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Oxidative stability of polyunsaturated fatty acid-enriched infant flours based on teff and cowpea: Impact of natural antioxidants from amaranth leaves and black rice bran

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ABSTRACT

From 4/6 months of age, complementary foods such as infant flours (IF) are introduced in the infant diet to supplement the nutritional intake of breast milk and/or infant formula that are no longer sufficient. However, the lipid profile and micronutrient content of IF are often unbalanced. In addition, the current lack of knowledge about oxidation mechanisms in low-moisture systems makes it difficult to control them in this type of food. The aim of this study was to better understand oxidation mechanisms in IF with optimized lipid profile for infant nutritional needs using climate smart crops, and with or without, the addition of vegetal sources of natural antioxidants. Five IF were formulated using climate smart crops, *i.e.* cowpea and teff flours, as sources of essential omega-3 fatty acids to complement soy flours. Four IF contained heat treated cowpea flour to evaluate the impact of lipoxygenases inactivation in this flour on global IF oxidative stability. Three IF were also fortified in long chain polyunsaturated fatty acid that are essential to support infant development to check whether this staple food could be good nutritional vector. Among these IF, two were enriched in natural antioxidant fractions with the addition of either powdered amaranth leaves or black rice bran, bringing carotenoids and phenolic compounds. The oxidative stability of IF was monitored over a 6-month storage period at 25 °C. The results showed that including teff and cowpea flours in the IF formulation enabled a balanced lipid profile (omega-6/omega-3 ratio close to 5) and ensured relatively good overall oxidative stability. However, 50 % of vitamin A was lost after 2 weeks of storage. Black rice bran and amaranth leaves acted as vitamin A stabilizers, delaying losses. This study unveiled that oxidation is triggered mainly during initial flour milling and propagated at a relatively slow rate over storage in these low moisture foods. Oxidation rate was related to the antioxidants composition and was faster in the presence of photosensitive compounds.

1. Introduction

Proper nutrition from birth to two years of age is essential to ensure comprehensive growth and development (Bourlieu et al., 2014; Bourlieu et al., 2017; Martorell, 2017). By around 6 months, breast milk alone no longer meets all infant's energy and nutrient requirements (WHO, 2021). At this age, the acquisition of certain digestive and neuromuscular maturity makes the consumption and assimilation of complex foods possible. Therefore, complementary foods are gradually introduced into the diet while breast milk remains the primary calorie source until 12 months (WHO, 2003; Cancalon et al., 2022). In many regions, especially in southern countries, infant flours (IF) are one of the first complementary foods introduced into the infant diet, with over 50 % of infants consuming them at around 6 months of age (Duffy et al.,

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2019; Lange et al., 2013; National Institute of Population Research and Training (NIPORT) et al., 2005; Roess et al., 2018).

In a previous study, our group collected data on the nutritional values and lists of ingredients in 96 IF marketed worldwide (Cancalon et al., 2023). Statistical analysis of this dataset revealed considerable variability in nutritional values due to the diversity of raw materials used. The main differences were between northern countries that mainly use cereals (wheat, oats, maize, rice), and southern countries that include legumes (soy, groundnuts) in IF formulations. Lipid content was particularly affected by these differences, with levels ranging from 0.2 to 18.8 g lipid/100 g IF. These results are in line with those of the analyses conducted by (Moustiés et al., 2019) on several IF from southern countries. (Dimaria et al., 2018) also showed that among 32 IF marketed in Benin, Burkina Faso, Ghana and Senegal only 8 had an adequate lipid content. Moreover, some commercial IF had ω6/ω3 ratios of over 30, exceeding international recommendations according to which the optimal range is between 5 and 15 (Moustiés et al., 2019). The imbalance in this ratio is particularly a result of an imbalance of the essential fatty acids (FA) content with an excess of linoleic acid (LA, C18:2 ω6) or insufficient of α-linolenic acid (ALA, C18:3 ω3). It can reduce the efficiency of the bioconversion of ALA into its longer-chain derivative, docosahexaenoic acid (DHA, C22:6 ω3), through competition with the metabolic pathways of synthesis and impact infant development. This imbalanced ω6/ω3 can be efficiently reduced by enriching staple food with climate smart crops naturally rich in omega 3. Our dataset, in line with the above-mentioned study by (Moustiés et al., 2019), highlighted the high prevalence of micronutrient overages, particularly of vitamins A and E. The aim of these voluntary overages is to compensate for any losses that occur during the storage of IF, thereby guaranteeing regulatory content until the expiry date despite the sensitivity of these compounds to oxidation and ignorance of the potential adverse effects of these neoformed oxidation compounds.

Indeed, lipid oxidation is a major concern in food products as it is one of the primary causes of alteration (Cancalon et al., 2022). While its mechanisms have been extensively studied in emulsified and bulk systems, few studies have focused on low-moisture systems like IF (Gumus & Decker, 2021). Thus there is a gap in current knowledge concerning the propagation of oxidation, which is assumed to differ from high moisture systems, based on different water activity and content. Water activity and content influence both the mass transfer and reactivity of the compounds (Nelson & Labuza, 1992). Lipid oxidation can occur *via* different chemical pathways, in processed products the most common is auto-oxidation (Schaich, 2005). However, the presence of intrinsic oxidative enzymes in some flours such as lipoxygenases and of numerous photo-sensitive compounds in plants could favor other oxidation pathways, such as enzymatic oxidation and photo-oxidation. Plant-based ingredients are also sources of a variety of natural antioxidant compounds that could offer protection against lipid oxidation. The antioxidant properties of leafy vegetables such as amaranth and some cereal bran are well known, and have been attributed in particular to the presence of respectively, carotenoids and a diversity of phenolic compounds (Fioroni et al., 2023; Huang & Lai, 2016; Santos et al., 2021; Sarker et al., 2020; Sarker & Oba, 2019). However, knowledge is currently lacking concerning the impact of their mechanisms of action in low moisture systems.

This study aimed to advance our understanding of lipid oxidation mechanisms in low-moisture systems as a function of various sources of exogenous natural antioxidants (leaves or bran) to IF presenting an optimized lipid profile thanks to the use of climate smart crops (Pinel et al., 2024). To this end, an IF composed of a mixture of soy but including also climate smart crops rich in essential fatty acids (LA and ALA), *i.e.* teff and cowpea flours, was formulated by linear programming to obtain a lipid profile that meets the needs of infants. This IF was then declined in several formulations to characterize and understand their mechanisms of oxidation. All IF were processed with similar granulometric profile to enable comparison of oxidative mechanisms which can be influenced by granulometry. An IF included heat-treated cowpea flours to inactivate lipoxygenase activity which is high in this flour source. Another IF was additionally fortified in long chain polyunsaturated fatty acid (LC-PUFA) including arachidonic acid (ARA, C20:4, ω6) and DHA. Two other IF were added with either plant or cereal fractions rich in exogenous natural antioxidants (black rice bran and powdered amaranth leaves) to improve both IF nutritional profile and oxidative stability. The evolution of the oxidation state was then evaluated during a 6-month storage period at 25 °C by monitoring primary and secondary oxidation products, fatty acid profile, vitamins E, A, and C.

