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Stability of omega-3 enriched milk powder in different commercial packages stored under accelerated conditions of temperature and relative humidity

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ABSTRACT

The effect of two storage conditions (34 °C/83% relative humidity (RH) and 43 °C/no RH control) on the stability of whole milk powder (WMP) with added omega-3 microcapsules packed in 4 different packages was evaluated. The packages were: flexible metallised polyethylene terephthalate laminated to low density polyethylene (PETmet/LDPE); flexible bioriented polypropylene laminated to polypropylene (BOPP/PP); three-piece metal can and three-piece composite can, whose body was composed of combined materials (lamination of plastic, cellulose and aluminium foil). The results showed that oxygen consumption in the package headspace was directly related to storage conditions. A lactose crystal formation occurred in the product in BOPP/PP stored at 34 °C/83% RH and in products in metal and composite cans stored at 43 °C. After 15 weeks of storage, microencapsulation was effective in maintaining the Brazilian label claim of "source of omega-3″, regardless of the type of packaging and storage condition.

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1. Introduction

Regular intake of marine fish or omega-3 polyunsaturated fatty acids (n-3 PUFA) from these fish, eicosapentaenoic (C20: 5 n-3, EPA) and docosahexaenoic (C22: 6 n-3, DHA) is known to be beneficial to consumer health. EPA and DHA are associated with a reduced risk of cardiovascular diseases (Bonaccio et al., 2017; Endo & Arita, 2016; Lajous et al., 2013) and some types of cancer (Apte, Cavazos, Whelan, & de Graffenried, 2013; Moore, Wang-Johanning, Chang, & Johanning, 2001), good neurological function (Innis, 2008) and bone health (Högström, Nordström, & Nordström, 2007).

EPA and DHA supplementation is recommended for pregnant women because it is related to a lower risk of preterm birth (Salvig & Lamont, 2011) and to a reduction in the occurrence of allergic diseases in childhood (Best, Gold, Kennedy, Martin, & Makrides, 2016). The consumption of these fatty acids during pregnancy is possibly linked to a substantial increase in the attention span of preschool children (Gould, Makrides, Colombo, & Smithers, 2014; Ramakrishnan et al., 2016). The importance of these fatty acids to cognitive function of the brain, when consumed by school-age children, has also been demonstrated by Sheppard and Cheatham (2013). Omega-3 consumption by healthy older adults has shown positive effects on visual acuity (Stough et al., 2012) and memorisation (Külzow et al., 2016).

The diet in western countries is characterised by an unbalanced consumption of essential fatty acids. In the last century, changes in socioeconomic conditions and consequently lifestyle has led to a decrease in the consumption of naturally occurring sources of n-3 PUFA (Simopoulos & Cleland, 2004). To supplement this nutrient deficiency, various food processing companies have reoriented their businesses to develop novel functional foods based on specific ingredients with positive health consequences (Niva, 2007).

Milk and dairy product consumption is often related to a healthy and balanced diet. Milk provides energy and nutrients needed for proper growth and development, and is crucial for bone mass

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formation. Epidemiological studies confirm the nutritional importance of milk in the human diet and reinforce the possible correlations between dairy consumption and reduction of various chronic conditions such as cardiovascular diseases, some cancers, obesity and diabetes (Pereira, 2014).

As milk is a staple food in the diet of children, pregnant women and elderly people (Upadhyay, Goyal, Kumar, Ghai, & Singh, 2014), fortification of milk powder with EPA and DHA is an alternative route for supplementing these important essential fatty acids. In this context, using technologies that can combine these ingredients and maintain their functional properties throughout the shelf life of the food is necessary.

EPA and DHA are highly susceptible to auto catalytic lipid oxidation that occurs via action of free radicals in the presence of molecular oxygen (Arab-Tehrany et al., 2012). In foods high in unsaturated lipids, auto-oxidation will occur even in mild conditions and is described by initiation, propagation and termination steps involving a series of complex chain reactions (Frankel, 2014). Microencapsulation of n-3 PUFAs has been shown to be efficient in minimising the oxidative deterioration to which these fatty acids are prone, allowing them to be used in various food systems (Kaushik, Dowling, Barrow, & Adhikari, 2015).

WMP is a highly nutritious food comprised mainly of lactose (38%), fat (27%), protein (26%) and ash (6%) (Chandan, Kilara, & Shah, 2009). Among the lipids of WMP, alpha-linolenic acid (C18: 3 n-3, ALA) represents n-3 PUFA naturally found in this food (Pereira, 2014). The main causes of WMP deterioration during storage are lactose crystallisation, caking, non-enzymatic browning and lipid oxidation (Thomas, Scher, Desobry-Banon, & Desobry, 2004).

In powdered milk manufactured by spray drying, amorphous lactose forms a continuous matrix in which proteins, fat and air vacuoles are dispersed (Aguilar & Ziegler, 1994). In this matrix, water is bound to proteins and lactose. The non-crystalline amorphous state of lactose is a “non-equilibrium" condition with a large driving force towards the crystalline equilibrium state. At temperature and water content exceeding the critical values for glass transition, molecular mobility increases rapidly, resulting in lactose crystallisation (Haque & Roos, 2005; Roos, 2009).

By studying the crystallisation kinetics of lactose, Clark, Paterson, Joe, and Mcleod (2016) demonstrated that the reactive process is characterised by an “all or nothing” event, as direct measurement of crystallisation kinetics is not possible. The authors observed that once the glass transition of sugar begins, crystallisation is completed quickly. The rapid crystallisation of lactose may be due to the auto catalytic effect of the moisture released during crystallisation. The water released by lactose crystallisation or other reactions (e.g., non-enzymatic browning), as well as water absorbed from the environment surrounding the food, will also favour the formation of liquid bridging, inter-particles that result in caking of food powders (Roos, 2009).

Lactose crystallisation in milk powder helps increase the rate of non-enzymatic browning and other deteriorative changes. Non-enzymatic browning (Maillard reaction) occurs between proteins and reducing sugars found in foods. In dairy products, the reaction begins by condensing lactose with amino acid residues and involves a variety of chemical reactions that, in advanced stages, will result in the formation of high molecular weight compounds called melanoidins (Thomas et al., 2004). Lactose crystallisation can also favour lipid oxidation (Shimada, Roos, & Karel, 1991).

Various authors have studied the effect of temperature on the occurrence of milk powder degradation reactions in milk formulas containing n-3 PUFA (Cheng et al., 2017; Garcia-Martínez et al., 2010; Gonzales, Naranjo, Leiva, & Malec, 2010; Romeu-Nadal, Chavez-Servin, Castellote, Rivero, & Lopez-Sabater, 2007; Thomsen, Lauridsen, Skibsted, & Risbo, 2005; Yazdanpanah & Langrish, 2013) and it is well known that the reaction rate is more rapid and pronounced at higher storage temperatures. Generally, studies are conducted with samples packed in non-commercial packages (glass vials, capsules or pouches made of plastic materials with non-quantified gas and water vapour barrier properties).

The aim of this study was to evaluate the effect of two accelerated storage conditions (34 °C/83% relative humidity (RH) and 43 °C/variable RH, as humidity was not controlled but monitored) on the stability of WMP with added omega-3 microcapsules (fish oil rich in EPA and DHA) and packaged in 4 commercially available packaging systems for a total of 15 weeks of storage.

2. Material and methods

2.1. Materials

Bovine WMP was produced by Itambé, Minas Gerais, Brazil. Powder contained approximately 23% total lipids and 90 mg 100 g^{-1} of ALA, α-linolenic acid (C18: 30 n-3). The moisture content was 3.6% and water activity was 0.305.

Fish oil microcapsules rich in EPA and DHA omega-3 fatty acids were produced by BASF, Denmark. The microcapsules contained 60% total lipids, 10.15 g 100 g^{-1} of DHA + EPA and 0.5 g 100 g^{-1} of ALA. The microcapsule moisture content was 1.4% and water activity was 0.252.

