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Comparison of Peroxide Value Methods Used for Semihard Cheeses

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The objective was to evaluate alternatives to the peroxide value method of choice in the dairy industry, the method issued by the International Dairy Federation. Furthermore, the study evaluated the feasibility of alternative solvents for extracting lipids and subsequent peroxide value determinations. Packaged cheeses were stored under illuminated display at 4 °C to obtain samples with various peroxide contents but with uniform gross composition. The hydroperoxide contents were measured during 3 weeks of storage by applying two lipid extraction methods, Folch and Bureau of Dairy Industry (BDI) extractions, and three different hydroperoxide extraction solutions [chloroform/methanol (7:3, v/v), hexane/2-propanol/methanol (5:7:2, v/v/v), and methanol/decanol/hexane (3:2:1, v/v/v)], prior to standard colorimetric measurements. Extraction yields of fat from Havarti cheeses using the Folch and BDI extraction methods were approximately 109 and 61%, respectively, of the yields obtained by the International Dairy Federation gravimetric reference method. Although differences in fat extraction yields were compensated for, significantly higher peroxide values resulted from the Folch extraction method than from the BDI extraction method. The peroxide values obtained by the three methods were all in the same range, and pronounced linear correlations between peroxide contents determined using the three solutions were noted (r^2 in the range of 0.951−0.983). Peroxide value levels were not significantly different in samples stored in the dark or exposed to light.

KEYWORDS: Peroxide value; cheese; analytical technique; substitution; lipid oxidation; light-induced oxidation; BDI; Folch extraction; extraction method

INTRODUCTION

Cheeses, exposed to light from both natural and artificial sources throughout processing, packaging, distribution and at retailers, are likely to undergo light-induced oxidation. Light exposure causes both formation of off-flavors, loss in nutritional value, and color changes, which rapidly impair product quality and marketability (*1*, *2*).

To date, measurements of peroxide values have been used for the determination of primary oxidation products in dairy foods (*3*−*12*). Although the peroxides are flavorless they may be used in any food as indicators of light-induced quality changes, which are detectable by consumers at a later stage. Peroxide values may be determined potentiometrically (*13*) or colorimetrically (*14*−*16*), by Fourier transform infrared spectroscopy (*17*), iodometrically (*18*), or by chromatographic separation using HPLC (*19*). Whatever principle is chosen for determining the peroxide content, all methods involve an extraction of the fat phase of the product, traditionally using chloroform (*15*, *20*).

The objective of the study was to evaluate alternatives to the peroxide value method of choice in the dairy industry, the method issued by the International Dairy Federation (IDF). Furthermore, the study encompassed evaluation of possible substitute solvents used for extracting lipids from the cheeses and for subsequent peroxide value determinations. In the present study, we have compared the actual extraction yield of the standard method and Bureau of Dairy Industry (BDI) extractions in which the para-casein of the cheese is dissolved by addition of sodium hydroxide and sodium hexametaphosphate, thereby liberating the fat globules (*21*) with the fat content being determined by the reference method of the IDF (*22*). The BDI method, which does not involve expensive laboratory equipment such as a centrifuge and vacuum evaporator, has previously successfully been applied to lipid extraction in yogurt (*23*) and milk powder (*24*). Additionally, we have compared three different methods for extracting the hydroperoxides from the fat phase prior to colorimetric measurement based on the standard oxidation by hydrogen peroxide/hydroperoxides of

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Fe(II) to Fe(III) and its subsequent reaction with thiocyanate forming a red complex quantified spectroscopically at 500 nm. Thus, we employed (i) chloroform/methanol (7:3, v/v), the solvent of the standard peroxide value procedure, (ii) methanol/decanol/hexane (3:2:1, v/v/v), and (iii) hexane/2-propanol/methanol (5:7:2, v/v/v). Peroxide value method ii was included because a European interlaboratory evaluation of the procedure was performed at the time of the experiment. Peroxide value method iii had proved to be suitable for peroxide determinations in walnuts (*25*) and other foods (results not published). Both methods involve substitution of chloroform with less critical solvents. All evaluations were carried out during a light storage experiment of cheeses from one batch only, thus providing samples with the same gross composition and identical levels of pro- and antioxidants but with different photoinduced peroxide contents. Realistic storage conditions and commercially applied packaging methodology were used to mimic real-life conditions.