2. Materials and methods

2.1. Materials

Teff (*Eragrostis tef*) and soy (*Glycine* max) flours were purchased from Le Moulin Des Moines (Krautwiller, France). Cowpea (*Vigna unguiculata*) flour and Amaranth (*Amaranthus*) leaves were purchased in local supermarkets (respectively Ô Sandaga and Le panier exotique, Montpellier, France). Black rice (*Oryza sativa*) was purchased from Bongran (Arles, France). Powders rich in ARA and DHA (ARASCO© from fungi and DHASCO© from microalgae) were a kind gift from DSM Nutritional products France (La Garenne Colombes, France). The vitamin and mineral complex (VMC), corresponded to a mixture of vitamin A (retinyl acetate, 6601.8 μg RA/g), vitamin E (α-tocopheryl acetate, 69.9 mg/g), vitamin C (sodium ascorbate, 385.4 mg/g), vitamin D (cholecalciferol, 88.6 μg/g) and iron (dried ferrous sulfate, 47.7 mg/g), and was a kind gift from DSM Nutritional Products South Africa (Isando, South Africa).

All analytical standards, reagents and solvents were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France) except protease - *B. licheniformis* (6 U/mg of protein 40 °C, pH 8.0 on casein) supplied by Megazyme (Neogen, Lansing, Michigan, United States).

2.2. Preparation of the materials

Cowpea flour was sieved to 700 μm. The coarsest fraction (> 700 μm) was ground using an MM500 ball mill (Retsch, Éragny, France) under cryogenic conditions. Grinding cycles were 30 s at 30 Hz with eight balls of 25 mm. The reground coarse fraction and the sieved fraction (< 700 μm) were mixed, then part of the cowpea flour was heat-treated for 10 min at 90 °C using a UF450 stove (Memmert, Schwabach, Germany).

Black rice bran was obtained by hulling black rice with a DMS 500N abrasive huller (Electra, Poudenas, France). Two hulling cycles were applied at a speed of 54 kg/h at 2000 rpm for the first cycle and 1000 rpm for the second. The resulting bran was then ground under cryogenic conditions with the MM500 ball mill (Retsch, Éragny, France) using a 30-s grinding cycle at 30 Hz with eight balls of 25 mm. A 10-min heat treatment at 105 °C in a UF55 stove (Memmert, Schwabach, Germany) was performed to inactivate the enzymes.

Amaranth leaf powder was obtained by freeze-drying amaranth leaves with a COSMOS (Cryotec, Saint-Gély-du-Fesc, France), followed by cryogenic grinding for 45 s at 30 Hz in the MM500 (Retsch, Éragny, France).

2.3. Formulation and assembly of the infant flours

The composition of IF was determined by linear programming according to a set of constraints to reach target nutritional values (Cancalon et al., 2023). Target values were established based on the composition of products on the market and international regulations concerning the composition of infant processed cereal-based foods. To improve their lipid profiles, the IF included climate smart omega-3 source flours, namely teff and cowpea flours. The composition of the reference IF, named OIF, is listed in Table 1. OIF was then divided into a panel of IF in order to monitor and explain their oxidative stability

Table 1
Composition of the IF and initial characterization of their vitamin contents (E, A and C) and their physico-chemical properties (PV, moisture and a_w).

	OIF	OIF_HT	OIF_HT_PUFA	OIF_HT_PUFA_BRB	OIF_HT_PUFA_AL
Composition (%w/w)					
Soy flour	24.7	24.7	23.6	22.9	22.9
Teff flour	33.7	33.7	32.1	31.2	31.2
Cowpea flour	41.5	41.5	39.6	38.5	38.5
ARA rich powder	–	–	1.6	1.6	1.6
DHA rich powder	–	–	2.9	2.8	2.8
Amaranth leaf powder	–	–	–	–	2.9
Black rice bran	–	–	–	2.9	–
VMC	0.2	0.2	0.2	0.2	0.2
Vitamin E content (µg/g lipids)					
Total	1909.9 ± 65.1^{a}	1885.0 ± 169.2^{a}	1764.0 ± 112.4^{ab}	1726.2 ± 59.9^{ab}	1581.9 ± 144.9^{b}
α-tocopherol	139.0 ± 8.5^{ab}	151.4 ± 27.7^{a}	126.8 ± 8.6^{ac}	115.4 ± 10.2^{bc}	103.7 ± 14.4^{c}
δ-tocopherol	573.1 ± 19.9^{a}	568.1 ± 51.2^{a}	533.2 ± 31.5^{ab}	501.3 ± 40.7^{ab}	544.3 ± 26.0^{b}
γ-tocopherol	1163.7 ± 47.1^{a}	1135.1 ± 89.3^{a}	1073.2 ± 73.0^{a}	1027.4 ± 38.7^{a}	949.2 ± 89.5^{a}
Vitamin A content (dry weight)					
Retinyl acetate (µg RA/g)	4.6 ± 0.1^{a}	6.3 ± 0.5^{a}	6.4 ± 1.1^{a}	4.8 ± 0.5^{a}	5.8 ± 1.2^{a}
Carotenoid (µg/g)	–	–	–	–	13.2 ± 0.9
α-carotene (µg/g)	–	–	–	–	0.9 ± 0.0
β-*carotene (µg/g)*	–	–	–	–	5.7 ± 0.3
Lutein (µg/g)	–	–	–	–	6.6 ± 0.4
Vitamin C content (mg/g of IF dry weight)					
Ascorbic acid	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	1.1 ± 0.0^{b}	1.0 ± 0.0^{c}	1.1 ± 0.0^{b}
Fatty acid (g/100 g of IF dry weight)					
Total lipids content	5.9 ± 0.2^{a}	5.8 ± 0.3^{ab}	6.6 ± 0.2^{ac}	6.4 ± 0.3^{c}	6.5 ± 0.1^{bc}
SFA	1.0 ± 0.1^{a}	0.9 ± 0.1^{a}	1.2 ± 0.0^{ab}	1.0 ± 0.1^{ab}	1.1 ± 0.0^{b}
MUFA	1.1 ± 0.1^{b}	1.0 ± 0.1^{b}	1.3 ± 0.0^{a}	1.2 ± 0.1^{a}	1.2 ± 0.0^{a}
PUFA	3.3 ± 0.2^{bc}	3.0 ± 0.2^{c}	3.6 ± 0.0^{a}	3.3 ± 0.1^{bc}	3.4 ± 0.1^{ba}
LA C18:2 ω6	2.8 ± 0.1^{a}	2.6 ± 0.2^{ab}	2.7 ± 0.0^{ab}	2.4 ± 0.1^{b}	2.5 ± 0.1^{b}
ALA C18:3 ω3	0.4 ± 0.0^{1}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{a}
ARA C20:4 ω6	–	–	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
DHA C22:6 ω3	–	–	0.3 ± 0.0^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}
ω6/ω3	6.7	6.6	3.9	4.0	3.7
Initial characterization					
Peroxide value (meqO_2/kg of lipids)	5.3 ± 1.1^{d}	3.9 ± 1.8^{d}	12.7 ± 1.9^{c}	19.8 ± 1.5^{b}	23.8 ± 1.1^{a}
Moisture (%)	7.3 ± 0.1^{a}	6.1 ± 0.1^{bc}	5.9 ± 0.1^{b}	6.7 ± 0.2^{cd}	6.3 ± 0.1^{bc}
a_w	0.52 ± 0.00^{a}	0.42 ± 0.01^{b}	0.43 ± 0.00^{b}	0.45 ± 0.00^{c}	0.44 ± 0.00^{d}
LOX activity (U/mg of IF)	202.9 ± 2.0^{a}	60.1 ± 23.5^{b}	–	–	–

VMC: Vitamin and mineral complex; SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; LA: Linoleic fatty acid; ALA: α-linolenic acid; ARA: Arachidonic acid; DHA: Docosahexaenoic acid; LOX: Lipoxygenase.
Different letters indicate a significant difference ($P < 0.05$) between IF.

according to different formulation parameters. Five different IF were formulated, processed and studied (the proportions of the raw materials in each are listed in Table 1):

- OIF was the reference flour based on a blend of teff, cowpea and soy flours.
- OIF_HT was the reference flour in which the cowpea flour was heat-treated, as described in the previous section.
- OIF_HT_PUFA was the reference flour in which the cowpea flour was heat-treated plus enriched with ARA- and DHA-rich powders.
- OIF_HT_PUFA_AL was the reference flour in which cowpea flour was heat-treated plus enriched with ARA- and DHA-rich powders and amaranth leaf powder.
- OIF_HT_PUFA_BRB was the reference flour in which cowpea flour was heat-treated plus enriched with ARA- and DHA-rich powders and black rice bran.

