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Analytical Methods

Oxidation of edible animal fats. Comparison of the performance of different quantification methods and of a proposed new semi-objective colour scale-based method

Francisco J. Méndez-Cid [a], José M. Lorenzo [b], Sidonia Martínez [a], Javier Carballo [a,*]

[a] Área de Tecnología de los Alimentos, Facultad de Ciencias, Universidad de Vigo, Campus Universitario s/n, 32004 Ourense, Spain
[b] Centro Tecnológico de la Carne de Galicia, c/ Galicia, 4, 32900 San Ciprián de Viñas, Ourense, Spain

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ABSTRACT

The agreement among the results determined for the main parameters used in the evaluation of the fat auto-oxidation was investigated in animal fats (butter fat, subcutaneous pig back-fat and subcutaneous ham fat). Also, graduated colour scales representing the colour change during storage/ripening were developed for the three types of fat, and the values read in these scales were correlated with the values observed for the different parameters indicating fat oxidation.

In general good correlation among the values of the different parameters was observed (*e.g.* TBA value correlated with the peroxide value: r = 0.466 for butter and r = 0.898 for back-fat). A reasonable correlation was observed between the values read in the developed colour scales and the values for the other parameters determined (*e.g.* values of r = 0.320 and r = 0.793 with peroxide value for butter and back-fat, respectively, and of r = 0.767 and r = 0.498 with TBA value for back-fat and ham fat, respectively).

1. Introduction

Oxidation (peroxidation) is the main reaction involved in fat degradation. Numerous primary and secondary products are formed as a result of fat oxidation, and the chemical nature of these compounds varies widely due to differences in molecular weight and polarity. Evaluation of the level of oxidation of fat is therefore not an easy task (Dobarganes & Márquez-Ruíz, 2003). Several different chemical and physical tests are available for this purpose (Giménez, Gómez-Guillén, Pérez-Mateos, Montero, & Márquez-Ruíz, 2011), ranging from the determination of classical indexes such as the peroxide value (PV) or the 2-thiobarbituric acid reactive substances (TBARs), to quantification of volatile and oxidized non-volatile compounds or the analysis of infrared spectra. Many of these methods require the use of complex and expensive equipment by highly trained and specialized analysts.

Various analytical techniques are used to evaluate lipid oxidation in foods; however, it is often not clear which method provides the most reliable information about the exact state of oxidation of the fats (St. Angelo, 1996). Moreover, modern industries and markets require rigorous controls and rapid decision-making (Trienekens & Zuurbier, 2008), and fast analytical techniques are often preferred over more accurate, but slower methods. Great importance is given to analytical methods being rapid, easy to perform and inexpensive (Tang, Lu, Zhao, & Wang, 2009).

During the ageing (storage/ripening) of animal fats, a change in colour to darker tones takes place in parallel with the fat oxidation processes. It should be possible to establish a relationship between the colour of the fat and the degree of oxidation. The colour changes could then be used to monitor the degradative changes in fat. Thus, the appreciation of fat auto-oxidation through the colour read in a scale could be a rapid, non-destructive and inexpensive method to monitor and appreciate the auto-oxidative changes in the fats.

According to the situation exposed, the aims of this work are to study the correlation between the results produced by different methods used to evaluate lipid oxidation in some fatty foods, in order to assess the consistency and accuracy of the results, and also to try to develop and test a simple semi-objective method based on comparison of the colour of the samples with those in a colour scale previously developed.

2. Materials and methods

2.1. Samples

Samples of butter, cured pig subcutaneous back-fat (lard) and subcutaneous fat from ham were obtained for analysis.

* Corresponding author.
E-mail address: carbatec@uvigo.es (J. Carballo).

