 top in the crystallization curve were also investigated (results not shown). Based on the statistical results, this difference was significant and with increased percentage of tallow into butter, the difference between the onset temperature of peak 1 and the temperature

Table 1. Isothermal behavior of butter fat crystallization in combination with different percentages of bovine tallow.

Tallow (%)	0	1.18	2.63	4.4	6.06	7.5	9.94	12.38	13.69	15
Onset peak1(°C)	18.12	15.27	18.18	15.46	14.95	16.76	18.35	18.40	17.15	21.62
Onset peak2 (°C)	12.04	12.00	11.79	11.71	11.02	11.38	11.67	11.99	11.69	11.76
Peak 2 top (°C)	5.67	4.34	3.15	5.00	3.48	4.10	4.80	5.32	5.02	4.63
Enthalpy2 (J/g)	29.94	37.27	27.09	29.52	24.60	11.61	42.12	49.97	34.33	38.16

Table 2. Analysis of variance according to response surface methodology for effect of different amounts of bovine tallow in butter on significant parameters of crystallization ($p < 0.05$).

Parameters	Prob>F ^a	%C.V	L_Pseudo equation ^b
Onset peak 1(°C)	0.0003	6.69	Onset peak 1(°C) = 20.98A ^c +18.02B-12.58AB (R ² =0.6502)
Onset peak 2 (°C)	0.0003	1.18	Onset peak 2 (°C) = 11.70A+12.03B-2.15AB+1.72AB(A-B)+4.96AB(A-B) ² (R ² =0.7499)
Peak 2 top (°C)	0.0009	10.63	Peak 2 top (°C) = 4.58A+5.53B-3.62AB+11.41AB(A-B) (R ² =0.6410)
Enthalpy 2 (J/g)	0.0038	21.01	Enthalpy 2(J/g) = -36.47A- 0.30B+53.38AB-66.26AB(A-B)-210.34AB(A-B) ² (R ² =0.6007)

^aValues of "Prob> F" less than 0.05 indicate model terms are significant; ^bThe 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; ^cA: added tallow coefficient and B: butter coefficient.

of peak 1 top has sharply increased and peak 1 expanded. The difference between the onset temperature of peak 1 and the temperature of peak 1 top has capability of estimation 85% about bovine tallow added to butter. The difference between the onset temperature of peak 2 and the temperature of peak 2 top in the crystallization curve were also studied. According to the statistical results, difference between the onset temperature of peak 2 and the temperature of the peak 2 top was significant and with increased percentage of tallow into butter, this difference increased but the effect of tallow on peak 1's becoming wider was higher than that on peak 2. The difference between the onset temperature of peak 2 and the temperature of the peak 2 top did not have ability to estimate bovine tallow added in butter. In the results obtained from DSC, two main peaks are seen. At exothermic peaks, with increase of bovine tallow in butter, the onset temperature of peak 1 increased and butter sample was crystallized at a higher temperature and faster than pure butter was. With increase of bovine tallow in butter, the exothermic peak 2 was crystallized at a lower temperature than pure butter was in a way that distance between peaks1 and 2 increased. In other researches, the isothermal behavior of pure butter and its mixture with palm oil in various concentrations have also been investigated, where shape of curve as well as the position of the crystallization peaks have changed with increase of palm oil content and temperature of peak 1 and distance between peaks 1 and 2 increased (Tomaszewska-Gras, 2016). In the study of olein and stearin palm oil fatty acids, it was found that the presence of higher saturated fatty acids in the sample is accompanied by a wider exothermic peak due to the widespread distribution of triacylglycerol, resulting in higher content of crystallized fat with a higher melting point (Zhang et al., 2013). With increase of bovine tallow in butter, the enthalpy changes of the exothermic peak 2 have increased. As shown in other studies, it has been observed that components with a higher melting point display faster crystallization (Che Man et al., 1999). Other researchers detected the adulteration of caprine body fat at 5% level in bovine milk fat (ghee) using a DSC technique that the midrange temperature of isothermal curves was significantly different that displaced the crystallization region and indicated the presence of foreign fats (Upadhyay et al., 2017).