In order to be representative of marketed products and to comply with regulations, all the flours were fortified by adding a VMC at 0.2 g/100 g of IF. For each IF, all the raw materials were assembled and mixed for 5 min in a Turbula T10B powder mixer (Willy A. Bachofen AG, Muttenz, Switzerland), with a three-dimensional movement of shaking, rotating and turning to ensure homogeneous mixing. Aliquots (20 g) of IF were placed in three-layer PET/ALU/PE Doypack® sachets and stored for 6 months at 25 °C. Samples were collected at the beginning of storage (M0), after two weeks' storage (M0.5), or after 1-, 2-, 3- and 6-months' storage (M1, M2, M3, M6). Once collected, the IF samples were stored at −20 °C until analysis.

2.4. Structural characterization

2.4.1. Particle size distribution

Particle size distribution was measured by laser light diffraction using an LS 13320 (Beckman Coulter, California, USA) and air as the carrier fluid. Measurements were taken at refractive indices of 1.54 (cellulose) and 1.00 (air). The volume-weighted mean diameter (D[3,2]) and a three-point specification of D10, D50 and D90 were measured. Data were collected using Adopt Software (Beckman Coulter, California, USA).

2.4.2. Confocal laser scanning microscopy (CLSM)

IF microstructure was observed on an inverted microscope using a

CLSM system (Leica SP8, Heidelberg, Germany). The microscope was operated with two He-Ne lasers (excitation at 543 and 633 nm) and an argon laser (excitation at 488 nm) that enabled multi-imaging of a sample. A 40× water-immersion objective was used for all images. Three fluorescent dyes were used to locate, respectively, (i) apolar lipids with Lipidtox® (0.2:100 v/v, λex 488 nm – λem 590 nm, Invitrogen), (ii) polar lipids with Rd-DOPE® (1:100 v/v, 16:0 Liss Rhod PE 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamin-N-(lissamine rhodamine B sulfonyl, λex 543 nm – λem 590 nm, Aventi polar lipids) and (iii) proteins with Fast green FCF (6:100 v/v, λex 633 nm – λem 655–755 nm, Invitrogen). The three dyes and 50 µL of ethanol were added in 100 mg of IF at least 24 h before observation. Marked IF were then deposited on a glass slide just before the observations.

2.5. Chemical characterization

2.5.1. Dry matter and a_w

Dry matter was determined by measuring the difference in mass after drying 1 g of IF at in a stove at 104 °C (Memmert, Schwabach, Allemagne) for 4 h. Water activity (a_w) was determined on 1 g of infant flour at 25 °C using a PRE aw-meter (Aqualab, Hopkins, USA).

2.5.2. Folch extraction

Total lipids were extracted using the Folch method with slight modifications (Moustiés et al., 2019). Briefly, 600 mg of IF were hydrated with 2.4 mL of ultrapure water for 10 min after which 360 µL 10 N HCL and 10 mL of Folch solvent mixture were added. The samples were mixed with an IKA T18 Ultra Turrax (IKA, Staufen, Germany). Three consecutive washes with 240 µL of 0.73 % sodium chloride and 2.4 mL Folch solvent mixture were performed, with 10 min of centrifugation at 1500 rpm with a Rotina 380R (Hettich, Westphalia, Germany) between each wash with. The lower phase was collected and is hereafter referred to as the Folch extract.

2.5.3. Fatty acid profile

The FA profiles were determined by gas chromatography (GC) following methylation of Folch extracts according to the NF T30–233 standard method with slight modifications. Briefly, 500 µL sodium methylate solution with phenolphthalein were added to 6 mg of Folch extracts previously evaporated under a nitrogen stream. After heating at 65 °C for 10 min, 500 µL chlorhydric methanol were added. The mixture was again heated at 65 °C for 10 min and 1 mL of hexane and water was added. The lower phase was collected and analyzed by GC after centrifugation at 1500 rpm for 5 min with a Rotina 380R (Hettich, Westphalia, Germany). An Agilent 8860 GC (Agilent Technologies, Santa Clara, USA) was equipped with a split injector (ratio of 1/20), a CP-Cil 88 Varian capillary column (50 m × 0.25 mm with 0.2 µm thick film; Chrompack, Mid-Delburg, Netherlands), and helium (1 mL/min) was used as carrier gas. Fatty acid methyl esters (FAME) were analyzed in a flame ionization detector using Agilent software (Agilent Technologies, Santa Clara, USA). The column temperature started at 150 °C, reached 225 °C with a rise of 5 °C/min and was held for 10 min. The injector and detector temperatures were 250 °C and 270 °C, respectively. FAME were identified using an external mixture of methyl ester standards.

2.5.4. Primary oxidation products

2.5.4.1. Peroxide value. The amounts of primary oxidation compounds monitored by measuring the peroxide value (PV), were determined according to (Ferreira da Silveira et al., 2021): 5 mg of Folch extract previously evaporated under a nitrogen stream were diluted with methanol/butanol (3:7 *v*/v) and deposited in a UV-Star 96-well COC F-bottom microplate (Greiner Bio-One, Courtaboeuf, France). Then, 2.5 µL of aqueous ammonium thiocyanate (300 mg/mL) and ferrous solution (0.144 mol/L) were added in each well. After incubating the microplate at 25 °C for 10 min under stirring at 1000 rpm in a microplate thermoshaker (Grant instruments Ltd. Shepreth, Cambridge, England), absorbances were measured at 500 nm using an Infinite M200 microplate reader (Tecan, Gröedig, Austria). Data were collected using Magellan software (Tecan, Gröedig, Austria). PV were determined using a standard calibration curve of cumene hydroperoxide and are expressed as $meqO_2$/kg oil.

2.5.5. Secondary oxidation products

Secondary oxidation products were determined by measuring volatile compounds *i.e.* hexanal and pentanal (Joly et al., 2022): 2 g of IF were placed in a 10-mL vial. The dynamic headspace (DHS) method was used to extract the volatile compounds using a Gerstel autosampler (Gerstel, Mülheim an der Ruhr, Germany); 1 µL of a 0.821 mg/mL 3-heptanol solution was added as internal standard. The samples were equilibrated for 10 min at 35 °C. The headspace was swept using a nitrogen flow at 25 mL/min stirred at 500 rpm and the volatile compounds were collected on a Tenax TA trap at 40 °C. Residual water was removed with an additional purge flow at 100 mL/min at 50 °C for 1 min. Desorption and analysis were carried out by gas chromatography coupled with mass spectrometry (GC–MS) using an Agilent 7890B GC (Agilent Technologies, Santa Clara, USA). Splitless desorption of volatile compounds was performed using an automatic thermal desorption unit maintained at 50 °C and then raised to 310 °C with a rise of 120 °C/min and held for 3 min. The desorbed compounds were cryofocused using a CIS4 cooled injection system in which the temperature started from 2 °C, reached 300 °C with a rise of 12 °C/s and was held for 3 min. A DB-Wax column (60 m × 250 µm × 0.25 µm) was used. The analyzer and source temperatures were 150 °C and 230 °C, respectively. Data were analyzed using MassHunter software version B.08.00. Volatile compounds were identified using the NIST08 library (Wiley, New Jersey, USA) and their semi-quantification as assessed using the peak area of the internal standard.

