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The Influences of Agglomeration and Storage on the Thermal Properties and Stability of Fats in Infant Formulas

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Abstract: Agglomeration is a technological process that is widely applied to obtain powdered products with the appropriate shape and particle size and different physical characteristics and stabilities. The purpose of this research was to study the influences of the composition and storage of powdered infant formulas on their thermal behaviours, as analysed by differential scanning calorimetry (DSC); fatty acid compositions, as determined by gas chromatography; and water activity and water content. This study investigated the influence of the storage time (six months) at temperatures of 20–22 °C and air humidities of 42–45% on powder mixtures and agglomerates. The isotherms of the agglomerates presented a shape and course similar to those of the isotherms of the mixtures from which they were obtained. The agglomeration process affected the stability of the fatty acids in the stored powdered infant formulas. The composition of the fatty acids changed during the storage process. The thermal properties of the powdered infant formulas were not significantly influenced by agglomeration. The compositions of the mixtures and agglomerates influenced the shape and course of the DSC diagrams. Using the DSC method, it was determined whether the fat was a natural component of the powder or it was added in the form of fatty acid preparations. Differences were observed between the shape and course of the DSC curves (heating and cooling) obtained for fresh and 6-month-stored mixtures and agglomerates.

Keywords: food powders; infant formulas; sorption isotherms; thermal properties



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1. Introduction

Mother's milk is thought to provide all the nutrients needed for normal growth and infants' digestive conditions. In the case in which breast milk is unavailable or insufficient, infant formula can guarantee infants' proper growth and development. The typical components of infant formula are carbohydrates, proteins, lipids, minerals, vitamins, and nucleotides. Infant formulas are composed of cow's milk and/or other ingredients suitable for infant feeding [1,2]. Whey protein and lactose, which are unique functional components of milk, can act as wall materials and create microencapsulated micronutrients during drying processes [3]. The process for adding bioactive substances, such as encapsulated vitamins, essential fatty acids, phenolic compounds, minerals, and enzymes, obtained by spray-drying, freeze-drying, extrusion gelation, coacervation, emulsification, and liposomal encapsulation, to breast milk and dairy milk has been discussed by Adinepour, Pouramin, Rashidinejad, and Jafari [4].

Spray-drying is known as a processing technology used in various food and nutraceutical applications [5,6]. This technology affects the physicochemical properties and stability of powder particles [7–9]. Powdered foods have the advantages of easy handling, transport, and storage and are characterised by an extended shelf life in comparison to fresh products. It should be mentioned that foods in the form of powders can have a high degree

of moisture absorption related to hygroscopicity [10,11]. Agglomeration is a technological process that allows a product to be obtained with the appropriate shape and particle size for fine-grained materials and physical characteristics different to those of powders. Agglomerated powders are more difficult to clump and remain flowable during storage, and they dissolve quickly and spontaneously. The use of powder agglomeration can reduce the environmental impact on unstable powder components. Low-moisture foods with a water activity of less than 0.85 are generally considered as being safer because of the fact that pathogens cannot grow under these conditions. It is worth mentioning that chosen pathogens can remain viable during storage and present potential health risks [12–14]. The fat content in milk can be different and influenced by the diet, breed, and lactation stage, which can result in higher contents of unsaturated fatty acids and medium-chain fatty acids [15,16]. Scanning electron microscopy (SEM), thermal analysis, and mid-infrared spectroscopy (MIR) are instrumental methods that can be applied to evaluate the quality of products submitted to the drying process [17,18].

Infant formula, which is a complex powder containing lactose, fat, and proteins, is produced in the process for spray-drying milk. [19]. Storage at an atmospheric relative humidity above 40%, influences the wettability and other physical properties of powdered formula [20]. These changes can affect the preparation time and lead to changes in the micronutrients' dispersion, which can lead to a reduction in the food quality when the formula is rehydrated and consumed by an infant [21].

