Lipid Composition and State of Oxidation of Fortified Infant Flours in Low-Income Countries Are Not Optimal and Strongly Affected by the Time of Storage

Célia Moustiès, Claire Bourlieu-Lacanal, Bruno Barea, Adrien Servent, Pascaline Alter, Marc Lebrun, Youna Morgane Hémery, Arnaud Laillou, Sylvie Avallone

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*Célia Moustiés[1], Claire Bourlieu[2], Bruno Barea[2], Adrien Servent[1], Pascaline Alter[1], Marc Lebrun[1], Youna Hemery[3], Arnaud Laillou[4], Sylvie Avallone[1,]**

Running title: Lipid composition of fortified infant flours

[11]QualiSud, Univ Montpellier, CIRAD, Montpellier SupAgro, Université d'Avignon, Université de la Réunion, Montpellier, France.

[2] IATE, INRA, CIRAD, Univ Montpellier, Montpellier SupAgro, Montpellier, France.

[3] Nutripass, Univ Montpellier, IRD, Montpellier SupAgro, Montpellier, France.

[4] UNICEF, Maternal Child Health and Nutrition section, Phnom Penh, Cambodia.

* Sylvie.avallone@supagro.fr

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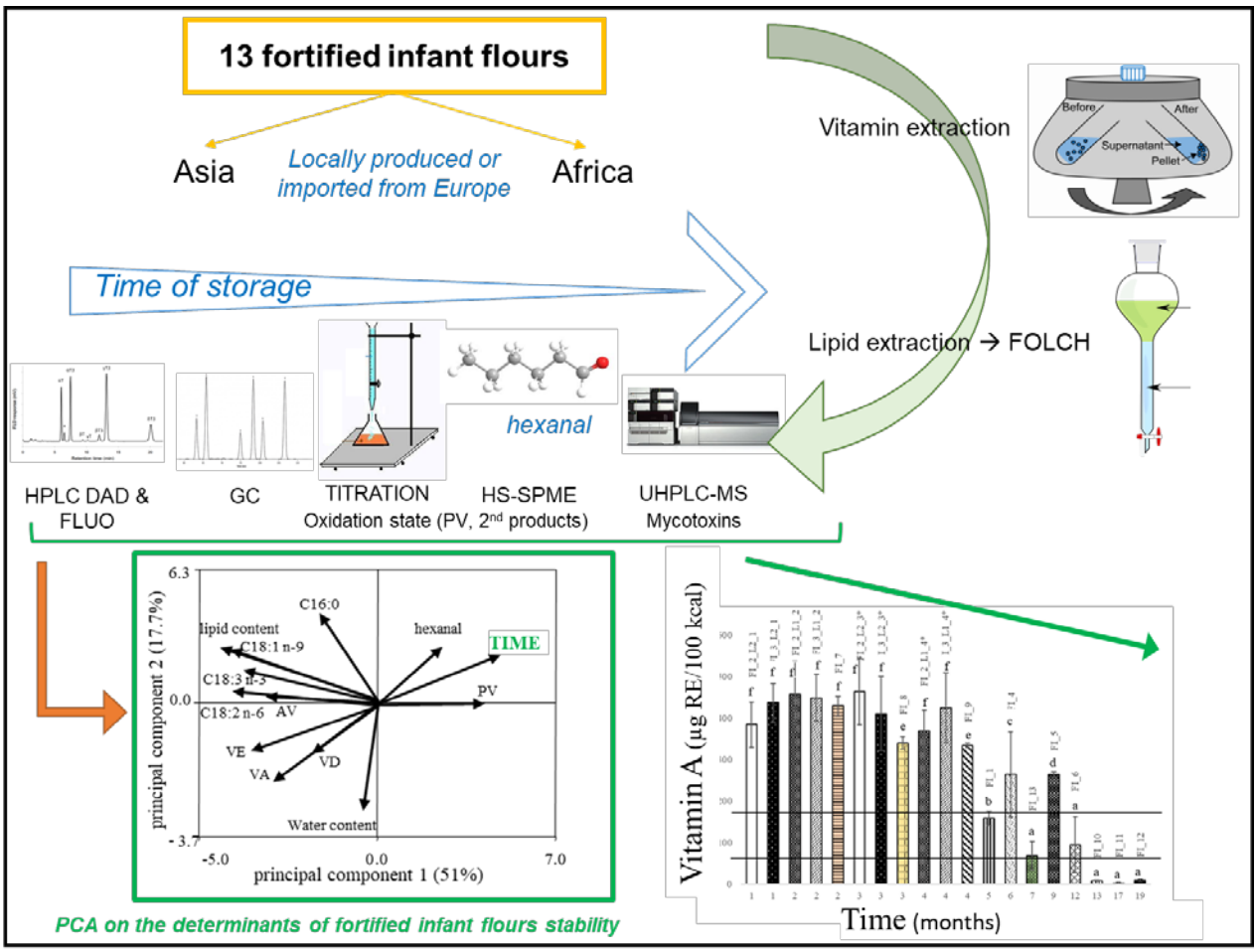
Graphical abstract



Legend of Graphical abstract

Main Conclusions: i) Fatty acid profiles $C_{18:2} > C_{18:1} > C_{16:0,}$ ii) *ω6/ω3* ratio could be optimized, iii) Overages in vitamins A, D and E , iv) Expiration date should be shortened to 12 months.
Abbreviations: GC: gas chromatography, DAD: diode array detector, FLUO: fluorometric detector, HPLC: high performance liquid chromatography, PCA: principal component analysis, PV: peroxide value, UHPLC-MS: ultra-high performance liquid chromatography – mass spectrometer.

Abbreviations used:

ALA: alpha-linolenic acid; Alu / PE: aluminium / polyethylene; a_w: water activity; C / Epox: cellophane / epoxy resin; CMV: mineral and vitamin complex; DAD: diode array detector; DHA: docosahexaenoic acid; EU: European Union; GC: gas chromatography; HPLC: high performance liquid chromatography; FA: fatty acid; FAME: fatty acid methyl ester; FFA: free fatty acid; FI: fortified infant flour; FM: fresh material; LA: linoleic acid; MUFA: monounsaturated fatty acid; n: number of repetitions; PE: polyethylene; PE / PP: polyethylene / polypropylene; PTFE: polytetrafluoroethylene; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; MCFA: medium chain fatty acid; MSMS: tandem mass spectrometry; PCA; Principal Component Analysis; OTR: oxygen transmission rate; RE: retinol equivalent; ROS: reactive oxygen species; UV: ultra violet; WVTR: water vapor transmission rate.

ABSTRACT

Food fortification is widely used to address the public health problem of nutrient deficiencies. Our purpose was to assess the lipid profile and nutritional quality of 13 fortified infant flours (FI) collected “in the field” in Africa and Asia after different periods of storage. The lipid content, fatty acid profiles, lipophilic vitamin content, lipid oxidation state (peroxide values, secondary oxidation products) were determined. To complete this panorama, mycotoxins in FI and packaging materials were characterized. The lipid content averaged 9.1 ± 3.5 g/100 g. Fatty acid profiles were dominated by linoleic (43.3 ± 8.8%), oleic (29.5 ± 7.4%) and palmitic acid (17.8 ± 6.7%) and resulted in average ω6/ω3 ratio of 12.2 ± 5.9 but with high values for some FI (maximal at 31.7). Very high overages in vitamins A, D and E were observed in FI stored for short periods (1 − 6 months) whereas FI stored for more than 12 months had insufficient vitamin content. Lipid oxidation was acceptable but for six FI presenting excessive peroxide values. Most products were contaminated by low amounts of mycotoxins but only two FI did not abide by the regulation. A strong correlation between peroxide values, hexanal content and time of storage was observed.

Practical applications: The expiration dates for fortified infant flours commercialized in low income countries should be shortened from 36 to 12 months so to guarantee the nutritional quality of these functional foods and abide by the fortified infant flour legislation. Indeed, FI quality significantly decreased over time of storage. The use of very barrier packaging materials must be generalized, although it is a necessary criterion but not sufficient to ensure the long term stability of FI. Special attention should be given to reduce lipophilic vitamin overages and improve their lipid profile, especially ω6/ω3 ratio, which has to be lower than 15.

1. INTRODUCTION

Nutrient deficiencies still affect two billion people worldwide, due to health issues, inappropriate diet, poverty, poor hygiene and sanitation [1]. In 2016, 155 million preschool-age children were still affected by stunting [1]. In Africa and South-East Asia, the diet of low-income populations is mainly based on starchy products and do not include enough nutrient-rich foods (*i.e.* fruits, vegetables, beans or animal products). These diets are lacking in essential micronutrients and in certain macronutrients, such as essential lipids, and children are particularly concerned by this nutritional issue. Lipids play different important roles, in particular energetic, structural and transport [2]. Moreover, adequate intakes in essential lipids are very important for visual acuity, brain development and lipid metabolism [2–5].

During the 1 000-days between a woman's pregnancy and her child's 2nd birthday, adequate micronutrient intakes are essential for child's cognitive and physical development. Indeed, during fetus and infant development, inadequate food intake strongly impacts the nutritional status, resulting in stunting with cognitive consequences and increased mortality [6]. As micronutrient deficiencies are public health issues, governments and international organizations have, for decades, implemented three main strategies to solve the problems: diet diversification, supplementation and food fortification [7]. Diet diversification has the advantages to stimulate the local economy by generating incomes for small farmers and to promote the consumption of diversified food with complementary nutritional profiles. But, in some contexts, the cost of a diversified diet seems too high and not reachable for people with low incomes [8]. Supplementation has the advantage of being very specific in the intervention and in terms of dose-adjustment, but expensive and with risk of toxicity, due to low compliance and self-prescription by users [9]. The use of food fortification is an alternative strategy which is considered as the most cost effective to eliminate micronutrient malnutrition [7]. The biological efficacy of fortified food products has been demonstrated in clinical trials. Under controlled environments, the conditions are perfect and the results are encouraging, but they do not reflect

the “real food system” in terms of conditions of storage in the supply chain of southern countries and practices at home.