2.5.6. Tocopherol contents

Four distinct tocopherol isomers (α, β, γ and δ) were quantified according to the ISO-FDIS 9936 standard (ISO, 2016). For this purpose, 4 mg of Folch extracts previously evaporated under a nitrogen stream were solubilized in hexane and analyzed using high performance liquid chromatography (HPLC) with an Ultimate 3000 system (Thermo Fisher Scientific, Waltham, USA). HPLC was equipped with a silica column (250 mm × 4.6 mm i.d., 5 µm, Delaware USA) and a fluorescence detector. Elution was performed in isocratic conditions using a mixture of hexane/dioxane (97:3 v/v) as mobile phase. The column temperature was maintained at 25 °C and the flow rate was 1.3 mL/min. Fluorescence detection was set at 296 and 330 nm for excitation and emission, respectively. The injection volume was 100 µL and the calibration curves were constructed using standard solutions of each tocopherol isomer.

2.5.7. Retinyl esters and carotenoid contents

Retinyl esters and carotenoids were quantified by HPLC. Extraction was adapted from the method described by (Moustiés et al., 2019) with slight modifications: 5 g of IF were hydrated with 100 mL ultrapure water for 10 min with magnetic stirring at 300 rpm, then 10 mL of the suspension were collected and 20 µL of protease - *B. licheniformis* (6 U/mg of protein 40 °C, pH 8.0 on casein) were added. The samples were placed in a water bath at 37 °C for 30 min with regular shaking, then 8 mL of an ethanol/hexane mixture (4:3, v/v) were added and mixed with an IKA T18 Ultra Turrax disperser (IKA, Staufen, Germany) for 30 s at 18,000 rpm. After 45 min of centrifugation at 4 °C at 8500*g* (Rotina 380R, Hettich, Westphalie, Germany), the hexanic phase was collected, evaporated under a nitrogen stream and mixed with 1 mL acetone. The extracts were filtered through a 0.2 µm Minisart SRP4 PTFE filter (Sartorius, Germany). 50 µL of extract were injected into a Thermo Scientific Ultimate 3000 HPLC system equipped with a YMC-30 column

(250 × 4.6 mm, YMC) and a photodiode array detector (Vanquish PDA, Thermo Scientific) with the injection method described in (Moustiés et al., 2019). Retinyl esters and carotenoids were identified by the combined use of their relative retention times and their absorption spectra, compared to analytical standards.

2.5.8. Ascorbic acid content

Vitamin C content was quantified by ascorbic acid determination with an UPLC system using trichloroacetic acid (TCA) in the presence of tris[2-carboxyethyl]phosphine (TCEP) as a reducing agent according to method ISO 20635:2018 (ISO, 2018). 2 g of IF were mixed with 16 mL of TCEP 25 % and 8 mL of TCA 15 %. The suspensions were homogenized for 30 s with an Ultra Turrax then 10 mL of ultra-pure water was added. After centrifugation for 10 min at 10,000 *g* at 20 °C with a Multifuge X1R (Thermo Fisher Scientific, Waltham, USA), 2 mL of supernatant was collected and mixed with 2 mL of sodium acetate solution (0.5 mol/L, pH 5.4) and 6 mL of mobile phase (sodium acetate solution (50 mmol/L, pH 5.4), decylamine (1.6 g/L), acetonitrile (1 %), TCEP (40 mg/L)) adjusted to pH 5.4. The samples and mobile phase were filtered through 0.2 µm RC. UPLC analysis was performed with an Acquity HClass system (Waters, Milford, USA) equipped with a BEH C18 column (1.7 µm, 2.1 mm × 50 mm) and a UV-VIS detector (PDA, Acquity Waters, Milford, USA). The injection volume was 5 µL. Elution was carried out under isocratic conditions at a flow rate of 0.35 mL/min. Column and injector temperatures were maintained at 25 °C and 10 °C, respectively. Detection was set at 265 nm. Ascorbic acid was identified and quantified using an analytical standard calibration curve.

2.5.9. Lipoxygenase activity

Lipoxygenase (LOX) activity was determined using a linoleic acid solution containing Tween 20 and an adjusted pH of 9.0 with NaOH as substrate (Axelrod et al., 1981; Pinel et al., 2024). 100 mg of IF were incubated with 2 mL of phosphate buffer (66.7 mM, pH 5.5) for 3 h at 4 °C under magnetic stirring. The samples were then centrifuged for 15 min at 5000*g* (Rotina 380R, Hettich, Westphalie, Germany) and filtered through 7–9 µm fast ashless Whatman paper. The samples were diluted several times with the phosphate buffer to determine enzymatic activity at different concentration: 8 to 15 µL of sample were placed in a Quartz 96-well microplate (Hellma Analytics, Paris, France), 34 µL of linoleic acid solution and 249.7 µL of phosphate buffer were added. Absorbance was read at 234 nm at 30 s intervals for 10 min using an Infinite M200 microplate reader (Tecan, Gröedig, Austria).

2.6. Statistical analysis

IF were stored in quadruplicate (one batch divided into four packages) and analyses were performed on each aliquot with two repeated measurements when possible. The results are presented as mean ± SD. Statistical significance was determined by one-way ANOVA using R software (R.2.13.0, http://cran.r-project.org). Differences were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Formulation and standardization of infant flours

IF were designed to have an optimized lipid profile. Teff, cowpea and soy flours were combined to obtain a balanced FA composition. The choice of raw materials was based on the composition of the IF produced and consumed in southern countries (Table 1) (Cancalon et al., 2023). LC-PUFA fortification of the IF was performed to further improve the lipid profile. However, as such modifications can increase sensitivity to lipid oxidation, different strategies were investigated to stabilize the newly formulated IF, including the effect of heat treatment of cowpea flour to inactivate lipoxygenases and the addition of exogenous antioxidant rich fractions (amaranth leaf powder and brown rice bran).

