

Analytical Methods

Volatile compounds and fatty acid profiles in commercial milk-based infant formulae by static headspace gas chromatography: Evolution after opening the packet

Jorge L. Chávez-Servín, Ana I. Castellote, M. Carmen López-Sabater*

Department of Nutrition and Food Science, Food Technology Reference Centre, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII s/n, E-08028 Barcelona, Spain

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Abstract

The evolution of the volatile compounds propanal, pentanal and hexanal, and fatty acid profiles were examined in 20 infant formula (IF) milk powders during storage at 25 °C for 70 days after their packaging was opened. Few changes were observed in the fatty acid content during storage, but significant losses were found in C18:2 *n* – 6 and C18:3 *n* – 3 for some formulae. All three volatiles increased during storage in all formulae, confirming oxidative stability decreases once packets were opened. Significant correlation ($p < 0.05$) was detected between hexanal content and oxidation of *n* – 6 PUFA, specifically C18:2 *n* – 6 losses, and between propanal content and oxidation of *n* – 3 PUFA, specifically from C18:3 *n* – 3 losses.

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1. Introduction

Lipid oxidation causes quality deterioration during manufacture and storage of lipid-containing foods. In the peroxidation of unsaturated fatty acids, lipid hydroperoxides are formed during the propagation phase. These primary compounds are unstable and rapidly decompose in the presence of trace elements to give a range of compounds, including alkoxy and alkyl radicals, aldehydes, ketones and a range of carboxyl compounds that form a complex mixture of secondary lipid oxidation products, which spoil infant formula (IF) milk powders. Traditionally, the peroxide value (POV) has been used to determine primary lipid oxidation products, and the thiobarbituric acid (TBA) assay for secondary oxidation products. Several protocols have been described for the determination of POV in milk products (FIL-IDF, 1991; Newstead &

Headifen, 1981). However, with complex foods such as IFs, a lipid extraction is required prior to POV measurement and this may introduce error and increase analysis time (Perkins, 1984). In contrast, the TBA test can be applied directly to the sample. However, the appropriateness of the TBA assay, especially when applied to milk and milk products, has been questioned (Ward, 1985). Researchers have focused their efforts on new technologies and methods for the evaluation of food lipid damage, that are simple, fast, reliable and sensitive, requiring less time and with minimal sample treatment.

Direct injection of fat into the heated injection port of a gas chromatograph and quantification of volatile substances originating from the thermal breakdown of lipid peroxides was a widely used approach for measuring rancidity in fatty foods (Dupuy, Fore, & Goldblat, 1973). Later automatic samplers for static or dynamic headspace gas chromatography were used, for the determination of volatile compounds produced by oxidation (Snyder, Franke, Selke, & Warner, 1988; Ulberth & Roubicek, 1993). The static head space gas chromatography (SHS-GC)

* Corresponding author. Tel.: +34 93 4024512; fax: +34 93 4035931.
E-mail address: mclopez@ub.edu (M.C. López-Sabater).

method reported by Romeu-Nadal, Castellote, and Lopez-Sabater (2004) for IF analysis is an easy, fast and reliable method for determining the main volatile compounds.

Many studies have reported the development of off-flavours in milk at a given storage time, usually at the end of the product's shelf-life (Cormier, Raymond, Champagne, & Morin, 1991; Vallejo-Cordoba & Nakai, 1994; Contarini & Pavolo, 2002). Volatile compounds such as hexanal and pentanal have been associated with the development of undesirable flavours and have been proposed as potential markers of fresh milk quality (Contarini & Pavolo, 2002; Karatapanis, Badeka, Riganakos, Savvaidis, & Kontominas, 2006; Kim & Morr, 1996; Marsili & Miller, 2003; Toso, Procida, & Stefanon, 2002). Concentrations of saturated aldehydes and hydrocarbons correlate well with sensory flavour (Hall & Andersson, 1985; Hall, Andersson, Lingnert, & Olofsson, 1985). A "cardboard-like" off-flavour is frequently associated with dehydrated milk products. This effect is highly correlated with the headspace concentration of hexanal (Hall et al., 1985). In stored UHT milk, concentrations of pentanal and hexanal are also related to the development of off-flavour (Rerkrai, Jeon, & Bassette, 1987). Several sampling techniques are currently available for the isolation and measurement of volatile compounds, such as gas chromatography (GC)–mass spectrometry-based electronic nose, solid-phase microextraction (SPM), GC/mass spectrometry (GC/MS), vacuum distillation, simultaneous steam distillation and extraction GC, static headspace (SHS), dynamic HS/ purge and trap GC, and direct thermal desorption (Cruwys, Dinsdale, Hawkes, & Hawkes, 2002; Contarini & Pavolo, 2002; Fenaille, Visani, Fumeaux, Milo, & Guy, 2003; Hardas, Danviriyakul, Foley, Nawar, & Chinachoti, 2002; Jung, Yoon, Lee, & Min, 1998; Kolb, 1999; Marsili, 1999a; Marsili, 1999b). The SHS technique requires minimal sample treatment and reduces artifactual volatile compound formation.

Many milk-based IF powders are supplemented with polyunsaturated fatty acids, such as arachidonic acid (C20:4, $n - 6$) (AA) and docosahexaenoic acid (C22:6, $n - 3$) (DHA), which are more susceptible to oxidation than linoleic acid (C18:2, $n - 6$) (LA), and may produce undesirable flavours and odours. However, LA is the main polyunsaturated fatty acid in IFs (Ulberth & Roubicek, 1995). The content of hexanal, which is a major breakdown product of LA oxidation (Frankel, 1993), has been used to follow the course of lipid oxidation and off-flavour development in foods (Dupuy et al., 1977). Pentanal and hexanal are volatile oxidation products of $n - 6$ PUFA and propanal of $n - 3$ PUFA (Romeu-Nadal et al., 2004). In spite of literature related to volatile content in milk (liquid and powdered), information about these compounds in IFs is scarce. van Ruth, Floris, and Fayoux (2005) studied the volatile profiles of 13 IFs by proton transfer reaction-mass spectrometry. Fenaille et al. (2006) measured the levels of secondary lipid oxidation products (malondialdehyde and hexanal) in relation to the processing conditions of IF, pasteurised and UHT milk samples.

When stored, IFs are usually protected from light and maintained at room temperature. However, because of the long storage life of these powders (usually 2 years), PUFAs can be oxidised, giving rise to a loss of nutritive value and to the generation of volatile compounds from peroxides. UV light induces lipid oxidation, therefore milk products such as IFs, once opened, are highly susceptible to lipid oxidation at room temperature, and light accelerates this process (Hardas, Danviriyakul, Foley, Nawar, & Chinachoti, 2000). There is a lack of information on the concentration and evolution of volatile compounds in commercial IFs. According to the manufacturers' instructions, once opened IFs should be used within a month. Generally, they are consumed before this time. However, when IFs are used for complementary feeds, the product could be stored longer. Therefore it would be of interest to study the evolution of volatiles not only during the one-month's life once opened, but also after this time, extending the analysis, for example, until 70 days after opening, a period greater than double the established time for formula consumption.

Here we measured and analysed the quantity of propanal, pentanal and hexanal in several brands of milk-based IF, as potential indicators of lipid oxidation and consequently formula decomposition. For this purpose we used SHS-GC, a simple and sensitive method developed in our laboratory (Romeu-Nadal et al., 2004). In addition, we evaluated oxidative stability of IFs by examining the evolution of these volatiles and the fatty acid profiles during the 70 days after package opening. It is hypothesised that oxidative stability decreases quickly once packets are opened, as a result of product exposure to the action of oxygen and light, and that IFs with major contents of longchain (LC-PUFAs) generate more volatiles, due to their higher susceptibility to oxidation. Correlations between fatty acid losses and volatiles increase were measured.

2. Materials and methods

2.1. Samples

Twenty branded milk-based powdered IFs were purchased from several markets. Table 1 indicates the general composition of the studied formulae, as stated on the product label.