According to the quartic statistical model obtained for different parts of the crystallization curve, the effect of different percentages of bovine tallow on the onset temperature of peak 1 and 2, the temperature of peak 2 top and enthalpy changes of peak 2 in the butter samples was also obtained and the results have been shown in Table 3.

The isothermal behavior of butter melting in combination with different percentages of bovine tallow was shown in Figure 1.

According to statistical method, it has been shown that addition of tallow fat has a significant difference at 95% level in the onset temperature of peak 1 and 2 and in the temperature of peak 1 and 2 top in the melting curves. According to statistical result for each factor in the melting curves, the L_Pseudo equation is obtained (Table 4), showing that both fat types of tallow and butter have a positive effect on the melting curve factors. In relation to the onset temperature of peak 1 and 2, and the temperature of peak 1 and 2 top, it has been shown that added tallow fat has a greater effect on increase in the amount of these temperatures than butter does. Regarding the value of R², the onset temperature of peak 2 and the temperature of peak 2 top in the melting curve have capability of estimation 78 and 80% about bovine tallow added to butter, respectively. In general, temperature of the melting curve peaks in the study of vegetable oils has shown a complex behavior (Tan & Man, 2000). Multiple endothermic peaks observed in DSC analysis of vegetable oils are due to unique features in distribution of TAG. In general, highly saturated TAGs are melted at higher temperatures compared to the highly unsaturated (Tan & Che Man, 2002).

According to the quadratic and linear statistical model obtained for peak 1 and 2, respectively, in the melting curve, the effect of different percentages of bovine tallow on the onset temperature of peak 1 and 2 and in the temperature of peak 1 and 2 top in the butter samples was also obtained and the results have been shown in Table 5.

According to the results of Figure 1, the difference between the onset temperature of peak 1 and the temperature of peak 1 top in the melting curves were also conducted (results not shown). According to the statistical results, this difference was significant

Table 3. The effect of different amounts of bovine tallow in butter on significant parameters of the crystallization curves. (According to the quartic statistical model).

Tallow (%)	Onset peak 1 (°C)	Onset peak 2 (°C)	Peak 2 top (°C)	Enthalpy 2 (J/g)
9.38	15.452	4.756	11.493	-28.610
6.06	15.031	3.746	11.343	-18.748
3.00	15.366	3.665	11.739	-28.750
2.63	15.376	3.769	11.801	-30.288
7.40	15.060	4.117	11.324	-19.786
9.94	15.620	4.922	11.573	-32.282
10.69	15.850	5.106	11.688	-37.344
2.31	15.366	3.883	11.851	-31.449
6.97	15.029	3.987	11.317	-18.988
6.63	15.020	3.891	11.320	-18.667
7.77	15.103	4.236	11.339	-20.829
14.34	15.767	4.909	11.862	-43.848
0.26	14.572	5.265	12.036	-31.744
8.07	15.150	4.335	11.357	-21.910
9.60	15.517	4.824	11.523	-30.014
11.02	15.947	5.172	11.739	-39.553
8.50	15.233	4.477	11.393	-23.786
8.79	15.298	4.570	11.421	-25.221
14.05	15.935	5.018	11.906	-45.866
10.34	15.741	5.025	11.633	-34.931
14.63	15.557	4.781	11.803	-41.151
9.15	15.390	4.687	11.464	-27.259
0.47	14.737	5.061	12.036	-32.614