Fortified infant flours (FI), mostly cereal-based, have been designed to fulfill the needs of infants and preschool children. FI are used in complement to breastfeeding from the age of six months, under the form of gruel or porridge, consumed on a daily basis. Most FI have adjusted lipid profile, and can be enriched in polyunsaturated fatty acids (PUFA). Enrichment in essential fatty acids (FA) − *i.e.* linoleic $C_{18:2}$ (ω6 family precursor) and linolenic $C_{18:3}$ (ω3 family precursor) FA − and frequently in long chain PUFA that are important for brain and vision development, such as docosahexaenoic acid (DHA, $C_{22:6}$, ω3 family) [4,10]. Indeed, lipids play several important functions for young children, as they supply most of the calories necessary for growth, contribute to the development of cerebral functions, and to lipophilic vitamin transport [2]. More specifically, DHA contributes to visual acuity and brain membrane accumulation during the child’s first two years of life. Unbalanced ω6/ω3 lipid input during infant growth do not support as satisfactorily infant development than balanced ones [4].

A previous study conducted in low-income countries, has reported that an adequate consumption of well-formulated fortified cereal-based complementary foods, has a positive impact on the nutritional status of young children [11]. The nutritional quality of many complementary foods sold in African and south east Asian countries have been assessed [12–14], but these studies focused either on the macronutrients, minerals and trace elements, or on the anti-nutrients, and none focused on lipid profile nor reported lipophilic vitamin contents. Moreover, unsaturated FA and lipophilic vitamins present poor chemical stability during food production, transportation and storage [15]. Vitamin A can be rapidly degraded, especially in food products stored in non-optimal conditions. High losses in Vitamin A have been reported in products stored at moderate temperatures (~40 °C) and when the packaging doesn’t display good oxygen and water vapor barrier properties [16,17]. Very recently, more than half of vitamin A fortified flours, used in 20 national fortification programs, did not meet the fortification

standards [18]. Performance data on fortification interventions are often missing and regular assessment of fortification programs in "field conditions" are needed [19,20].

The aim of the present work was to characterize 13 FI, sold and consumed on a daily basis, in four countries in Africa and Asia. FI were collected "in the field" after different storage periods. Lipid composition (*i.e.* total and essential fatty acids, lipophilic vitamins), lipid oxidation (peroxide and acid values, volatile compounds), mycotoxins contents and barrier properties of packaging materials were evaluated.

2. MATERIALS AND METHODS

2.1 Collection and Storage of Fortified Infant Flours

Thirteen FI consumed on a daily basis were collected in pharmacies, groceries and markets in four countries in Africa and South east Asia (Table 1). The products were sampled after being stored in "field conditions" in the real supply chain from 1 to 19 months. Storage time is thus the period of time elapsing from the manufacture to the collection (purchase). Products were then shipped to our laboratory (Montpellier, France). The products were produced either locally or imported.

In order to obtain a representative sample, several unit-doses were mixed (achieved average mass ~ 105 - 150 g). At opening, products were immediately analyzed for vitamin profiles, dry matter and water activity. The rest of the samples was vacuum-packed in distinct bags and frozen at -80 °C for further analyses (mycotoxins, total and neutral lipids extraction and characterization, volatile compounds).

Each product was labelled with a code FI_X, where X corresponds to a specific brand. The characteristics of the products in terms of formulation, packaging and duration of storage since production date are reported in Table 1.

For two FI brands (FI_2 and FI_3), two distinct batches were characterized. These four products were tested rapidly at reception (*i.e.* after 1 or 2 month "in field" storage) or stored for two more months in the laboratory at ambient temperature (~20 °C) and again characterized. For these two

brands, the products were labelled as follows FI_X_LY_Z to indicate that the FI was produced by a brand (X) with a number of batch (LY) and a specific duration of storage in month (Z). Products that were stored for two more months under controlled conditions in the laboratory are noted with an asterisk (*): FI_2_L2_3*, FI_2_L1_4*, FI_3_L2_3*, FI_3_L1_4*.

In total thirteen brands of FI were purchased in local groceries and markets in Vietnam, Burkina Faso, Madagascar and Benin. Their production place, time of storage, packaging and composition are detailed in **Table 1**. The collected products can be considered as representative of the ratio (imported/local) of local products in the supply chain. This ratio, imported/local, was of 3/13. The three imported products had all been produced in the European Union.

FI were formulated with five to 13 ingredients and limited amounts of additives (except in FI_6). FI were based on cereals, either local ones such as millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*) in African FI, but also wheat (*Triticum*) in three products. In most FI, lipids were mainly brought by peanuts (*Arachis hypogaea*), some oleo-proteaginous such as soy (*Glycine max*) (present in 8 FI) or sesame (*Sesamum indicum*) and milk. 38% of FI included added "fats" with no other precision. 85% of the formulas included sugar and 23% added salt. Lipophilic vitamins were brought under the form of mineral and vitamin complex (CMV).

2.2 Materials and Chemicals

Solvents (ethanol, diethylic oxide, hexane, chloroform, methanol, acetone, tertbutylethylether), reagents (potassium hydroxide, phenolphatlein, methylate sodium, hydrochloric methanol, potassium iodide, thiodene, sodium thiosulfate) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). FAMEs external calibration standards (Mixture ME 100) were obtained from Larodan (Sweden). HPLC standards (α-tocopherol, δ-tocopherol, γ-tocopherol, β-tocopherol, retinol palmitate, retinol acetate, vitamin D_2 and D_3) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France) and Biopharm Rhône Ltd (Glasgow, UK). The polytetrafluoroethylene (PTFE) membranes were obtained from Sartorius (Palaiseau, France). The water used in all the experiments was mQ water (HPLC grade).

2.3 Dry Matter and Water Activity Determination

The dry matter (DM) contents were determined in triplicate by drying 3 g of the samples at 105 °C in Memmert heat chamber (modell 100 Schwabach, Germany) until constant weight following method 44-15A [21]. Samples and their dehydrated residues were precisely weighted on a Mettler AE166 balance (Viroflay, France). Water activity (a_w) was determined at 25 °C on approximately 1 g with a water activity meter 4TE AQUALAB (Hopkins, USA).

2.4 Determination of Vitamin A and D

The vitamin profiles were determined by liquid chromatography and specific detection by DAD (vitamin A and D) as previously described [22]. The UV detection allowed us to quantify two forms of vitamin A (acetate and palmitate) and two forms of vitamin D (cholecalciferol and ergocalciferol).

One gram of flour was mixed with 20 mL water and gently shaken for 10 min [23]. Samples were homogenized in 16 mL of ethanol/hexane (4:3, v/v) for 30 seconds at 30 000 rpm (Ultra-Turrax, Prolabo, France) and then centrifuged (30 min, 4 °C, 13 000 g) (Heraeus Multifuge X1R, Thermo FIher Scientific, Villebon sur Yvette, France). Afterward, the upper layer was transferred into amber glass tubes and dried with a Genevac during 25 min at 30 °C (EZ-2 series, Genevac LTD, Sp Scientific, England). The dried residues were dissolved in 2 mL of acetone, filtered at 0.45 µm PTFE minisart SRP4 membrane (Sartorius) and analyzed by liquid chromatography (Agilent System 1200 series, Massy, France). The column was a polymeric C_{30} (4.6 mm i.d. × 250 mm, 5 µm particle size, YMC, Inc Wilmington NC) and the injection volume was 20 µL. The mobile phase was based on the blend of two mixes at a flow rate of 1 mL/min and the temperature was 25 °C. Mix A was constituted of methanol and water, 60:40 (v/v), and mix B was constituted of methanol, methyl tert-butyl-ether and water, 28.5:67.5:4.0 (v/v/v). The gradient applied progressed from 100% to 0% (A/B) over a period of 43 min and the column was washed 5 min with ethyl acetate at the end of each run. Chromatograms were

recorded with a UV-visible photodiode array detector (Agilent Technologies 1200 series) at the wavelength of maximum absorption of the vitamin A and D in the mobile phase (*i.e.* 325 and 265 nm respectively). External calibration was realized weekly with standard solutions of the pure chemical in acetone on the range of 0.5 to 25 mg/L. Each sample was analyzed with five repetitions. Vitamin A activity was expressed in retinol equivalents (RE) [24]:

1 µg retinol equivalent = 1.146 µg retinol acetate = 1.832 µg retinol palmitate.

2.5 Determination of Vitamin E

Vitamin E was determined on the same extract that had been obtained for vitamin A and D quantification. Fluorescence was used to detect and quantify four distinct tocopherols (α, β, δ and γ). HPLC analysis was performed with an Agilent System 1290 series (Massy, France) equipped with a C_{18} column (250 mm × 4.6 mm i.d., 5 µm, HALO®-5 column, AMT, Wilmington, Delaware, USA) and a fluorescence detector. The mobile phase consisted of ethanol/methanol (40:60, v/v) in isocratic conditions. The temperature of the column was maintained at 25 °C and flow rate was 0.8 mL/min. Fluorescence detection was set at 296 nm for excitation and 330 nm for emission. The injection volume was 20 µL and the calibration curves were realized with standard solutions from 0.1 to 1.5 mg/L. Each sample were analyzed with five repetitions.