The characteristics of the packages used in this study were represented in Table 1.

2.2. Preparation and packaging of samples

WMP and omega-3 microcapsules were blended in two stages: the premixture and the final blend. In the premixture stage, the total amount of omega-3 microcapsules was added to 20% (w/w) of the total amount of WMP. The pre-mixture stage was performed in a Kitchenaid ProLine Mixer (model KSSS; Kitchenaid, Benton Harbot, MI, USA) with mixer blades, mixing for 240 s at 58 revolutions per min. The final blend was made by adding the premixture to the remainder of WMP wherein the ingredients were blended for approximately 200 s in a horizontal paddle blender (model MH201; Consolid, Poá, São Paulo, Brazil). The final composition of the product was 96.9% WMP and 3.1% omega-3 microcapsules.

The quality of the mixture was evaluated by the standard deviation (SD) and coefficient of variation (CV) of the concentration of EPA + DHA in samples collected at 5 different points equidistant from the paddle blender. The method of analysing the concentration of EPA and DHA is described in section 2.7. The mixture presented a SD equal to 0.01 μg of EPA + DHA 100 μg^{-1} of mixture and CV equal to 3.38%.

Portions of 400 g of the product obtained were packed in the 4 packaging systems (Table 1). The products were packaged in atmospheric air so that it was possible to quantify the oxygen consumption in headspace of the packages over time in both storage conditions.

The closure of the flexible plastic packages was evaluated by the colour solution penetration test (erythrosine 0.15%), based on the ability of a low surface tension solution to penetrate small defects and microholes, according to the procedure described by Arndt, 2001. The closure for cans (metal and composite) was evaluated by cross-sectional cuts of the seaming, which was observed in a WACO VSM II image analysis system (Accuseam 2000 Automatic Vision Seam System version 3.0; WACO, Chicago, IL, USA) according to the methodology proposed by Dantas, Anjos, Segantini, and Gatti (1996). The quality and airtightness of the closure system were evaluated by helium leak testing with a mass spectrometer detector probe (Spectrum 5000; BOC Edwards, Crawley, UK) adapted from the ASTM E499/E499 M methodology (ASTM, 2017b). No closure gaps were identified. The product packaged in the containers was stored under two accelerated conditions of temperature and RH, i.e., 34 ± 1 °C/83 ± 5% RH and 43 ± 1 °C/variable RH, as RH was not controlled, although it was constantly monitored, varying from 16 to 57.8%.

At day 0 and every 3 weeks thereafter, up to a total of 15 weeks storage, different samples were taken for each time point and analysis and the various analyses were conducted.

2.3. Package headspace oxygen content

The packages were analysed for headspace oxygen content at the initial time (day 0), immediately packaging, and every 3 weeks thereafter for a total of 15 weeks storage time. The oxygen analysis was performed using an Agilent gas chromatograph (model 7890; Agilent Technologies Inc., Wilmington, DE, USA) and results were analysed by the ChemStation/Agilent program, version B 03.01, based on standard curves made with calibration gases. This gas was collected using a hermetic syringe through a septum fixed on the different packages (Sarantópoulos et al., 2017). Three packages of each type were analysed every 3 weeks.

2.4. Water activity

Water activity was determined for 3 product samples of each package/storage condition every 3 weeks using Aqua Lab equipment (model 4TEV, Decagon, Pullman, USA) at a constant temperature (25.0 ± 0.3 °C).

2.5. Moisture content

Samples were weighed and dried in an air circulation oven (Fanem - 515/4 – C; Guarulhos, Brazil) at a temperature of 102 °C until constant residual weight was obtained between consecutive weighings. The first weighing was performed 4 h after oven temperature stabilisation (ISO/IDF, 1993). Four product samples of each package/storage condition were analysed every 3 weeks.

2.6. Colour (b^* value)

Colour was measured instrumentally using a Konica Minolta portable colourimeter (Chroma Meter CR-410; Reston, VA, USA), with CIELAB scale. Approximately 5 g of product were packed in a black circular container and ten readings of the b^* values were measured for each product sample and each package/storage condition every 3 weeks. The b^* value can vary from yellowness (+) to blueness (−). An increase in the b^* value indicates a colour change towards yellow and brown.

2.7. Concentration of n-3 fatty acids (PUFA)

To determine EPA (C20: 5 n-3) and DHA (C22: 6 n-3) concentrations, omega-3 fatty acids from the omega-3 microcapsules and ALA (C18: 3 n-3), omega-3 fatty acid found in WMP and in the omega-3 microcapsules, 5 samples of the product of each package/storage condition at each analysis period were subjected to extraction of the lipid fraction using the method described by Bligh and Dyer (1959). The extracts of each product/type of packaging/storage condition were mixed to composite a blend. After the solvent evaporated, the extract blends were subjected to esterification of the fatty acids, transforming them into methyl esters using the Hartman and Lago (1973) method. The composition of fatty acids (including EPA, DHA and ALA) was determined by gas chromatography (model 7890 G344A; Agilent Technologies Inc.) with a flame ionisation detector (GC-FID), by comparing the retention times of the fatty acids of the samples with those obtained for fatty acids standards (Supelco 37 Component FAME Mix; Bellefonte, PA, USA) (Firestone, 2014). Quantifications were performed by normalisation and transformation of the area for g 100 g^{-1}.

2.8. Morphology and microstructure

Surface morphology of the blended product was visualised using scanning electron microscopy (SEM). The samples were fixed on metal surfaces and then gold coated (four coating cycles, Baltec Sputter Coater SDC 50; Balzers, Liechtenstein) and observed through a scanning electron microscope JEOL JSM-5800LV (JEOL Ltd., Tokyo, Japan) capturing electronic images at different magnifications and using an electron beam at 10 kV.

2.9. Statistical analysis

The results of package headspace oxygen content, water activity, moisture content, colour (b^* value) and concentration of fatty acids EPA (C20: 5 n-3), DHA (C22: 6 n-3) and ALA (C18: 3 n-3), were statistically analysed by comparing the means. When the results demonstrated normality (Shapiro–Wilk or Anderson-Darling tests) and homoscedasticity (equal variances, Levene or Bartlett tests), analysis of variance (ANOVA; Tukey's multiple paired comparisons test) was used. In the case of normality and different variances, Welch's ANOVA and the paired multiple comparison tests of T2 and Games-Howell were used. In the cases of non-normality, the Kruskal–Wallis non-parametric test and Dunn's paired multiple comparison test were used. The tests were performed using the XLSTAT statistical program (version 17.06.36749, Addinsoft, Paris, France).

3. Results and discussion

Samples of product in the different packages and storage conditions were evaluated for oxygen concentration in the package headspace, water activity, moisture content, EPA (C20: 5 n-3), DHA (C22: 6 n-3) and ALA (C18: 3 n-3) concentrations and colour (b^* value), immediately after processing the mixture of ingredients (day 0) and every 3 weeks for 15 weeks total. The morphology and microstructure of the product were evaluated by SEM at day 0 and at 12 weeks.

The initial results of package headspace oxygen content did not present a statistically significant difference ($p < 0.05$) between the 4 packages and were equal to 20.73% (PETmet/LDPE), 20.66% (BOPP/PP), 20.61% (metal can) 20.67% (composite can).

Reduction in headspace oxygen content was observed throughout the storage period at 34 °C/83% RH (Table 2) in all packages. Results obtained at 15 weeks of storage were lower than those measured at day 0 ($p < 0.05$) and results showed a statistically significant difference between packages at the end of the study (week 15) ($p < 0.05$).

At 43 °C, although a slight increase in the percentage of package headspace oxygen was observed in week 3 (increase of 1.24% in relation to day 0), the product in BOPP/PP was shown to be stable through 15 weeks (Table 2). In the other analysis periods, there was no statistically significant difference ($p < 0.05$). This was expected due to the high OTR of BOPP/PP, which allowed gas exchange between the package headspace and external environment. Thus, even though oxygen was being consumed due to oxidative reactions, oxygen permeation to the inside of the package maintained 20–21% oxygen concentration in the BOPP/PP headspace through 15 weeks.