2.2. Storage

All IFs were opened on the same day; approximately in the 5–9 month of their shelf-lives. In addition, IFs were opened three times every day thereafter; each time the powder was stirred in the original packet to maintain uniform exposure to environment and two scoops of powder were discarded, thereby simulating normal storage and preparation. We kept the IFs at room temperature (25 °C: min 23 °C, max 25.5 °C), and the contents were analysed at 0, 15, 30, 50 and 70 days. The volatile compound content was determined on-line. We placed aliquots of approximately 15 g

Table 1
Composition of infant milk-based formulae as stated on product label

| Formula | Lipid ^a | Protein ^a | Carbohydrate ^a | Main ingredients ^b |
|---------|--------------------|----------------------|---------------------------|---|
| 1 | 23.1 | 12.8 | 58.3 | Skim milk, lactose, starch, palm olein, demineralised whey milk, colza, coconut and sunflower oils, soybean lecithin. |
| 2 | 24.0 | 12.5 | 58.6 | Demineralised whey milk, skim milk, palm olein, maltodextrin, colza, palm and corn oils, soybean lecithin. |
| 3 | 24.0 | 12.5 | 58.6 | Demineralised whey milk, palm olein, starch, skim milk, corn syrup, colza, coconut and corn oils. |
| 4 | 24.0 | 12.0 | 58.7 | Demineralised whey milk, palm oil, skim milk, maltodextrins, vegetal oils (colza, sunflower). |
| 5 | 24.5 | 11.0 | 59.7 | Skim milk, vegetal fat matters (palm, coconut, colza and sunflower), demineralised whey milk, maltose, maltodextrins, soybean lecithin. |
| 6 | 27.5 | 13.9 | 52.4 | Skim milk, lactose, glucose syrup, vegetal oils (palm, colza, corn and coconut). |
| 7 | 28.0 | 11.6 | 56.0 | Demineralised whey milk, vegetable oils, skimmed milk powder, lactose, soybean lecithin and monoglycerides of fatty acids. |
| 8 | 28.2 | 11.3 | 60.5 | Whey powder, vegetable oils, skimmed milk, lactose, galacto-oligosaccharides, polyfructose, fish oil. |
| 9 | 24.4 | 11.7 | 58.6 | Demineralised whey milk, milk powder (partially demineralised), vegetable oils (palm, colza, sunflower), lactose. |
| 10 | 27.0 | 11.5 | 55.0 | Skim milk, fat milk, lactose, lactose, vegetable oils (palm, sunflower, colza) milk proteins. |
| 11 | 25.4 | 12.1 | 50.0 | Demineralised whey milk, vegetable oils (palm, coconut, soybean), lecithin. |
| 12 | 23.9 | 11.2 | 60.0 | Milk protein, skim milk, whey protein milk, vegetable oils (palm, coconut, colza, sunflower), lactose, carob flour, glucose syrup. |
| 13 | 24.0 | 12.5 | 55.6 | Demineralised whey milk, palm olein, starch, skim milk, corn syrup, colza, coconut and corn oils. |
| 14 | 29.0 | 11.0 | 56.0 | Lactose, skim milk, palm oil, whey milk protein concentrate, coconut and soybean oils, vegetable oil rich in oleic acid, soybean lecithin. |
| 15 | 29.0 | 11.0 | 56.0 | Lactose, skim milk, palm oil, whey milk protein concentrate, coconut and soybean oils, vegetable oil rich in oleic acid, soybean lecithin. |
| 16 | 26.4 | 11.5 | 56.4 | Hydrolyzed whey milk protein minerals reduced, vegetable oils (palm olein, soybean, coconut, sunflower, high oleic acid), lactose, corn maltodextrin. |
| 17 | 26.0 | 11.5 | 57.7 | Hydrolyzed whey milk protein minerals reduced, corn syrup, vegetable oils (palm olein, canola, coconut, sunflower, high oleic acid). |
| 18 | 28.0 | 11.0 | 56.0 | Skim milk, lactose, vegetal oils, fractionated milk protein (α -lactoalbumin), soybean lecithin, LC-PUFA (arachidonic and docosahexaenoic acid). |
| 19 | 26.0 | 9.5 | 58.0 | Skim milk, vegetable oils (palm, coconut, sunflower, soybean, high oleic acid), LC-PUFA (arachidonic and docosahexaenoic acid), lactose, maltodextrin, milk proteins, soybean lecithin. |
| 20 | 26.0 | 10.7 | 58.3 | Lactose, vegetable oil, skim milk, maltodextrin, serum protein, egg phospholipids. |

^a Expressed as g/100 g of powder.

^b Ingredients are listed in the order in which they appear on the label.

IF in amber glass flasks, sealed them under nitrogen and kept them at $-80\text{ }^{\circ}\text{C}$ until fatty acid measurements.

2.3. Chemicals

Deionised water was purified through a Milli-Q system (Millipore, Bedford, MA). Standards of propanal (97% pure), pentanal (97% pure) and butyl acetate (99% pure), were purchased from Aldrich (Steinheim, Germany); hexanal (98% pure), SupelcoTM 37 component fatty acid methyl esters mix, and tridecanoic acid (C13:0), used as the fatty acid internal standard, were purchased from Sigma (St. Louis, MO). Boron trifluoride in methanol (20% w/v), *n*-hexane, sodium chloride and anhydrous sodium sulphate were from Merck (Darmstadt, Germany), sodium methylate was obtained from Fluka (Buchs, Switzerland), and dry methanol from Panreac (Barcelona, Spain).

2.4. Instruments

For the analysis of volatiles, we used a Shimadzu gas chromatograph system model GC-14 A, with flame ionisation detector and split-splitless injector (Shimadzu, Kyoto,

Japan). We used a SupelcowaxTM-10 fused silica capillary column (30 m \times 0.25 mm \times 0.25 μm film thickness) from Supelco (Bellafonte, PA). The aldehydes were separated isothermally at $75\text{ }^{\circ}\text{C}$. The injector and detector temperatures were 185 and $200\text{ }^{\circ}\text{C}$, respectively; with a split ratio of 1:20; helium was used as carrier gas, at a linear velocity of 20.39 cm/s. Data acquisition was performed using HP GC Chemstation software for Windows (Hewlett-Packard).

For the fatty acid analysis, we used a Shimadzu GC-2010 gas chromatograph equipped with a flame ionisation detector and a Shimadzu AOC-20i autoinjector. The fatty acid methyl esters (FAMES) were separated on a fast capillary column (10 m \times 0.10 mm i.d.), coated with BPX70 cross-linked stationary phase (0.20 μm film thickness) from SGE (SGE Europe Ltd., Milton Keynes, United Kingdom).

2.5. Analytical determinations

2.5.1. Measurement of volatile compounds

Volatile compounds in the IFs were measured by static headspace gas chromatography (Romeu-Nadal et al.,

2004). Approximately 500 mg of IF powder were weighed into a 10 ml headspace vial, then 2.5 ml of Milli-Q water and 0.5 ml of butyl acetate internal standard (1 µg/ml) were added. The vials were sealed with silicone rubber PTFE caps. Samples were homogenised for 1 min, using a vortex. Samples were equilibrated at 60 °C for 15 min in a 2t® vial heater (model VH 6200), then 500 µl were removed by a static headspace sampler MHS 123. Sampling time was 30 s. The gas volume sample was then injected in the GC system. Propanal, pentanal and hexanal were identified by comparison of retention times with those of the standards, and were quantified by interpolation in a calibration curve, within a range of 0.03–0.70 µg/ml. Analyses were performed in quadruplicate.

2.5.2. Fatty acid analysis

Fatty acid profiles were analysed by fast GC after derivatisation to FAMES. IF samples were prepared as described by Lopez-Lopez, Lopez-Sabater, Campoy-Folgooso, Rivero-Urgell, and Castellote-Bargallo (2002), using sodium methylate and boron trifluoride in methanol, thereby obtaining the FAMES in *n*-hexane for subsequent injection into the gas chromatograph. Fast GC analysis was carried out following the method of Bondia-Pons, Castellote, and López-Sabater (2004). Injection was in split mode, with a split ratio of 1:100. The injection volume was 1 µl. The injector and detector temperatures were kept at 250 °C and 270 °C respectively. The temperature programme was as follows: initial temperature 60 °C, increasing at 50 °C/min until 175 °C (holding for 1 min), then, increasing at 20 °C/min until 250 °C (holding for 0.5 min). Data acquisition and processing were performed with the Shimadzu Chemstation software for GC. Analyses were carried out in triplicate.

2.6. Statistical analysis

For statistical analysis, we used one-way analysis of variance, as well as multiple comparisons, using the Tukey HSD procedure for each volatile, storage time and formula brand. Pearson's correlations were removed between content of volatile compounds and fatty acids. Data on volatiles and fatty acids were subjected to principal component analysis (PCA). Statistical analysis was performed using the SPSS package for Windows version 12 (SPSS, Chicago, IL). The level of statistical significance was set at 5% for all analyses.

3. Results and discussion

3.1. Volatile content

In our 20 IF samples, which differed in brand and type, the only volatiles found were propanal, pentanal and hexanal (Fig. 1). The composition data of the major components of the IFs, such as lipid, protein and carbohydrate (Table 1), were subjected to a PCA (Fig. 2a). As expected,

this analysis showed that the global composition of the IF samples was very similar. Only formulae 6 and 11 showed slight differences, which were the result of lower carbohydrate content, 52.4% and 50%, respectively, compared with the remaining samples. However, the volatile contents of the brands differed markedly (Tables 2–4); a fact that is not surprising, considering that the studied IFs came from different manufacturers and contain different raw materials. In newly-opened (time 0) formulae 2, 6, 9, 12, 13, 17 and 20, we did not detect propanal (Table 2), while the remaining IFs registered values between 0.420 and 1.72 mg/kg. However, at 70 days of storage, all IFs contained between 0.319 and 3.45 mg/kg of propanal. This compound is a product of the oxidation of *n* – 3 polyunsaturated fatty acids. In spite of the variability of the results, a clear increase was observed.