Differential scanning calorimetry (DSC) is one of the methods of thermal analysis based on measuring changes in the differential heating rate and heat flow between the test material and the reference sample subjected to the same controlled temperature changes. The method also involves measuring the quantity of energy absorbed or emitted during sample heating and cooling or while the sample is kept at a constant programmed temperature [22]. DSC provides a detailed analysis of the thermal properties of food powders and has been tested on the crystallisation behaviour of powders [23].

The objective of this study was to analyse the influences of the composition and storage of powdered infant formulas on their thermal behaviour, fatty acid composition, water activity, and water content. Differential scanning calorimetry, measurements of water content and activity, and gas chromatography were applied for the analyses of the quality and shelf life of the studied products.

2. Materials and Methods

2.1. Materials

The powders used in this study were as follows: skim milk powder produced by District Cooperative Dairy in Koło, Poland, containing essential polyunsaturated fatty acids, Ropufa 10 n-3; food powder S/SD and Ropufa 10 n-6 distributed by DSM Nutritional Products Co., Ltd. in Mszczonów, Poland; and lactose and whey protein powder distributed by Hortimex Co., Ltd. in Konin, Poland, containing casein. The compositions of the mixtures and agglomerates are presented in Tables 1 and 2, respectively. Additionally, to mixtures M2 and M3, calcium citrate and magnesium citrate were added in amounts of 0.6% and 0.4%, respectively.

Table 1. The compositions of the mixtures of infant formulas.

Mixture	Skim Milk Powder (%)	Whole Milk Powder (%)	Protein (%)	Lactose (%)	Essential Polyunsaturated Fatty Acids (%)	Different Additives (%)
M1	-	47	β -lactoglobulin (10)	10	n-6: 8.5 n-3: 4.5	maltodextrin (20)
M2	14.5	-	β -lactoglobulin (5)	39	n-6: 34.0 n-3: 6.5	-

Table 1. Cont.

Mixture	Skim Milk Powder (%)	Whole Milk Powder (%)	Protein (%)	Lactose (%)	Essential Polyunsaturated Fatty Acids (%)	Different Additives (%)
M3	9.5	-	casein (10)	39	n-6: 34.0 n-3: 6.5	-

The n-3 preparation is ω -3 fatty acids microencapsulated using maltodextrin. The n-6 preparation is ω -6 fatty acids microencapsulated using maltodextrin.

Table 2. The compositions of the agglomerated infant formulas.

Agglomerate	Type of Mixture	Wetting Liquid (%)
A1	M1	15% lactose
A2	M2	Water
A3	M3	20% maltodextrin

2.2. Mixing, Agglomeration, and Drying

Technological processes, such as mixing, wet agglomeration, and drying, were involved in the production of the powder mixtures.

A fluidised bed agglomerator of the STREA 1 type produced by Niro-Aeromatic A.G., Bubendorf, Switzerland was applied for these processes. As the wetting liquids (binder solutions), a 15% lactose solution, a 20% maltodextrin solution, and water were used. A powdered sample (300 g) was placed in the product container and fluidised with the use of an upwards flowing air stream at a temperature of 50 °C for the inlet's fluidising air entering the bed. During the process of agglomeration, the fluidising air flow was increased regularly for the correct fluidisation of the enlarged agglomerates. After using up the wetting liquid, the product was dried for 15 min at 50 °C [23].

The thermal properties of the mixtures and agglomerates were examined after a storage period of six months. The mixtures and agglomerates were stored in plastic containers, with lids made of the same material, at a temperature of 20–22 °C. The relative humidity of the air was 42–45%.

2.3. Powder Properties

A total of 1 g of the sample was dried at 105 °C for 4 h to determine the water content by measuring the mass loss. The water activity was analysed by applying a Rotronic HydroLab C1 (Rotronic AG, Bassersdorf, Switzerland) at a temperature of 24 ± 1 °C. Laser diffraction using a Cilas particle size analyser 1190 (Cilas, Orleans, France) was employed to define the particle size distribution. These results are presented as the median particle size (D_{50}) [24].