Table 1
Main characteristics of evaluated packages and their closure systems.[a]

Package	Description	OTR	WVTR	
PETmet/LDPE	Metallised polyethylene terephthalate laminated to low density polyethylene, heat sealed (Coverplast, São Paulo, Brazil)	0.731 ± 0.06	1.189 ± 0.10	
BOPP/PP	Biaxially oriented polypropylene laminated to polypropylene, heat sealed (Coverplast, São Paulo, Brazil)	1197 ± 10.53	4.160 ± 0.17	
Metal can	Three-piece can (body, top and bottom) composed entirely of metallic material (tinplate) sealed with aluminum foil-based laminate and plastic cover (Itambé, Minas Gerais, Brazil)	<0.005*	<0.0001*	
Composite can	Three-piece can whose body was composed of combined materials, characterised by the lamination of plastic, cellulose and aluminum foil. The bottom and top was made of tinplate. The top was sealed with aluminum foil-based laminate and plastic cover	<0.005*	<0.0001*	

[a] Abbreviations are: OTR, oxygen transmission rate; WVTR, water vapour transmission rate. For PETmet/LDPE and BOPP/PP, OTR [values, the mean of 2 determinations ± standard deviation, expressed in cm^3 (STP) m^{-2} day^{-1}] was measured at 23 °C, 1 atm of partial gas pressure gradient (ASTM, 2017a) using an Oxtran model 2/20 (Mocon Inc., Brooklyn Park, MN, USA).; WVTR (values, the mean of 4 determinations ± standard deviation, expressed in g water m^{-2} day^{-1}) was measured at 38 °C and 90% RH (ASTM, 2013) using a Permatran model W3/31 (Mocon Inc.). For cans, OTR [values, the mean of 2 determinations ± standard deviation, expressed in cm^3 (STP) $package^{-1}$ day^{-1}] was measured at 23 °C, 1 atm of partial gas pressure gradient (ASTM, 2014) using an Oxtran model 2/60 (Mocon Inc.); WVTR (values, the mean of 3 determinations ± standard deviation, expressed in g water $package^{-1}$ day^{-1}) was measured at 38 °C and 90% RH (ASTM, 1995) using an oven (model VC 0057; Votsch, Balingen, Germany) and an analytical balance Toledo (model XP 504; Mettler, Greifensee, Switzerland)). An asterisk indicates results corresponding to the detection limit of the equipment and analytical conditions.

A significant reduction ($p < 0.05$) in the package headspace oxygen was observed in PETmet/LDPE at week 6 of storage at 43 °C (Table 2). At week 15, the PETmet/LDPE headspace oxygen was reduced to 12.05%. The OTR of the PETmet/LDPE was about 1000-fold lower than that found in BOPP/PP (Table 1), which justifies the lower oxygen content in PETmet/LDPE.

In the cans (metal and composite), the reduction in headspace oxygen content was significant until week 9 of storage at 43 °C (Table 2) ($p < 0.05$). At week 15, oxygen content remained stable and similar to that observed in week 9. These results are consistent with the gas sealing and impermeability of the can materials that did not allow oxygen permeation. Oxidative reactions as lipid and protein oxidation consumed the oxygen found in cans headspace, reaching levels close to zero after 6 weeks of storage.

The initial Aw (day 0) of the product was 0.313, while the moisture content was 3.54 ± 0.04%.

In storage at 34 °C/83% RH, product in BOPP/PP showed an increase in Aw and moisture content (Tables 3 and 4) due to the water vapour permeability of this packaging material (Table 1) and the high RH conditions. A peak Aw in week 9 suggests that in addition to water vapour sorption, reactions were occurring to release water that was previously complexed to the food matrix. The increase in Aw is consistent with lactose crystal formation and initiation of the Maillard reaction for the product in this package, as both phenomena are associated with water release.

Table 2
Oxygen content (%, v/v) in the package headspace over time in storage condition of 34 °C/83% RH and 43 °C.[a]

Storage condition	Package	Storage period (weeks)					
		0	3	6	9	12	15
34 °C/83% RH	PETmet/LDPE	20.73 ± 0.06 ^{aA}	21.17 ± 0.13 ^{bA}	19.59 ± 0.12 ^{bB}	18.44 ± 0.27 ^{bC}	17.34 ± 0.4 5 ^{bD}	16.87 ± 0.11 ^{dD}
	BOPP/PP	20.66 ± 0.06 ^{aB}	21.69 ± 0.05 ^{aA}	20.56 ± 0.21 ^{aB}	19.88 ± 0.05 ^{aC}	18.41 ± 0.31 ^{aD}	18.64 ± 0.05 ^{aD}
	Metal can	20.61 ± 0.04 ^{aB}	21.16 ± 0.09 ^{bA}	19.07 ± 0.15 ^{cC}	18.92 ± 0.03 ^{bC}	17.89 ± 0.18 ^{abD}	17.44 ± 0.16 ^{cE}
	Composite can	20.67 ± 0.16 ^{aB}	21.41 ± 0.12 ^{bA}	19.53 ± 0.18 ^{bC}	19.51 ± 0.08 ^{aC}	18.54 ± 0.16 ^{aD}	18.23 ± 0.09 ^{bD}
43 °C	PETmet/LDPE	20.73 ± 0.06 ^{aA}	19.57 ± 0.38 ^{bA}	14.98 ± 1.85 ^{bB}	14.76 ± 1.08 ^{bBC}	13.53 ± 1.18 ^{bBC}	12.05 ± 0.24 ^{bC}
	BOPP/PP	20.66 ± 0.06 ^{aB}	21.90 ± 0.09 ^{aA}	20.48 ± 0.08 ^{aB}	20.53 ± 0.05 ^{aB}	20.73 ± 0.08 ^{aB}	20.42 ± 0.32 ^{aB}
	Metal can	20.61 ± 0.04 ^{aA}	18.98 ± 0.39 ^{bB}	12.93 ± 0.46 ^{bC}	0.65 ± 0.18 ^{dD}	0.74 ± 0.12 ^{cD}	0.40 ± 0.27 ^{dD}
	Composite can	20.67 ± 0.16 ^{aA}	19.73 ± 0.21 ^{bB}	15.81 ± 0.38 ^{bC}	2.79 ± 0.57 ^{cD}	1.60 ± 0.04 ^{cE}	2.17 ± 0.15 ^{cDE}

[a] Values are the mean of four determinations ± standard deviation; values in a column for samples for the same storage period followed by the same superscript lowercase letter and values in a row for storage periods for the same sample followed by the same uppercase superscript letter do not differ from each other at the 95% confidence level ($p < 0.05$).