Vitamin E activity is expressed in α - tocopherol equivalent (α-TE) in mg for 100 g [25] and calculated as follow:

$$\alpha - TE = (1 \times \alpha - \mathrm{T}) + (0.7 \times \alpha - \mathrm{TA}) + (0.4 \times \beta - \mathrm{T}) + (0.2 \times \gamma - \mathrm{T}) + (0.1 \times \delta - \mathrm{T}) \quad (1)$$

With α -TE: tocopherol equivalent, T: tocopherol, TA: tocopherol acetate, α: α-tocopherol, β : β-tocopherol, γ: γ-tocopherol, δ: δ-tocopherol.

2.6 Determination of Lipid Content and Fatty Acid composition

The lipid content was determined gravimetrically in triplicate [26] and each extract was then analyzed in duplicate. Briefly, lipids were extracted from thoroughly homogenized food

samples using chloroform/methanol (2:1, v/v). 15 g of sample was rehydrated during 10 min in distilled water and, thereafter, dispersed in a sample/solvent ratio of 1:37 (w/v) for 1 min at 10 000 rpm (Ultra-Turrax T8, IKA Labortechnick, Staufen, Germany). NaCl 0.73% (w/v) was added to the organic phase with thorough shaking. After dephasing, the lower phase was filtered and retrieved. Then, the sample was washed again with NaCl 0.58% (w/v). Once clear, the organic layer was filtered and collected. Solvent was evaporated with a rotavapor at 40 °C until constant weight and residual lipids were precisely weighted (Laborota 400-efficient, Heidolph, Schawabach, Germany).

Fatty acid methyl esters (FAMEs) were obtained by methylation of lipids (~ 200 mg) with 3 mL of sodium methylate for 10 min at 65 °C in heated mantle with saponification sticks under reflux. Then, 3 mL of chloride methanol were added and samples were heated again for 10 min at 64.7 °C. FAMEs were extracted with 8 mL of hexane, washed with 10 mL of distilled water. 5 µl of samples were analyzed by gas chromatography (Focus GC, Thermo electron corporation, Massachusetts, USA) equipped with a split injector (ratio of 1/20), a CP-Cil 88 Varian capillary column (50 m × 0.25 mm with a 0.2 µm film thickness; Chrompack, Middelburg, The Netherlands) and helium 1 mL/min as the carrier gas. FAMEs were analyzed by flame ionization detector and ChromCard software data system (version 2005; Thermo FIher Scientific, Massachusetts, USA).

The column temperature started from 150 °C, then reached 150 °C to 225 °C with a rise of 5 °C/min and was kept at 225 °C during 10 minutes. These chromatographic conditions allowed a correct and rapid separation of all FAMEs from $C_{10:0}$ to $C_{24:1}$. FAMEs were identified using as external standards a mixture of methyl esters (Mixture ME 100, Larodan, Sweden). The injector and detector temperatures were 250 °C and 270 °C respectively. FA contents were expressed in g per 100 g of fresh product FA contents were expressed in g per 100 g of fresh product, taking into account the total lipid content determined by Folch extraction.

2.7 Peroxide Value Determination

The peroxide values (PV) were determined by iodometric titration [27] with slight modifications. For each FI, average values were calculated from six determinations (three extractions analyzed in duplicate). Approximately 200 mg of oil was dissolved in 1 mL of chloroform. After addition of 1.5 mL of acetic acid and 0.1 mL of saturated potassium iodide, the solution was shaken for 1 min and placed 5 min in the dark. After addition of 7.5 mL of distilled water and iodine indicator, the solution was titrated with $Na_2S_2O_3$ 0.002N until the color disappeared. A blank determination was made, and the PV was calculated using the following equation:

$$PV = (Vs - Vb) \times N \times \frac{100}{W} \qquad (2)$$

With PV: mEqO_2/kg of lipid, Vs: volume of $Na_2S_2O_3$ used in sample (mL), Vb: volume of $Na_2S_2O_3$ used in the blank sample (mL), N: normality of $Na_2S_2O_3$ (mEq/ mL), and W: weight of lipids (g).

2.8 Acid Value Determination

The acid values (AV) were determined by titrimetry [28] and average values were obtained from six determinations (three extractions analyzed in duplicate). A precise quantity of lipid (~200 mg) were dissolved in 0.4 mL ethanol/diethyl oxide (50:50, v/v). After addition of phenolphthalein, the solution was titrated with KOH 0.01N until the pink color persisted for at least 10 seconds. A blank determination was made, and the AV was expressed in oleic acid equivalent and calculated using the following equation:

$$AV = (Vs - Vb) \times N \times \frac{282.46}{10 \times W} \qquad (3)$$

With AV: % lipid (acid oleic eq%), Vs: volume of KOH used in sample (mL), Vb: volume of KOH used in the blank sample (mL), N: normality of KOH, 282.46: molecular weight of oleic acid (g/mol), and W: weight of lipids (g).

2.9 Volatile Compound Determination

For each sample, the volatile compounds were extracted in triplicate using headspace solid-phase microextraction gaz chromatography [29]. Extraction and analysis were carried out using

divinylbenzene/carboxen/polydimethylsiloxane fiber (Supelco, Bellefonte, Pennsylvania, USA), and a tandem gas chromatograph 6890 / MSD 5973 (Agilent Technologies, Palo Alto, USA) / Gerstel Multipurpose Sample MPS-2. Sample (1 g) was placed in headspace vials (10 mL) with addition of benzyl butyrate (5 µL/100 mL) as internal standard for semi quantification. Samples were left to equilibrate for 15 min and trapped 60 min at 60 °C. DB-Wax UI (J&W Scientific Folsom, CA, USA) fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film) was used with a 1.2 mL/min constant flow of hydrogen. The temperature program for elution was: 3 °C/min from 40 °C to 170 °C, then 10 °C/min up to 240 °C and held for 10 min. The injection was performed in splitless mode injector at 250 °C. Mass spectrum were recorded in EI+ mode at 70eV within 40 to 350 Da. Analyzer and source temperatures were 150 °C and 250 °C respectively.

Data were analyzed with MassHunter version B. 06.00 (Agilent Technologies, Palo Alto, USA). Peak identification was realized comparing mass spectra with those of the NIST 14 database. Co-injection of alkanes from C_8 to C_{20} (Sigma-Aldrich) was used for Kovats retention indice calculations and their comparison with the literature (flavornet and pherobase websites and NIST). The contents of the volatile compounds were expressed in mg equivalent of internal standard (*i.e.* benzyl butyrate) as previously described [30].

2.10 Determination of Mycotoxins

Five grams of sample was homogenized with 20 mL of acetonitrile/water/ acetic acid (50:50:1, v/v/v). After 10 min of gentle stirring, salts were added as follow: $MgSO_4$ (4 g), NaCl (1 g), SCTD (1 g), SCDS (0.5 g). Samples were again stirred for 10 min and centrifuged (3000 rpm, 10 min). A mix of internal standard was added to 200 µl of supernatant and the overall extract was evaporated under nitrogen at 40 °C. Residue was diluted with 1 mL water/acetic acid 0.5%. The extract was filtered with 0.45 µm PTFE minisart SRP4 membrane (Sartorius). The extracts were analyzed by ultra-high performance liquid chromatography [31] and injection volume was 20 µL. The pump model was a LC 20 AD Shimadzu (Kyoto, Japan) with an autosampler model:

SIL 20 A XR Shimadzu and a column Kinetex 2.6 µm XB-C18 100A (50 mm × 2.1 mm, Phenomenex Inc., California, USA). The oven temperature was 50 °C. The mobile phase A was water/acetic acid 0.5%, and mix B was isopropanol/acetic acid 0.5% at a flow rate of 0.4 mL/min. The elution gradient started from 10% of eluent B, to 55% in 1.5 min, then 85% at 2 min and decreased to 80% in 0.5 min and a stabilization step of 2% during seven min. The tandem mass spectrometry (MSMS) conditions with system LCMS 8040 Shimadzu (Kyoto, Japan) were ionization ESI (positive and negative), MRM mode, ion source temperature (for desolvation line) was set at 250 °C and 400 °C for heater block, gases nebulization at 3 L/min and gases drying at 15 L/min.

Standard calibration curves were established with several mycotoxins (aflatoxins, ochratoxins, fumonisins, trichotecenes, zearalenon, beauvericins) (Biopharm Rhône Ltd, Glasgow, UK). The contents were expressed in µg/100 g of FI and analyses were performed in triplicate.

2.11 Packaging Characterization

Packaging was characterized by Fourier Transform Infrared Spectroscopy (Table 1). Spectra were recorded using a Nexus 5700 spectrometer (Thermo Electron Corp., Massachusetts, USA) equipped with the diamond ATR Smart Orbit Accessory inserted. Spectral data were accumulated from 128 scans with a resolution of 4 cm^{-1} in the range 800–4000 cm^{-1}. All spectra pretreatments were performed using Omnic v7.3 and TQ Analyst v7.3 softwares (Thermo Electron Corp., Massachusetts, USA). Oxygen and water permeabilities were obtained from the literature for standard polymers [32]. Oxygen transmission rate (OTR) represents the quantity of O_2 which goes through the packaging per day and water vapor transmission rate (WVTR) represents the quantity of H_2O which diffuses through the packaging per day. OTR and WVTR were calculated according to the packaging surface, the permeability coefficient of the material and the time of storage of the product [32].