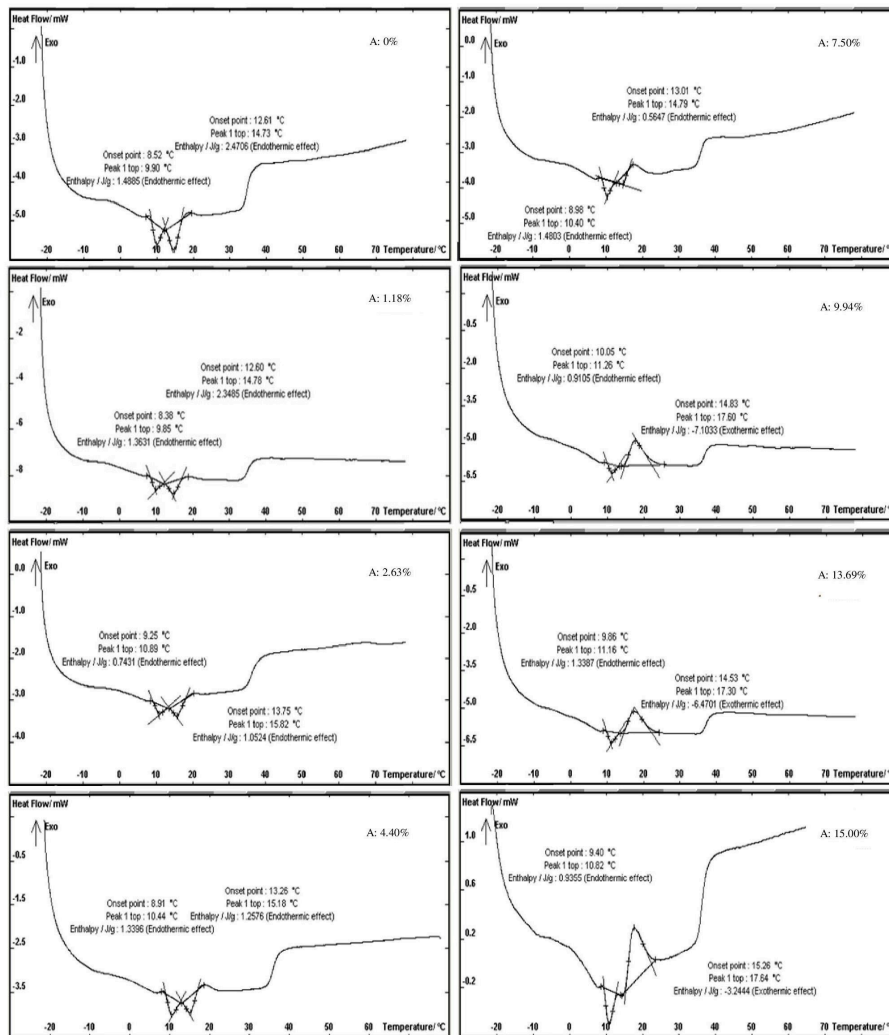


Figure 1. Isothermal behavior of butter melting in combination with different percentages of bovine tallow.

Table 4. Analysis of variance according to response surface methodology for the effect of different amounts of bovine tallow in butter on significant parameters of the melting curves ($p < 0.05$).

Parameters	Prob>F ^a	% C.V	L_Pseudo equation ^b
Onset peak 1(°C)	0.0020	3.57	Onset peak 1(°C) = 9.47A ^c + 8.51B + 1.76AB ($R^2 = 0.5864$)
Peak 1 top (°C)	0.0014	2.87	Peak 1 top (°C) = 10.86A + 9.94B + 2.32AB ($R^2 = 0.6401$)
Onset peak 2(°C)	0.0001	3.55	Onset peak 2(°C) = 15.09A + 12.68B ($R^2 = 0.7812$)
Peak 2 top (°C)	0.0001	3.47	Peak 2 top (°C) = 17.61A + 14.74B ($R^2 = 0.8086$)

^aValues of "Prob> F" less than 0.05 indicate model terms are significant; ^bThe 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; ^cA: added tallow coefficient and B: butter coefficient.

Table 5. The effect of different amounts of bovine tallow in butter on significant parameters of the melting curves (According to the quartic statistical model).

Tallow (%)	Onset peak 1 (°C)	Onset peak 2 (°C)	Peak 1 top (°C)	Peak 2 top (°C)
9.38	11.058	16.531	9.522	14.185
6.06	10.869	15.896	9.319	13.653
3.00	10.494	15.310	8.980	13.162
2.63	10.435	15.238	8.928	13.102
7.40	10.973	16.152	9.422	13.868
9.94	11.067	16.640	9.540	14.276
10.69	11.070	16.783	9.555	14.396
2.31	10.383	15.178	8.883	13.052
6.97	10.944	16.071	9.392	13.799
6.63	10.918	16.006	9.367	13.745
7.77	10.995	16.223	9.445	13.927
14.34	10.916	17.482	9.503	14.982
0.26	9.994	14.785	8.551	12.723
8.07	11.011	16.281	9.463	13.976
9.60	11.062	16.574	9.530	14.221
11.02	11.067	16.847	9.559	14.450
8.50	11.030	16.364	9.485	14.045
8.79	11.041	16.418	9.499	14.091
14.05	10.939	17.426	9.515	14.935
10.34	11.070	16.715	9.549	14.339
14.63	10.893	17.537	9.491	15.028
9.15	11.052	16.488	9.514	14.149
0.47	10.039	14.826	8.589	12.757