2.4. Sorption Isotherms

The static gravimetric method was used to determine the water vapour adsorption isotherms. The samples were placed in desiccators and stored for 3 months at a temperature of 25 °C and at a relative humidity ranging from 0.0 to 0.9. Saturated salts, such as CaCl_2 , LiCl , CH_3COOK , MgCl_2 , K_2CO_3 , $\text{Mg}(\text{NO}_3)_2$, NaNO_2 , NaCl , $(\text{NH}_4)_2\text{SO}_4$, and BaCl_2 , with water activities of 0, 0.113, 0.225, 0.329, 0.438, 0.529, 0.648, 0.753, 0.810, and 0.903, respectively, were used as hygroscopic factors. Before the analysis, the moisture content was measured by drying the powder samples at 50 °C for 24 h [20,23,25].

2.5. Fatty Acid Composition/GC Analysis

The analysed fats were converted to volatile acids and methyl esters of fatty acids. The derivatisation process included two main stages: saponification and esterification. To determine the fatty acid profile, a YL6100 GC Clarity gas chromatograph equipped with a

flame-ionisation detector and a BPX 70 capillary chromatographic column, 60 m long, with an internal diameter of 0.25 mm and a film thickness of 0.25 μm , was involved. Nitrogen was used as the carrier gas. For the separation of the fatty acid methyl esters, the following specific thermal protocol was implemented:

- initial temperature of 70 $^{\circ}\text{C}$, maintained for 0.5 min;
- gradual temperature increase from 70 $^{\circ}\text{C}$ to 160 $^{\circ}\text{C}$ at a rate of 15 $^{\circ}\text{C}/\text{min}$;
- temperature increase from 160 $^{\circ}\text{C}$ to 200 $^{\circ}\text{C}$ at a rate of 1.1 $^{\circ}\text{C}/\text{min}$;
- temperature increase from 200 $^{\circ}\text{C}$ to 225 $^{\circ}\text{C}$ at a rate of 30 $^{\circ}\text{C}/\text{min}$;
- final temperature of 225 $^{\circ}\text{C}$, held for 1 min;
- the injector temperature was set to 225 $^{\circ}\text{C}$ and the detector temperature to 250 $^{\circ}\text{C}$.

The qualitative identification of the fatty acids in the analysed oils was carried out by comparing the retention times with those of the standards. The percentage of fatty acids was determined by calculating the area under individual peaks [26–28]. The study was performed in two repetitions.

The changes in the fatty acid content were calculated according to Formula (1).

$$FACC = \frac{(F_1 - F_2) * 100\%}{F_1} \quad (1)$$

$FACC$ —fatty acid content change;

F_1 —the content of the fatty acid in the fresh sample;

F_2 —the content of the fatty acid in the stored sample.

2.6. Differential Scanning Calorimetry—Phase Transitions and Crystallisation Curves

The infant formula powders were studied using DSC (DSC, TA Instruments Q 200, New Castle, DE, USA) in a normal pressure cell. The cell was purged with dry nitrogen at 50 mL/min. An empty pan was used as a reference in every test. The food powders (10–15 mg) were hermetically sealed in aluminium pans (volume: 30 μL). The samples were heated from -60°C up to 300°C at a heating rate of $5^{\circ}\text{C min}^{-1}$. The DSC technique was used to obtain heat flow (W/g) versus temperature curves. The DSC test for the crystallisation was performed by cooling the samples from 20 to -90°C at a cooling rate of $2^{\circ}\text{C min}^{-1}$. All the analyses were completed in triplicate [22–24].

2.7. Statistical Analysis

The data were reported as the mean $\pm$ standard deviation. One-way ANOVA was performed using Statgraphics Plus, version 5.1 (Statistical Graphics Corporation, Warrenton, VA, USA). Differences were considered to be significant at a p -value of 0.05, according to Tukey's multiple range test. The experimental design was carried out with three replications [29]. The t -test was used to determine whether the differences between the fresh and stored powder samples were statistically significant.

3. Results and Discussion

3.1. Powdered Infant Formula Properties

When determining the raw material composition of the mixtures, the fat content in the finished products was taken into account. The fat present in the mixtures came mainly from the powdered milk and n-3 and n-6 fatty acid preparations. The agglomeration process improves the physicochemical properties of the modified milk powder. Mixtures M1 and M2 were subjected to an agglomeration process using various wetting liquids. One agglomerate was obtained from the M1 mixture. Two agglomerates were obtained from the M2 mixture, while no agglomerate was obtained from the M3 mixture.