2.12 Statistical Analysis

If not stated otherwise, all characterizations were conducted at least in triplicate. Analysis of variance (one-way ANOVA), followed by LSD post hoc tests were performed using Statgraphics plus 5.1 (Virginia, USA) to identify significant differences between samples. Differences were considered statistically significant for p value <0.05. Still using the same software, correlations between the biochemical composition, time of storages, oxidation markers, oxygen and water transfer rates were evaluated using Principal Component Analysis (PCA).

3. RESULTS

3.1 Dry Matter and Water Activity.

The water contents of the FI ranged from 1.78 ± 0.02% to 6.45 ± 0.30% and a_w from 0.06 ± 0.01 to 0.41 ± 0.01 (**Table 2**).

3.2 Lipid Content and Fatty Acid Profiles

A high heterogeneity was observed among FI in terms of lipid contents which averaged 9.1 ± 3.5 g/100 g but ranged from 1.5 to 15.1 g/ 100 g (**Table 2**). The FI produced in Europe had a lower lipid content in comparison to the locally produced FI (Average 2.9 ± 1.8 versus 10.3 ± 2.6 g/100 g respectively). European Union directive [33] requires a maximal lipid content of 3.3 g per 100 Kcal. For the imported products, the highest lipid content was 5.0 ± 1.4 g/100 g corresponding to 1.3 ± 0.4 g/100 Kcal (caloric equivalent: 1 g of lipid = 9 Kcal). Despite this heterogeneity, all the FI were in agreement with the European legislation for the maximal lipid contents.

The FA profiles in the FI were characterized by GC (**Figure 1** and **Table 2**). The main FA were, by decreasing order, linoleic acid $C_{18:2}$ *n*-6, oleic acid $C_{18:1}$ *n*-9 and palmitic acid $C_{16:0}$. Their average percentages were respectively 43.3 ± 8.8%, 29.5 ± 7.4% and 17.8 ± 6.7% of total lipids. FI contained on average 22.0 ± 7.3% saturated FA (SFA) with important discrepancies between

FI since the lowest value for SFA was 15.7% (FI_3) and highest reached 40.9% (FI_6). Most FI contained a high amount of PUFA (47.8 ± 9.1%), again with discrepancies (MIN= 18.7%, MAX= 60.6%). The ω6/ω3 ratios of FI averaged 12.2 ± 5.9 and were globally in agreement with the international recommendations which indicates an optimal ratio range between five and 15 [3]. However, it is important to underline that for two FI (FI_6 and FI_7), the ratios exceeded largely these recommendations (21.2 ± 3.8 and 31.7 ± 1.0 respectively).

Two FI (FI_2 and FI_3) were analyzed after being stored over two months in the laboratory. The main fatty acids (*i.e.* $C_{16:0}$, $C_{18:1}$ *n*-9, $C_{18:2}$ *n*-6) did not evolve significantly with the time of storage over this limited period of time (**Table S1**).

3.3 Lipophilic Vitamin Profiles

The lipophilic vitamin contents in FI are displayed according to their time of storage (**Figure 2**). Most of the FI were not consistent with the European directive [33]. For all lipophilic vitamins a significant "time of storage effect" was observed whatever the FI brand or formulation: vitamin contents decreased with time of storage ($p<0.001$). Globally, the use of vitamin overages, that led to contents above the upper recommended limits, was far more frequent than insufficient contents. Whatever the vitamin, 13 out of 19 analyzed FI were above the upper limits and the excess reached up to 15, 35 and 10 times the recommended upper limits for vitamin A, D and E, respectively. Average amount of vitamin A for FI stored from one to six months reached 378.8 ± 88.0 µg/100 Kcal corresponding to an overage of 210% compared to the authorized upper limit and dropped down to an average value of 100.9 ± 116.6 µg/100 Kcal for FI stored for more than six months (six products). Similarly, values were quite homogeneous with important overages up to five-month-storage, for vitamin D (average value = 13.5 ± 5.2 µg/100 Kcal corresponding to an overage of 450% compared to the authorized upper limit) and for vitamin E (average value= 4.3 ± 2.7 µg/100 Kcal corresponding to an overage of 143%

compared to the authorized upper limit). After five months, averaged values dropped down to 3.5 ± 6.3 µg/100 Kcal and 1.4 ± 1.5 µg/100 Kcal, for vitamin D and E respectively.

Seven out of 19 analyzed products were below the lower limits and most of them were close to the expiry date (time of storage > 12 months): three FI for vitamin A, six for vitamin D and four for vitamin E. All the imported products, *i.e*, FI produced in Northern countries and shipped to Africa (FI_10, FI_11, FI_12) were included in this set of seven products and had an insufficient vitamin content.

When assessing compliance with the nutritional composition indicated on product label, we observed that vitamin contents were not always detailed. Moreover, the products were most of the time not compliant with the contents indicated on the label. To be more precise, for vitamin A, six products out of 13 had a content indicated on the label and two products only were compliant with indications. Regarding vitamin D, four products out of 13 had a content indicated on the label and none was compliant with indications. Regarding vitamin E, three products out of 13 had a content indicated on the label and one product only was compliant with indications. When comparing the two products stored over two months in the laboratory at ambient temperature (~20 °C), no significant difference was observed for any of the vitamins. A short time of storage, at moderate temperature in the first months after production did not alter the vitamin profile of these fortified foods. However, it also is important to note that these products were packed with multilayer aluminum [34].

3.4 Acid Values and Peroxide Values

The acidity values of all the products were acceptable (average value= 0.06 ± 0.04%, range= [0.01; 0.16]%). Indeed, the values were below the maximum limit of 0.6% of total lipids as defined officially [35] (**Figure 3**). Regarding peroxide contents, five products did not meet the international recommendations, *i.e.* were above the limit of 10 meq O_2 per kg of lipids [35,36]. These products were those which had been stored for the longest period (*i.e.* nine months and more). Peroxide contents increased significantly over the time of storage ($p<0.001$).

When comparing the two products stored over two months in the laboratory (FI_2 and FI_3), the time of storage significantly increased the acid and peroxide values ($p<0.001$), despite multilayer aluminum packaging.

3.5 Volatile Compounds

The main volatile oxidation compounds identified were saturated aldehydes and, by decreasing order, hexanal, pentanal and nonanal (**Table 3**). These three volatile compounds significantly increased over the time of storage of FI ($p<0.001$). The highest content was reached with hexanal in the product stored the longest time ($1.35e^{-08} \pm 2.19e^{-09}$ mg eq benzyl butyrate/100 g in FI_12 after a 19-month-storage). When comparing the two products stored over two months in the laboratory (FI_2, FI_3) an increase in hexanal was observed.

3.6 Determination of Mycotoxins

In our experiments, trichotecene, zearalenone, ochratoxin A, beauvericin were not detected. Low levels of fumonisins (FB1 and FB2) and aflatoxin were detected in 14 FI and only four FI were free of mycotoxins (**Table 3**). Samples were considered contaminated if the mycotoxin levels exceeded the EU limit [37] which corresponds to: 0.1 µg/kg for aflatoxin B1 and 200 µg/kg for fumonisins. Among the 19 analyzed products, only one product reached the limit for aflatoxin AFB1 (FI_6, 4.2 ± 0.2 µg/kg) and another for fumonisins (FI_8, FB1= 198.1 ± 3.5 µg/kg and FB2= 40.6 ± 1.1 µg/kg).

3.7 Packaging Characterization

The packaging materials used to commercialize the FI were by decreasing frequency of use: aluminum/polyethylene multilayer, polyethylene, polyethylene/polypropylene multilayer and cellophane/epoxy resin (**Table 1**).

OTR and WVTR were sometimes equal to zero if packaging is structured by an aluminum thin layer which is a very good barrier to oxygen and water transfer **(Table 4)**. Such very good

packaging materials were used for seven FI out of 13, particularly for FI stored over long periods of time (> 12 months, *i.e.* FI_10, FI_11 and FI_12).

3.8 Determinants of the Degradation of Lipids and Vitamins

The key determinants of the stability of FI were explored by a correlation approach using Principal Component Analyses (PCA) with the biochemical composition, oxidation level, OTR and WVTR. However, this first analysis was not satisfying because several variables were not well projected in two or three dimensions. It was not surprising because of the great discrepancy between the product formulations, the packaging types and conditions of storage (duration, temperature, etc…). In order to obtain a better representation of the variability of the data, OTR and WVTR data were excluded and a new PCA was realized. As expected for moisture sorption isotherm of starchy products, a positive correlation (0.78) between a_w and water content was observed. As a consequence, only water content was integrated in the PCA (**Figure 4**).

The three components extracted explained 79% of the variability of the data set. The representation of the variables along the first component accounted for 51% of the variability of the data, the second for 18%, and the third one for 10%. Despite the design of the study, triggering high variability in FI's formulation, packaging materials and conditions of storage, the set of analyzed products presented variables very well correlated (negatively or positively) with time of storage. The contents in vitamins A and E were negatively correlated with the time of storage and the correlation was rather strong with $R = 0.86$ for vitamin A and $R = 0.85$ for vitamin E. The peroxide content and the time of storage were positively correlated ($R = 0.81$). The content in volatile compounds was not correlated to the lipid content of the FI.

