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The Stabilizing Effect of Natural and Synthetic Antioxidant on Mutton Tallow- Evaluation of DSC and its Comparison with Rancimat

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ABSTRACT: The antioxidant activities of TBHQ, BHT and tocopherol mixtures at different concentrations (0, 100, 200, 400 mg/kg) have been evaluated by the application of Differential Scanning Calorimetry (DSC) and Rancimat apparatus. Rancimat apparatus that measures the secondary oxidation products was set at 110 ˚C with an air flow of 18-20 L/h to measure the induction period (IP) of the samples. The DSC method was concerned with the oxidation of the samples in an oxygen flow DSC cell, while the cell temperature was set at isothermal temperature of 150˚C. Oxidative induction time (T_0), and the maximum of the peak (T_{max}) were improved with increasing antioxidant concentrations. According to the results, tocopherols mixture was the most efficient antioxidant, followed by TBHQ and BHT. Furthermore, it was indicated that there is a good agreement (P<0.0001) between both methods and values obtained. In conclusion, the DSC method is a fast, convenient and reliable method to predict the antioxidant activities.

Keywords: *Antioxidant, DCS, Mutton Tallow, Oxidative Induction Time, Rancimat.*

Introduction

The stability and consequently the quality of edible oils and fats are affected by the oxidation chain reactions. This is a complex phenomenon that causes off-flavor and reduces the nutritional value of the oil accordingly (Arain *et al*., 2009; Giuffrida *et al*., 2007). In order to prevent or retard the oxidation reactions, natural and synthetic antioxidants might be employed. However, due to the possible side effects of the synthetic antioxidants, the use of natural antioxidants, compounds naturally present and consumed by man for years are preferred (Suja *et al*., 2004; Renuka Devi *et al*., 2000).

Evaluation of the stability referred to the induction period is an indicator to define the time before a dramatic increase in the oxidation reaction has occured (Tan *et al*., 2002). Many methods have been used to determine the oxidative stability. The Schaal Oven Test (OST) and Active Oxygen Methods (AOM) have been widely applied to evaluate the oil stability (Wan, 1995).

Currently, oxidative stability of oils and fats can be determined by two commercially available equipments recommended by AOCS (AOCS, 1992). The Rancimat and Oxidative Stability Instrument (OSI) are

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manufactured by Metrohm Ltd. (Herisau, Switzerland) and Omniom Inc., respectively (Akoh, 1994).

Rancimat apparatus is based on the work of Pardun and Korall (1972) involving continuous measurement of water conductivity of receiving volatiles from heated oil through which a constant stream of controlled filtered air is passed (Ghavami and Gharachorloo, 2006; Farhoosh, 2007).

Thermal analysis is an accelerated method, which determines the oil stability and the antioxidant activity (Kowalski, 1993). DSC and pressurized DSC are considered as promising methods (Tan *et al*., 2001; Kowalski, 1989; Che Man & Tan, 1999). The end point of DSC was taken at the time that the rapid exothermic reaction of oil and oxygen has occurred (Ramezan *et al*., 2015; Pardauli, 2011). Therefore, mutton tallow a natural substrate deficient in natural antioxidants such as tocopherols was chosen as a substrate to evaluate the antioxidant activities and compare the results obtained by both DSC technique and Metrohm Rancimat method.

Materials and Methods

Freshly rendered dried mutton tallow was isolated by washing, drying, freezing, mincing and finally melting the tail end of the animal under vacuum using rotary evaporator. Tocopherol mixture (E oil super 60; α, β, γ and δ tocopherols) was purchased from Riken vitamin company (Tokyo, Japan). All the used chemicals and solvents were of analytical grade purchased from Merck chemical company (Darmstadt, Germany).

Gas liquid chromatography was used to determine the component fatty acids. Fatty acids were converted into their methyl esters using transesterification methods of oils with sodium methoxide as alkali catalyst according to AOCS method (1995a). An Agilent- technologies 6890 N gas chromatograph equipped with flame ionization detector and BPX capillary column and temperature Programming was employed to determine the fatty acid composition according to AOCS method (1995b).