To enable good understanding of oxidative mechanisms all IF were standardized in terms of vitamin E and A. All IF had equivalent vitamin E contents averaging 1773.4 ± 132.4 µg/g lipids, γ-tocopherol was the main isomer averaging 1069.7 ± 85.8 µg/g lipids (Table 1). The IF were fortified with vitamin A in the form of retinyl acetate and had equivalent contents averaging 5.8 ± 0.7 µg RA/g IF. OIF_HT_PUFA_AL also contained carotenoids (α and β-carotene and lutein), due to the addition of amaranth leaf powder. Vitamin C, in the form of ascorbic acid, was supplied either by adding VMC or ARA and DHA powders containing ascorbic acid as an antioxidant. Non-enriched IF with LC-PUFA (OIF and OIF_HT) contained 0.6 ± 0.0 mg ascorbic acid/g IF, whereas IF enriched with LC-PUFA, contained almost twice as much, with an average 1.1 ± 0.1 mg ascorbic acid/g IF. Heat treatment of cowpea flour reduced both moisture and a_w, which averaged 6.3 ± 0.3 % and 0.436 ± 0.015, respectively, compared with 7.3 ± 0.1 % and 0.523 ± 0.003 in OIF containing cowpea flour that had not been heat-treated. All IF had standardized FA profiles with equivalent saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FA contents averaging 1.0 ± 0.1, 1.2 ± 0.2 and 3.4 ± 0.2 g/100 g IF, respectively. The ω6/ω3 ratios of 6.6 decreased to an average of 3.9 with the addition of LC-PUFA. These ratios are in line with those cited in international recommendations indicating an optimal ratio ranging from 5 to 15 for infants. It represents an improvement over commercial IF, whose ratios have been reported to reach 30 (Moustiés et al., 2019). These imbalances are linked to an excess of LA and low ALA content in certain raw materials (*e.g.* millet flour ω6/ω3 ratio is over 40). Based on the labels on the raw materials, protein and carbohydrate contents were estimated at 23.0 and 49.3 %, respectively. Macro- and micronutrient contents depend to a large extent on the raw materials used, the nutritional composition of flours consequently varies from one study to another. (Araro et al., 2020) reported that the protein and carbohydrate contents of complementary food based on sweet potato, brown teff and dark red kidney beans ranged from 3.65 % to 14.25 % and 67.10 % to 83.96 %, respectively, but that the lipid contents did not match recommendations. The authors concluded that it was necessary to optimize the lipid fraction of IF.

The granulometry of the IF was standardized and was similar in all systems. Fig. 1.a shows the typical particle size distribution of OIF corresponding to a multimodal distribution with 3 modes centered on 18, 96 and 269 µm, respectively. A similar particle size distribution was measured in all IF with an average specific surface area of 1294 ± 23 cm^2/mL. The organization of the compounds was assessed by CLSM as shown in Fig. 1.b for OIF. The results showed a homogeneous distribution of proteins and lipids in the grains, with a higher concentration on the surface, indicated by greater fluorescence intensity. On the other hand, amphiphilic compounds were mainly located at the periphery of the grains.

3.2. Oxidative stability of infant flours enriched in essential fatty acids

The impact of introducing ω3 essential FA-carrier flours on IF oxidative stability was assessed using an OIF and OIF_HT storage test. Cowpea which, in our study, represented 41.5 % of IF contains large amounts of ALA (15.3 ± 1.6 %) but has a high LOX activity. In their study comparing the LOX activity of 14 legumes, (Chang & McCurdy, 1985) classified cowpea in the category of legumes with the highest LOX activity (> 2000 U/mg flour). LOX is ubiquitous in both the plant and animal kingdoms, and is expressed at particularly high levels in legumes. LOX are responsible for the enzymatic oxidation pathway of PUFA, LA is generally the main substrate. LOX activity promotes the formation of hydroxyperoxides that are further degraded into metabolites such as jasmonic acid and contribute to plant defense against biotic or abiotic stresses. Several strategies are used to inactivate LOX, including heat treatment (Shi et al., 2020). The cowpea flour in OIF_HT was heat-treated to limit this oxidation pathway and reduced LOX activity more than three-fold compared to non-heat-treated IF. OIF had an activity of

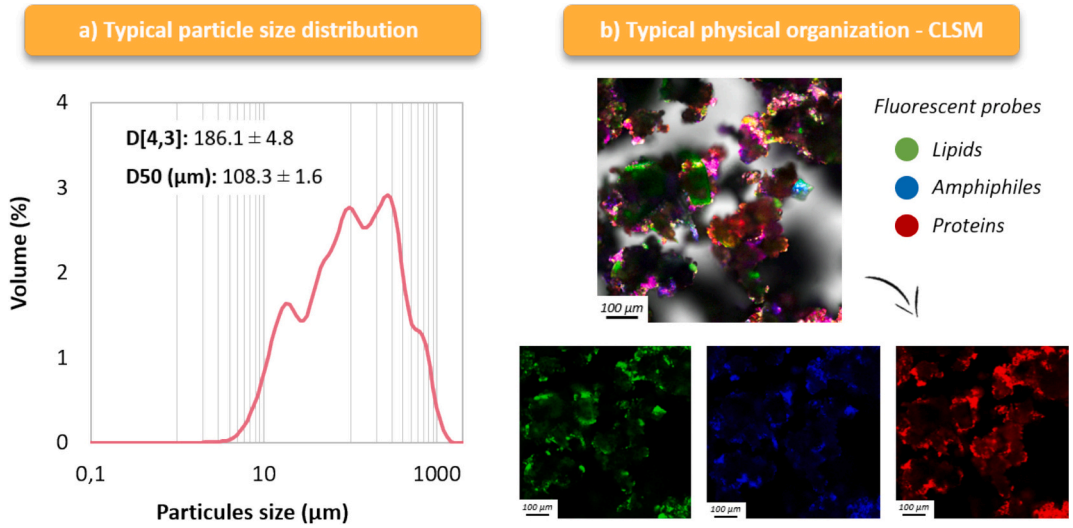

Fig. 1. a) Typical particles size distribution by laser light granulometry b) and physical structure by Confocal Laser Scanning Imaging (CLSM) of IF with the example of OIF.

202.9 ± 2.0 U/mg of flour compared with 60.1 ± 23.5 U/mg of flour for OIF_HT (Table 1). The LOX activity of soy, which accounted for 24.7 % of IF in our study, was also investigated by other authors who reported 2370 U/mg of flour at pH 6.9 (Chang & McCurdy, 1985). However, the commercial soy flour used to formulate the IF in our study was roasted, thus inhibiting the action of the enzyme in this raw material.

OIF and OIF_HT both exhibited relatively good overall oxidative stability, except for vitamin A, which underwent significant degradation in a short period of time. Fig. 2a and b show that heat treatment had no significant effect on tocopherol loss. Tocopherol content remained stable, but significant losses were observed in the two IF after 6 months of storage and reached an average of 23.6 % in OIF and OIF_HT. Considering isomer reactivity more specifically, α-tocopherol was completely degraded after 6 months of storage in both IF (Fig. 2a and b). Tocopherol losses were mainly associated with loss of γ-tocopherol (the main isomer), while δ-tocopherol remained stable throughout storage. The reactivity of tocopherol isomers is related to the nature of the matrix (Barouh et al., 2021). Indeed, a previous study by our group on the oxidative stability of liquid infant follow-on formulas showed that γ-tocopherol was the most stable isomer, while α-tocopherol was oxidized. (Athanasiadis et al., 2023) also demonstrated that α-tocopherol is more susceptible to oxidation in sunflower oil and olive pomace oil. However, (Descalzo et al., 2021) showed that α-tocopherol remained stable throughout the storage period of pecans, while γ-tocopherol decreased by 24 % after 300 days of storage at 20 °C.

The ascorbic acid content remained stable throughout storage in both IF, suggesting that it is not involved in pro- or antioxidant reactions, despite its free radical scavenging capacity (Ma et al., 2023). Barden et al. (2015) reported the lipid oxidation lag phase can be extended by using hydrophobic antioxidants in low-moisture crackers. Ascorbic acid does not appear to be mobilizable probably due to its hydrophilic nature.