and with increased percentage of tallow to butter, difference between the onset temperature of endothermic peak 1 and the temperature of peak 1 top increased and the peak 1 in the melting curves was wider. This difference between the onset temperature of endothermic peak 1 and the temperature of peak 1 top has capability of estimation 85% about bovine tallow added in butter. Difference between the onset temperature of endothermic peak 2 and the temperature of peak 2 top was also studied. According to the statistical results, the difference between the onset temperature of endothermic peak 2 and the temperature of peak 2 top was significant too, and with increased percentage of tallow into butter, this difference increased and the peak became wider. Difference between the onset temperature of the peak 2 top and the temperature of the peak 2 top was capable of estimation to 85% of bovine tallow in butter. According to results obtained from the effect of addition of bovine tallow into butter on peaks' being wider during DSC heating, it is shown that the absence of a sharp peak in the melting curve indicates a wide range of melting temperatures, depending on combination of TAG, as well. Evidence has

already been provided that melting of TAG compounds can simultaneously occur during heat in the DSC (Tan & Man, 2000). Therefore, at a specific temperature range, melting of two or more TAG structures can simultaneously occur, leading to a broad or overlapping melting curve. In endothermic curves, it is also observed that by increase of bovine tallow in butter, the melting temperature of butter samples in the endothermic peaks 1 and 2 increased and blended butter sample melted at a lower rate than pure butter did. In general, in relation to milk fat, in melting curve, two main peaks were also observed that peak 1 in the distance between temperatures of 0-20 °C was affected by the oleic section and peak 2 in the distance between temperatures of 20-40 °C was affected by the stearic section (Heyen et al., 1999). According to studies, melting curves are more complex than crystallization curves and are wide and overlapping with each other (Zhang et al., 2013).

In one study, DSC along with multivariate statistical methods identified 100% of cheeses adulterated with vegetable oil that the melting temperature and peak increased as vegetable fat

increased (Lara et al., 2017). The effects of spread between peaks are due to the large distribution of TAG (Liu et al., 2010). The melt profiles represent the amount of crystalline fat and the occurrence of the transfer of polymorphs (Fredrick et al., 2008). The first thermal thermogram peak is related to the melting of the shape of α , while the last peak is related to the melting of the β shape, and the middle peak indicates the melting of the shape of β' (Garti et al., 1989). Busfield & Proschogo also analyzed α , β and β' structures from the thermal thermograms of palm stearin (Busfield & Proschogo, 1990).

The crystalline structure of pure butter and blended butter with 7.5 and 15% bovine tallow was obtained in isothermal conditions using a polarized optical microscope

at 20, 10 and -20 °C (Figure 2). As shown in Figure 2, at -20 °C, the crystalline particles in the pure butter sample are sharper and have more needles than the blended butter with 7.5 and 15% bovine tallow. At 10 °C, with increase of bovine tallow up to 15%, the crystalline particles indicate a denser net than pure butter does, which indicates strong interactions between crystals (Zhang et al., 2013). When the temperature reaches 20 °C, the pure butter sample forms clear needle crystals, while the butter sample in combination with the bovine tallow shows smaller crystals. At temperatures of 20 °C, there are independent and larger crystals compared to the other two temperatures. As the temperature rises, the crystal net structure is gradually loosened and the crystal net becomes more transparent.