Free fatty acids (FFA), Iodine (IV), Peroxide (PV) and Anisdine (AV) values of the samples were determined according to AOCS method (AOCS, 1992).

The antioxidants activities were evaluated by Metrohm Rancimat apparatus model 743(Herisau/ Switzerland). The analysis was performed as defined in AOCS Official Method, cd 12b-92 (1992). The instrument was operated at 110 °C with an air flow rate of 18-20 L/h. The induction period was identified by a sharp change in the slope on the chart. A tangent was drawn from the slope to intersect the extension of the baseline and the distance of this intersection from the start was considered as a measure of the induction time.

The antioxidant activities were also determined by Setaram instrumentation 131 differential scanning calorimeter, 69300 (France). The equipment was calibrated with pure indium and the baseline obtained with an empty aluminum pan. Mutton tallow samples of 5.0±0.5 mg with different antioxidant concentrations were weighed into the open aluminum pans and placed in the sample chamber. The isothermal temperature was programmed at 150 °C and then purified oxygen (99.8%) was passed through the sample enclosure at 50 mL/min. The DSC induction time (T_0) of the oxidation reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm.

All the experiments were performed in triplicate orders and the data were analyzed with Minitab 16 software. Person correlations compared the values obtained for DSC induction times (T_0) and Rancimat values. These correlations were applied because they measure the strength and

direction of their linear relationship, describing the direction and degree to which variable is linearly related to others.

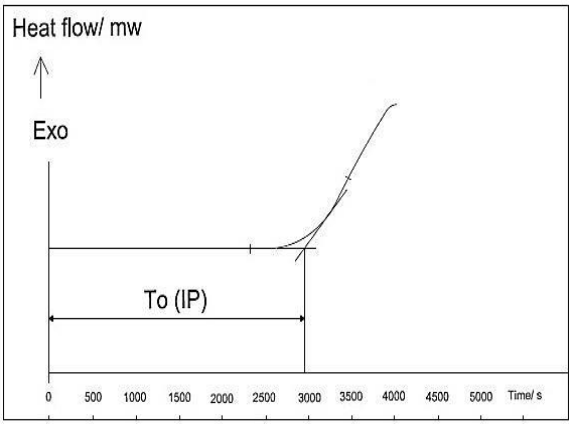

Fig. 1. A typical example of differential scanning calorimetry for mutton tallow with added 100 mg/kg tocopherol mixture. Isothermal curve at 150 ^{0}C with oxygen (99.8%) flowing at 50 mL/min.

Results and Discussion

Table 1 presents the fatty acid compositions of mutton tallow, where oleic acid followed by palmitic and stearic acids were the predominant fatty acids in respective decreasing order. The reason for selection of tallow as the substrate is due to its relative low induction period and the absence of natural antioxidants namely tocopherols. Tallow has a high concentration of palmitic and stearic acids and moderate amount of oleic acid that are quite resistant to oxidation reaction, it is therefore quite ideal for the evaluation of antioxidant activity due to the deficiency of natural antioxidants and consequently low induction period.

Figure 1 is a typical example of a model representing the induction time; T_0 and T_{max} calculated by DSC, the exothermic curve was obtained, when atmospheric oxygen (99.8%) was flowing at 50 mL/min. The oxidative induction time (T_0) of the oxidation reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exotherm. The T_0 obtained by DSC curve extrapolation, ranged between 9.48 and 93.94 min was significantly lower *($P<0.05$)* than those obtained by the Rancimat method (Table 2). These results are thoroughly in agreement with the results reported by Tan *et al.* (2002) and Velasco *et al.* (2004).