Monitoring of retinyl acetate content revealed significant losses (58.5 in OIF and 43.7 % in OIF_HT) within 15 days of storage (Fig. 2a and b). Subsequently, retinyl acetate content remained relatively stable in OIF up to 6 months, and the total loss at the end of storage was 65.0 %. In OIF_HT, a more progressive loss of retinyl acetate was observed up to 6 months of storage, finally reaching 76.5 %. This result suggests that the initiation of vitamin A oxidation probably occurs during processing, although in our systems, it was added after heat treatment. In non-heat-treated oat grains, (Lampi et al., 2015) showed that lipid hydrolysis occurred immediately after milling, which favored the propagation of lipid oxidation during storage. The mixing process applied in our study, using a three-dimensional movement to ensure homogeneous raw materials, promotes contact between oxidizable lipids including vitamin A and enzymes, minerals and air. As vitamin A is particularly sensitive to heat, light and contact with oxygen (higher reactivity than vitamin E), this compound is degraded primarily (Palace et al., 1999). In their study on the stability of fortified wheat flour (Hemery et al., 2018) reported vitamin A losses of up to 85 % after 3 months of storage in PET/ALU bags under different conditions: 25 °C or 40 °C, with 65 % or 85 % relative humidity.

Fig. 3a and b show changes to primary and secondary oxidation products assessed by measuring PV and volatile compounds (hexanal and pentanal), respectively. Primary oxidation compounds remained stable over the 6-month storage period in OIF and OIF_HT, with PV below the regulatory limit in oils of 10 meqO_2/kg. Concerning volatile compounds, only a relatively low level of hexanal was detected in OIF after 6 months of storage. Therefore, despite the significant degradation of vitamin A, both peroxide value and volatile compounds remained stable and quite low. This suggests that oxidation mainly occurs through the autoxidation of vitamin A, which is known to form a wide variety of oxidized compounds not derived from peroxyl radicals, explaining the minimal evolution of peroxide and volatile compounds. The low amount of generated peroxyl radicals is expected to be neutralized by antioxidants, likely tocopherols, as a gradual loss of total tocopherol content was measured (Wei et al., 2021).

Finally, monitoring changes in the FA profile in both IF revealed a non-significant loss of PUFA up to 3 months and a limited but significant loss of on average 9.2 % PUFA at the end of 6 months of storage (Fig. 4a). This loss was attributed to a loss of LA in OIF. However, no significant loss of LA occurred in OIF_HT. As mentioned above, LA is the main substrate for LOX, its loss in OIF could therefore possibly have occurred *via* the enzymatic oxidation pathway.

3.3. *Impact of fortifying infant flours in LC-PUFA on oxidative stability*

Both DHA and ARA play an essential role in infant cognitive and retinal development (Agostoni, 2008; Carlson et al., 2019; Gil et al., 2003; Makrides et al., 1995). These LC-PUFA are derived by endogenous biosynthesis from precursor FA, *i.e.* LA for ω6 and ALA for ω3. In our study, OIF_HT was fortified with DHA and ARA (from respectively, algal and fungal sources) to further optimize the lipid profile for infant needs. As expected, LC-PUFA fortification modified the FA profile by lowering the ω6/ω3 ratio (Fig. 4b). LA content decreased during storage to a limited extent and at a pace similar to that in OIF (11.4 % of loss at the

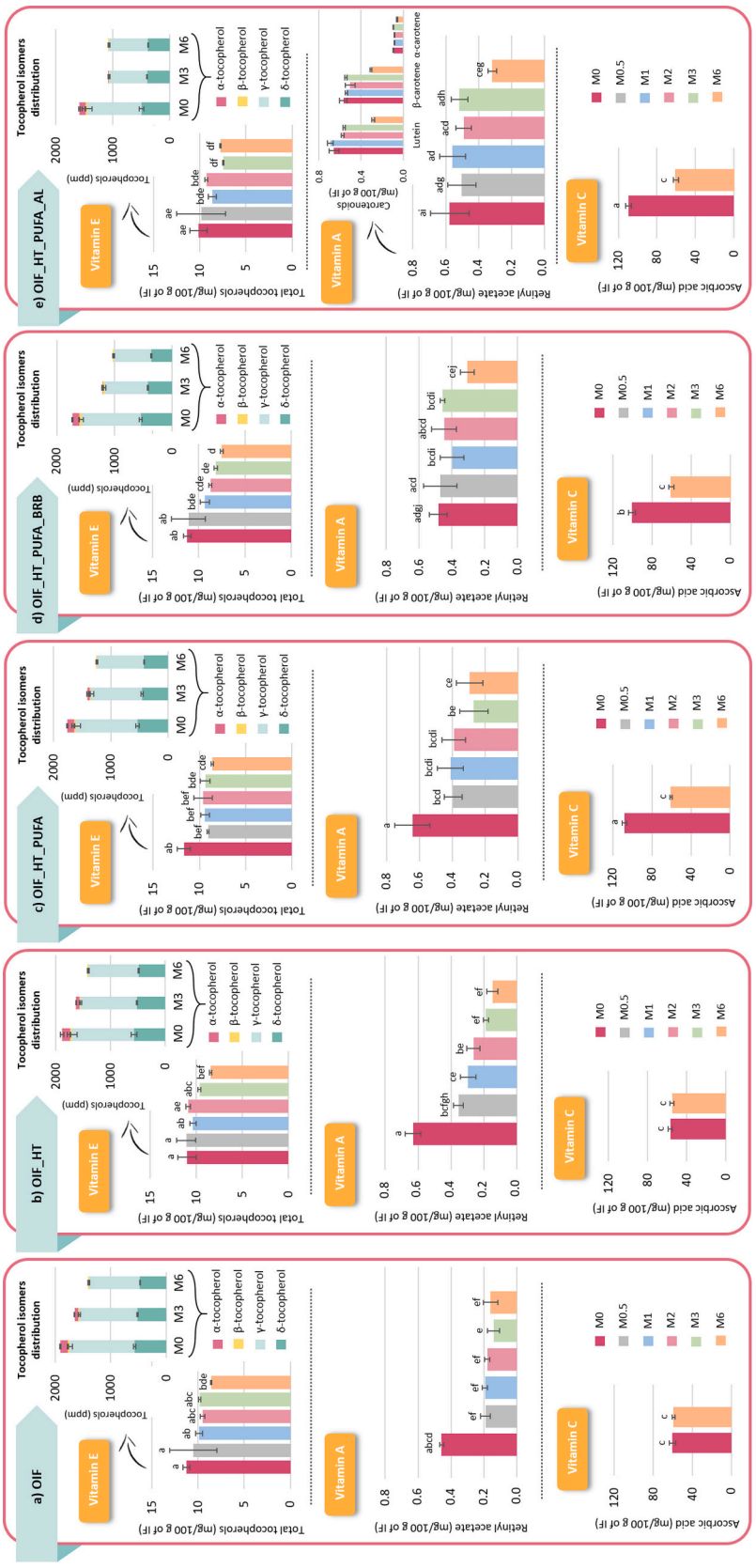

Fig. 2. Changes in vitamin E, A and C contents during storage.
Different letters indicate a significant difference ($P < 0.05$) between IF and time.

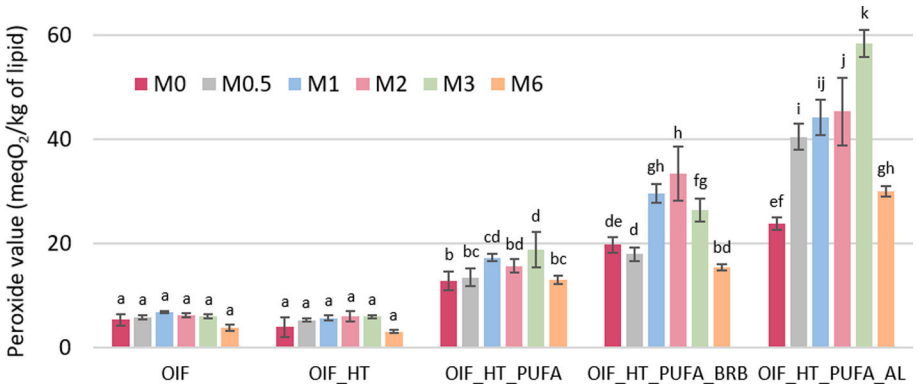

Fig. 3. Evolution of peroxide value over the 6-month storage period.
Different letters indicate a significant difference ($P < 0.05$) between IF and time.