Table 3
Product Aw in different packages over time in storage condition of 34 °C/83% RH and 43 °C.[a]

Storage condition	Package	Storage period (weeks)					
		0	3	6	9	12	15
34 °C/83% RH	PETmet/LDPE	0.313 ± 0.003 aAC	0.322 ± 0.002 bAB	0.328 ± 0.001 bAB	0.322 ± 0.001 bAB	0.318 ± 0.002 aAB	0.342 ± 0.001 bBD
	BOPP/PP	0.313 ± 0.003 aD	0.334 ± 0.001 aBC	0.383 ± 0.001 aB	0.575 ± 0.001 aC	0.581 ± 0.002 bCD	0.616 ± 0.001 aA
	Metal can	0.313 ± 0.003 aAB	0.306 ± 0.004 cBC	0.308 ± 0.003 cABC	0.315 ± 0.002 cA	0.300 ± 0.002 aC	0.316 ± 0.003 dA
	Composite can	0.313 ± 0.003 aAB	0.297 ± 0.002 dAB	0.297 ± 0.001 dAC	0.314 ± 0.004 cAB	0.300 ± 0.003 aAB	0.322 ± 0.002 cBD
43 °C	PETmet/LDPE	0.313 ± 0.003 aA	0.286 ± 0.003 bB	0.305 ± 0.003 cA	0.293 ± 0.006 dB	0.273 ± 0.004 aC	0.269 ± 0.001 cC
	BOPP/PP	0.313 ± 0.003 aC	0.316 ± 0.001 aC	0.305 ± 0.002 cD	0.324 ± 0.002 cB	0.311 ± 0.003 bCD	0.353 ± 0.003 bA
	Metal can	0.313 ± 0.003 aE	0.315 ± 0.004 aE	0.484 ± 0.002 aC	0.468 ± 0.002 aD	0.499 ± 0.002 aB	0.511 ± 0.001 aA
	Composite can	0.313 ± 0.003 aE	0.318 ± 0.001 aDE	0.323 ± 0.002 bD	0.453 ± 0.002 bC	0.502 ± 0.002 aB	0.515 ± 0.001 aA

[a] Values are the mean of four determinations ± standard deviation; values in a column for samples for the same storage period followed by the same superscript lowercase letter and values in a row for storage periods for the same sample followed by the same uppercase superscript letter do not differ from each other at the 95% confidence level ($p < 0.05$).

Table 4
Product moisture content (%) in different packages over time in storage condition of 34 °C/83% RH and 43 °C.[a]

Storage condition	Package	Storage period (weeks)					
		0	3	6	9	12	15
34 °C/83% RH	PETmet/LDPE	3.56 ± 0.05 aC	4.21 ± 0.05 bAB	4.56 ± 0.17 bcA	4.32 ± 0.03 bcAB	4.03 ± 0.15 bB	4.48 ± 0.15 aA
	BOPP/PP	3.56 ± 0.05 aE	4.41 ± 0.04 aD	4.99 ± 0.08 abC	5.27 ± 0.06 acB	5.28 ± 0.08 aB	5.46 ± 0.02 aA
	Metal can	3.56 ± 0.05 aD	3.90 ± 0.03 cB	4.02 ± 0.05 cA	4.07 ± 0.02 bcA	3.81 ± 0.03 cC	3.80 ± 0.01 bC
	Composite can	3.56 ± 0.05 aC	3.90 ± 0.04 cAB	3.99 ± 0.10 cA	4.00 ± 0.03 bA	3.74 ± 0.11 cB	3.80 ± 0.05 bB
43 °C	PETmet/LDPE	3.56 ± 0.05 aAB	3.43 ± 0.01 cC	3.48 ± 0.05 dBC	3.59 ± 0.04 dA	3.45 ± 0.03 cBC	3.45 ± 0.05 bBC
	BOPP/PP	3.56 ± 0.05 aC	3.76 ± 0.02 bB	3.86 ± 0.03 cA	3.94 ± 0.02 cA	3.54 ± 0.04 bC	3.46 ± 0.06 bC
	Metal can	3.56 ± 0.05 aC	3.89 ± 0.07 aB	4.32 ± 0.01 bA	4.43 ± 0.10 aA	4.34 ± 0.04 aA	4.33 ± 0.02 aA
	Composite can	3.56 ± 0.05 aD	3.96 ± 0.02 aC	3.97 ± 0.08 aBC	4.14 ± 0.07 bB	4.37 ± 0.03 aA	4.41 ± 0.10 aA

[a] Values are the mean of four determinations ± standard deviation; values in a column for samples for the same storage period followed by the same superscript lowercase letter and values in a row for storage periods for the same sample followed by the same uppercase superscript letter do not differ from each other at the 95% confidence level ($p < 0.05$).

At 34 °C/83% RH, 15 weeks, an increase of Aw in the product in PETmet/LDPE was lower than in BOPP/PP, however this increase was significant when compared with day 0 ($p < 0.05$). In metal and composite cans, no statistically significant difference ($p < 0.05$) was observed in Aw values at day 0 and at week 15 (Table 3).

In week 15, moisture content of products in metal and composite cans was lower than in BOPP/PP ($p < 0.05$) at 34 °C/83% RH (Table 4). The cans are characterised by the closure sealing and the lack of water vapour permeation through the packaging material, which justifies the results obtained. Over time, small differences in moisture content of the products in the cans in relation to day 0 could be due to the balance of the food with the RH of the package headspace.

At 43 °C, samples in metal cans showed an increase in Aw from 0.313 to 0.484 in week 6 of storage. Similar results were observed for samples in composite cans (increase from 0.313 to 0.453) in week 9 under equal storage conditions (Table 3). At 15 weeks, the Aw values of product in metal cans (0.515) and composite cans (0.511) were statistically equal to and greater than the Aw of samples in BOPP/PP (0.353) and PETmet/LDPE (0.269) ($p < 0.05$), respectively (Table 3). Thomsen et al. (2005) also showed an increase in Aw (from 0.23 to 0.46) in milk powder packed in hermetically sealed glass vials and stored at 45 °C for 147 days. The authors reported that the same did not occur in product stored at 35 °C. The authors attributed increased Aw to lactose crystallisation.

A recent study by Cheng et al. (2017) demonstrated a significant increase in Aw in infant formulas supplemented with PUFAs stored in closed containers after 48 days of storage at 55 °C and after 2 days of storage at 70 °C. The authors correlated the phenomenon to the degradation of lactose and proteins, which release water as a product of the reactions.

In the storage condition at 43 °C, product in BOPP/PP presented higher moisture content than product in PETmet/LDPE (Table 4) from week 3 to week 12 of storage ($p < 0.05$). The WVTR of the BOPP/PP film was approximately 3.5 times greater than PETmet/LDPE film at 38 °C and 90% RH (condition in which the materials were characterised for WVTR according to Table 1). A decline in moisture content of the sample in BOPP/PP was observed after week 6 of storage (Table 4). This may have occurred as a function of the RH variation of the chamber at 43 °C during the study. Although the RH of this chamber was not controlled, monitoring of this parameter showed a peak of 57.8% in week 6 and values always below 45.6% RH from week 9 of storage.

The increase in moisture of product in BOPP/PP at 43 °C by week 6 may have accelerated lactose crystallisation and, consequently, release of water previously bound to the amorphous structure of the sugar since Aw of the product in this package increased from week 12 of storage (Table 3), even considering the reduction in moisture content, which also justifies the colour change (increase in the b* value) (Table 5) for product in BOPP/PP at week 12.

At day 0, the product b* value was 20.20 (Table 5). In both storages (34 °C/83% RH and 43 °C), the non-enzymatic darkening, as shown by the changing in b* values throughout storage time, showed a similar pattern to Aw variation (Table 3).