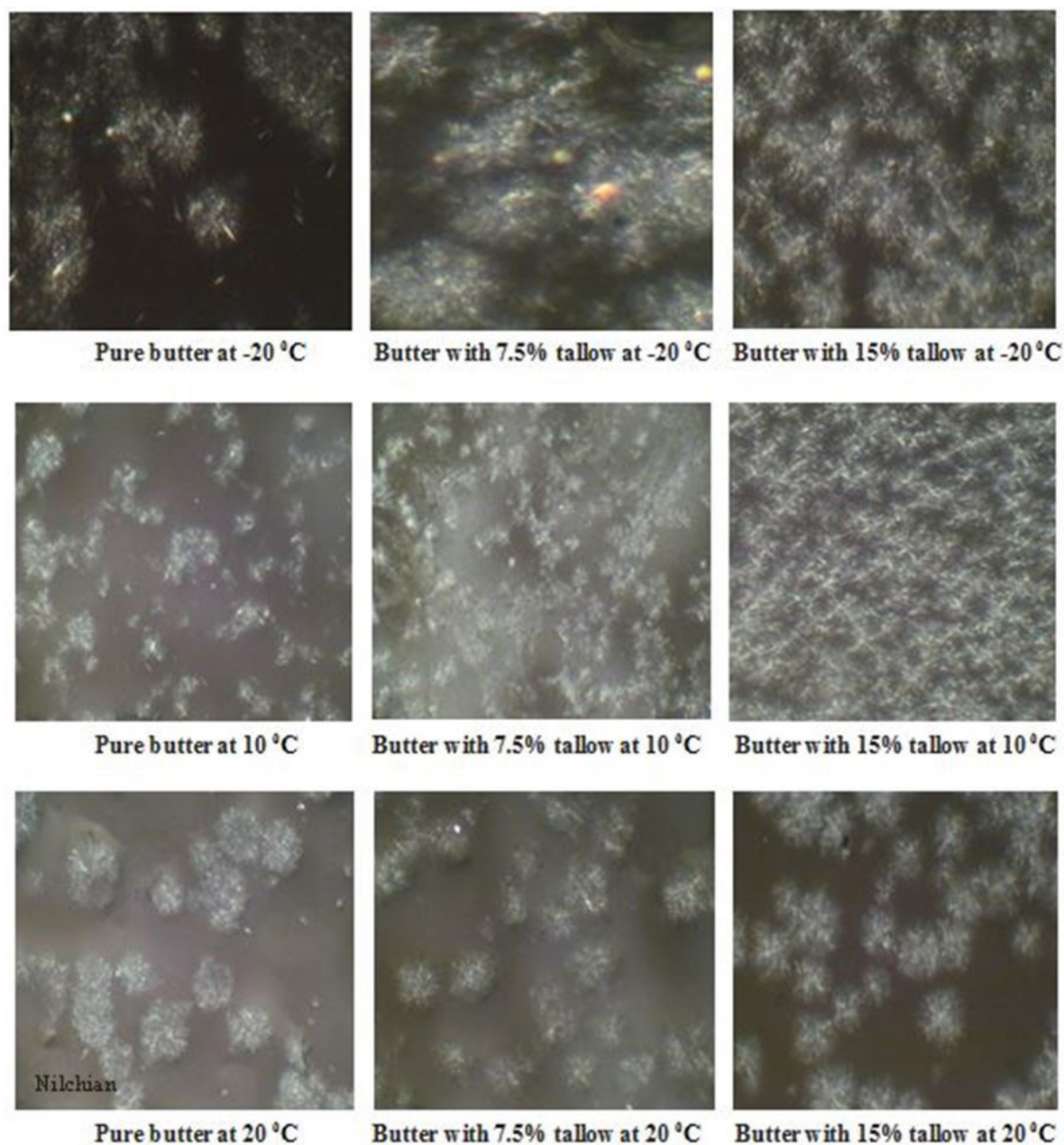


Figure 2. The crystalline structure of samples by polarized optical microscope (200 \times) at 20, 10 and -20 °C.

4 Conclusion

The results of this study showed that the peaks resulting from the isothermal behavior of the samples are mainly related to the distribution of TAG and because of the polymorphism changes. The difference for fatty acids in the butter and bovine tallow samples has led to a different isothermal behavior in treatments. The morphology of crystals has also varied in different treatments and temperatures. In general, the study of the melting curve and the crystallization behavior of pure butter has made it possible to distinguish it from butter blended with bovine tallow. Therefore, the study of DSC parameters such as temperature and peaks enthalpy can be an effective method to describe butter in combination with animal fat, and can be used simply and quickly before TAG analysis.

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