4. DISCUSSION

The composition of FI sold in low-income countries was not always adequate in terms of fatty acid composition, lipophilic vitamins content, peroxide value, and mycotoxins content. The nutritional profiles of the 13 FI greatly differed depending on the formulation and on the

location of the producing unit. The FI produced in Africa and in Asia contained three times more lipids than the products from Northern countries. Such difference can be explained by the fact that children in developing countries need to cover a higher level of macronutrients with these fortified foods than children from northern countries that have access to a large choice of food to cover their needs. Indeed, the daily diet of children from southern countries is poorly diversified and can generate nutrients and caloric deficiencies.

Even if the FI lipid content greatly differs, most of them had a similar lipid profiles with three predominant fatty acids (*i.e.* $C_{18:2}$ *n*-6 followed by $C_{18:1}$ *n*-9 and $C_{16:0}$). Lipids are crucial macronutrients that support most of the growth during the first six months of life and represent 40 to 60% of caloric requirements. Then, lipid need gradually decreases to ~35% of the total energy intake at 24 months. Palmitic acid, the third major FA in the fortified FI, plays an important role in infant nutrition [2]. It is the main saturated FA in breast milk. Nevertheless, its bioavailability will depend on food matrix composition (content in Ca_2^+) and on its position on the triacylglycerol backbone. In breastmilk, palmitic acid is located in *sn-2* position in the triacylglycerol, the latter position will favor its absorption and role in the bone mineralization [2]. In vegetable oils used in our fortified FI, palmitic acid is predominantly located in external positions [2]. We can thus suspect that the consumption of formulas with high contents of saturated fat from vegetable oils or palm olein will not favor $C_{16:0}$ absorption, but can be counterbalanced by complementary breast feeding until 24 months.

Globally, ω6/ω3 ratios were high in fortified FI and sometimes excessive compared to the recommended range (5 - 15). Furthermore, the amounts of essential FA (linoleic acid $C_{18:2}$ *n*-6 and α-linolenic acid $C_{18:3}$ *n*-3) present in the FI were sometimes too high. These FA represent approximately 43.3 ± 8.8% and 2.8 ± 0.9% of the total FA in the fortified FI, while it represents only 10.5 - 12.7% and 0.72 - 0.95% of the total FA in human milk [3]. Linoleic acid (LA) and α-linolenic acid (ALA) intake recommendations for six-month-old-infant to five-year-old-children are 3.0 - 4.5% and 0.5 - 0.6% of energy intake respectively [38], which corresponds to 8.1 - 25.2 Kcal/day and 1.4 - 3.4 Kcal/day. If the FI analyzed in our study were consumed on a

daily basis, LA intakes would be higher than the nutritional recommendations [38]. For a daily consumption of 100 g flours, an average 29.7 ± 11.7 Kcal would be attributed to LA, which is higher than the recommendation, while ALA intakes would be satisfying.

Unbalanced LA intake can decrease blood DHA and cerebral lipids of infants [4]. Excessive intakes of ALA and LA can limit DHA biosynthesis by a competitive inhibition of PUFA bioconversion [3,5]. Conversely, a decrease in ALA contents in infant food complement while maintaining $C_{18:2\ n\text{-}6}/C_{18:3\ n\text{-}3}$ ratio below 5 could promote DHA biosynthesis. Unbalanced ω6/ω3 ratios during infancy and childhood have long term consequences on adipogenesis and lipid metabolism [3,5].

In most FI, over fortification in vitamins was observed to ensure that vitamins would still remain in the product until its consumption whatever the storage conditions. However, consumption of large amounts of vitamin A over a long period of time can induce liver damage, bone abnormalities and vomiting [39]. Furthermore, in most developing countries, children aged 6 - 59 months, are supplemented twice a year with a dose of 100 000 UI. The daily intakes from diet should not exceed 900 µg which is well above the mean requirement of about 200 µg/day for infants [39]. In our experiments, seven FI could be at risk of excessive contents. Risk of hypervitaminosis (D or E) were already documented [39,40]. In addition, one can wonder about the nature and safety of the lipophilic vitamins overages degradation products. This aspect should be further investigated.

Globally, a strong effect of time of storage was observed on the set of FI despite heterogeneous conditions of storage in the supply-chain. This strong effect of time of storage is an important outcome of our study. For the FI produced in Europe and purchased in Africa with time of storage > 12 months, the vitamin contents were below the recommended amounts. Two hypothesis can be formulated: either the vitamins were not adequately added during the industrial production but this hypothesis is so unlikely that it can be rejected, or the temperature during shipping and storage have activated chemical degradation within the products. There was a clear trend of vitamin degradation and peroxide generation over the storage period

(between one and 19 months) in the “real supply chain”. The use of high barrier packaging material (ALU / PE) did not modify this trend. An overall adequacy of the FI, with acceptable nutritional profiles and low levels of degraded lipids, was observed up to 12 months, which could be a reasonable expiration date. According to the climatic conditions in tropical countries, the average temperatures are high and the temperature control within supply chain is not always possible. Indeed, vitamin A is susceptible to light and oxygen exposure while vitamin E is more stable. For instance, 90% degradation of vitamin A degradation was observed in fortified wheat flour after a three-month storage at 40 °C [16]. On the contrary, Vitamin E has been reported to be stable over 70 days even after opening a unit dose of infant milk [41].

According to their a_w, the studied FI can be altered during storage mainly by hydrolysis reactions (a_w>0.25) and enzymatic activity (a_w>0.35). On the contrary, very low a_w (<0.2) favors very high oxidation rate so the ideal window in terms of a_w is narrow probably (0.2 - 0.3). In our set of FI, three products were in this window and six products had a_w that exceeded 0.35.

With regards to peroxide and acidity values, they were higher when the storage duration increases, but the products were still acceptable for human consumption up to nine months of storage whatever the packaging used. The degradation reactions are rapid, especially in food matrices in powder form and at low a_w.

FI have a powdery structure, with a high surface area between the product and atmosphere which makes them an ideal matrix for the propagation of lipid oxidation [42]. Moreover, reactive oxygen species (ROS) can initiate the oxidation of PUFA and, as a consequence, induce the production of hydroperoxides as well as other oxidation compounds such as aldehydes (hexanal, pentanal and propanal) [43] breakdown on *n*-6 FA (hexanal, pentanal) and *n*-3 FA (propanal) [44]. These molecules are good markers of infant formula oxidation and participate to the off flavor development [45].

5. CONCLUSIONS

The panorama of fortified infant foods collected “in the field” after one to 19 months of storage indicated that their lipid profile and nutritional quality could be improved. Indeed, in most FI stored less than six months, lipophilic vitamin overages were observed. Long time of storages (> 12 months) have deleterious impact on the fortified food nutritional profile despite the use of protective packaging. The expiration dates should be shortened in order to guarantee an optimal quality. In addition, ω6/ω3 ratios should be maintained below 15. Such recommendation could improve the biological efficacy of fortified FI and better help preventing deficiencies. In perspectives, it would be very interesting to develop a mathematical model that could predict the degradation of lipophilic vitamins in complex matrices stored under different environmental conditions. This model would help optimizing vitamin levels and limiting overages, so that the level of added vitamins is acceptable from production up to consumption of fortified food products. Such model would improve significantly the vitamin profile of fortified foods and would constitute a helpful tool to abide by the regulation and fulfill infant needs.

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7. CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

8. PERMISSION STATEMENTS

The manuscript ***does not*** contain experiments using animals.

The manuscript ***does not*** contain human studies.

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Table 1. Characteristics of the fortified infant flours.

Code	Purchase place (production place)*	Storage duration (months)/ Expiration date (months)	Packaging material	Ingredients	Producer typology
FI_1	Burkina Faso	5 / 6	PE†	Mil, soy, peanut, sugar, iodized salt, industrial amylase, CMV$.	Local (national producer)
FI_2	Madagascar	1-2-3-4 / 12	Alu / PE††	Corn, rice, soy, peanut, sugar, salt, vitamins, minerals	Local (national producer)
FI_3	Madagascar	1-2-3-4 / 12	Alu / PE††	Corn, rice, soya, peanut, sugar, salt, vitamins, minerals	Local (national producer)
FI_4	Benin	6 / 24	PE†	Cereal sprouts, soy germs, fats, sugar, CMV$	Local (national producer)
FI_5	Benin	9 / 24	PE†	Corn, mil, tubers, soy, sugar, fats, full milk, carrots, , vanilla, CMV$	Local (national producer)
FI_6	Benin (Ghana)	12 / 12	Alu / PE††	Wheat flour, skimmed milk, sugar, palm olein, calcium carbonate, maltodextrin, vitamins, , ferrous fumarate, vanillin, zinc sulfate, bifidus bacteria, sodium chloride, potassium iodide	Local (multi-national producer)
FI_7	Benin	2 / 6	PE / PPΔ	Rice, corn, milk, sugar, CMV$	Local (national producer)
FI_8	Benin	3 / 6	PE†	Corn, sorghum, soy, sugar, CMV$	Local (national producer)
FI_9	Benin	4 / 24	PE†	Cereals, soy, tubers, fats, sugar, CMV$	Local (national producer)
FI_10	Benin (France)	13 / 18	C/ Epox□	Flours (wheat, rice, corn), sugar, CMV$	Imported (multi-national producer)
FI_11	Benin (France)	17 / 36	Alu / PE††	Corn starch, rice flour, maltodextrins, tapioca, sunflower lecithin, CMV$	Imported (multi-national producer)

FI_12	Benin (France)	19 / 36	Alu / PE[††]	Flours (wheat, corn, rice), maltodextrins, sugar, caramel, vanillin, sunflower lecithin, CMV[$]	Imported (multi-national producer)
FI_13	Vietnam	7 / 12	Alu / PE[††]	Sticky rice, soy, green bean, sesame, whole milk powder, vegetal fat, CMV[$], amylase, yeast, vanillin, salt	Local (national producer)

[*]the place of production is indicated when different from the place of purchase.
[$]CMV: mineral and vitamin complex.
[†]PE: polyethylene.
Alu / PE: aluminum/ polyethylene.
[Δ]PE/ PP: polyethylene/polypropylene.
[□]C / Epox: cellophane/ epoxy resin.