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Samples of cow butter were supplied by Mantequera de Tineo, S. A. (Tineo, Asturias, Spain). A total of 60 samples comprising butter immediately after manufacture without sodium chloride or with 2.1% sodium chloride, and after storage for 1, 3, 6 or 9 months at different temperatures (4 or 12 °C) were analyzed. The distribution of samples was as follows. Unsalted butter: immediately after manufacture (n = 4); stored at 4 °C for 1 month (n = 4); stored at 4 °C for 3 months (n = 3); stored at 4 °C for 6 months (n = 3); stored at 4 °C for 9 months (n = 3); stored at 12 °C for 1 month (n = 4); stored at 12 °C for 3 months (n = 3); stored at 12 °C for 6 months (n = 3); and stored at 12 °C for 9 months (n = 3). Salted butter: immediately after manufacture (n = 4); stored at 4 °C for 1 month (n = 4); stored at 4 °C for 3 months (n = 3); stored at 4 °C for 6 months (n = 3); stored at 4 °C for 9 months (n = 3); stored at 12 °C for 1 month (n = 4); stored at 12 °C for 3 months (n = 3); stored at 12 °C for 6 months (n = 3); and stored at 12 °C for 9 months (n = 3).

Sixty pieces of pig subcutaneous back-fat (lard) (fresh or salted with two salt levels and ripened for different lengths of time at different temperatures) were analyzed. Samples were manufactured in the pilot plant of the Centro Tecnológico de la Carne de Galicia (Ourense, Spain). Pieces of dorsal back-fat (2 kg each) were dry-salted (for 2 or 4 days) and later ripened for 1, 3, 6 or 9 months at two different temperatures (8 or 12 °C) and 75% relative humidity. The distribution of samples was as follows: Fresh (immediately after quartering and before salting) (n = 4). Salted for two days: ripened at 8 °C for 1 month (n = 4); ripened at 8 °C for 3 months (n = 4); ripened at 8 °C for 6 months (n = 3); ripened at 8 °C for 9 months (n = 3); ripened at 12 °C for 1 month (n = 4); ripened at 12 °C for 3 months (n = 4); ripened at 12 °C for 6 months (n = 3); and ripened at 12 °C for 9 months (n = 3). Salted for 4 days: ripened at 8 °C for 1 month (n = 4); ripened at 8 °C for 3 months (n = 4); ripened at 8 °C for 6 months (n = 3); ripened at 8 °C for 9 months (n = 3); ripened at 12 °C for 1 month (n = 4); ripened at 12 °C for 3 months (n = 4); ripened at 12 °C for 6 months (n = 3); and ripened at 12 °C for 9 months (n = 3).

For butter and subcutaneous back-fat, samples within the same group of characteristics and ripening/storage conditions belonged to three or four different batches manufactured on different days.

A total of 60 pieces of ripened ham produced by different manufacturers were bought from local markets for analysis of the subcutaneous fat. Both Serrano and Iberian hams were obtained; the latter were produced from extensively or intensively reared pigs fed a commercial diet or with acorns. The hams were ripened for variable periods of between 9 and 36 months. The distribution of the samples was as follows: hams from Iberian pigs fed compound feed, ripened for 12 months (n = 6); hams from Iberian pigs fed compound feed, ripened for 17 months (n = 4); hams from Iberian pigs fed compound feed, ripened for 27 months (n = 6); hams from extensively farmed Iberian pigs fed with acorns, ripened for 24 months (n = 8); hams from extensively farmed Iberian pigs fed with acorns, ripened for 36 months (n = 6). Serrano hams (Landrace x Large White pig) ripened for 9 months (n = 6); Serrano hams ripened for 12 months (n = 9); Serrano hams ripened for 16 months (n = 5); Serrano hams ripened for 18 months (n = 6); Serrano hams ripened for 23 months (n = 4).

Once in the laboratory, after measurement of the colour parameters, samples were minced for further analysis.

All samples were analyzed by determining the parameters listed below in order to establish correlations between the results produced by the different tests.

2.2. Analytical methods

Total chlorides in samples were quantified according to the Charpentier-Volhard official method (ISO 1841-1:1996) (ISO, 1996), and expressed as g/100 g of sample.