The particle size distribution plays a special role because it both indicates a relationship with the functional properties of the powder and determines the characteristics of the material during further operations and processes, e.g., pneumatic transport, dosing, and packaging [30].

Figure 1 shows the particle size distributions of mixtures M1, M2, and M3, differing in the contents of the milk powder and lactose.

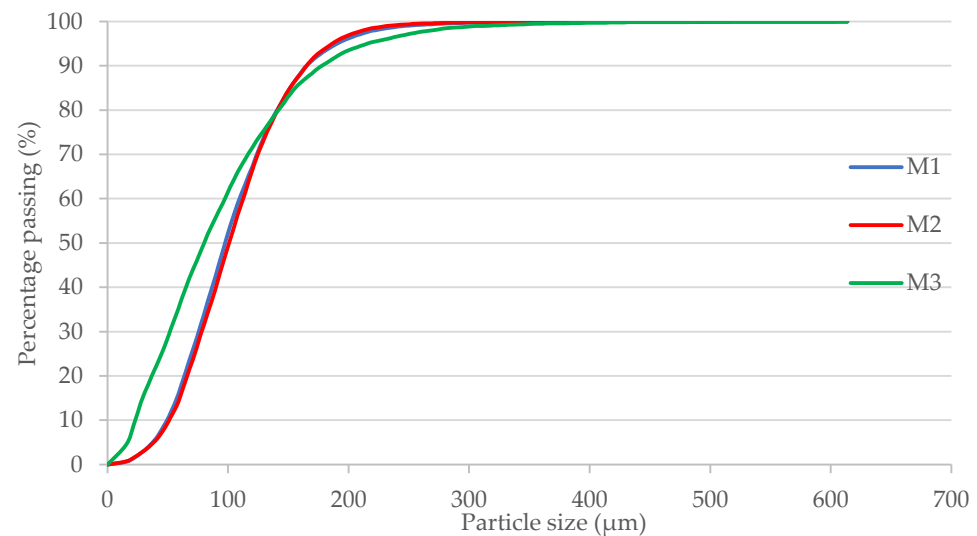


Figure 1. Particle size distributions of powdered infant formulas (mixtures).

The values of the median diameters, D_{50} , from the particle size distributions for the volume fractions of the tested mixtures were 83.2 μm for mixture M3 and 101.7 μm for the M1 and M2 mixtures (Figure 1). Mixture M3 was characterised by a lower particle diameter. Its main ingredients were lactose and casein (Table 1). Mixtures M1 and M2 were characterised by different ingredient compositions. The particle size distributions of these mixtures were the same (Figure 1). They were characterised by a higher median diameter, D_{50} . The values of the median diameters, D_{90} , from the particle size distributions of the tested mixtures ranged from 164.2 for the M1 and M2 mixtures to 181.9 μm for the M3 mixture. Mixtures M2 and M3 were characterised by similar ingredient compositions. Whey proteins (5%) were added to the M2 mixture and casein (10%) to the M3 mixture. The particle size distributions of the individual mixtures were influenced by the particle sizes of the components of these mixtures. There was no significant effect of the addition of n-3 and n-6 fatty acid preparations on the particle size distributions of the mixtures.

The particle size distribution of the powdered infant formula changed after the agglomeration process.

In Figures 2 and 3, the influence of the agglomeration of the mixtures on their particle size distribution can be observed. The material ingredient compositions of the agglomerates and wetting liquids are presented in Table 2. The differences between the median diameters, D_{50} and D_{90} , of the mixtures and agglomerates were statistically significant, while the differences between the agglomerates obtained from the same mixture using different wetting liquids (maltodextrin and water) were statistically insignificant. The type of wetting liquid (maltodextrin or water) had no influence on the particle size distribution of the agglomerates (Figure 3).