MATERIALS AND METHODS

Cheeses were stored in fluorescent light or in the dark. At the time of sampling, Folch (existing) and BDI (alternative) extractions were applied. Subsequently, peroxide values were determined by either the standard IDF method (chloroform/methanol) or modified IDF methods (methanol/decanol/hexane or hexane/2-propanol/methanol).

Packaging and Storage of Cheeses. Sliced Havarti cheeses (targeted at 55% fat in dry matter) were obtained from Arla Foods amba (Viby J, Denmark) as part of its standard production. The round slices had a diameter of 9 cm and a thickness of 3 mm. Each sample was packaged in conventional packaging materials consisting of a burgundy polystyrene base, a transparent polyester dome, and a polyester barrier layer used to seal the dome, which faces the light source. The oxygen transmission rate (OTR) was determined by Kristensen et al. (*12*) to be 0.034 cm^3 per package (24 h, 23 °C, 0/50% RH) according to standard methods (*26*). The OTR of the barrier layer was 60 $cm^3\ m^{-2}$ (24 h, 23 °C, 5/95% RH) (Amcor Flexibles Europe, Lyngby, Denmark). Kristensen et al. (*12*) determined the light transmission of the dome in the interval 200−800 nm, using a Cintra 40 spectrometer (GBC Scientific Equipment Pty Ltd, Dandenong, Victoria, Australia) equipped with an integrating sphere detector. Only very limited amounts of light were transmitted through the packaging material at wavelengths shorter than 320 nm (cutoff of the material). Furthermore, the transmission spectra revealed that the dome was transparent with transmission >80% in the range of 350−520 nm, which is critical with respect to photooxidation in dairy products (*1*).

Cheeses were packaged in modified atmospheres consisting of ∼25% CO_2 and 75% N_2 at a local dairy plant, and the residual oxygen content was below 0.1% at the time of the experiment. Gas composition, expressed as percent O_2 and percent CO_2, was determined using a CheckMate 9900 gas analyzer (PBI Dansensor, Ringsted, Denmark) prior to lipid extractions. The sliced cheeses were stored at ∼3.5 °C in a display counter at retail conditions. Samples were exposed to 1000 lx fluorescent light (Philips TLD 18W/830 New Generation, Philips, Eindhoven, The Netherlands), 24 h a day, 7 days a week. Half of the packages were covered with black plastic to protect the cheeses from light. The samples were rotated regularly to minimize possible temperature differences in the display cabinets. Sampling took place at 0, 1, 3, 6, and 21 days of storage, each day withdrawing four samples stored in the dark and four samples exposed to fluorescent light. Only the top slices were used for the analyses because they represent the worst-case scenario. The top slices of four packages were shredded and pooled prior to the lipid extractions to obtain sufficient sample material.

Characterization of the Cheeses. Characterization of the product was carried out using standard methods to include total fat (*22*), fatty acid composition (*27*), total protein (*28*), total solids (*29*), and ash (*30*). Potentiometric pH measurements were carried out on the cheese prior to departure from the dairy plant. Entire samples were used for characterization of the cheeses.