Table 1. Fatty acid composition and chemical characteristics of Iranian mutton tallow [a]

Fatty acids (%)				IV(g I_2/100 g oil)	PV (meq/ kg oil)	AnV	TxV	FFA (%)
C14:0	2.56	C18:1t[b]	3.55	47.07± 0.14	0.8± 0.03	2.16± 0.2	3.76± 0.08	0.17± 0.02
C14:1	1.20	C18:2	2.34					
C16:0	19.53	C18:2t	1.90					
C16:1	1.90	C18:3	0.40					
C17:0	3.40	C20:0	0.50					
C17:1	1.17	C20:1	0.13					
C18:0	18.42	C22:0	0.30					
C18:1	34.10	Others	8.54					

[a] Each value in the table represents the mean value± standard deviation of triplicate analyses
[b] Trans fatty acid
Abbreviation: IV: Iodine value; PV: peroxide value; AnV: anisidine value; Tx V: Totox value; FFA: free fatty acids

Table 2. Rancimat induction period (*IP*) and Differential Scanning Calorimetry (DSC) oxidative induction time (T_0) values of Iranian mutton tallow with different antioxidants (mg/ kg) concentrations[a]

Samples	Induction period (min)[b] 110 °C	DSC T_0 (min) 150 °C	DSC T_{max} (min) 150 °C
Mutton tallow	248.1±4.7 ^{Ja}	9.483±1.20 ^{Hb}	13.97±2.23 ^{Fb}
MT+ 100 *(mg/ kg)* BHT[c]	483.5±3.5 ^{Ia}	11.546±0.88 ^{Hb}	15.48±1.16 ^{FEb}
MT+ 200 *(mg/ kg)* BHT	590.0±2.8 ^{Ha}	16.884±0.16 ^{Gb}	21.06±0.38 ^{DEb}
MT+ 400 *(mg/ kg)* BHT	672.5±1.8 ^{Ga}	20.884±1.01 F^{Gb}	26.87±0.36 ^{Dc}
MT+ 100 *(mg/ kg)* TBHQ	1840.0±11.3 ^{Fa}	21.612±0.39 ^{Fb}	34.83±0.42 ^{Cb}
MT+ 200 *(mg/ kg)* TBHQ	2149.8±49.2 ^{Ea}	28.499±0.53 ^{Eb}	37.18±1.90 ^{Cb}
MT+ 400 *(mg/ kg)* TBHQ	2308.6±12.2 ^{Da}	33.615±0.19 ^{Db}	41.27±0.51 ^{Cb}
MT+ 100 *(mg/ kg)* Tocopherol mixture	3786.0±8.5 ^{Ca}	49.703±0.42 ^{Cb}	70.77±2.21 ^{Bb}
MT+ 200 *(mg/ kg)* Tocopherol mixture	3938.1±11.5 ^{Ba}	68.256±0.62 ^{Bb}	75.85±2.58 ^{Bb}
MT+ 400 *(mg/ kg)* Tocopherol mixture	4209.6±3.4 ^{Aa}	93.948±3.12 ^{Ab}	104.13±2.75 ^{Ab}

[a] Each value in the table represents the mean value± standard deviation of three measurements.
[b] Means within each column with different letters (A-J) are significantly ($p<0.05$) different. Means within each row with different letters (a-c) are significantly ($p<0.05$) different.
[c] MT, Mutton tallow

The difference between DSC and Rancimat is due to the lower sample size required for DSC (5.0±0.5 mg) against the Rancimat method (3.0±0.5 g). Moreover, the surface to volume ratio should be considered as an important parameter. In DSC method, pure oxygen (99.8%) is passed through the sample, while in Rancimat normal air is applied after passing through the air filter. All the added antioxidants to the substrate increased both T_0 and T_{max}. The results indicated that tocopherols mixture is more efficient than TBHQ and BHT at different concentrations (Table 2), but one has to understand that the activity of the antioxidant particularly tocopherols is concentration dependent. Figure 2, represents the oxidation curve for mutton tallow and the three selected antioxidants at the same concentration (100 mg/kg). There were good correlations between DSC T_0 and Rancimat induction period results. Pearson correlation coefficient matrixes between DSC T_0 and Rancimat values for each antioxidant are tabulated in Table 3, and the results obtained from the values indicated superb correlations between the two methods (Table 4).