The surface moistening of particles with water has a significant impact on the increase in the particle size distribution during agglomeration, while the carbohydrate content in the wetting liquid has a lower impact. This was demonstrated in research by Bika et al. [31] on the agglomeration process of lactose and mannitol using ethyl alcohol and water as wetting solutions. Additives to the moisturising solutions included, among others, hydroxypropyl cellulose, polyvinylpyrrolidone, and selected surfactants. A similar relationship was demonstrated by Palzer [32], who examined the influence of the glassy state of amorphous food powders (e.g., maltodextrin, tomato powder, coffee, and baby formula) on the possibility of their agglomeration [33].

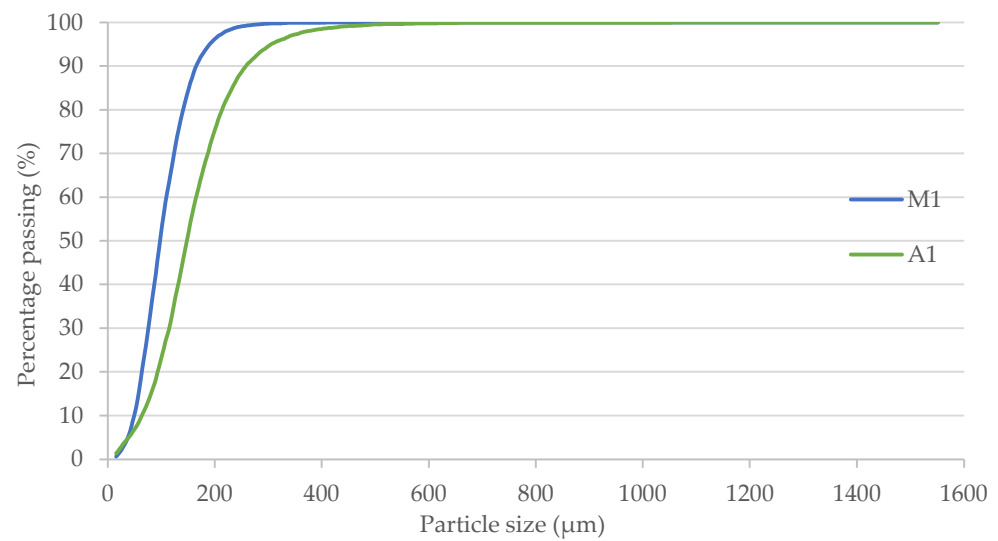


Figure 2. Particle size distributions of powdered infant formulas in mixture M1 and agglomerate A1.

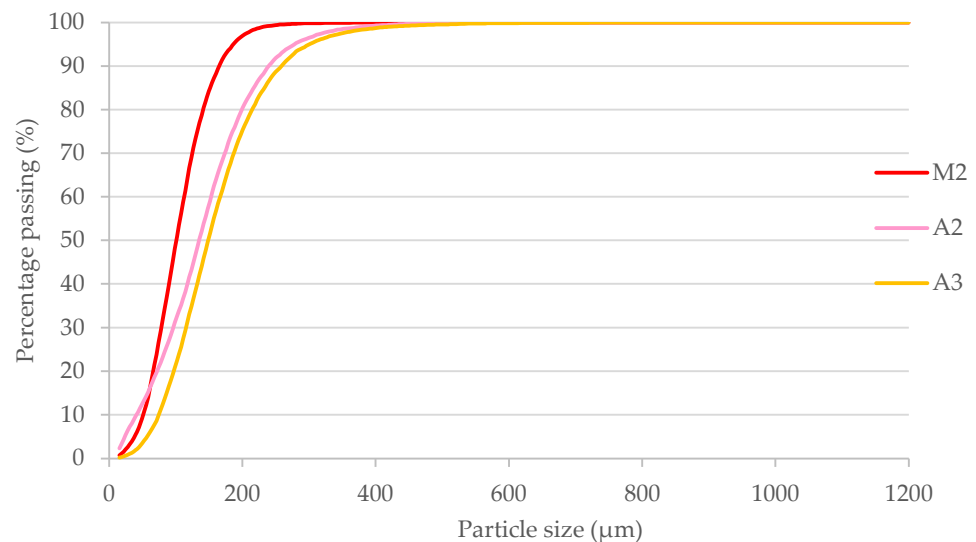


Figure 3. Particle size distributions of powdered infant formulas in mixture M2 and agglomerates A2 and A3.