Lipid Extraction Using a Modified Folch Extraction. The lipids were extracted using modified Folch extractions (*31*). The lipid phase was extracted from 2.5 g of cheese using a 50 mL chloroform/methanol solution (2:1, v/v) followed by homogenization with an Ultra Turrax (Jankel & Kunkel IKA-Labortechnik, Staufen, Germany). To the homogenate was added 10 mL of 1 mM $CaCl_2$ prior to further mixing for 10 s. The resulting mixture was centrifuged at 3000 rpm for 30 min in a Heraeus labofuge A (Kendro, Newtown, CT), after which the chloroform phase was transferred to an evaporation flask. To the remaining aqueous phase was added 30 mL of chloroform, and this mixture was homogenized and centrifuged for 30 min. The four extracts, which underwent the same treatment, were finally pooled. The chloroform phase was dried off using a vacuum evaporator, and the lipid phase was redissolved in 2−3 mL of chloroform, nitrogen flushed, and stored at −20 °C awaiting the peroxide measurements. The chloroform was evaporated prior to peroxide determinations.

Lipid Extraction Using BDI Reagent. A modified Singsaas and Hadland (*21*) BDI method was used. In a mortar, 2.5 g of shredded cheese was mixed with 0.4 g of sodium hexametaphosphate, and 10 mL of 0.1 N NaOH was slowly added, followed by 40 mL of 50 °C distilled water, resulting in a uniform cheese slurry. The slurry was subsequently transferred to a 100 mL volumetric flask and heated at 100 °C for 15 min until the fat surfaced. Care was taken to avoid shaking of the volumetric flask when 6 mL of 1 N HCl was added, and the flask was left at room temperature for 15 min. Subsequently, 20 mL of BDI reagent (30 g of Triton-X and 70 g of sodium hexametaphosphate diluted to 1000 mL with water) was added. The flask was swung and placed in boiling water for another 10 min until a clear lipid phase appeared. The bottle was placed at room temperature, and boiling distilled water was added up to 2−3 cm from the top of the bottle. After 5 min, when the lipid phase was clearly separated, the lipid phase was transferred to a brown flask, redissolved in 2−3 mL of chloroform, nitrogen flushed, and stored at −20 °C awaiting the peroxide measurements. The chloroform was evaporated prior to peroxide determinations.

Peroxide Value Method A. The colorimetric method of Shantha and Decker (*20*)/International Dairy Federation (*15*) was used employing an HP 8453 UV−visible spectrophotometer (Hewlett-Packard, Palo Alto, CA) when measuring A_{500}. Results, expressed as milliequivalents per kilogram of lipid, are means of triplicate determinations.

Peroxide Value Method B. The principles described in IDF Standard 74A (*15*) were applied. The only modification was substitution of the chloroform/methanol (7:3, v/v) with a solution of methanol/decanol/hexane (3:2:1, v/v/v), as described by Van Reusel (*32*). Results, expressed as milliequivalents per kilogram of lipid, are means of triplicate determinations.

Peroxide Value Method C. The principles described in IDF Standard 74A (*15*) were applied. The only modification was substitution of the chloroform/methanol (7:3, v/v) with a solution of hexane/2-propanol/methanol (5:7:2, v/v/v), as described by Jensen et al. (*25*). Results, expressed as milliequivalents per kilogram of lipid, are means of triplicate determinations.

RESULTS AND DISCUSSION

Characterization of the Cheeses. The Havarti cheeses contained 36.6% total fat. Protein and dry matter contents were determined to be 21.7 and 62.9%, respectively, resulting in a fat in dry matter content amounting to 58.2%. Ash content totaled 2.4%, and the pH was 5.36. The characterization indicated that the products were well within the expected gross composition range.

Lipid Extractions. The IDF reference method for determination of the fat content of cheeses is based on hydrochloric acid hydrolysis followed by multiple extractions with ethanol, diethyl ether, and petroleum ether. Compared to the IDF reference method, the standard Folch extraction procedure for determination of peroxide value gave an average extraction yield, which was 108.7% of the reference numbers, whereas the average extraction yield for the BDI method was merely