Table 2. Water content, a_w, lipid contents and fatty acid profiles of the fortified infant flours.

Sample	a_w*	Water (g/100 g)*	Lipids (g/100 g)*	SFA (%)$	MUFA (%)$	PUFA (%)$	ω6/ω3$
FI_1	0.22 ± 0.00	3.91 ± 0.16	13.6 ± 0.4^{f}	19.8 ± 0.1^{bc}	34.1 ± 0.1^{ef}	46.2 ± 0.2^{d}	8.5 ± 1.5^{a}
FI_2_L2_1	0.35 ± 0.00	5.72 ± 0.27	11.7 ± 3.5^{def}	16.7 ± 0.2^{a}	32.6 ± 0.5^{def}	50.7 ± 0.2^{g}	8.2 ± 0.5^{a}
FI_2_L1_2	0.33 ± 0.01	4.98 ± 0.09	8.7 ± 0.2^{def}	17.7 ± 0.2^{a}	33.1 ± 0.3^{def}	49.3 ± 0.6^{g}	8.2 ± 0.6^{a}
FI_2_L2_3*	0.38 ± 0.00	5.95 ± 0.10	9.9 ± 0.9^{def}	17.5 ± 0.1^{a}	32.4 ± 0.4^{def}	50.3 ± 0.3^{g}	8.8 ± 4.8^{a}
FI_2_L1_4*	0.33 ± 0.00	5.92 ± 0.25	10.0 ± 1.2^{def}	17.4 ± 0.1^{a}	33.8 ± 0.6^{def}	49.6 ± 0.1^{g}	9.1 ± 1.0^{a}
FI_3_L2_1	0.32 ± 0.00	5.09 ± 0.19	15.1 ± 3.2^{ef}	17.5 ± 0.2^{a}	33.8 ± 1.1^{f}	48.8 ± 0.7^{ef}	8.3 ± 1.0^{a}
FI_3_L1_2	0.33 ± 0.00	5.44 ± 0.67	10.6 ± 1.3^{ef}	15.7 ± 0.1^{a}	40.3 ± 0.5^{f}	48.5 ± 0.2^{ef}	8.2 ± 0.6^{a}
FI_3_L2_3*	0.31 ± 0.00	5.66 ± 0.29	10.3 ± 0.4^{ef}	17.5 ± 0.1^{a}	33.7 ± 0.1^{f}	48.8 ± 0.2^{ef}	9.2 ± 1.5^{a}
FI_3_L1_4*	0.35 ± 0.00	4.89 ± 0.16	9.8 ± 0.4^{ef}	17.8 ± 0.1^{a}	34.6 ± 0.7^{f}	48.2 ± 0.1^{ef}	9.1 ± 1.3^{a}
FI_4	0.18 ± 0.01	3.20 ± 0.12	8.8 ± 1.5^{cdef}	18.3 ± 0.3^{abc}	30.1 ± 0.7^{cd}	51.7 ± 0.3^{gh}	10.0 ± 1.1^{bc}
FI_5	0.15 ± 0.01	1.99 ± 0.13	9.0 ± 0.3^{cdef}	20.4 ± 2.5^{c}	29.6 ± 2.1^{c}	50.2 ± 1.0^{fg}	11.6 ± 4.0^{d}
FI_6	0.20 ± 0.00	2.96 ± 0.13	10.2 ± 0.4^{cdef}	40.9 ± 0.5^{h}	42.8 ± 0.9^{g}	18.7 ± 0.8^{a}	21.2 ± 3.8^{h}
FI_7	0.40 ± 0.01	6.37 ± 0.08	8.8 ± 3.5^{cdef}	23.6 ± 0.3^{d}	34.7 ± 0.3^{f}	41.7 ± 0.4^{c}	31.7 ± 1.0^{i}
FI_8	0.40 ± 0.00	6.45 ± 0.30	6.6 ± 0.7^{abcd}	17.7 ± 0.1^{ab}	29.5 ± 0.3^{c}	52.8 ± 0.2^{h}	13.5 ± 5.9^{e}
FI_9	0.16 ± 0.01	3.07 ± 0.16	7.7 ± 0.2^{bcde}	18.1 ± 0.1^{ab}	28.8 ± 0.0^{c}	53.2 ± 0.1^{h}	10.7 ± 1.8^{cd}
FI_10	0.40 ± 0.01	5.49 ± 0.02	5.0 ± 1.4^{abc}	25.7 ± 0.4^{de}	14.1 ± 0.2^{a}	60.6 ± 0.6^{i}	14.8 ± 5.4^{f}
FI_11	0.28 ± 0.00	4.46 ± 0.52	1.5 ± 0.4^{a}	32.3 ± 0.4^{f}	20.4 ± 0.2^{b}	47.4 ± 0.7^{de}	16.4 ± 5.9^{g}
FI_12	0.41 ± 0.01	5.75 ± 0.14	2.5 ± 1.1^{ab}	27.1 ± 2.9^{e}	13.6 ± 9.3^{a}	59.3 ± 6.5^{i}	14.9 ± 0.4^{f}
FI_13	0.06 ± 0.01	1.78 ± 0.02	13.5 ± 0.7^{f}	36.3 ± 0.3^{g}	30.6 ± 0.6^{cde}	33.0 ± 0.3^{b}	8.9 ± 3.5^{ab}
Average	0.29 ± 0.1	4.69 ± 1.44	9.1 ± 3.6	22.0 ± 7.3	30.6 ± 7.5	47.8 ± 9.1	12.2 ± 5.9
min	0.06	1.78	1.5	15.7	13.6	18.7	8.2
max	0.41	6.45	15.1	40.9	42.8	60.6	31.7

Number of repetitions (n), *n = 3, $ n=6.
a_w: water activity.
SFA: saturated fatty acid. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid.
Data are presented as mean ± standard deviation for 100 g of product.
Values with distinct lowercase superscript letters in the same column are significantly different ($p \leq 0.05$).

Table 3. Major volatile compounds detected and contents in fumonisins and aflatoxin of fortified infant flours (n=3).

	Hexanal (mg eqBB/100g)*	Pentanal (mg eqBB/100g)*	Nonanal (mg eqBB/100g)*	Fumonisin FB1 (µg/kg)	Fumonisin FB2 (µg/kg)	Aflatoxin AFB1 (µg/kg)
FI_1	$5.38e^{-09} \pm 1.77e^{-09}$	$6.83e^{-10} \pm 2.23e^{-10}$	$4.35e^{-09} \pm 6.47e^{-10}$	-	-	-
FI_2_L2_1	$2.73e^{-0} \pm 1.19e^{-09ab}$	$6.27e^{-10} \pm 3.13e^{-10a}$	$6.03e^{-10} \pm 2.16e^{-10\,a}$	113.1 ± 3.6^{b}	16.4 ± 0.4^{a}	-
FI_2_L1_2	$2.49e^{-09} \pm 4.10e^{-10a}$	$4.89e^{-10} \pm 9.95e^{-11a}$	$6.45e^{-10} \pm 9.04e^{-11a}$	122.3 ± 2.0^{b}	32.0 ± 1.2^{a}	-
FI_2_L2_3*	$2.86e^{-09} \pm 2.48e^{-10ab}$	$5.25e^{-10} \pm 1.41e^{-11a}$	$6.53e^{-10} \pm 1.33e^{-10a}$	115.7 ± 3.0^{b}	-	-
FI_2_L1_4*	$3.54e^{-09} \pm 5.39e^{-10b}$	$6.15e^{-10} \pm 8.28e^{-11a}$	$6.05e^{-10} \pm 4.83e^{-11a}$	118.5 ± 2.1^{b}	-	-
FI_3_L2_1	$9.69e^{-10} \pm 1.46e^{-10c}$	$1.89e^{-10} \pm 1.87e^{-11b}$	$3.80e^{-10} \pm 3.12e^{-11b}$	121.9 ± 3.6^{bc}	-	-
FI_3_L1_2	$1.00e^{-09} \pm 3.90e^{-10c}$	$2.43e^{-10} \pm 1.07e^{-10bc}$	$1.63e^{-10} \pm 2.06e^{-10b}$	117.5 ± 3.0^{bc}	-	-
FI_3_L2_3*	$1.97e^{-09} \pm 5.38e^{-10d}$	$4.55e^{-10} \pm 1.69e^{-10c}$	$3.54e^{-10} \pm 3.18e^{-10b}$	114.5 ± 3.2^{bc}	-	-
FI_3_L1_4*	$2.95e^{-09} \pm 1.18e^{-09d}$	$3.78e^{-10} \pm 9.76e^{-11c}$	$3.22e^{-10} \pm 3.09e^{-10b}$	126.7 ± 4.4^{bc}	-	-
FI_4	$7.74e^{-10} \pm 9.36e^{-11}$	$1.59e^{-10} \pm 2.41e^{-11}$	$7.06e^{-10} \pm 9.42e^{-11}$	123.6 ± 4.9^{c}	37.7 ± 1.0^{c}	-
FI_5	$2.69e^{-09} \pm 5.97e^{-10}$	$4.50e^{-10} \pm 1.49e^{-10}$	$1.83e^{-09} \pm 8.73e^{-11}$	134.7 ± 4.4^{d}	30.1 ± 0.8^{c}	-
FI_6	$2.29e^{-09} \pm 5.40e^{-10}$	$2.70e^{-10} \pm 4.98e^{-11}$	$1.24e^{-09} \pm 3.49e^{-10}$	-	-	4.2 ± 0.2^{a}
FI_7	$2.71e^{-09} \pm 8.89e^{-10}$	$3.10e^{-10} \pm 1.07e^{-10}$	$2.88e^{-09} \pm 6.19e^{-10}$	133.5 ± 3.3^{d}	-	-
FI_8	$9.63e^{-09} \pm 3.24e^{-09}$	$1.21e^{-09} \pm 4.14e^{-10}$	$3.81e^{-09} \pm 6.16e^{-10}$	198.1 ± 3.5^{e}	40.6 ± 1.1^{c}	-
FI_9	$9.91e^{-10} \pm 3.35e^{-10}$	$1.82e^{-10} \pm 4.51e^{-11}$	$6.72e^{-10} \pm 1.62e^{-10}$	-	-	-
FI_10	$5.64e^{-09} \pm 1.92e^{-09}$	$9.28e^{-10} \pm 3.99e^{-10}$	$3.97e^{-10} \pm 8.00e^{-12}$	105.2 ± 3.1^{a}	-	-
FI_11	$2.63e^{-09} \pm 3.65e^{-10}$	$4.35e^{-10} \pm 6.75e^{-11}$	$1.33e^{-10} \pm 1.92e^{-11}$	116.0 ± 1.9^{b}	30.3 ± 0.7^{c}	-
FI_12	$1.35e^{-08} \pm 2.19e^{-09}$	$2.14e^{-09} \pm 4.17e^{-10}$	$1.22e^{-09} \pm 1.02e^{-10}$	-	-	-
FI_13	$1.28e^{-08} \pm 1.67e^{-10}$	$2.86e^{-09} \pm 3.00e^{-10}$	$9.90e^{-10} \pm 6.71e^{-11}$	-	-	-