The particle size compositions of the M1 mixture and the agglomerate obtained from it are shown in Figure 2. The diameter D_{50} for the M1 mixture was 95.6 μm , and for the A1 agglomerate, it was 151.1 μm (Figure 2). The diameter D_{90} for the M1 mixture was 163.7 μm , while for the A1 agglomerate, it was 249.8 μm . The particle size distributions of the M2 mixture and the agglomerates obtained from it are shown in Figure 3. The diameter D_{50} for the M2 mixture was 101.7 μm , while for the agglomerates it was 132.6 (A2) and 144.9 μm (A3) (Figure 3). The D_{90} diameter for the M2 mixture was 163.4 μm , while for the agglomerates it was 231.3 (A2) and 262.7 μm (A3).

A difference was found in the median particle sizes of the mixtures and agglomerates. The type of wetting liquid had no influence on the particle size distributions of the powdered infant formulas. Similar results were obtained in previous studies [22,23].

3.2. Water Content and Activity of Powdered Infant Formula

The water content in food may change during technological processing [34]. For the fresh powdered mixtures, the water content ranged from 2.89 (M2) to 3.62 (M1) g of H_2O /100 g of product, and for the agglomerates, it ranged from 2.69 (A3) to 4.61 g of

H₂O/100 g of product (A1). After six months of storage, the water content increased for all the studied samples (Table 3).

Table 3. Water contents and water activities of fresh and stored mixtures and agglomerates of infant formulas.

Sample	Water Content (g H ₂ O/100 g Product)		Water Activity (-)	
	Storage Duration (Months)			
	0	6	0	6
M1	3.62 ^a ± 0.03	4.07 ^b ± 0.04	0.215 ^a ± 0.002	0.264 ^b ± 0.003
M2	2.89 ^a ± 0.01	3.36 ^b ± 0.03	0.298 ^a ± 0.004	0.349 ^b ± 0.001
M3	2.93 ^a ± 0.00	3.19 ^b ± 0.03	0.241 ^a ± 0.004	0.306 ^b ± 0.004
A1	4.61 ^a ± 0.06	4.89 ^b ± 0.05	0.111 ^a ± 0.001	0.195 ^b ± 0.002
A2	3.42 ^a ± 0.02	3.86 ^b ± 0.11	0.235 ^a ± 0.002	0.278 ^b ± 0.003
A3	2.69 ^a ± 0.00	3.05 ^b ± 0.04	0.221 ^a ± 0.004	0.282 ^b ± 0.004

Values represent means ± standard deviations. Data denoted by the same lowercase letters are not statistically different ($\alpha = 0.05$) in terms of different rows ($p < 0.05$) by Tukey's test.

The mixture M2 was characterised by the highest water activity among the mixtures. Among the fresh agglomerates, A2 was characterised by the highest water activity (0.235). After a six-month storage period, the water activity of all the samples increased (Table 3).

3.3. Sorption Isotherms

Figure 4 presents the water adsorption isotherms for the mixtures of the powdered infant formulas. The course of the isotherms for all the mixtures was very similar.