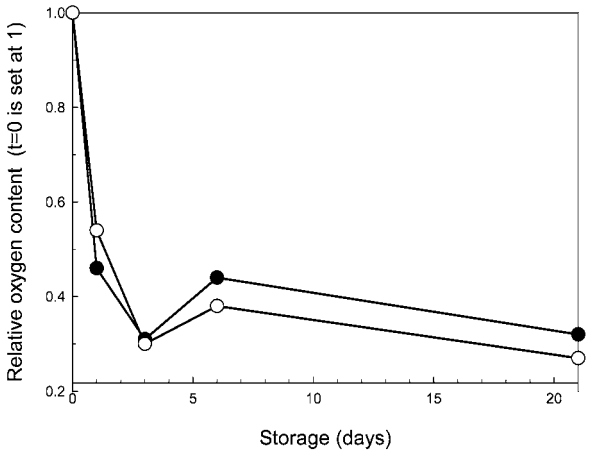

Figure 1. Oxygen contents in the headspace of packaged Havarti cheese measured at 0, 1, 3, 6, and 21 days of storage in the dark (●) or at 1000 lx (○) using a Checkmate gas analyzer (PBI Dansensor, Ringsted, Denmark).

60.4% of the reference method numbers. These results are most likely attributable to the more pronounced polar nature of the Folch solvent, thus extracting nonfat components, whereas the poor result of the BDI extraction is probably due to insufficient liberation of fat from the fat globules of the cheese matrix. Thus, the BDI method may be suitable for lipid extractions despite the low extraction yields. However, evaluations of fatty acid profiles for extracts from both methods should be performed in order to provide firm conclusions with respect to the superiority of one method to the other. This was attempted, but instrumental breakdown made it impossible. However, recent experiments indicated that the best results were obtained when the BDI method rather than the Folch method was applied for extraction of milk fat from yogurt (*23*).

Gas Composition. Figure 1 depicts the headspace oxygen concentration in samples exposed to light and stored in the dark. As may be seen, oxygen levels decrease rapidly from their initial content (0.1%) during the initial period of storage to 30% of the original content after 3 days, subsequently leveling off at an equilibrium of ~25% of the original content. In light of the concentration gradient from package to surroundings, the observed decrease cannot be due to diffusion but is ascribed to oxidation in the cheese. Interestingly, the rate of decrease of oxygen content is similar for cheeses exposed to light and stored in the dark, in contrast to the expectation of a higher oxygen consumption rate for cheeses exposed to light. Notably, the time resolution used in the present study is too high to differentiate between oxygen consumptions, indicating that initial reactions are very rapid. Changes in carbon dioxide revealed no significant effect of storage in the dark, at 1000 lx, or of storage time (results not shown).

Peroxide Value. For the peroxide determination methods evaluated, a sharp decline in peroxide values was seen within the first 1−2 days of storage followed by a more constant level of peroxide values (**Figure 2**).

This consistent pattern was somewhat surprising; an initial increase in peroxide content followed by a decrease upon degradation to secondary lipid oxidation products was expected, as observed in the case of storage of, for example, milk powder (*10*). Moreover, a higher content of peroxides was expected for cheeses exposed to light than for cheeses stored in the dark, in contrast to the nonsignificant time traces recorded. If one takes into account the results of **Figure 1**, the results may indicate a mechanism including (i) a rapid formation of hydroperoxides