Pentanal was not detected in seven (2, 4, 6, 9, 12, 13 and 20) of the twenty formulae at time 0, and in one (IF 20) of the 20 after 15 days: thereafter it increased like propanal (Table 3). In the remaining 13 newly opened IFs, pentanal content ranged from 0.250 to 1.72 mg/kg and increased constantly during storage to 0.025–4.74 mg/kg after 70 days. Finally, hexanal content, which was not found in four IFs (4, 9, 12 and 13) at time 0, (Table 4) ranged from 0.026 to 5.11 mg/kg. Hexanal content also increased during storage, to 0.345 mg/kg–14.7 mg/kg at 70 days.

The increase in these three volatiles is consistent with the findings of other studies, e.g., Karatapanis et al. (2006), which evaluated the changes in volatiles of whole pasteurised milk during storage. These authors did not report the concentrations of these compounds but showed only increasing/decreasing trends for each compound, which they related to storage under fluorescent light for 7 days. Ulberth and Roubicek (1995) monitored oxidative deterioration of milk powder by HS-GC and reported hexanal (from 10 µg/kg to 106 µg/kg) as the main volatile during storage at room temperature under direct light for 130 days at 30 °C. They also reported headspace volatiles in a milk-based IF stored in air at 40 °C, with the major compounds, in order of amount, being hexanal, heptanal and pentanal. Kim and Morr (1996) used dynamic HS to monitor volatile compounds in commercial fluid milk stored in Pyrex test tubes and exposed to fluorescent light. In their study the major volatiles were hexanal, pentanal, dimethyl disulfide, 2-butanone and 2-propanol. Earlier reports (Cadwallader & Howard, 1997; Cladman, Scheffer, Goodrich, & Griffiths, 1998) also show that the most common volatile compounds in light-oxidised milk were pentanal and hexanal. Although the profile of volatiles in IFs differ, these two compounds were detected in our IF samples. The concentrations of the volatiles measured after 70 days of storage were submitted to PCA (Fig. 2c). Hexanal, pentanal and propanal were used as variables. The two extracted components explained 95.93% of the variance (cumulative percentage). Component 1 explained 82.42% of the variance, and in the graph (Fig. 2c) one group of samples is located near to the *x*-axis on the left side, with values of the *y*-axis

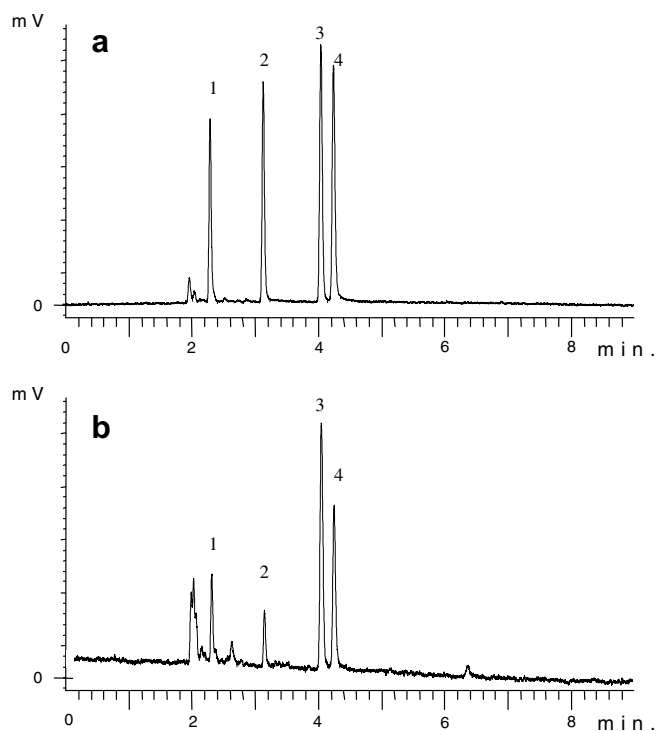


Fig. 1. Typical chromatograms of volatile compounds by HS GC-FID. Peaks: 1, propanal; 2, pentanal; 3, butyl acetate (I.S.); 4, hexanal. (a) Standards and (b) infant formula.

close to zero. The first extracted group (IFs 1, 2, 3, 6, 8, 9, 12, 13, 15, 17 and 20) showed lower concentrations of volatiles, in comparison with the remaining IFs (5, 7, 10, 11, 14, 16, 18 and 19). This graph shows that IF 4 is separate from the two groups as a result of possessing the highest concentration of propanal (11.0 mg/kg). IF 4 showed a significant reduction ($p < 0.05$) of $n - 3$ PUFA, comparing time 0 vs 70 days (Table 5), and propanal formation is consistent with this observation.

Pentanal and hexanal are volatile oxidation products of $n - 6$ PUFA. Gaafar (1991) determined volatiles in UHT milk stored for 24 weeks at room temperature, using a direct static HS-GC method, and reported hexanal levels up to 100 $\mu\text{g}/\text{kg}$. In another study on unspecified milk-based products purchased from supermarkets, hexanal values of 240–290 $\mu\text{g}/\text{kg}$ were recorded by dynamic HS-GC (Park & Goins, 1992). Also, in fresh whole milk powder, thin-layer chromatographic analysis of derivatised carbonyl components detected an increase in hexanal from 90 $\mu\text{g}/\text{kg}$ to 1190 $\mu\text{g}/\text{kg}$ over a storage period of 16 months at 37 °C in air (Boon, Keen, & Walker, 1976). Reported hexanal concentrations in stored milk products vary considerably, depending among other factors, on the analytical technique used, raw material, manufacturing conditions, formula composition, metal ion content, packaging materials, as well as time and storage conditions (Bassette & Jeon, 1983).

Flavour strongly affects the infant acceptance of IF (Mennella & Beauchamp, 1998; Mennella, Griffin, & Beau-

champ, 2004). Packaging (can, bag) directly prevents the development of light-induced off-flavour and lipid oxidation (Karatapanis et al., 2006; Schroder, Scott, Bland, & Bishop, 1985; Vassila, Badeka, Kondyli, Savvaidis, & Kontominas, 2002). We assumed that once IF packets are opened, the powder is exposed to light and oxygen and therefore is more susceptible to oxidative reactions. This hypothesis was confirmed by the constant production of propanal, pentanal and, more markedly, hexanal, in the formulae.

The formation of light-activated flavour in milk and dairy products is due to photosensitized oxidation of lipids and amino acid sulphur groups. Light, oxygen and substrate are required for this reaction (Foote, 1968, 1991; Kim & Morr, 1996). The development of light-activated flavour depends on the wavelength and intensity of the light, exposure time, product temperature and the light transmission properties of the container. The IFs we tested were packed in cans or bags and were therefore protected from light and sunlight and had a controlled atmosphere, containing approximately >0.2% of residual oxygen.

To show the overall behaviour of the volatiles found, we plotted a graph which presents the average of volatiles detected in the IFs along storage. Although great variability in this content was detected, the graph is an easy way to show the overall evolution of these compounds, independently of brand and composition of formulae (Fig. 3). We determined the POV in ten IF samples through storage and related these values to headspace hexanal content. Low correlations were found when lineal data ($r^2 = 0.467$) and logarithmically transformed data ($r^2 = 0.667$) were used. Ulberth and Roubicek (1995) reported a highly significant relationship between hexanal content and POV ($r^2 = 0.931$), when these values were logarithmically transformed. This finding can be attributed to the fact that they used a steam-distillation HS-GC procedure. In this technique, not only is the free aldehyde content of the headspace gas above the sample determined, but also the aldehyde content resulting from the thermally induced breakdown of lipid peroxides. Therefore, a high relationship between hexanal content and POV was found. The method used in our study is presented as a rapid way to evaluate the extent of oxidation of IFs by determining free aldehyde content.