Cheng et al. (2017) evaluated b* values in infant formulas stored at different temperatures and they also found a similar pattern of colour change and Aw variation in formulas at 55 °C. However, below this temperature, at 25 °C and 40 °C, the colour changes observed by the authors were minimal. The relationship between the intensity of yellow colour and Aw in milk powder has previously been pointed out by Stapelfeldt, Nielsen, and Skipsted (1997) in a stability study of powdered milk stored with different Aw controlled at 45 °C. Thomsen et al. (2005) observed no change in the b* values in milk powder samples stored in hermetically sealed vials at 37 °C. For samples stored at 45 °C a difference in the b* value was observed after 71 days of storage while at 55 °C, the change in

Table 5
Product colour (b* value) in different packages over time in storage condition of 34 °C/83% RH and 43 °C.[a]

Storage condition	Package	Storage period (weeks)					
		0	3	6	9	12	15
34 °C/83% RH	PETmet/LDPE	20.20 ± 0.08 aE	20.41 ± 0.01 cC	22.63 ± 0.04 cB	20.41 ± 0.02 dC	25.17 ± 0.01 aA	21.29 ± 0.01 cD
	BOPP/PP	20.20 ± 0.08 aF	20.70 ± 0.03 bE	22.96 ± 0.04 aB	22.81 ± 0.01 aC	20.82 ± 0.02 dD	28.13 ± 0.01 aA
	Metal can	20.20 ± 0.08 aF	20.70 ± 0.02 bE	22.92 ± 0.02 bA	21.50 ± 0.02 bB	21.34 ± 0.02 cD	21.40 ± 0.01 bC
	Composite can	20.20 ± 0.08 aF	20.80 ± 0.02 aE	22.92 ± 0.03 bA	20.93 ± 0.01 cD	21.87 ± 0.06 bB	21.24 ± 0.01 cC
43 °C	PETmet/LDPE	20.20 ± 0.08 aF	20.87 ± 0.02 bcE	24.18 ± 0.06 abA	21.08 ± 0.02 dD	22.19 ± 0.02 dB	21.65 ± 0.01 dC
	BOPP/PP	20.20 ± 0.08 aF	20.61 ± 0.01 cE	22.6 ± 0.02 cC	21.27 ± 0.01 cD	23.01 ± 0.01 cB	26.95 ± 0.02 cA
	Metal can	20.20 ± 0.08 aF	22.13 ± 0.02 aE	31.51 ± 0.07 aD	32.05 ± 0.03 aC	38.85 ± 0.02 aA	36.70 ± 0.01 bB
	Composite can	20.20 ± 0.08 aF	21.94 ± 0.02 abE	23.25 ± 0.02 bcD	27.17 ± 0.01 bC	36.59 ± 0.02 bB	42.15 ± 0.02 aA

[a] Values are the mean of ten determinations ± standard deviation; values in a column for samples for the same storage period followed by the same superscript lowercase letter and values in a row for storage periods for the same sample followed by the same uppercase superscript letter do not differ from each other at the 95% confidence level ($p < 0.05$).

colouration could be observed at 7 days of storage. The authors stated that increases in the b* values quantify the formation of final products of the Maillard reaction (melanoidines) and confirmed that darkening occurred simultaneously with the increase in Aw.

Saltmarch, Vagnini-Ferrari, and Labuza (1981) showed that the darkening rate at 45 °C increased rapidly in Aw 0.33, with a peak between Aw 0.44 and 0.53. The maximum rate of non-enzymatic browning coincided with the intense lactose crystallisation, which was observed by the authors using SEM. Kim, Saltmarch, and Labuza (1981) observed that the lactose crystallisation in whey powder (stored at 35 °C, closed pouches) rapidly increased Aw and accelerated non-enzymatic browning as compared with the reaction rate observed in samples stored at the same temperature, open and exposed to the environment, where Aw was constant.

ALA, as well as EPA plus DHA concentrations were expressed in milligrams of fatty acids per 100 g of product (Fig. 1).

The content of EPA plus DHA at day 0 was 324 mg 100 g^{-1} (Fig. 1a and b), while the initial concentration of ALA was 103 mg 100 g^{-1} (Fig. 1c and d).

In storage at 34 °C/83% RH, at the end of the 15-week study the concentration of EPA plus DHA ranged from 347 mg 100 g^{-1} (PETmet/LDPE sample) to 382 mg 100 g^{-1} (composite can sample). Intermediate values were observed in BOPP/PP and metal can samples (360 mg 100 g^{-1} and 357 mg 100 g^{-1} respectively), with no statistically significant difference between these samples ($p < 0.05$).

At 43 °C, 15 weeks, the BOPP/PP sample presented the lowest value for EPA plus DHA content (336 mg 100 g^{-1}), while values obtained for the metal can (365 mg 100 g^{-1}), PETmet/LDPE and composite can samples (both 370 mg 100 g^{-1}) did not differ significantly ($p < 0.05$).

Although the EPA plus DHA content showed a slight fluctuation during 15 weeks in both storage conditions, the difference between the analyses, when significant, may have been due to a variation in concentration of these fatty acids in the product.

This study was conducted in Brazil where a minimum of 40 mg of EPA plus DHA per 100 mL of ready-to-eat product is required for the product to be labelled "source of omega-3" (Brasil, 2012). The 100-mL portion ready-to-eat is prepared with 13 g of powder (WMP with added omega-3) and 90 mL of water. At 15 weeks of storage, all samples remained "source of omega-3" (EPA plus DHA ≥ 40 mg 13 g^{-1}) according to Brazilian legislation.

Up to week 3 of storage, the ALA concentration of products in BOPP/PP (109 mg 100 g^{-1}), metal can (106 mg 100 g^{-1}) and composite can (108 mg 100 g^{-1}) remained stable and without

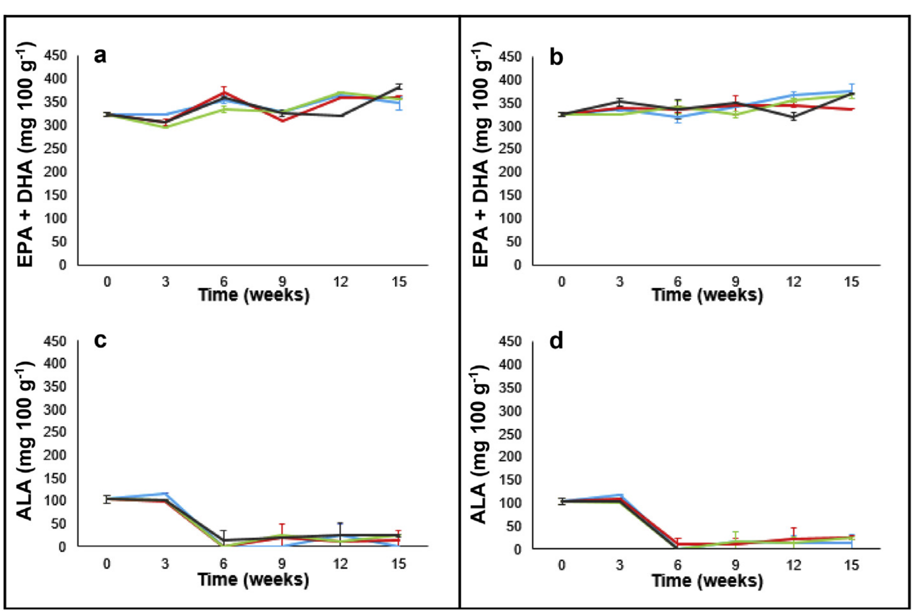

Fig. 1. EPA plus DHA (a, b) and ALA (c, d) concentration (mg 100 g^{-1}) of the product in different packages over time in storage conditions of 34 °C/83% RH (a,c) and 43 °C (b,d): blue line, PETmet/LDPE; red line, BOPP/PP; green line, composite can; black line, metal can. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

statistically significant differences ($p < 0.05$) at 34 °C/83% RH. The PETmet/LDPE sample presented ALA content of 116 mg 100 g^{-1} (Fig. 1c). As of week 3, the ALA concentration of the products in the different packages reduced about 70–90% in relation to the initial content and was not able to be detected in some samples at different analysis periods (Fig. 1c).

At 34 °C/83% RH, 15 weeks, the ALA concentration was undetectable for the PETmet/LDPE sample. The values of ALA concentration were 25 mg 100 g^{-1} and 24 mg 100 g^{-1} in the cans (metal and composite), with no statistically significant difference ($p < 0.05$), whereas the content of this fatty acid in samples in BOPP/PP was 14 mg 100 g^{-1} (Fig. 1c).