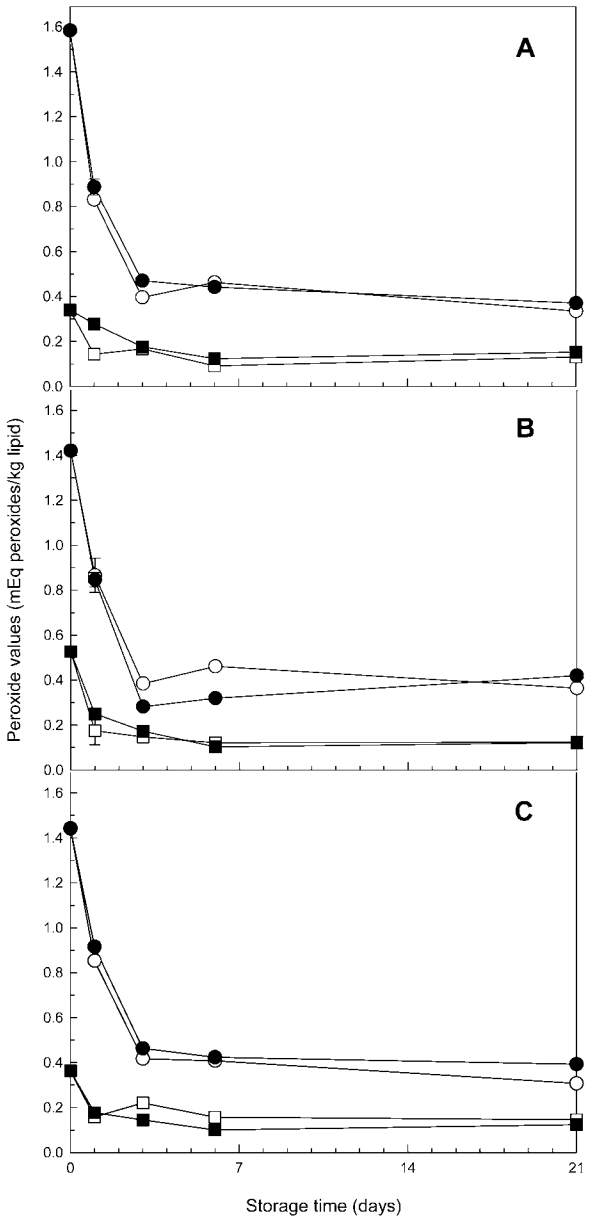

Figure 2. Peroxide values during storage at 4 °C of Havarti cheeses in the dark (solid symbols) or at 1000 lx (open symbols) after Folch extraction (circles) or BDI extraction (squares) and determination of peroxides in (A) chloroform/methanol (7:3, v/v), (B) methanol/decanol/hexane (3:2:1, v/v/v), or (C) hexane/2-propanol/methanol (5:7:2, v/v/v), respectively.

to a certain level determined by the low residual oxygen content available followed by (ii) temperature-dependent cleavage of hydroperoxides with one pool being transformed to secondary oxidation products within the first 3−7 days and a rather stable pool not being transformed within the evaluated storage period. However, the present study was not designed to resolve the above, and further studies involving more rapid methods are required to elucidate initial kinetics. All of the peroxide values determined for samples to which the Folch method had been applied were significantly higher than those for the BDI-extracted samples. For all peroxide extractions, this difference was higher than what could be accounted for by the variance in extraction yields (39.8 vs 22.1%), indicating a higher peroxide extracting capability of the Folch extraction including peroxides too polar to be extracted with the pure lipid in the BDI method.

Peroxide numbers determined using the three solvents, chloroform/methanol (7:3, v/v), methanol/decanol/hexane (3:2:1,