3.2. Fatty acid profiles

Fatty acid profiles were determined at times 0, 15, 30, 50 and 70 days after opening IF packets. The concentrations recorded were submitted to a PCA (Fig. 2b), using as data variables MUFA, PUFA, LC-PUFA, $n - 3$ PUFA, $n - 6$ PUFA, $n - 6$ LC-PUFA and SFA contents. The two extracted components explained 71.05% of the variance (cumulative percentage). The IFs showed similar compositions, except IFs 4, 8, and 12, and 11, 15 and 16. IFs could also be differentiated if they were supplemented with AA and DHA. Due to the low differences between fatty acids

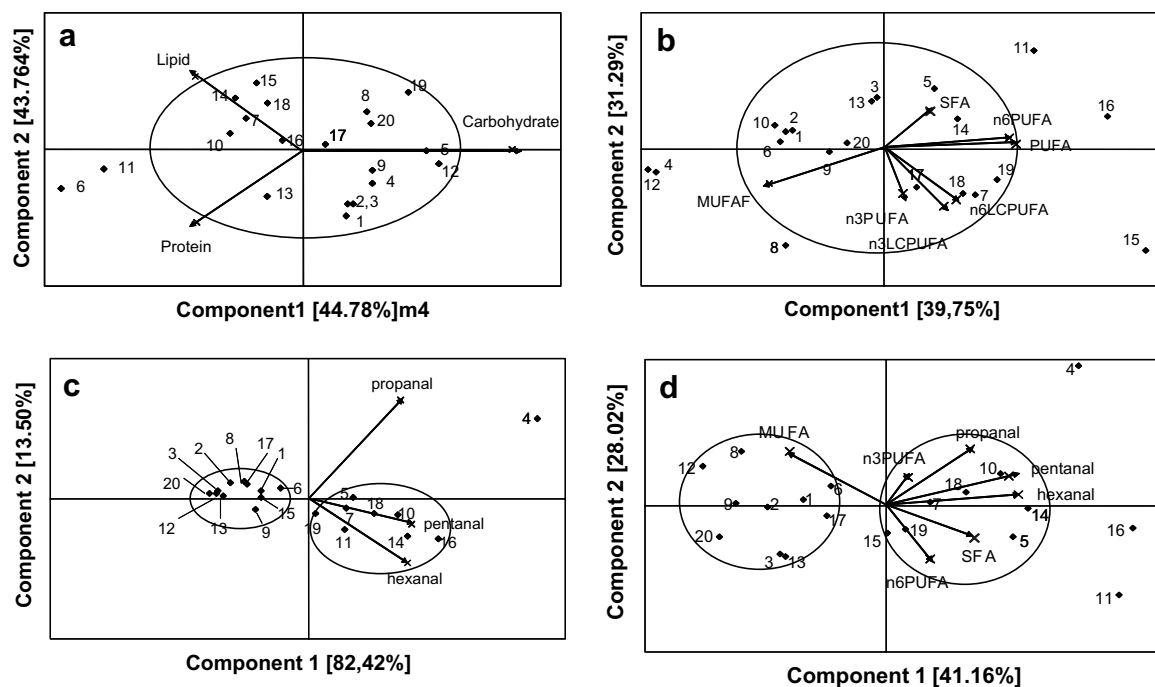


Fig. 2. Principal component analysis of: (a) the compositional data (lipid, protein and carbohydrate), (b) the fatty acid composition (SFA, MUFA, and PUFA), (c) volatile contents after 70 days of storage (propanal, pentanal and hexanal) and (d) volatiles and fatty acid composition, of the 20 IFs.

Table 2
Evolution of propanal in infant milk-based formulae once opened and stored at 25 °C

| Formula | Storage (days) | | | | |
|---------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | 0 | 15 | 30 | 50 | 70 |
| | Mean ^A ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 1 | 0.608 ± 0.002 ^a | 0.766 ± 0.039 ^b | 0.971 ± 0.002 ^c | 1.03 ± 0.025 ^c | 1.44 ± 0.105 ^d |
| 2 | ND ^a | ND ^a | ND ^a | ND ^a | 1.12 ± 0.173 ^b |
| 3 | 0.428 ± 0.006 ^a | 0.429 ± 0.023 ^a | 0.518 ± 0.018 ^a | 0.516 ± 0.005 ^a | 0.500 ± 0.002 ^a |
| 4 | 0.665 ± 0.004 ^a | 3.93 ± 0.022 ^b | 8.7 ± 0.061 ^c | 9.68 ± 0.009 ^d | 11.0 ± 0.395 ^c |
| 5 | 1.163 ± 0.029 ^a | 1.71 ± 0.045 ^b | 2.67 ± 0.154 ^c | 2.52 ± 0.291 ^c | 3.24 ± 0.040 ^d |
| 6 | ND ^a | 0.886 ± 0.122 ^b | 1.14 ± 0.115 ^c | 1.57 ± 0.105 ^d | 2.01 ± 0.219 ^c |
| 7 | 1.72 ± 0.154 ^a | 1.98 ± 0.078 ^a | 2.58 ± 0.234 ^b | 2.35 ± 0.258 ^b | 2.65 ± 0.377 ^b |
| 8 | 0.420 ± 0.015 ^a | 0.667 ± 0.005 ^b | 0.779 ± 0.009 ^b | 0.802 ± 0.011 ^c | 1.47 ± 0.126 ^d |
| 9 | ND ^a | ND ^a | ND ^a | ND ^a | 0.719 ± 0.061 ^b |
| 10 | ND ^a | 1.65 ± 0.004 ^a | 2.09 ± 0.055 ^a | 2.25 ± 0.215 ^b | 3.45 ± 0.106 ^c |
| 11 | 0.746 ± 0.018 ^a | 1.16 ± 0.036 ^b | 1.24 ± 0.005 ^b | 1.55 ± 0.085 ^b | 1.93 ± 0.196 ^c |
| 12 | ND ^a | ND ^a | ND ^a | 0.322 ± 0.001 ^b | 0.333 ± 0.006 ^b |
| 13 | ND ^a | ND ^a | 0.590 ± 0.009 ^b | 0.321 ± 0.006 ^b | 0.319 ± 0.011 ^b |
| 14 | 0.852 ± 0.023 ^a | 1.342 ± 0.022 ^b | 1.82 ± 0.199 ^c | 2.04 ± 0.448 ^c | 2.20 ± 0.404 ^c |
| 15 | 0.700 ± 0.049 ^a | 0.696 ± 0.005 ^a | 0.893 ± 0.034 ^a | 1.02 ± 0.078 ^b | 1.12 ± 0.083 ^b |
| 16 | 1.25 ± 0.027 ^a | 2.90 ± 0.039 ^b | 2.85 ± 0.225 ^b | 2.97 ± 0.127 ^b | 2.94 ± 0.005 ^b |
| 17 | ND ^a | ND ^a | ND ^a | 0.329 ± 0.001 ^b | 1.42 ± 0.045 ^c |
| 18 | 1.79 ± 0.123 ^a | 2.11 ± 0.086 ^a | 2.50 ± 0.099 ^b | 2.88 ± 0.064 ^b | 2.88 ± 0.263 ^b |
| 19 | 0.669 ± 0.001 ^a | 1.45 ± 0.046 ^b | 1.54 ± 0.086 ^b | 1.96 ± 0.137 ^c | 1.87 ± 0.094 ^c |
| 20 | ND ^a | ND ^a | 0.285 ± 0.010 ^b | 0.320 ± 0.001 ^b | 0.321 ± 0.004 ^b |

^A Values are expressed in mg/kg of powder as the mean ± standard deviation ($n = 4$). Means in the same row with different letters are significantly different ($p < 0.05$).

over time, we report only the initial fatty acid profiles (Table 5). Unexpectedly, only two fatty acids studied exhibited significant decreases at the end of the study, the first being C18:2 $n - 6$ in nine formulae: IF5 (from 16.48% to 16.38%), IF7 (from 16.79% to 16.42%), IF11

(from 16.89% to 16.75%), IF12 (from 11.20% to 10.96%), IF14 (from 18.59% to 18.41%), IF15 (from 18.34% to 18.26%), IF16 (from 21.14% to 21.06%), IF19 (from 17.48% to 17.32%) and IF20 (from 16.29% to 15.98%), on average decreases were $0.18\% \pm 0.10$. The other fatty

Table 3
Evolution of pentanal in infant milk-based formulae once opened and stored at 25 °C