Fat from the samples was extracted following the procedure described by Folch, Lees, and Sloane-Stanley (1957). Briefly, five g of sample were homogenized with 100 mL of a mixture of trichloromethane:methanol (50:50; v/v) in a Sorvall Omni Mixer 17150 homogenizer (Ivan Sorwall Inc., Newtown, CONN, USA) for 4 min. The resulting mixture was filtered and then placed in a separation funnel to which 20 mL of distilled water were added. After vigorous agitation and subsequent resting, two phases were separated. The polar phase (methanol + water) was discarded and the organic phase (fat dissolved in trichloromethane) was collected in an Erlenmeyer flask to which 0.2 g of anhydrous sodium sulphate were added. After agitation and filtration to remove the sodium sulphate residue, the mixture was placed in a round bottom flask, and the trichloromethane was finally evaporated at 55 °C in a BÜCHI R-210 Rotavapor® evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland). The fat samples were stored at −80 °C for no longer than one month until analysis.

The iodine, saponification and peroxide indexes were determined following the Spanish Official Standards UNE 55.013, UNE 55.012 and UNE 55.023, respectively (Spanish Government Presidency, 1977). The refractive index was measured with a Standard Abbe refractometer ER-2S (Shibuya Optical Co., Ltd., Saitama, Japan). The fat was firstly melted at 40 °C in a water bath. Next, a drop was placed on the sample stage for measurement. Refractometer was thermostated by circulating water bath at 40 °C. The TBA value was measured according to the method of Tarladgis, Watts, Younathan, and Dugan (1960), with some modifications. Briefly, 10 g of sample were homogenized with 50 mL of distilled water in a Sorvall Omni Mixer 17150 homogenizer (Ivan Sorwall Inc., Newtown, CONN, USA) for 2 min. The mixture was transferred to a round bottom flask and subsequently 47.5 mL of distilled water, and 2.5 mL of 4 N hydrochloric acid to adjust the pH to a value of approximately 1.5, were added. Glass beads and a few drops of an antifoaming agent were also added. The flask was placed in a distillation equipment, in which the mixture was boiled until 50 mL of distillate were collected. Five mL of the distillate were then mixed in a vial with 5 mL of a 0.02 M thiobarbituric acid solution. The vial was labelled and heated in a water bath at 70 °C for 40 min. The vial was then cooled in a water bath and the absorbance was read at 532 nm. The reading was finally interpolated on a calibration curve (absorbance vs. concentration) prepared with malondialdehyde. The TBA values were expressed as mg malondialdehyde/kg sample.

All parameters were determined at least in duplicate in each fat sample.

Colour measurements were obtained with a portable colorimeter (Chroma Meter Cr-400, Konica Minolta Sensing, Inc., Osaka, Japan). The CIELAB space (CIE, 1978) is represented by lightness (L^*), redness (a^*) and yellowness (b^*). The L^* values (from 0 to 100 units) represent the lightness (a lower value indicates a darker colour, black: $L^* = 0$ and white: $L^* = 100$); a^* values represent the balance between red (>0) and green (<0), and b^* values represent the balance between yellow (>0) and blue (<0) (Chen, Zhu, Zhang, Niu, & Du, 2010). The a^*, b^* and L^* values were used to calculate the chroma, hue angle, ΔE and E values. Chroma (C^*) describes the brightness or vividness of colour (colour saturation starting from a value of 0); $C^* = (a^{*2} + b^{*2})^{0.5}$. Hue angle ($H^\circ$) describes the hue or colour angle (colour of sample as defined by its location in a 360° axis; 0 or 360° = red, 90° = yellow, 180° = green and 270° = blue); $H^\circ = \text{arctg}\ b^*/a^*$. ΔE describes the colour difference ($\Delta E = [(L^* - L^*ref)^2 + (a^* - a^*ref)^2 + (b^* - b^*ref)^2]^{1/2}$). The E value represents enhancement of the fraction of redness relative to those of yellowness and lightness; this parameter was calculated using an equation rewritten according to Liu, Fan, Chen, and Thayer (2003) ($E = a^*/b^* + a^*/L^*$). The instrument was