Fig. 4. Changes in the IF PUFA profiles.
Different letters indicate a significant difference ($P < 0.05$) between time in an IF.

end of 6 months of storage). In addition, this fortification was expected to reduce oxidative stability due to the high sensitivity of LC-PUFA to lipid oxidation, but the results showed no reduction of ARA and DHA contents in OIF_HT_PUFA. While fatty acid loss might be more subtle, quantifying lipid oxidation compounds and the loss of tocopherol content provides a reliable way to assess the overall quality and stability of a food product (Shahidi & Zhong, 2010; Frankel, 2005).

A loss of 22.6 % of total tocopherols was measured after 15 days of storage, mainly associated with a loss of γ-tocopherol (Fig. 2c). From then on, tocopherol contents remained stable throughout storage, with a loss of 26.4 % measured after 6 months.

The dynamics of retinyl acetate loss in OIF_HT_PUFA was similar to that observed in OIF_HT, with significant losses over the first 15 days (Fig. 2c); retinyl acetate content then remained stable until the third month of storage, and reached a total loss of 54.2 % at the end of 6 months of storage. However, in OIF and OIF_HT the loss of vitamin A

after storage was more significant, suggesting that PUFA may have acted as scavengers of reactive oxygen species, thereby reducing their availability and protecting vitamin A from oxidation.

OIF_HT_PUFA showed a higher level of PV post-processing than OIF_HT (Fig. 3). PV then underwent limited changes over time up to 6 months of storage. Concerning secondary oxidation compounds, only relatively low level of hexanal was detected after 6 months of storage (Table 2). These results reinforce the hypothesis that oxidation initiation occurs mainly during processing which promotes contact between unsaturated lipids and oxidation-initiating factors (enzymes, minerals and oxygen), and that the propagation rate is then slow due to the limited diffusion of compounds in low moisture systems (Wei et al., 2021). An a_w range of around 0.2 to 0.4 leads to the formation of a monolayer of bound water that has a protective effect by limiting direct contact with oxygen due to its lower solubility in water than in oil fractions (Cuvelier et al., 2017). Ascorbic acid, which is hydrophilic, is probably located at the level of the water monolayer. Monitoring of ascorbic acid content showed a loss of 44.3 % after storage (Fig. 2.c). Thus, a two-fold increase in its content leads to greater losses. This compound could become pro-oxidant with the increase in its concentration or is potentially more mobilized for oxygen quenching than for its metal chelation properties. Indeed, (Barden & Decker, 2016) pointed out that the antioxidant effect *via* metal chelation is restricted in low-moisture foods because of the limited mobility of transition metals, resulting in their minimal contribution to pro-oxidant mechanisms.

3.4. Introducing an exogenous antioxidant rich fraction impacted oxidation mechanisms and induced different pathway of oxidation depending on the fraction

OIF_HT_PUFA was enriched in natural antioxidant rich fractions to improve its stability. Black rice bran for its wide range of phenolic compounds were added to OIF_HT_PUFA_BRB (Santos et al., 2021) and amaranth leaf powder for its carotenoid content to OIF_HT_PUFA_AL.

The addition of an exogenous antioxidant rich fraction delayed retinyl acetate losses up to 3 months of storage. Only 36.8 % of retinyl acetate loss was measured in OIF_HT_PUFA_BRB after 6 months of storage, suggesting black rice bran has a strong stabilizing effect on vitamin A (Fig. 2d). Several authors have reported the vitamin A-stabilizing agent properties of cereal bran including wheat, oat and rice (Rohfritsch et al., 2021; Rosa et al, 2013). Van Wayenbergh et al. (2020) showed that rice bran effectively improves the stability of vitamin A in the form of retinyl palmitate during long-term storage. A 35 % retention of vitamin A was observed after 8 weeks of storage at 60 °C with 68 % relative humidity in the presence of rice bran, whereas in the absence of bran, it was completely degraded after less than one week. The same authors showed that the efficacy of cereal bran as an antioxidant was enhanced by heat-treated leading to bran lipase inactivation and stabilization against extensive lipolysis (Van Wayenbergh et al., 2023). Their efficacy was not associated with a single compound, but rather with a wide range of antioxidant compounds that could act synergistically, such as tocopherols and phenolic compounds, of which high levels are present in cereal bran (Van Wayenbergh et al., 2023). The authors also reported that wheat bran was more effective than rice bran, due to its higher content of phenolic compounds, despite the significant ester-linked with polysaccharides or conjugated with the mono- or oligosaccharide fraction. In our study, we used black rice bran, which is richer in antioxidant compounds: with a total of 89 phenolic compounds belonging to flavonoids (52 %), phenolic acids (33 %), other polyphenols (8 %), lignans (6 %) and stilbenes (1 %) (Santos et al., 2021).

Similarly, vitamin A stabilization results were demonstrated in OIF_HT_PUFA_AL, with a retinyl acetate loss of only 44.9 % after 6 months of storage (Fig. 2e). The addition of amaranth leaf powder also provided carotenoids, including two carotenes (α- and β-carotene) and a xanthophyll (lutein). These compounds are known for their antioxidant capacity, particularly in emulsified systems (Cancalon et al., 2023; Dimakou & Oreopoulou, 2012), and were probably responsible for the improved vitamin A stability in OIF_HT_PUFA_AL. The proximity of the compounds in the system due to their equivalent polarity gives carotenoids their protective effect. The antioxidant capacity of β-carotene is associated with its singlet oxygen quenching or free radical scavenging effect. Studies in emulsified systems have also shown that carotenoids, and in particular β-carotene, act synergistically with tocopherols to promote regeneration (Barouh et al., 2021; Heinonen et al., 1997). Lutein, with its system of 11 conjugated double bonds, also has important antioxidant properties, thereby improving vitamin A stability. Changes in carotenoid contents showed that the degradation of carotenes only started in the sixth month of storage, and reached losses of 37.6 % and 46.0 % for α and β-carotene, respectively. In the case of lutein, losses were observed in three stages: content remained stable for one month, followed by stable loss averaging 13.5 % up to the third month of storage, and finally a significant accumulated loss of 56.4 % after 6 months.