The ALA concentration in samples at 43 °C was similar to that observed in samples conditioned at 34 °C/83% RH, i.e., until week 3. In the metal and composite cans, the ALA concentration remained stable (101 mg 100 g^{-1} and 104 mg 100 g^{-1}) and was similar to that at day 0 (103 mg 100 g^{-1}) (Fig. 1d). At the same time, samples in BOPP/PP and PETmet/LDPE showed an increase in ALA concentration (109 mg 100 g^{-1} and 116 mg 100 g^{-1}, respectively. In week 6 to week 15, the ALA content was reduced by 60%–100% relative to the concentration of this fatty acid at day 0. At 43 °C, the composite can sample had no detectable value for ALA at week 15, while the PETmet/LDPE sample presented an ALA content of 13 mg 100 g^{-1}. Samples in BOPP/PP and in metal can presented results that did not differ significantly (25 mg 100 g^{-1} and 23 mg 100 g^{-1}, respectively) ($p < 0.05$) (Fig. 1d).

Garcia-Martínez et al. (2010) studied the lipid stability of infant formulas supplemented with PUFAs of vegetal origin (blend of vegetable oils) stored in plastic packaging without modified atmosphere at 25 °C, 30 °C and 37 °C. No significant changes were observed in relation to the lipid profile extracted from infant formula during the storage period, however the authors reported that samples stored at 37 °C had a rancid flavour.

Romeu-Nadal et al. (2007) evaluated the oxidative stability of the lipid fraction in formulas made with whole milk powder and PUFAs. The formulas were packed in sealed aluminium foil packages and stored at 25 °C and 37 °C for 15 months. Among the formulations evaluated, product supplemented with 0.83% of fish oil microcapsules showed stable levels for DHA over the period studied in both storage temperatures.

In the present study, regardless of storage temperature or packaging, while EPA plus DHA concentration proved to be stable for 15 weeks, ALA concentration reduced to values close to zero. Therefore, microencapsulation of fish oil high in EPA and DHA had a protective effect on omega-3 fatty acids.

The SEM images showed that the integrity of the omega-3 microcapsules was preserved from the beginning (Fig. 2) to the end of the study (Fig. 3). Although the images presented in this work were taken at week 12, similar patterns of omega-3 microcapsules characteristics were observed in the last analysis period (week 15).

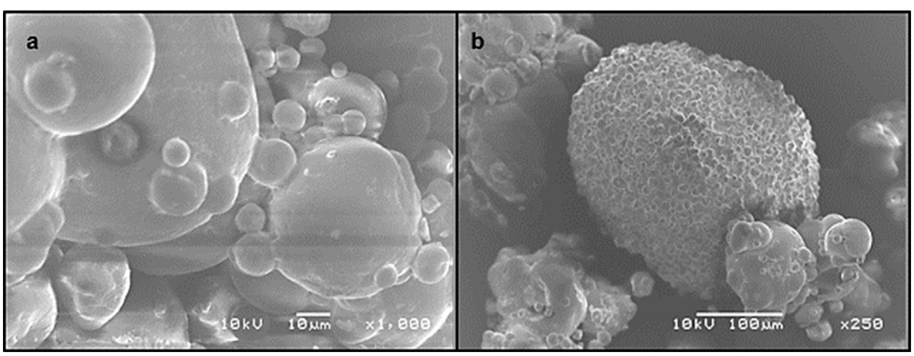

Fig. 2. Surface and microstructure morphology (SEM) of samples at the beginning of the study (day 0), where a = WMP particles, 1000 × magnification, 10 kv, bar = 10 μm and b = omega-3 microcapsules, 250 × magnification, 10 kv, bar = 100 μm.

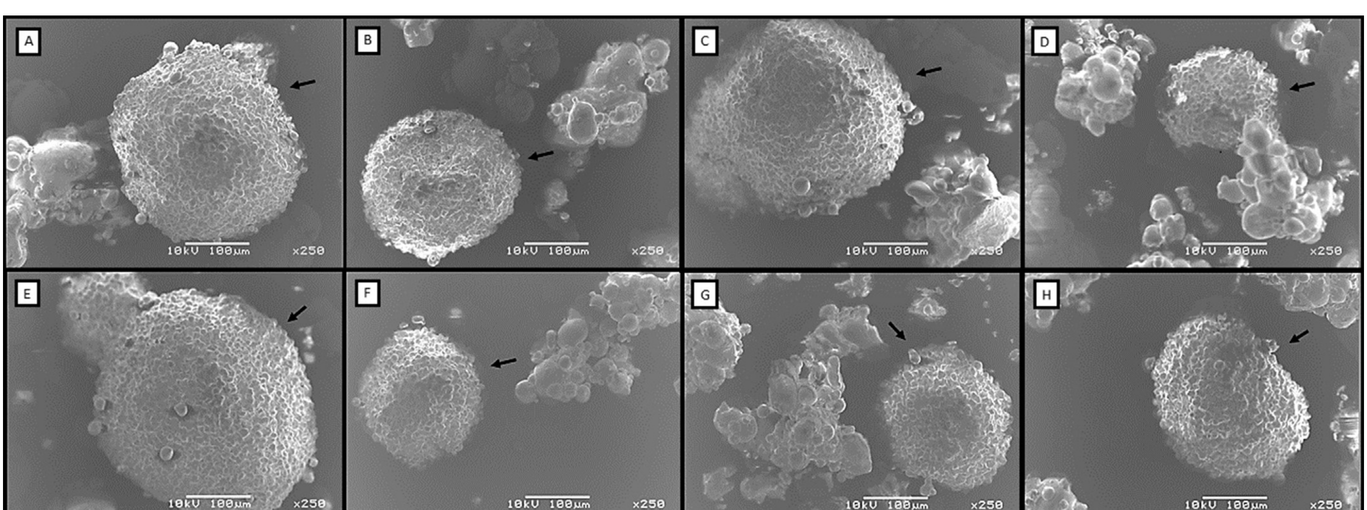

Fig. 3. Surface and microstructure morphology (SEM) of samples at week 12 of storage at 34 °C/83% RH (A, B, C and D), and 43 °C (E, F, G and H) where A and D = samples in PETmet/LDPE, B and E = samples in BOPP/PP, C and F = samples in metal can and D and H = samples in composite can. 250 × magnification, 10 kv, bar = 100 μm. The arrows show the omega-3 microcapsules.

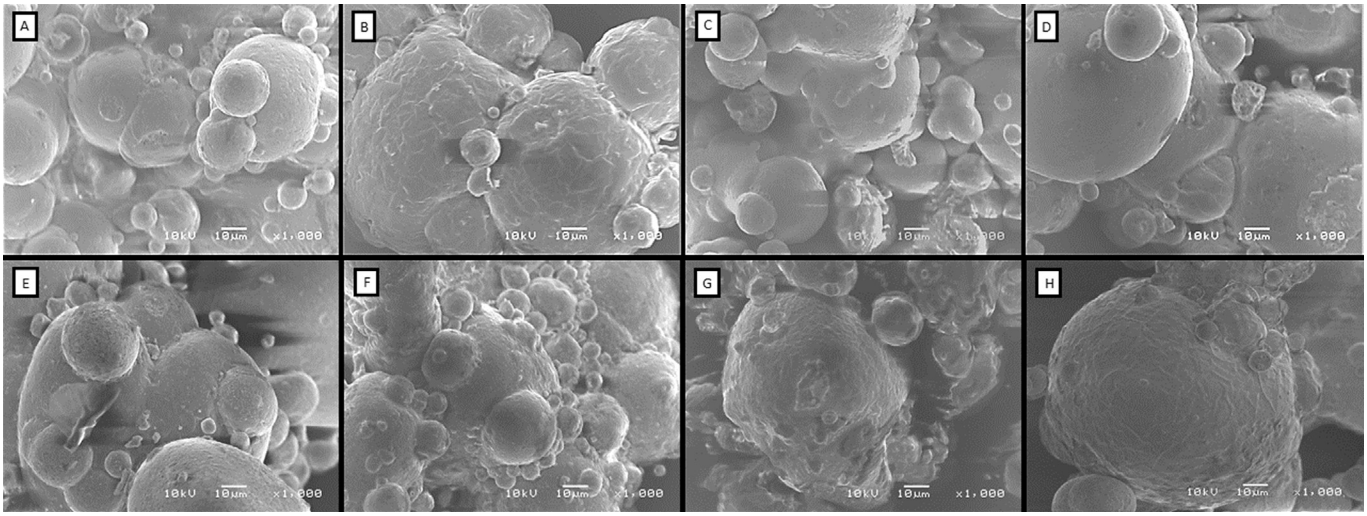

Fig. 4. Surface and microstructure microscopy morphology (SEM) of samples in week 12 of 34 °C/83% RH (A, B, C and D), and 43 °C (E, F, G and H) where A and D = samples in PETmet/LDPE, B and E = samples in BOPP/PP, C and F = samples in metal can and D and H = samples in composite can. 1000 × magnification, 10 kv increase, bar = 10 µm.