Conclusion

In this study Iranian tail end mutton tallow with and without antioxidants at different concentrations were investigated using Metrohm Rancimat and DSC methods. Both methods confirmed good correlations between the results obtained regarding the investigated substrate with and without added natural and synthetic antioxidant. The results also indicated that tocopherols mixture exhibited better antioxidant activity if compared with the synthetic antioxidants; TBHQ and BHT. Moreover, it was revealed that DSC method is an accurate, reliable and fast method which could be considered as a suitable alternative for the evaluation of the antioxidant activities in oils and fats. Therefore, DSC method might be recommended as an accelerated method to assess the oxidative stability due to its time saving, simplicity and the fact that small quantity of substrate might be required to carry out the tests.

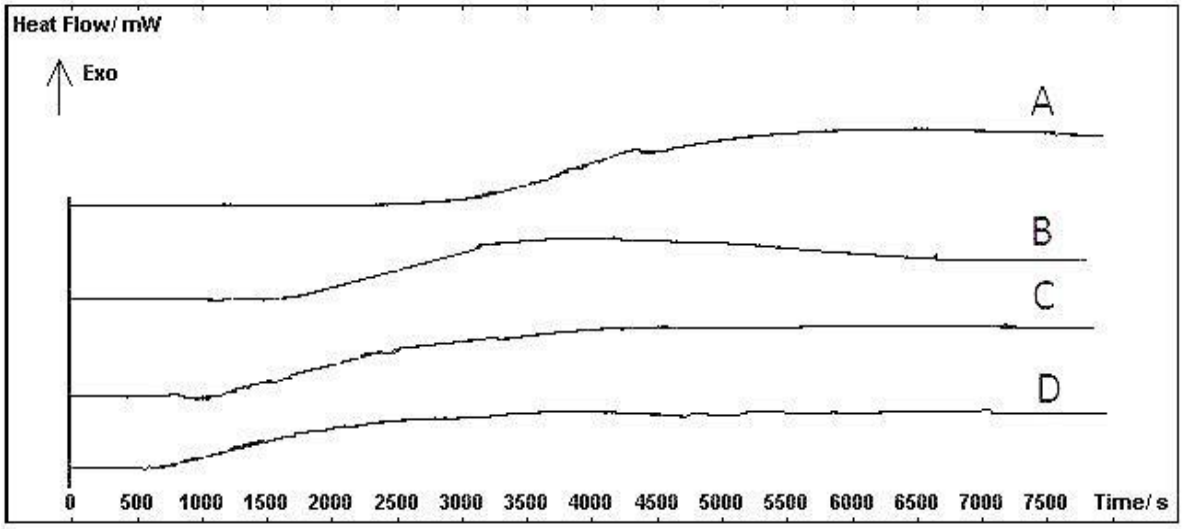

Fig. 2. DSC oxidation curves of Iranian mutton tallow with added antioxidants at concentration of 100 mg/kg. Isothermal curve at 150 ^{0}C with oxygen flowing at 50 mL/min. A: tocopherol mixture; B: TBHQ; C: BHT; D: mutton tallow without antioxidant.

Table 3. Pearson correlation coefficient matrix between DSC (T_0) and Rancimat values for three antioxidants

	DSC(T_0) 150 °C
Rancimat 110 °C, BHT	0.989
Rancimat 110 °C, TBHQ	0.983
Rancimat 110 °C, Tocopherol mixture	0.993

Table 4. Relationship between Rancimat values and DSC oxidative induction time (T_0) for the three antioxidants

Indicator (Y)	Indicator (x)	Regression equation	*p*-value
Rancimat 110, BHT	DSC (T_0), BHT	T_0 ($_{\text{Rancimat110}}$) = 257 + 19.8 $T_{0(\text{DSC 150})}$	0.0001
Rancimat 110, TBHQ	DSC (T_0), TBHQ	T_0 ($_{\text{Rancimat110}}$) = 1010 + 39.0 $T_{0(\text{DSC 150})}$	0.0001
Rancimat 110, TM [a]	DSC (T_0), TM	T_0 ($_{\text{Rancimat110}}$) = 3302 + 9.57 $T_{0(\text{DSC 150})}$	0.0001

[a] Significance at 0.0001 level ($p<0.0001$). DSC150, DSC at isothermal temperature 150 °C; Rancimat 110, Rancimat at isothermal temperature 110 °C.
[b] TM, tocopherol mixture

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