*: mg equivalent benzyl butyrate / 100 g fresh product.
Data are presented as mean ± standard deviation for 100 g or kg of fresh product.
Values with distinct lower case superscript letters in the same column are significantly different ($p \leq 0.05$).
-: not detected.

Table 4. Oxygen (OTR) and Water Vapor Transmission (WVTR) rates of fortified infant flours packaging.

Code	Packaging	OTR* (cm^3/mm^2)	WVTR$ (g/mm)

FI_1	PE[†]	17.46	0.03
FI_2	Alu / PE[††]	0.00[**]	0.00[**]
FI_3	Alu / PE[††]	0.00[**]	0.00[**]
FI_4	PE[†]	13.87	0.02
FI_5	PE[†]	13.87	0.02
FI_6	Alu / PE[††]	0.00[**]	0.00[**]
FI_7	PE / PP[Δ]	12.14	0.03
FI_8	PE[†]	20.96	0.03
FI_9	PE[†]	13.87	0.02
FI_10	C/ Epox[□]	0.00[**]	0.02
FI_11	Alu / PE[††]	0.00[**]	0.00[**]
FI_12	Alu / PE[††]	0.00[**]	0.00[**]
FI_13	Alu / PE[††]	0.00[**]	0.00[**]

[*]OTR: oxygen transmission rate.
[$]WVTR: water vapor transmission rate.
[†]PE: polyethylene.
[††]Alu / PE: aluminum/ polyethylene.
[Δ]PE / PP: polyethylene/polypropylene.
[□]C / Epox: cellophane/ epoxy resin.
[**]Aluminum multilayer packaging are considered as total oxygen and water barriers.

Figure captions

Figure 1. Main fatty acids of the fortified infant flours. The products are classified according to increasing time of storage from the left to the right (range X-Y g/100g of matrix). (n=6).

Figure 2. Content in vitamins of the fortified infant flours according to the time of storage: different letters above each bars indicate significant difference ($p<0.05$) (µg RE/100 Kcal; µg/100 Kcal; mg α TE/100 Kcal). The products are classified according to increasing time of storage from the left to the right. (n=5).

The limits are defined by straight lines:

Lower and upper limits of vitamin A: 60 - 180 µg RE/100 Kcal

Lower and upper limits of vitamin D: 1 - 3 µg/100 Kcal

Lower and upper limits of vitamin E: 0.5 – 3.0 mg α TE/100 Kcal

Figure 3. Acid and peroxide values of the fortified infant flours according to the time of storage. Different letters above each bars indicate significant difference ($p<0.05$).

The limits are defined by straight lines:

Maximal limit of peroxide value: 10 $mEqO_2$/kg of lipid

Maximal limit of acid value: 0.6% lipid

Figure 4. Principal Component Analysis of the determinants of the stability of the fortified infant flours (VE : vitamin E ; VA : vitamin A ; VD : vitamin D ; PV : peroxide value ; AV: acid value).

Figure 1.

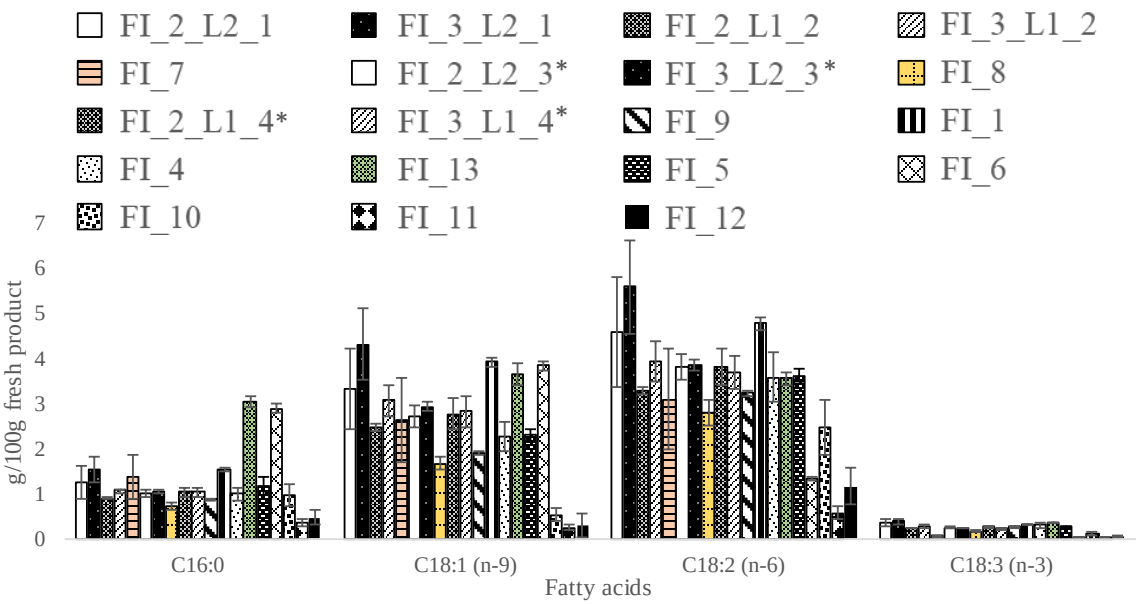
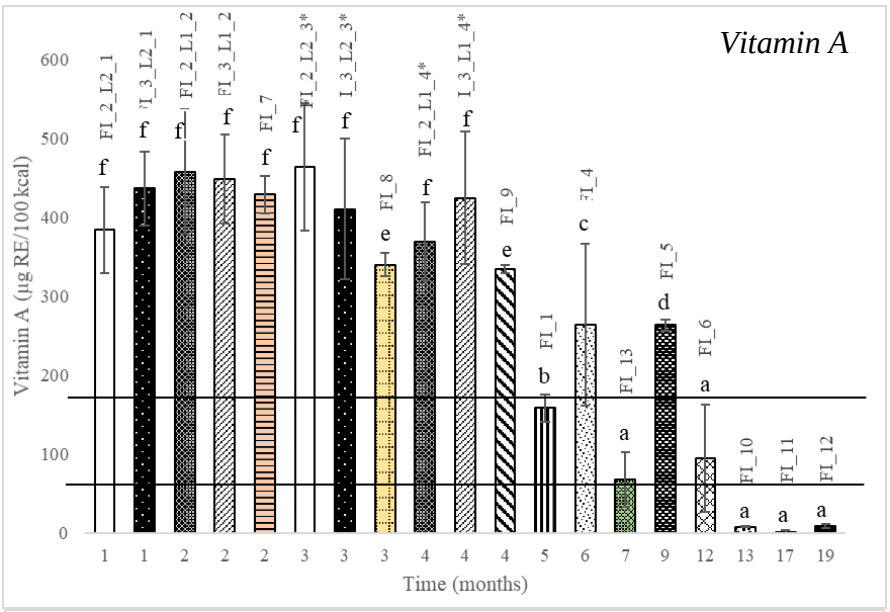
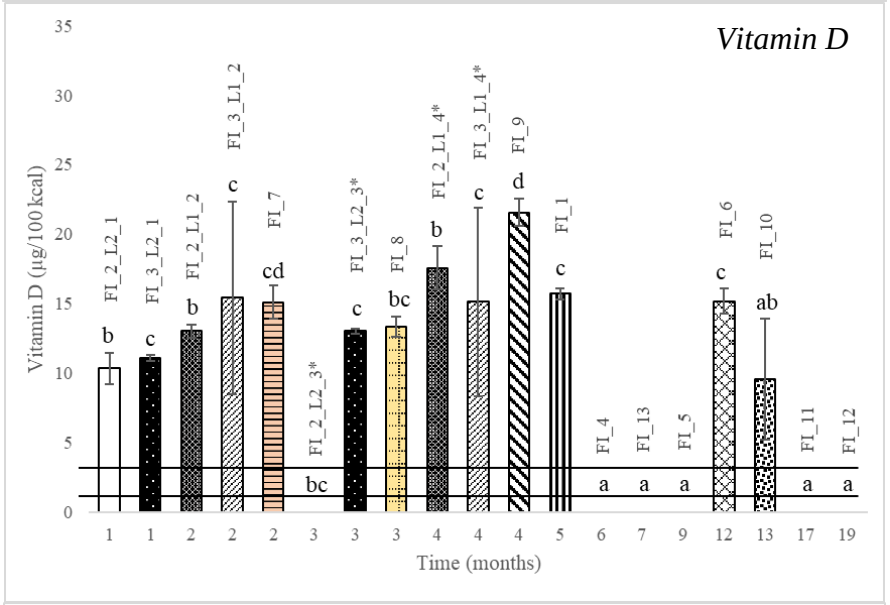