At the beginning of the study, WMP had rounded shapes and polydispersed particles. Few clusters were observed and the surface was relatively smooth and regular (Fig. 2a).

In week 12, at 34 °C/83% RH, changes in the particle surface characteristics of WMP were mainly observed in the BOPP/PP sample (Fig. 4B). The surface of the particles became rough due to the lactose crystallisation.

Similar images to those in the present study were published by Murrieta-Pazos et al. (2011). The authors evaluated the particle surface characteristics of WMP samples whose Aw varied from 0.11 to 0.97 and found that above Aw 0.43, the surface of the particles became irregular, which corresponded to the formation of lactose crystals. The crystals were not visible because they were located under the particle surface of WMP, which is composed mainly of a thin layer of fat (Murrieta-Pazos et al., 2011; Ozkan, Walisinghe, & Chen, 2002). At 34 °C/83% RH at week 12, Aw of the product in BOPP/PP was 0.581, while product in the other packages showed Aw always below 0.320.

At 43 °C, week 12, Aw of products in metal and composite packages were 0.499 and 0.502 respectively. The products in flexible packaging presented Aw equal to 0.311 (BOPP/PP) and 0.273 (PETmet/LDPE). SEM images for the products in metal can and composite can packaging are shown in Fig. 4G and H, respectively. The surface roughness of the canned samples can be observed in both images and, as the BOPP/PP product at 34 °C/83% RH, changes were presumably related to formation of lactose crystals.

Though the groove formations were less defined than those visualised for samples in cans, at 43 °C, product in PETmet/LDPE and BOPP/PP (Figs. 4E and F, respectively) also presented an irregular surface pattern at week 12.

In both storage conditions, images showed formation of clusters of milk powder particles that suggest the appearance of caking. The phenomenon may have occurred due to the increase in moisture content and Aw and probably also due to the migration of fat from the interior of the milk powder matrix to the surface, similar to that observed by Zhong et al. (2017) while heating WMP by radiofrequency energy.

4. Conclusion

Lactose crystallisation was inferred as the phenomenon of greater interference in the stability of WMP supplemented with omega-3 in different commercial packages and stored at 34 °C/83% RH and at 43 °C/without control of RH. The degradation of lactose was related to increasing moisture content and Aw in the BOPP/PP package due to the high WVTR, mainly in the storage condition of high RH (34 °C/83% RH). At 43 °C, the Tg of lactose was probably reached and crystallisation occurred in product in metal and composite cans, even though these packages were impermeable to water vapour. The change in the vitreous state of lactose promoted the release of water bound to the amorphous structure of the lactose. The sealing (hermetic) and high water vapour barrier of the cans prevented the water (released by lactose crystallisation) from permeating the packaging materials, favouring the increase in Aw and, consequently, increased non-enzymatic browning (b* value). The surface roughness of BOPP/PP samples (34 °C/83% RH) and metal and composite cans (43 °C) make it clear that lactose crystals are formed under the surface of WMP particles.

The difference between the storage conditions was also relevant in the occurrence of lipid oxidation, since at 43 °C a higher consumption of oxygen can be seen. Flexible packages were permeable to oxygen (the OTR of the BOPP/PP package is about 1000 × greater than that of the PETmet/LDPE package) so that higher contents of oxygen were kept in the headspace of these packages than in the metal and composite cans headspace. In both storage conditions and in all samples evaluated, ALA fatty acid, present mainly in milk powder, was degraded by the presence of oxygen by week 3 of storage, while the microencapsulated EPA and DHA fatty acids maintained satisfactory levels throughout the study so that the product maintained the labelling claim "source of omega-3", demonstrating that microencapsulation had a protective effect on fatty acids from fish oil.

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References

Aguilar, C. A., & Ziegler, G. R. (1994). Physical and microscopic characterization of dry whole milk with altered lactose content. 2. Effect of lactose crystallization. *Journal of Dairy Science, 77*, 1198–1204.

Apte, S. A., Cavazos, D. A., Whelan, K. A., & de Graffenried, L. A. (2013). A low dietary ratio of omega-6 to omega-3 Fatty acids may delay progression of prostate cancer. *Nutrition and Cancer, 65*, 556–562.

Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., & Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. *Trends in Food Science & Technology, 25*, 24–33.

Arndt, G. W., Jr. (2001). Examination of flexible and semirigid food containers for integrity. In *Food & drug administration. Bacteriological analytical manual online*. Silver Spring, MD: FDA. https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm072703.htm.

ASTM. (1995). *ASTM D 4279-95: Standard test methods for water vapor transmission of shipping containers-constant and cycle methods*. West Conshohocken, PN, USA: ASTM International.

ASTM. (2013). *ASTM F 1249-13: Standard test methods for water vapor transmission rate through plastic film and sheeting using a modulated infrared sensor*. West Conshohocken, PN, USA: ASTM International.

ASTM. (2014). *ASTM F1307-14: Standard test method for oxygen gas transmission rate through dry packages using a coulometric sensor*. West Conshohocken, PN, USA: ASTM International.

ASTM. (2017a). *ASTM D 3985-17: Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor*. West Conshohocken, PN, USA: ASTM International.

ASTM. (2017b). *ASTM E499/E499M-17: Standard practice for leaks using the mass spectrometer leak detector in the detector probe mode*. West Conshohocken, PN, USA: ASTM International.

Best, K. P., Gold, M., Kennedy, D., Martin, J., & Makrides, M. (2016). Omega-3 long-chain PUFA intake during pregnancy and allergic disease outcomes in the offspring: A systematic review and meta-analysis of observational studies and randomized controlled trials. *American Journal of Clinical Nutrition, 103*, 128–143.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*, 911–917.

Bonaccio, M., Ruggiero, E., Di Castelnuovo, A., Costanzo, S., Persichillo, M., De Curtis, A., et al. (2017). Fish intake is associated with lower cardiovascular risk in a mediterranean population: Prospective results from the moli-sani study. *Nutrition Metabolism and Cardiovascular Diseases, 27*, 865–873.

Brasil. (2012). *Agência Nacional de Vigilância Sanitária. Resolução RDC nº 54, de 12 de novembro de 2012. Regulamento Técnico sobre informação nutricional complementar*. Ministério da Saúde. http://portal.anvisa.gov.br/documents/%2033880/2568070/rdc0054_12_11_2012.pdf/c5ac23fd-974e-4f2c-9fbc-48f7e0a31864.

Chandan, R. C., Kilara, A., & Shah, N. P. (2009). *Dairy processing and quality assurance*. London, UK: John Wiley & Sons.

Cheng, H., Ru-Gang, Z., Erichsen, H., Soerensen, J., Petersen, M. A., & Skibsted, L. H. (2017). High temperature storage of infant formula milk powder for prediction of storage stability at ambient conditions. *International Dairy Journal, 73*, 166–174.

Clark, Z., Paterson, A. H. J., Joe, R., & Mcleod, J. S. (2016). Amorphous lactose crystallisation kinetics. *International Dairy Journal, 56*, 22–28.

Dantas, S. T., Anjos, V. D. A., Segantini, E., & Gatti, J. A. B. (1996). *Assessment of metallic packaging quality: steel and aluminium. Brazil*.