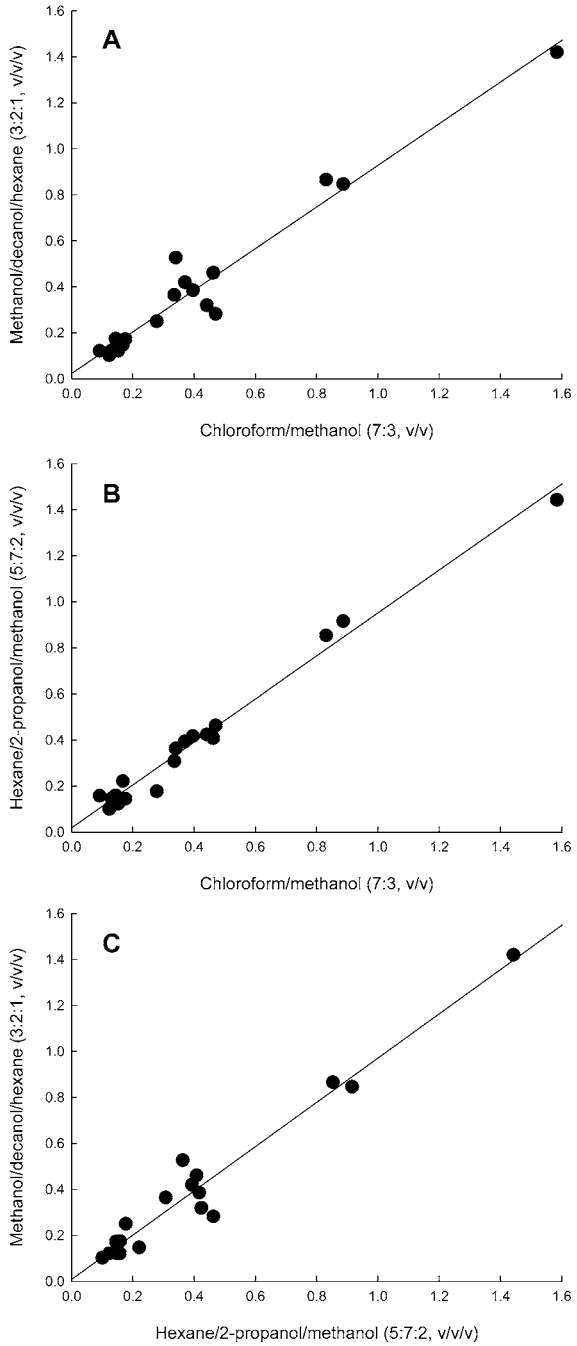

Figure 3. Correlations between peroxide values obtained using (A) chloroform/methanol (7:3, v/v) vs methanol/decanol/hexane (3:2:1, v/v/v), $r^2 = 0.951$; (B) chloroform/methanol (7:3, v/v) vs hexane/2-propanol/methanol (5:7:2, v/v/v), $r^2 = 0.983$; and (C) hexane/2-propanol/methanol (5:7:2, v/v/v) vs methanol/decanol/hexane (3:2:1, v/v/v), $r^2 = 0.953$.

v/v/v), and hexane/2-propanol/methanol (5:7:2, v/v/v), respectively, were in the same range. Moreover, the peroxide values obtained using the different solvents correlated well with each other with regression coefficients from 0.951 to 0.983 (**Figure 3**).

The Folch method requires only small sample amounts. However, the method is time-consuming, and expensive equipment is required (centrifuge and vacuum evaporator). Additionally, chloroform is used. On the other hand, the BDI method is less time-consuming than the Folch extraction, requires no expensive equipment, and does not involve the use of chloroform. The drawback of the BDI method is that it requires larger sample amounts than the Folch extraction.

In conclusion, the nontoxic BDI method may be used as an attractive alternative to the Folch extractions. However, further investigations of the fatty acid profiles obtained when the two extraction methods are applied are required. The extraction yield obtained may be explained by the polarity of the extraction solvents. Furthermore, the standard solvent, chloroform/methanol (7:3, v/v), may be substituted by either methanol/decanol/hexane (3:2:1, v/v/v) or hexane/2-propanol/methanol, 5:7:2, v/v/v), and accordingly it is possible to achieve chloroform-free steps throughout the analysis. Differences in peroxide values for samples stored in the dark or exposed to light were not evident. This may be attributable to rapid photooxidative reactions that the present study was not designed to resolve; a short time exposure study may further elucidate these aspects. Including analysis of secondary oxidation products may also be a suitable approach if the oxidative reactions progress rapidly and may be more appropriate as they are generally better correlated to sensory analysis than are primary oxidation product results (*33*).

ABBREVIATIONS USED

BDI, Bureau of Dairy Industry; IDF, International Dairy Federation; OTR, oxygen transmission rate.

SAFETY

Chloroform is a mutagen agent; hence, precaution must be taken when peroxide values are determined. Skin contact with Triton-X and sodium hexametaphosphate should be avoided. The use of the remainder of the organic solvents should take place under fume hood conditions.

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