FI_2_L2_1
FI_3_L2_1
FI_2_L1_2
FI_3_L1_2
FI_7
FI_2_L2_3*
FI_3_L2_3*
FI_8
FI_2_L1_4*
FI_3_L1_4*
FI_9
FI_1
FI_4
FI_13
FI_5
FI_6
FI_10
FI_11
FI_12
g/100g fresh product
0
1
2
3
4
5
6
7
C16:0
C18:1 (n-9)
C18:2 (n-6)
C18:3 (n-3)
Fatty acids

Figure 2.





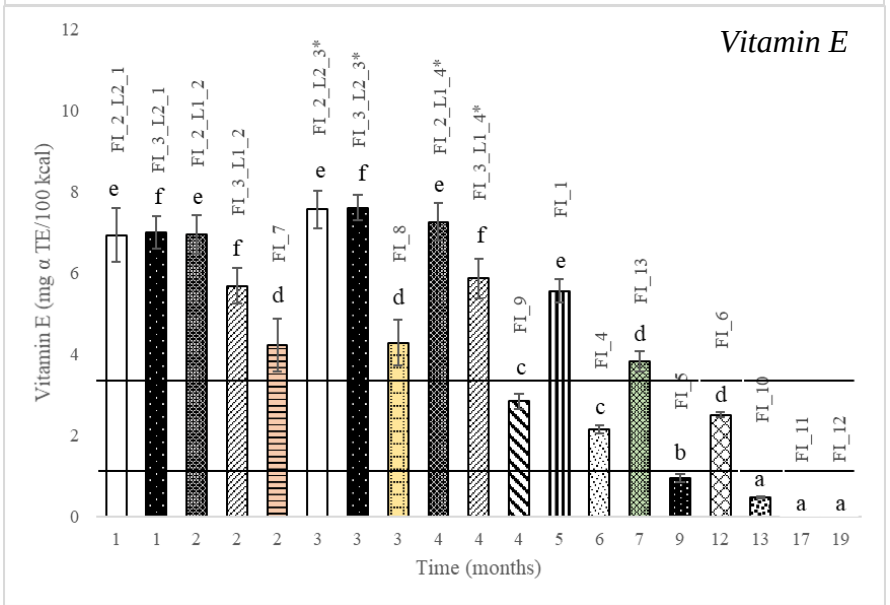


Figure 3.

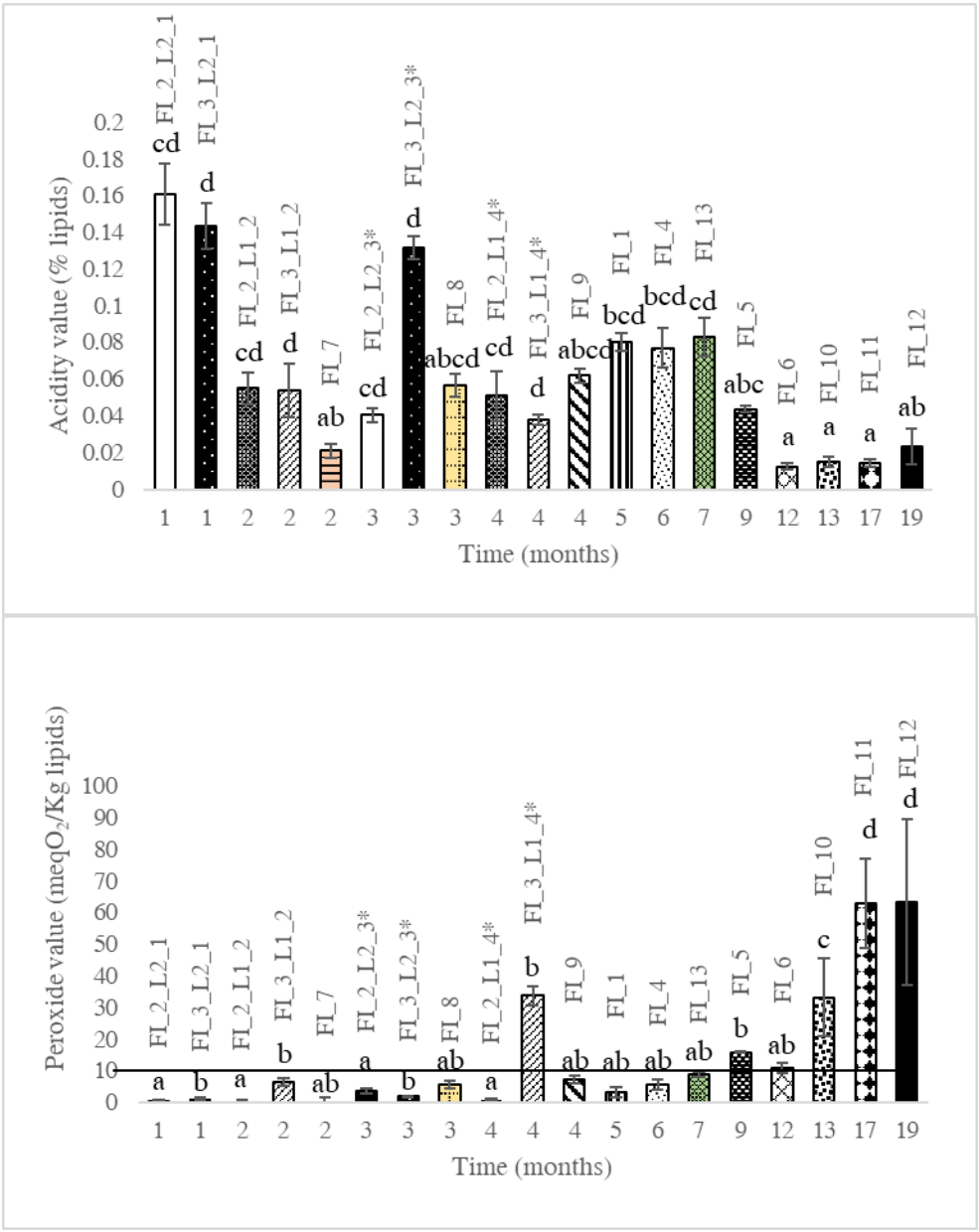
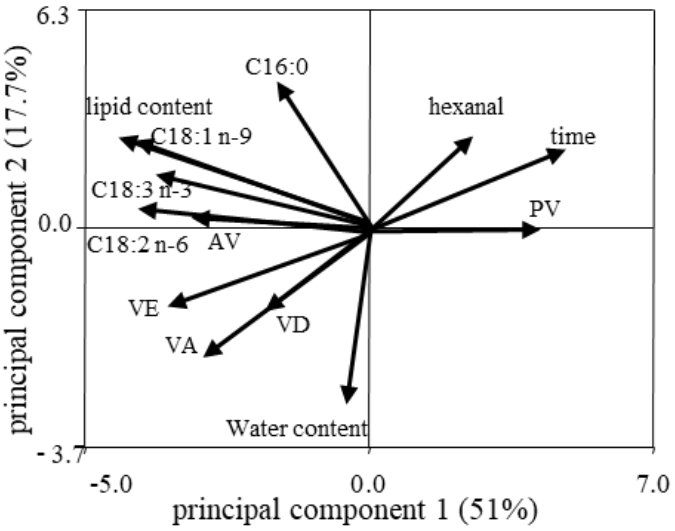

Acidity value (% lipids)
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
FI_2_L2_1
FI_3_L2_1
FI_2_L1_2
FI_3_L1_2
FI_7
FI_2_L2_3*
FI_3_L2_3*
FI_8
FI_2_L1_4*
FI_3_L1_4*
FI_9
FI_1
FI_4
FI_13
FI_5
FI_6
FI_10
FI_11
FI_12
cd d cd d ab cd d abcd cd d abcd bcd bcd cd abc a a a ab
1 1 2 2 2 3 3 3 4 4 4 5 6 7 9 12 13 17 19
Time (months)
Peroxide value (meqO2/Kg lipids)
100
90
80
70
60
50
40
30
20
10
0
a b a b ab a b ab a b ab ab ab ab b ab d d
1 1 2 2 2 3 3 3 4 4 4 5 6 7 9 12 13 17 19
Time (months)

Figure 4.


6.3
0.0
- 3.7
-5.0
0.0
7.0
principal component 2 (17.7%)
principal component 1 (51%)
C16:0
lipid content
C18:1 n-9
C18:3 n-3
C18:2 n-6
AV
hexanal
time
PV
VE
VD
VA
Water content