Endo, J., & Arita, M. (2016). Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. *Journal of Cardiology, 67*, 22–27.

Firestone, D. (2014). *Official methods and recommended practices of the American oil Chemists Society* (6th ed.). Urbana, IL, USA: AOCS.

Frankel, E. N. (2014). *Lipid oxidation* (2nd ed.). Cambridge, UK: Woodhead Publishing Limited.

Garcia-Martínez, C., Rodríguez-Alcalá, L. M., Marmesat, S., Alonso, L., Fontecha, J., & Márquez-Ruiz, G. (2010). Lipid stability in powdered infant formula stored at ambient temperatures. *International Journal of Food Science and Technology, 45*, 2337–2344.

Gonzales, A. P., Naranjo, G. B., Leiva, G. E., & Malec, L. S. (2010). Maillard reaction kinetics in milk powder: Effect of water activity at mild temperatures. *International Dairy Journal, 20*, 40–45.

Gould, J. F., Makrides, M., Colombo, J., & Smithers, L. G. (2014). Randomized controlled trial of maternal omega-3 long-chain PUFA supplementation during pregnancy and early childhood development of attention, working memory, and inhibitory control. *American Journal of Clinical Nutrition, 99*, 851–859.

Haque, M. K., & Roos, Y. H. (2005). Crystallization and X-ray diffraction of spray-dried and freeze-dried amorphous lactose. *Carbohydrate Research, 340*, 293–301.

Hartman, L., & Lago, R. C. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice, 22*, 494–495.

Högström, M., Nordström, P., & Nordström, A. (2007). n− 3 Fatty acids are positively associated with peak bone mineral density and bone accrual in healthy men: the NO_2 Study. *American Journal of Clinical Nutrition, 85*, 803–807.

Innis, S. M. (2008). Dietary omega 3 fatty acids and the developing brain. *Brain Research, 1237*, 35–43.

ISO/IDF. (1993). *Dried milk and dried cream: Determination of water content. International dairy Federation standard 26A*. Brussels, Belgium: International Dairy Federation.

Kaushik, P., Dowling, K., Barrow, C. J., & Adhikari, B. (2015). Microencapsulation of omega-3 fatty acids: A review of microencapsulation and characterization methods. *Journal of Functional Foods, 19*, 868–881.

Kim, M. N., Saltmarch, M., & Labuza, T. P. (1981). Non-enzymatic browning of hygroscopic whey powders in open versus sealed pouches. *Journal of Food Processing and Preservation, 5*, 49–57.

Külzow, N., Witte, A. V., Kerti, L., Grittner, U., Schuchardt, J. P., Hahn, A., et al. (2016). Impact of omega-3 fatty acid supplementation on memory functions in healthy older adults. *Journal of Alzheimer's Disease, 51*, 713–725.

Lajous, M., Willett, W. C., Robins, J., Young, J. G., Rimm, E., Mozaffarian, D., et al. (2013). Changes in fish consumption in midlife and the risk of coronary heart disease in men and women. *American Journal of Epidemiology, 178*, 382–391.

Moore, N. G., Wang-Johanning, F., Chang, P. L., & Johanning, G. L. (2001). Omega-3 fatty acids decrease protein kinase expression in human breast cancer cells. *Breast Cancer Research and Treatment, 67*, 279–283.

Murrieta-Pazos, I., Gaiani, C., Galet, L., Cuq, B., Desobry, S., & Scher, J. (2011). Comparative study of particle structure evolution during water sorption: Skim and whole milk powders. *Colloids and Surfaces B Biointerfaces, 87*, 1–10.

Niva, M. (2007). 'All foods affect health': Understandings of functional foods and healthy eating among health-oriented Finns. *Appetite, 48*, 384–393.

Ozkan, N., Walisinghe, N., & Chen, X. D. (2002). Characterization of stickiness and cake formation in whole and skim milk powders. *Journal of Food Engineering, 55*, 293–303.

Pereira, P. C. (2014). Milk nutritional composition and its role in human health. *Nutrition, 30*, 619–627.

Ramakrishnan, U., Gonzalez-Casanova, I., Schnaas, L., DiGirolamo, A., Quezada, A. D., Pallo, B. C., et al. (2016). Prenatal supplementation with DHA improves attention at 5 y of age: A randomized controlled trial. *American Journal of Clinical Nutrition, 104*, 1075–1082.

Romeu-Nadal, M., Chavez-Servin, J. L., Castellote, A. I., Rivero, M., & Lopez-Sabater, M. C. (2007). Oxidation stability of the lipid fraction in milk powder formulas. *Food Chemistry, 100*, 756–763.

Roos, Y. H. (2009). Solid and liquid states of lactose. *Advanced Dairy Chemistry, 3*, 17–33.

Saltmarch, M., Vagnini-Ferrari, M., & Labuza, T. P. (1981). Theoretical basis and application of kinetics to browning in spray-dried whey food systems. *Progress in Food & Nutrition Science, 5*, 331–344.

Salvig, J. D., & Lamont, R. F. (2011). Evidence regarding an effect of marine n-3 fatty acids on preterm birth: A systematic review and meta-analysis. *Acta Obstetricia et Gynecologica Scandinavica, 90*, 825–838.

Sarantópoulos, C. G. L., Alves, R. M. V., Coltro, L., Padula, L., Teixeira, F. G., & Moreira, C. Q. (2017). *Embalagens plásticas flexíveis principais polímeros e avaliação de propriedades*. Chapt. 11 (2nd ed.). Brasil: CETEA, ITAL.

Sheppard, K. W., & Cheatham, C. L. (2013). Omega-6 to omega-3 fatty acid ratio and higher-order cognitive functions in 7-to 9-y-olds: A cross-sectional study. *American Journal of Clinical Nutrition, 98*, 659–667.

Shimada, Y., Roos, Y., & Karel, M. (1991). Oxidation of methyl linoleate encapsulated in amorphous lactose-based food model. *Journal of Agricultural and Food Chemistry, 39*, 637–641.

Simopoulos, A. P., & Cleland, L. G. (2004). Omega-6/omega-3 essential fatty acid ratio: The scientific evidence,. *Journal of Human Nutrition and Dietetics, 17*, 165–166.

Stapelfeldt, H., Nielsen, B. R., & Skibsted, L. H. (1997). Effect of treatment, water activity and storage temperature on the oxidative stability of whole milk powder. *International Dairy Journal, 7*, 331–339.

Stough, C., Downey, L., Silber, B., Lloyd, J., Kure, C., Wesnes, K., et al. (2012). The effects of 90-day supplementation with the omega-3 essential fatty acid docosahexaenoic acid (DHA) on cognitive function and visual acuity in a healthy aging population. *Neurobiology of Aging, 33*, 824.e1–824.e3.

Thomas, M. E., Scher, J., Desobry-Banon, S., & Desobry, S. (2004). Milk powders ageing: Effect on physical and functional properties. *Critical Reviews in Food Science and Nutrition, 44*(5), 297–322.

Thomsen, M. K., Lauridsen, L., Skibsted, L. H., & Risbo, J. (2005). Temperature effect on lactose crystallization, Maillard reactions, and lipid oxidation in whole milk powder. *Journal of Agricultural and Food Chemistry, 53*, 7082–7090.

Upadhyay, N., Goyal, A., Kumar, A., Ghai, D. L., & Singh, R. (2014). Preservation of milk and milk products for analytical purposes. *Food Reviews International, 30*, 203–224.

Yazdanpanah, N., & Langrish, T. A. (2013). Comparative study of deteriorative changes in the ageing of milk powder. *Journal of Food Engineering, 114*, 14–21.

Zhong, Y., Wu, Y., Zheng, Y., Zhu, H., Liu, Z., & Jiao, S. (2017). Assessment of radio frequency heating on composition, microstructure, flowability and rehydration characteristics of milk powder. *Food Science and Technology, 37*. Article 18316.