| Formula | Storage (days) | | | | |
|---------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | 0 | 15 | 30 | 50 | 70 |
| | Mean ^A ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 1 | 0.167 ± 0.006 ^a | 0.613 ± 0.013 ^b | 0.777 ± 0.010 ^b | 0.729 ± 0.018 ^b | 1.25 ± 0.096 ^c |
| 2 | ND ^a | 0.382 ± 0.013 ^b | 0.438 ± 0.042 ^b | 0.489 ± 0.026 ^b | 0.684 ± 0.069 ^c |
| 3 | 0.250 ± 0.012 ^a | 0.331 ± 0.001 ^a | 0.414 ± 0.007 ^a | 0.438 ± 0.019 ^a | 0.405 ± 0.013 ^a |
| 4 | ND ^a | 2.18 ± 0.131 ^b | 4.00 ± 0.142 ^c | 4.51 ± 0.480 ^d | 4.74 ± 0.547 ^d |
| 5 | 1.17 ± 0.046 ^a | 1.73 ± 0.018 ^b | 2.16 ± 0.382 ^c | 2.34 ± 0.880 ^c | 2.50 ± 0.372 ^c |
| 6 | ND ^a | 1.31 ± 0.025 ^b | 1.03 ± 0.088 ^b | 1.54 ± 0.040 ^b | 1.43 ± 0.278 ^b |
| 7 | 1.72 ± 0.088 ^a | 1.76 ± 0.021 ^a | 1.98 ± 0.128 ^a | 2.34 ± 0.026 ^a | 2.27 ± 0.236 ^a |
| 8 | 0.444 ± 0.028 ^a | 0.615 ± 0.023 ^a | 0.805 ± 0.030 ^b | 0.855 ± 0.050 ^b | 0.910 ± 0.085 ^b |
| 9 | ND ^a | 0.413 ± 0.010 ^b | 0.437 ± 0.010 ^b | 0.498 ± 0.001 ^b | 0.509 ± 0.027 ^b |
| 10 | 0.436 ± 0.006 ^a | 0.452 ± 0.013 ^a | 2.558 ± 0.241 ^b | 3.109 ± 0.566 ^c | 3.292 ± 0.644 ^c |
| 11 | 0.559 ± 0.020 ^a | 2.47 ± 0.039 ^b | 4.07 ± 0.121 ^c | 1.78 ± 0.172 ^b | 1.68 ± 0.258 ^b |
| 12 | ND ^a | 0.301 ± 0.023 ^b | 0.378 ± 0.026 ^b | 0.452 ± 0.041 ^b | 0.443 ± 0.018 ^b |
| 13 | ND ^a | 0.346 ± 0.022 ^b | 0.528 ± 0.065 ^b | 0.526 ± 0.058 ^b | 0.519 ± 0.069 ^b |
| 14 | 1.62 ± 0.030 ^a | 3.32 ± 0.090 ^b | 3.50 ± 0.010 ^b | 4.04 ± 0.472 ^c | 4.69 ± 0.121 ^d |
| 15 | 0.477 ± 0.003 ^a | 0.985 ± 0.002 ^b | 1.12 ± 0.211 ^b | 0.946 ± 0.176 ^b | 1.26 ± 0.193 ^b |
| 16 | 1.56 ± 4.478 ^a | 4.48 ± 0.449 ^b | 4.53 ± 0.449 ^b | 3.92 ± 0.072 ^b | 4.69 ± 0.315 ^b |
| 17 | 0.337 ± 0.011 ^a | 0.644 ± 0.006 ^b | 0.576 ± 0.033 ^b | 0.697 ± 0.007 ^b | 0.954 ± 0.172 ^b |
| 18 | 1.70 ± 0.021 ^a | 2.30 ± 0.252 ^b | 2.91 ± 0.481 ^b | 2.80 ± 0.212 ^b | 3.05 ± 0.501 ^c |
| 19 | 0.296 ± 0.010 ^a | 1.06 ± 0.032 ^b | 1.47 ± 0.216 ^b | 1.69 ± 0.124 ^c | 1.54 ± 0.003 ^c |
| 20 | ND ^a | ND ^a | 0.022 ± 0.001 ^b | 0.025 ± 0.001 ^b | 0.025 ± 0.001 ^b |

^A Values are expressed in mg/kg of powder as the mean ± standard deviation ($n = 4$). Values in the same row with different letters are significantly different ($p < 0.05$).

Table 4
Evolution of hexanal in infant milk-based formulae once opened and stored at 25 °C

| Formula | Storage (days) | | | | |
|---------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | 0 | 15 | 30 | 50 | 70 |
| | Mean ^A ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| 1 | 0.701 ± 0.026 ^a | 1.09 ± 0.027 ^b | 1.52 ± 0.005 ^c | 1.77 ± 0.034 ^d | 2.66 ± 0.076 ^c |
| 2 | 0.026 ± 0.002 ^a | 0.088 ± 0.005 ^a | 0.129 ± 0.011 ^b | 0.443 ± 0.021 ^c | 0.345 ± 0.009 ^c |
| 3 | 0.157 ± 0.008 ^a | 0.330 ± 0.018 ^a | 0.431 ± 0.007 ^b | 0.437 ± 0.006 ^b | 0.527 ± 0.045 ^b |
| 4 | ND ^a | 0.561 ± 0.004 ^b | 4.53 ± 0.012 ^c | 10.7 ± 0.497 ^d | 14.7 ± 1.294 ^c |
| 5 | 3.96 ± 0.178 ^a | 5.51 ± 0.070 ^b | 7.17 ± 0.248 ^c | 7.22 ± 0.088 ^c | 9.61 ± 0.211 ^d |
| 6 | 0.783 ± 0.021 ^a | 1.96 ± 0.177 ^b | 2.32 ± 0.099 ^c | 3.49 ± 0.236 ^d | 3.91 ± 0.208 ^d |
| 7 | 6.23 ± 0.558 ^a | 9.44 ± 0.247 ^b | 8.75 ± 0.996 ^b | 10.9 ± 1.228 ^b | 10.7 ± 1.417 ^b |
| 8 | 0.511 ± 0.007 ^a | 0.747 ± 0.024 ^a | 0.950 ± 0.033 ^b | 0.913 ± 0.107 ^b | 0.953 ± 0.053 ^b |
| 9 | ND ^a | 0.343 ± 0.002 ^b | 0.403 ± 0.008 ^b | 0.529 ± 0.054 ^b | 6.33 ± 0.213 ^c |
| 10 | 0.494 ± 0.001 ^a | 4.44 ± 0.376 ^b | 6.67 ± 0.530 ^c | 8.68 ± 0.490 ^d | 13.8 ± 0.453 ^c |
| 11 | 1.03 ± 0.012 ^a | 1.94 ± 0.354 ^b | 2.77 ± 0.323 ^c | 3.95 ± 0.010 ^d | 14.4 ± 0.681 ^e |
| 12 | ND ^a | 0.249 ± 0.007 ^b | 0.116 ± 0.003 ^b | 0.412 ± 0.035 ^b | 0.612 ± 0.003 ^c |
| 13 | ND ^a | 0.259 ± 0.034 ^b | 0.486 ± 0.061 ^c | 0.513 ± 0.024 ^c | 0.686 ± 0.003 ^c |
| 14 | 3.55 ± 0.270 ^a | 7.46 ± 0.120 ^b | 7.34 ± 0.591 ^b | 10.4 ± 0.496 ^c | 12.8 ± 0.103 ^c |
| 15 | 0.312 ± 0.011 ^a | 1.72 ± 0.153 ^b | 1.90 ± 0.230 ^b | 2.33 ± 0.134 ^c | 3.31 ± 0.297 ^d |
| 16 | 4.09 ± 0.117 ^a | 13.2 ± 0.24 ^b | 12.52 ± 0.41 ^b | 13.5 ± 0.403 ^b | 16.4 ± 0.411 ^b |
| 17 | 0.250 ± 0.019 ^a | 0.991 ± 0.055 ^b | 1.10 ± 0.168 ^b | 1.26 ± 0.170 ^c | 1.34 ± 0.059 ^c |
| 18 | 5.11 ± 0.097 ^a | 6.92 ± 0.774 ^b | 8.82 ± 0.447 ^c | 10.7 ± 0.475 ^c | 11.8 ± 0.499 ^c |
| 19 | 0.860 ± 0.045 ^a | 4.99 ± 0.251 ^b | 6.16 ± 0.316 ^c | 8.56 ± 0.036 ^d | 10.1 ± 0.052 ^c |
| 20 | 0.665 ± 0.036 ^a | 0.819 ± 0.051 ^a | 0.852 ± 0.108 ^a | 1.07 ± 0.048 ^b | 1.10 ± 0.021 ^b |

^A Values are expressed in mg/kg of powder as the mean ± standard deviation ($n = 4$). Values in the same row with different letters are significantly different ($p < 0.05$).

acid to decrease was C18:3 $n - 3$ in three formulae: IF4 (from 1.72% to 1.58%), IF7 (from 1.38% to 1.31%) and IF12 (from 1.72% to 1.57%).

PUFAs, in particular, are easily attacked by free radicals that react with their double bonds, thereby yielding

products, such as short-chain aldehydes. The major susceptibility of fatty acids to oxidation is thought to be directly dependent on their degree of unsaturation, and thus DHA, EPA and AA, which are the most unsaturated FAs in IFs, would be most susceptible to oxidation. The major satu-