Like for OIF_HT_PUFA, the results showed higher post-processing PV than for non-enriched with LC-PUFA IF (Fig. 3). For OIF_HT_PUFA_BRB, the changes were not as clear-cut, and remained relatively stable during the 6 months of storage, despite a slight increase. A clear increase in PV was measured in the presence of the powdered amaranth leaves in OIF_HT_PUFA_AL. Furthermore, monitoring of volatile compounds showed the presence of relatively low levels of hexanal after 3 months of storage in OIF_HT_PUFA_BRB and the presence of both hexanal and pentanal in OIF_HT_PUFA_AL. A slight increase was measured after 6 months of storage, with higher overall content of volatile compound in OIF_HT_PUFA_AL. These results suggest that the addition of amaranth leaves destabilized the system by accelerating the production of primary and secondary oxidation compounds. These observations may be attributed to the presence of chlorophyll in the amaranth leaves, whose content depends on the species and has been reported to range from 428.17 to 735.54 mg/kg fresh weight (Sarker et al., 2024). Indeed, a negative correlation between chlorophyll content and oxidative stability has already been demonstrated in rapeseed and coconut oils (Li et al., 2019; Rukmini & Raharjo, 2010). Chlorophyll is capable of initiating photo-oxidation even at very low levels (*i.e.* < 0.1 mg/kg). Moreover, the rate of photo-oxidation is up to 1000 to 1500 times faster than autoxidation (Ghanbari et al., 2012). Thus, initiation of this oxidation pathway probably occurred during processing following exposure to light (IF stored in light-barrier bags), leading to electronic excitation of chlorophyll. The excitation energy favors the conversion of ground state triplet oxygen ($^3O_2$) to highly reactive excited state singlet oxygen ($^1O_2$), which promotes lipid oxidation and consequently favors the formation of oxidation compounds.

Our results showed similar tocopherol loss that reached a rate of 33.2 % in OIF_HT_PUFA_BRB and of 23.2 % in OIF_HT_PUFA_AL after 6 months of storage (Fig. 2d and e). Like in other IF, tocopherol losses were associated with loss of the γ-tocopherol isomer and of the totality of α-tocopherol isomer. Tocopherols are probably mobilized for their free radical scavenging and oxygen quenching properties. In addition, they are able to act synergistically with other compounds for example, with phenolics and carotenoids by combining their properties or regenerating

Table 2
Evolution of volatile compounds over the 6-month storage period.

	ng eq. 3-heptanol/g of IF			
	Hexanal		Pentanal	
	M3	M6	M3	M6
OIF	*N.D.*	6.3 ± 1.2^{ac}	*N.D.*	*N.D.*
OIF_HT	*N.D.*	*N.D.*	*N.D.*	*N.D.*
OIF_HT_PUFA	*N.D.*	4.6 ± 0.7^{ad}	*N.D.*	*N.D.*
OIF_HT_PUFA_BRB	4.1 ± 0.7^{a}	11.1 ± 0.9^{cb}	*N.D.*	*N.D.*
OIF_HT_PUFA_AL	5.9 ± 1.3^{ab}	12.8 ± 2.0^{b}	38.3 ± 3.7^{a}	47.8 ± 4.4^{b}

Different letters indicate a significant difference ($P < 0.05$) between IF and time.

effects (Ma et al., 2023). Our results also revealed ascorbic acid losses of 39.9 % in OIF_HT_PUFA_BRB and of 45.1 % in OIF_HT_PUFA_AL, similar to losses in OIF_HT_PUFA suggesting that the addition of antioxidant fraction has no influence on this compound.

Finally, our results showed a loss of LA in OIF_HT_PUFA_BRB after 6 months for whereas the FA profile in OIF_HT_PUFA_AL did not change at all during storage (Fig. 4b).

Overall, our results show that phenolic compounds are particularly effective in limiting oxidation in granular systems, in contrast to (Barden et al., 2015), who reported that lipid oxidation was delayed more effectively by more hydrophobic antioxidants in low-moisture systems.

The antioxidant capacity of pigmented rice brans (red and black), as well as leafy vegetables, particularly amaranth leaves, has been extensively reported in the literature (Catarino et al., 2019; Fioroni et al., 2023; Jiménez-Aguilar & Grusak, 2017) and in some our previous work (Pinel et al., 2024; Santos et al., 2021). The reported values vary depending on the analytical method used (*e.g.*, DPPH, FRAP, ORAC, ABTS), the species studied, as well as factors such as preservation method and maturity stage. Nevertheless, these plant-based ingredients consistently exhibit high antioxidant activity. For example, Catarino et al. (2019) reported values of 180.8 μmol TE/g and 266.7 μmol TE/g (dry weight) for *Amaranthus hybridus* using the DPPH and FRAP methods, respectively. Jiménez-Aguilar & Grusak (2017) measured antioxidant capacities ranging from 38 to 90 μmol TE/g (fresh weight) using the ORAC method on 15 leafy *Amaranthus* species. Regarding black rice bran, Santos et al. (2021) determined EC_{50} values using the DPPH assay, expressed as the concentration of rice bran extract (mg/mL) required to scavenge 50 % of DPPH radicals (*i.e.*, the effective concentration at which 50 % of the maximum antioxidant activity is achieved), which were of 0.26 and 0.18 mg/mL depending on the deep eutectic solvent (DES) used for extraction. The authors highlighted that black rice bran extracts exhibited a higher ability to reduce DPPH radicals compared to other pigmented rice bran (red rice bran extracts) very likely due to their higher polyphenol content.

Taken together, these results suggest that oxidation initiation occurs mainly in a relatively short period of time and depends on both processing conditions and on the composition of IF. IF could be good carriers of both essential FA and LC-PUFA in the presence of endogenous fiber-associated antioxidant rich fractions and adequately controlled processing conditions.

4. Conclusion

The formulation of an IF containing climate smart crops such as cowpea and teff flours resulted in an improved lipid profile characterized by a balanced ω6/ω3 ratio in line with recommendations. In addition, micronutrient levels were adjusted in line with international regulations to avoid overages. These IF showed relatively good oxidative stability except for vitamin A, of which more than 50 % was lost in the first two weeks of storage. While heat-treatment of cowpea to inactivate lipoxygenases had fairly limited effects, the addition of LC-PUFA impacted the initial oxidation level of IF, but remained afterwards relatively stable during storage. However, a 50 % loss was observed during storage for vitamin C in IF enriched with LC-PUFA. Initiation of oxidation occurs mainly during milling process, emphasizing the importance of controlling the quality of the raw materials, as well as pre-milling (roasting or other heat-treatment), milling and post-processing conditions (heat-treatment, mixing conditions). The propagation rate of oxidation is relatively slow in low moisture systems due to the limited diffusion of the compounds. However, the vitamin loss and the formation of oxidation compounds are influenced by the addition of antioxidant rich fractions. In the absence of an antioxidant rich fraction, vitamin A was rapidly lost. The stability of vitamin A was improved with the addition of black rice bran, which provides phenolic compounds, and amaranth leaf powder, which supplies carotenoid compounds. Oxidation pathways also appear to be impacted by the addition of antioxidants, particularly in the presence of photosensitive compounds such as chlorophyll provided by amaranth leaf powder. The loss of vitamin A and oxidation pathway switches require further investigation to understand the interaction between antioxidants, unsaturated lipids and oxidation initiation factors in granular systems. The addition of black rice bran appears to be a promising strategy to improve both the oxidative stability and the nutritional profile of infant flours enriched with LC-PUFA. However, further sensory evaluation and cost analysis are necessary to assess the feasibility of incorporating black rice bran at the tested levels in commercial food formulations.

CRediT authorship contribution statement

Mathilde Cancalon: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Youna Hemery:** Writing – review & editing, Validation, Methodology. **Noémie Dormoy:** Investigation. **Nathalie Barouh:** Writing – review & editing, Methodology. **Bruno Baréa:** Resources. **Erwann Durand:** Writing – review & editing. **Reine Barbar:** Methodology, Investigation. **Carole Antoine-Assor:** Investigation. **Valérie Micard:** Writing – review & editing. **Leslie Lhomond:** Investigation. **Adrien Reau:** Investigation. **Pierre Villeneuve:** Writing – review & editing, Supervision. **Claire Bourlieu-Lacanal:** Writing – review & editing, Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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