Table 5
Fatty acid profiles of the studied infant milk-based formulae immediately after opening the packet (% wt/wt)

| Fatty acids | IF1 | IF2 | IF3 | IF4 | IF5 | IF6 | IF7 | IF8 | IF9 | IF10 |
|----------------------|------------------------|--------------|--------------|--------------|---------------|--------------|---------------|--------------|--------------|--------------|
| | Mean ^a ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| C4:0 | Tr | Tr | Tr | 0.05 ± 0.01 | Tr | Tr | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.59 ± 0.01 |
| C6:0 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.12 ± 0.00 | 0.04 ± 0.00 | 0.13 ± 0.00 | 0.10 ± 0.00 | 0.12 ± 0.00 | 0.13 ± 0.03 | 0.03 ± 0.00 | 0.52 ± 0.01 |
| C8:0 | 0.53 ± 0.01 | 0.52 ± 0.01 | 1.32 ± 0.03 | 0.06 ± 0.00 | 1.66 ± 0.03 | 1.27 ± 0.01 | 1.36 ± 0.01 | 1.67 ± 0.00 | 0.04 ± 0.00 | 0.44 ± 0.02 |
| C10:0 | 0.59 ± 0.01 | 0.58 ± 0.01 | 1.15 ± 0.03 | 0.12 ± 0.04 | 1.40 ± 0.01 | 1.08 ± 0.00 | 1.12 ± 0.00 | 1.37 ± 0.00 | 0.10 ± 0.00 | 0.97 ± 0.01 |
| C12:0 | 7.63 ± 0.22 | 7.56 ± 0.07 | 9.13 ± 0.18 | 0.43 ± 0.08 | 11.14 ± 0.13 | 8.99 ± 0.04 | 9.19 ± 0.01 | 11.04 ± 0.03 | 0.28 ± 0.00 | 1.33 ± 0.02 |
| C14:0 | 3.08 ± 0.07 | 3.16 ± 0.19 | 4.06 ± 0.07 | 1.25 ± 0.14 | 4.71 ± 0.05 | 3.87 ± 0.03 | 3.99 ± 0.01 | 4.57 ± 0.01 | 0.94 ± 0.01 | 4.38 ± 0.03 |
| C14:1 | 0.16 ± 0.03 | 0.15 ± 0.03 | 0.14 ± 0.02 | 0.11 ± 0.02 | 0.18 ± 0.01 | 0.10 ± 0.01 | Tr | Tr | Tr | 0.41 ± 0.00 |
| C15:0 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.09 ± 0.02 | 0.04 ± 0.00 | 0.04 ± 0.01 | 0.04 ± 0.00 | 0.07 ± 0.01 | 0.08 ± 0.01 | 0.41 ± 0.00 |
| C16:0 | 26.30 ± 0.01 | 26.31 ± 0.06 | 23.44 ± 0.09 | 36.03 ± 0.19 | 25.20 ± 0.13 | 24.50 ± 0.03 | 21.77 ± 0.01 | 17.62 ± 0.07 | 30.72 ± 0.11 | 31.15 ± 0.04 |
| C16:1 <i>n</i> – 7 | 0.18 ± 0.01 | 0.18 ± 0.00 | 0.25 ± 0.03 | 0.34 ± 0.08 | 0.20 ± 0.05 | 0.20 ± 0.01 | 0.20 ± 0.00 | 0.27 ± 0.03 | 0.26 ± 0.04 | 0.92 ± 0.06 |
| C17:0 | 0.09 ± 0.00 | 0.09 ± 0.00 | 0.11 ± 0.02 | 0.14 ± 0.03 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.09 ± 0.02 | 0.11 ± 0.03 | 0.13 ± 0.05 | 0.29 ± 0.00 |
| C17:1 | 0.06 ± 0.01 | 0.06 ± 0.00 | 0.08 ± 0.00 | 0.09 ± 0.02 | 0.07 ± 0.00 | 0.06 ± 0.01 | 0.05 ± 0.00 | 0.07 ± 0.00 | 0.07 ± 0.03 | 0.13 ± 0.01 |
| C18:0 | 3.91 ± 0.06 | 3.89 ± 0.03 | 3.35 ± 0.04 | 3.55 ± 0.13 | 3.26 ± 0.01 | 2.92 ± 0.03 | 3.98 ± 0.00 | 3.16 ± 0.02 | 3.54 ± 0.15 | 5.97 ± 0.02 |
| C18:1 <i>n</i> – 9 | 40.16 ± 0.27 | 40.17 ± 0.09 | 37.66 ± 0.19 | 43.93 ± 0.86 | 33.24 ± 0.14 | 40.00 ± 0.09 | 38.42 ± 0.03 | 43.44 ± 0.11 | 43.50 ± 0.06 | 37.10 ± 0.06 |
| C18:2 <i>n</i> – 6 | 14.78 ± 0.05 | 14.75 ± 0.05 | 16.98 ± 0.05 | 11.20 ± 0.34 | *16.48 ± 0.04 | 14.07 ± 0.05 | *16.79 ± 0.03 | 12.92 ± 0.01 | 17.76 ± 0.13 | 12.93 ± 0.01 |
| C18:3 <i>n</i> – 6 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.13 ± 0.00 | 0.04 ± 0.01 | 0.05 ± 0.00 | 0.10 ± 0.00 | 0.07 ± 0.00 | 0.15 ± 0.01 | 0.09 ± 0.00 | 0.05 ± 0.00 |
| C18:3 <i>n</i> – 3 | 1.60 ± 0.02 | 1.60 ± 0.01 | 1.24 ± 0.01 | *1.72 ± 0.04 | 1.34 ± 0.01 | 1.71 ± 0.01 | *1.38 ± 0.00 | 1.82 ± 0.01 | 1.32 ± 0.02 | 1.44 ± 0.01 |
| C20:0 | 0.33 ± 0.01 | 0.34 ± 0.02 | 0.29 ± 0.01 | 0.30 ± 0.04 | 0.25 ± 0.01 | 0.30 ± 0.01 | 0.26 ± 0.00 | 0.29 ± 0.01 | 0.34 ± 0.01 | 0.26 ± 0.01 |
| C20:1 <i>n</i> – 9 | 0.27 ± 0.01 | 0.27 ± 0.00 | 0.28 ± 0.00 | 0.28 ± 0.02 | 0.24 ± 0.01 | 0.35 ± 0.01 | 0.15 ± 0.01 | 0.38 ± 0.00 | 0.31 ± 0.00 | 0.29 ± 0.01 |
| C20:2 <i>n</i> – 6 | 0.03 ± 0.00 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.06 ± 0.00 |
| C20:3 <i>n</i> – 6 | Tr | Tr | Tr | Tr | Tr | Tr | 0.03 ± 0.00 | Tr | Tr | Tr |
| C20:4 <i>n</i> – 6 | ND | ND | ND | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.01 ± 0.00 | 0.31 ± 0.00 | 0.08 ± 0.00 | ND | 0.04 ± 0.00 |
| C22:0 | 0.11 ± 0.01 | 0.11 ± 0.00 | 0.13 ± 0.01 | 0.08 ± 0.01 | 0.15 ± 0.01 | 0.10 ± 0.01 | 0.29 ± 0.00 | 0.26 ± 0.00 | 0.19 ± 0.00 | 0.14 ± 0.00 |
| C20:5 <i>n</i> – 3 | ND | ND | ND | ND | ND | ND | 0.05 ± 0.01 | 0.04 ± 0.00 | ND | ND |
| C24:0 | 0.09 ± 0.01 | 0.09 ± 0.00 | 0.08 ± 0.00 | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.08 ± 0.01 | 0.14 ± 0.00 | 0.13 ± 0.00 | 0.11 ± 0.00 | 0.07 ± 0.00 |
| C22:6 <i>n</i> – 3 | ND | ND | ND | ND | ND | ND | 0.16 ± 0.00 | 0.29 ± 0.00 | ND | ND |
| (1) SFA | 42.73 ± 0.24 | 42.75 ± 0.10 | 43.22 ± 0.16 | 42.20 ± 0.30 | 48.11 ± 0.09 | 43.35 ± 0.04 | 42.36 ± 0.01 | 40.46 ± 0.07 | 36.52 ± 0.11 | 46.53 ± 0.02 |
| (2) MUFA | 40.82 ± 0.30 | 40.83 ± 0.07 | 38.40 ± 0.20 | 44.75 ± 0.80 | 33.93 ± 0.10 | 40.71 ± 0.10 | 38.82 ± 0.03 | 44.21 ± 0.08 | 44.24 ± 0.05 | 38.91 ± 0.02 |
| (3) PUFA | 16.45 ± 0.07 | 16.42 ± 0.05 | 18.38 ± 0.05 | 13.05 ± 0.50 | 17.96 ± 0.04 | 15.94 ± 0.06 | 18.82 ± 0.02 | 15.33 ± 0.01 | 19.24 ± 0.15 | 14.57 ± 0.00 |
| <i>n</i> – 3 PUFA | 1.60 ± 0.02 | 1.60 ± 0.01 | 1.24 ± 0.01 | *1.76 ± 0.05 | 1.39 ± 0.01 | 1.74 ± 0.01 | *1.59 ± 0.01 | 2.14 ± 0.01 | 1.36 ± 0.01 | 1.49 ± 0.01 |
| <i>n</i> – 6 PUFA | 14.85 ± 0.05 | 14.82 ± 0.05 | 17.14 ± 0.05 | 11.29 ± 0.36 | *16.57 ± 0.04 | 14.20 ± 0.05 | *17.23 ± 0.03 | 13.18 ± 0.01 | 17.88 ± 0.14 | 13.08 ± 0.01 |
| <i>n</i> – 3 LC-PUFA | Tr | Tr | Tr | Tr | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.21 ± 0.01 | 0.32 ± 0.00 | 0.04 ± 0.01 | 0.04 ± 0.00 |
| <i>n</i> – 6 LC-PUFA | 0.03 ± 0.00 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.06 ± 0.01 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.37 ± 0.00 | 0.12 ± 0.01 | 0.03 ± 0.01 | 0.10 ± 0.00 |

(continued on next page)

Table 5 (continued)

| Fatty acids | IF11 | IF12 | IF13 | IF14 | IF15 | IF16 | IF17 | IF18 | IF19 | IF20 |
|----------------------|------------------------|---------------|--------------|---------------|---------------|---------------|--------------|--------------|---------------|---------------|
| | Mean ^a ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD | Mean ± SD |
| C4:0 | Tr | 0.05 ± 0.01 | Tr | Tr | Tr | 0.05 ± 0.00 | 0.03 ± 0.00 | 0.03 ± 0.00 | Tr | Tr |
| C6:0 | 0.17 ± 0.00 | 0.04 ± 0.00 | 0.12 ± 0.00 | 0.11 ± 0.00 | 0.11 ± 0.00 | 0.13 ± 0.01 | 0.12 ± 0.00 | 0.13 ± 0.00 | 0.10 ± 0.01 | 0.06 ± 0.00 |
| C8:0 | 2.00 ± 0.01 | 0.06 ± 0.00 | 1.35 ± 0.04 | 1.36 ± 0.02 | 1.34 ± 0.02 | 1.37 ± 0.03 | 1.24 ± 0.01 | 1.36 ± 0.02 | 1.40 ± 0.06 | 0.49 ± 0.01 |
| C10:0 | 1.61 ± 0.01 | 0.12 ± 0.04 | 1.18 ± 0.04 | 1.08 ± 0.01 | 1.08 ± 0.00 | 1.13 ± 0.00 | 1.06 ± 0.01 | 1.13 ± 0.00 | 1.20 ± 0.04 | 0.54 ± 0.01 |
| C12:0 | 13.31 ± 0.10 | 0.43 ± 0.08 | 9.33 ± 0.39 | 9.00 ± 0.07 | 9.24 ± 0.03 | 9.00 ± 0.01 | 8.35 ± 0.05 | 9.04 ± 0.03 | 9.76 ± 0.29 | 6.68 ± 0.09 |
| C14:0 | 5.55 ± 0.07 | 1.25 ± 0.14 | 4.12 ± 0.16 | 3.94 ± 0.04 | 4.15 ± 0.00 | 4.07 ± 0.01 | 3.78 ± 0.02 | 4.09 ± 0.04 | 4.12 ± 0.09 | 2.78 ± 0.05 |
| C14:1 | Tr | 0.11 ± 0.02 | 0.14 ± 0.03 | Tr | Tr | 0.04 ± 0.00 | Tr | Tr | 0.15 ± 0.01 | 0.16 ± 0.01 |
| C15:0 | 0.04 ± 0.00 | 0.09 ± 0.02 | 0.05 ± 0.00 | 0.06 ± 0.00 | 0.12 ± 0.07 | 0.12 ± 0.00 | 0.11 ± 0.00 | 0.16 ± 0.04 | 0.03 ± 0.00 | 0.04 ± 0.00 |
| C16:0 | 27.06 ± 0.10 | 36.03 ± 0.19 | 23.29 ± 0.06 | 22.47 ± 0.04 | 22.58 ± 0.04 | 23.71 ± 0.05 | 25.47 ± 0.04 | 21.88 ± 0.05 | 21.74 ± 0.02 | 25.52 ± 0.78 |
| C16:1 <i>n</i> – 7 | 0.31 ± 0.01 | 0.34 ± 0.08 | 0.26 ± 0.03 | 0.36 ± 0.08 | 0.33 ± 0.10 | 0.35 ± 0.02 | 0.38 ± 0.13 | 0.38 ± 0.06 | 0.12 ± 0.02 | 0.32 ± 0.04 |
| C17:0 | 0.14 ± 0.01 | 0.14 ± 0.03 | 0.10 ± 0.01 | 0.19 ± 0.12 | 0.23 ± 0.01 | 0.10 ± 0.00 | 0.14 ± 0.08 | 0.24 ± 0.04 | 0.06 ± 0.01 | 0.09 ± 0.01 |
| C17:1 | 0.08 ± 0.00 | 0.09 ± 0.02 | 0.08 ± 0.00 | 0.13 ± 0.01 | 0.13 ± 0.07 | 0.11 ± 0.04 | 0.11 ± 0.06 | 0.19 ± 0.04 | 0.04 ± 0.00 | 0.03 ± 0.00 |
| C18:0 | 3.88 ± 0.12 | 3.55 ± 0.13 | 3.36 ± 0.05 | 3.83 ± 0.15 | 3.73 ± 0.07 | 3.91 ± 0.02 | 3.60 ± 0.06 | 4.11 ± 0.03 | 3.67 ± 0.07 | 3.82 ± 0.20 |
| C18:1 <i>n</i> – 9 | 26.81 ± 0.03 | 43.93 ± 0.86 | 37.49 ± 0.51 | 36.35 ± 0.14 | 34.85 ± 0.08 | 32.05 ± 0.03 | 37.48 ± 0.16 | 37.94 ± 0.08 | 37.55 ± 0.42 | 40.93 ± 0.43 |
| C18:2 <i>n</i> – 6 | *16.89 ± 0.02 | *11.20 ± 0.04 | 16.98 ± 0.02 | *18.59 ± 0.08 | *18.34 ± 0.15 | *21.14 ± 0.04 | 15.11 ± 0.11 | 16.48 ± 0.10 | *17.48 ± 0.04 | *16.29 ± 0.03 |
| C18:3 <i>n</i> – 6 | 0.08 ± 0.00 | 0.04 ± 0.01 | 0.13 ± 0.00 | 0.07 ± 0.01 | 0.10 ± 0.01 | 0.09 ± 0.00 | 0.12 ± 0.01 | 0.06 ± 0.00 | 0.03 ± 0.00 | 0.08 ± 0.00 |
| C18:3 <i>n</i> – 3 | 1.48 ± 0.02 | *1.72 ± 0.04 | 1.23 ± 0.00 | 1.50 ± 0.02 | 1.90 ± 0.01 | 1.86 ± 0.01 | 1.57 ± 0.01 | 1.40 ± 0.01 | 1.19 ± 0.01 | 1.00 ± 0.02 |
| C20:0 | 0.23 ± 0.01 | 0.30 ± 0.04 | 0.28 ± 0.04 | 0.27 ± 0.01 | 0.30 ± 0.01 | 0.27 ± 0.02 | 0.29 ± 0.00 | 0.25 ± 0.02 | 0.24 ± 0.01 | 0.26 ± 0.01 |
| C20:1 <i>n</i> – 9 | 0.13 ± 0.00 | 0.28 ± 0.02 | 0.27 ± 0.01 | 0.17 ± 0.00 | 0.28 ± 0.00 | 0.16 ± 0.00 | 0.37 ± 0.00 | 0.17 ± 0.00 | 0.13 ± 0.00 | 0.27 ± 0.00 |
| C20:2 <i>n</i> – 6 | Tr | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.05 ± 0.00 | 0.03 ± 0.00 | Tr | Tr | 0.02 ± 0.00 | 0.03 ± 0.00 |
| C20:3 <i>n</i> – 6 | Tr | Tr | Tr | Tr | Tr | Tr | Tr | Tr | 0.03 ± 0.00 | 0.01 ± 0.00 |
| C20:4 <i>n</i> – 6 | ND | 0.01 ± 0.00 | ND | ND | 0.54 ± 0.00 | ND | 0.21 ± 0.00 | 0.32 ± 0.00 | 0.31 ± 0.00 | 0.13 ± 0.00 |
| C22:0 | 0.11 ± 0.00 | 0.08 ± 0.01 | 0.12 ± 0.01 | 0.22 ± 0.00 | 0.18 ± 0.00 | 0.15 ± 0.00 | 0.16 ± 0.00 | 0.29 ± 0.00 | 0.27 ± 0.01 | 0.25 ± 0.00 |
| C20:5 <i>n</i> – 3 | ND | ND | ND | ND | 0.05 ± 0.00 | ND | 0.04 ± 0.00 | 0.07 ± 0.00 | 0.04 ± 0.01 | 0.02 ± 0.00 |
| C24:0 | 0.07 ± 0.00 | 0.06 ± 0.01 | 0.08 ± 0.01 | 0.11 ± 0.00 | 0.12 ± 0.00 | 0.09 ± 0.00 | 0.09 ± 0.00 | 0.12 ± 0.04 | 0.12 ± 0.01 | 0.11 ± 0.00 |
| C22:6 <i>n</i> – 3 | ND | ND | ND | ND | 0.27 ± 0.00 | ND | 0.19 ± 0.00 | 0.16 ± 0.01 | 0.17 ± 0.00 | 0.07 ± 0.00 |
| (1) SFA | 54.17 ± 0.02 | 42.20 ± 0.30 | 43.39 ± 0.48 | 42.64 ± 0.18 | 43.18 ± 0.09 | 44.11 ± 0.03 | 44.43 ± 0.11 | 42.83 ± 0.08 | 42.72 ± 0.39 | 40.65 ± 0.67 |
| (2) MUFA | 27.33 ± 0.02 | 44.75 ± 0.80 | 38.23 ± 0.48 | 37.12 ± 0.07 | 35.58 ± 0.10 | 32.70 ± 0.01 | 38.33 ± 0.03 | 38.69 ± 0.02 | 38.00 ± 0.43 | 41.70 ± 0.42 |
| (3) PUFA | 18.50 ± 0.01 | 13.05 ± 0.50 | 18.38 ± 0.02 | 20.24 ± 0.12 | 21.24 ± 0.16 | 23.19 ± 0.04 | 17.24 ± 0.12 | 18.48 ± 0.10 | 19.28 ± 0.06 | 17.65 ± 0.25 |
| <i>n</i> – 3 PUFA | 1.53 ± 0.02 | *1.76 ± 0.05 | 1.23 ± 0.00 | 1.55 ± 0.02 | 2.22 ± 0.00 | 1.92 ± 0.00 | 1.80 ± 0.01 | 1.63 ± 0.01 | 1.40 ± 0.02 | 1.09 ± 0.02 |
| <i>n</i> – 6 PUFA | *16.97 ± 0.02 | *11.29 ± 0.06 | 17.15 ± 0.02 | *18.69 ± 0.10 | *19.02 ± 0.16 | *21.27 ± 0.04 | 15.44 ± 0.11 | 16.85 ± 0.10 | *17.88 ± 0.04 | *16.56 ± 0.04 |
| <i>n</i> – 3 LC-PUFA | 0.05 ± 0.00 | 0.04 ± 0.01 | Tr | 0.05 ± 0.01 | 0.31 ± 0.00 | 0.06 ± 0.00 | 0.23 ± 0.00 | 0.23 ± 0.01 | 0.21 ± 0.01 | 0.09 ± 0.00 |
| <i>n</i> – 6 LC-PUFA | Tr | 0.06 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.58 ± 0.00 | 0.03 ± 0.00 | 0.21 ± 0.00 | 0.32 ± 0.00 | 0.36 ± 0.00 | 0.18 ± 0.00 |

(1) Saturated fatty acids, (2) monounsaturated fatty acids, (3) polyunsaturated fatty acids; ND = not detected. Tr = Trac < 0.01%.

^a Values are the mean of three measurements.

* Significant difference ($p < 0.05$) between immediately after opening the packet (T0) vs 70 days after opening the packet.

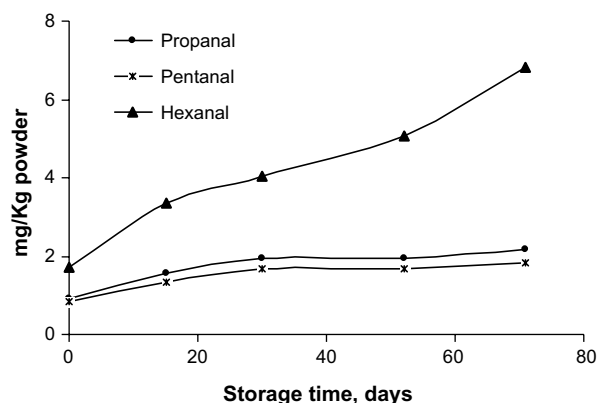


Fig. 3. Means of headspace volatile aldehydes produced during IF storage at room temperature after opening packets and determined by HS GC-FID.

rated fatty acid (SFA) in our IFs was C16:0, in the range 17.62–36.15%; the major monounsaturated fatty acid (MUFA) was C18:1 $n-9$ in the range 26.81–44.11%, and the major LC-PUFA was C18:2 $n-6$ in the range 10.96–21.14%. The powders that simultaneously showed the highest concentrations of propanal, pentanal and hexanal were IFs 4, 7, 10, 14, 16 and 18 (Tables 2–4). On the basis of this observation, we expected that all these IFs were supplemented with LC-PUFA. However, this was not the case for IFs 4, 10, 14 and 16. Hexanal and pentanal arose as a result of the oxidation of $n-6$ PUFA. The samples with major $n-6$ PUFA content were IF 3 (17.14%), hexanal 0.527 mg/kg; IF 7 (17.23%), hexanal 10.7 mg/kg; IF 9 (17.14%), hexanal 6.33 mg/kg; IF 13 (17.15%), hexanal 0.68 mg/kg; IF 14 (18.69%), hexanal 12.8 mg/kg; IF 15 (19.02%), hexanal 3.31 mg/kg; IF 16 (21.27%), hexanal 16.4 mg/kg; and IF 19 (17.88%), hexanal 10.1 mg/kg.

Table 6

Pearson's correlation coefficients between losses of selected fatty acids and volatile contents after storage

| Fatty acids ^a | Hexanal | Pentanal | Propanal |
|--------------------------|---------|----------|----------|
| C17:1 | 0.001 | -0.006 | -0.031 |
| C18:0 | 0.069 | 0.135 | 0.153 |
| C18:1 $n-9$ | 0.022 | -0.055 | -0.130 |
| C18:2 $n-6$ | *0.499 | 0.185 | 0.305 |
| C18:3 $n-6$ | 0.021 | 0.149 | 0.253 |
| C18:3 $n-3$ | 0.127 | 0.235 | *0.544 |
| C20:0 | 0.039 | 0.265 | 0.115 |
| C20:1 $n-9$ | 0.018 | 0.247 | 0.116 |
| C20:4 $n-6$ | 0.042 | 0.105 | 0.024 |
| C20:5 $n-3$ | -0.129 | -0.012 | -0.025 |
| C22:6 $n-3$ | -0.070 | 0.030 | -0.009 |
| SFA | 0.140 | 0.286 | 0.207 |
| MUFA | -0.257 | -0.395 | -0.349 |
| PUFA | 0.221 | 0.201 | 0.382 |
| $n-3$ PUFA | 0.145 | 0.267 | *0.543 |
| $n-6$ PUFA | 0.406 | 0.178 | 0.310 |
| $n-3$ LC-PUFA | -0.129 | -0.003 | -0.040 |
| $n-6$ LC-PUFA | -0.022 | -0.076 | -0.100 |

^a Difference resulting from initial fatty acid content minus final fatty acid content.

* Significant Pearson's correlation at $p < 0.05$.

Therefore, no clear relation between $n-6$ PUFA content and hexanal increase was observed. In the same way, propanal came from $n-3$ PUFA. The IFs with major $n-3$ PUFA content were IF 4 (1.76%), propanal 11.0 mg/kg; IF 6 (1.74%), propanal 2.01 mg/kg; IF 8 (2.14%), propanal 1.47 mg/kg; IF 12 (1.76%), propanal 0.33 mg/kg; IF 15 (2.22), propanal 1.12 mg/kg; IF 16 (1.92%), propanal 2.94 mg/kg; and IF 17 (1.80%), propanal 1.42 mg/kg. In the same manner, no clear relation between $n-3$ PUFA and propanal increase was observed. The content of volatile compounds after 70 days of storage and MUFA, SFA, $n-3$ PUFA and $n-6$ PUFA contents were used as variables for the PCA. The two extracted components explained 69.19% of the variance (cumulative percentage). IFs 4, 11 and 16 were observed to be separated from the rest (Fig. 2d). IF 4, due to the high content of propanal (as stated above), pentanal and hexanal, while formulae 11 and 16 exhibit the highest content of hexanal and $n-6$ PUFA. These last two formulae showed a significant reduction in $n-6$ PUFA content, as a result of the reduction in LA content during storage (Table 5).

In general, marked increases in volatile evolution, with no obvious change in fatty acid profiles, were observed. However, we noted a significant reduction ($p < 0.05$) of C18:2, $n-6$ (the major $n-6$ PUFA in IFs) in IFs 5, 7, 11, 12, 14, 16, 19 and 20. A positive relationship between propanal content, and the difference from initial and final value of $n-3$ PUFA was observed (Table 6), indicating that the increase in propanal is directly related to the oxidation of $n-3$ PUFA, specifically from C18:3 $n-3$. Also a significant positive correlation between the difference from the initial to final C18:2 $n-6$ and hexanal content was observed. This finding indicates that the hexanal content derives mostly from the degradation of C18:2 $n-6$.

4. Conclusions

Significant losses were found in C18:2 $n-6$ and C18:3 $n-3$ of some formulae; however, no significant decreases were observed in the rest of the fatty acids, including C20:4 $n-6$ and C22:6 $n-3$. Contrary to expectations, the observed stability of the fatty acid profiles in the studied formulae 70 days after opening the packets, (even in the samples where there were found significant losses), is noteworthy. To gain better information on volatile formation and evolution in IFs, the same basal IF composition should be used, varying only in the fatty acid content. Hexanal could be used as a rapid indicator of formula oxidation. This aldehyde is proposed as a potential marker to evaluate freshness and overall oxidation quality in IFs.

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