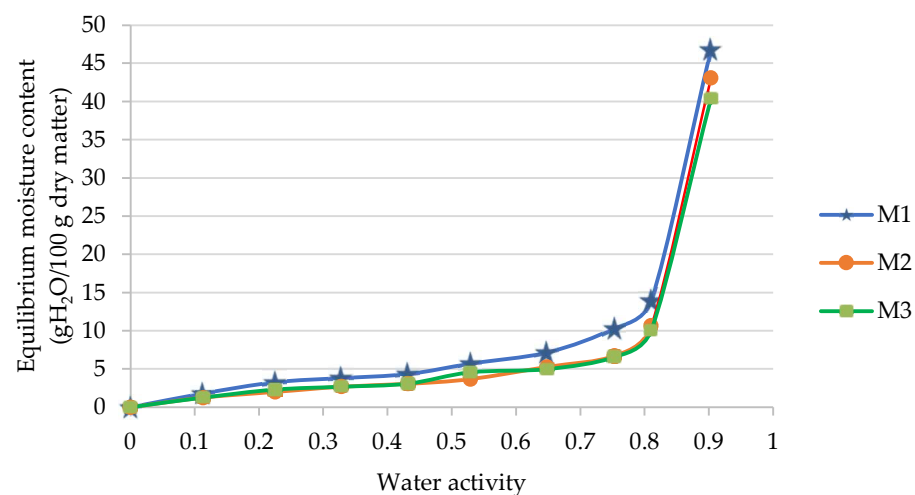


Figure 4. Sorption isotherms of powdered infant formulas (mixtures).

There were no statistically significant differences between the water contents despite the differences in the compositions of the mixtures. The amounts of water adsorbed by the M2 and M3 mixtures were at a similar level. In the M2 and M3 mixtures, the main ingredient was lactose (Table 1). The statistical differences were insignificant in the course and shape of the isotherms for all the mixtures. For all the mixtures, after reaching a water activity of 0.75, there was a sharp increase in the amount of adsorbed water (Figure 4). The lowest level of the water content at a water activity of 0.75 was observed in the M3 mixture, amounting to 6.6 g of H₂O/100 g of DM, and the highest was observed in the M1 mixture, amounting to 10.35 g of H₂O/100 g of DM (Figure 4).

Figures 5 and 6 present the influences of agglomerations using water, lactose, and maltodextrin on the courses of the water vapor adsorption isotherms for the powdered infant formulas. There was no significant effect of the wetting liquid on the amount of water absorbed by the agglomerates. At a water activity of 0.75, the A2 agglomerate (water wetting liquid) reached a level of absorbed water of 7.2 g of H₂O/100 g of DM (Figure 6). At a water activity of 0.75, the A1 agglomerate (lactose wetting liquid) reached a level of absorbed water of 9.8 g of H₂O/100 g of DM (Figure 5), while the powder agglomerated with maltodextrin, A3, reached a level of absorbed water of 7.0 g of H₂O/100 g of DM (Figure 6). Tham et al. [20] obtained sorption isotherms for three types of infant formulas with contents of lactose above 50%. The sorption isotherms for all the powders were characterised by a similar shape and course.

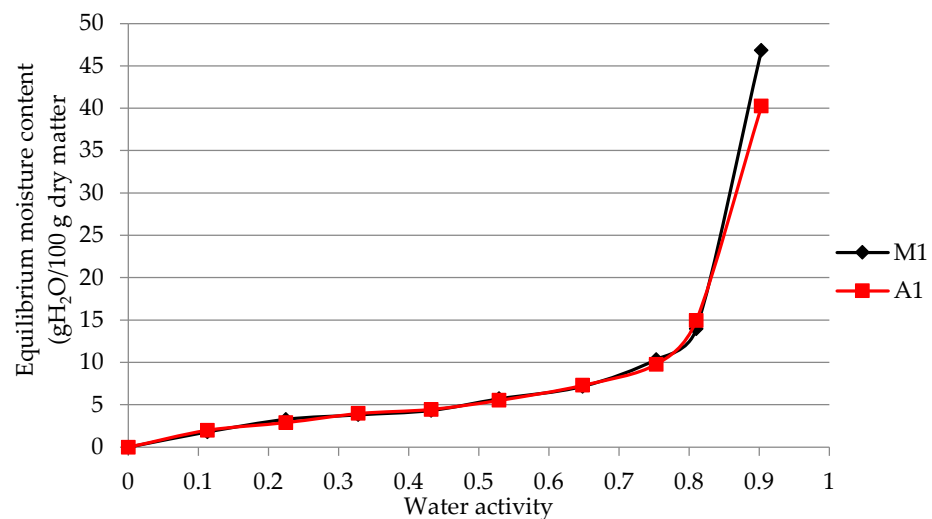


Figure 5. Sorption isotherms of mixture M1 and agglomerate A1.

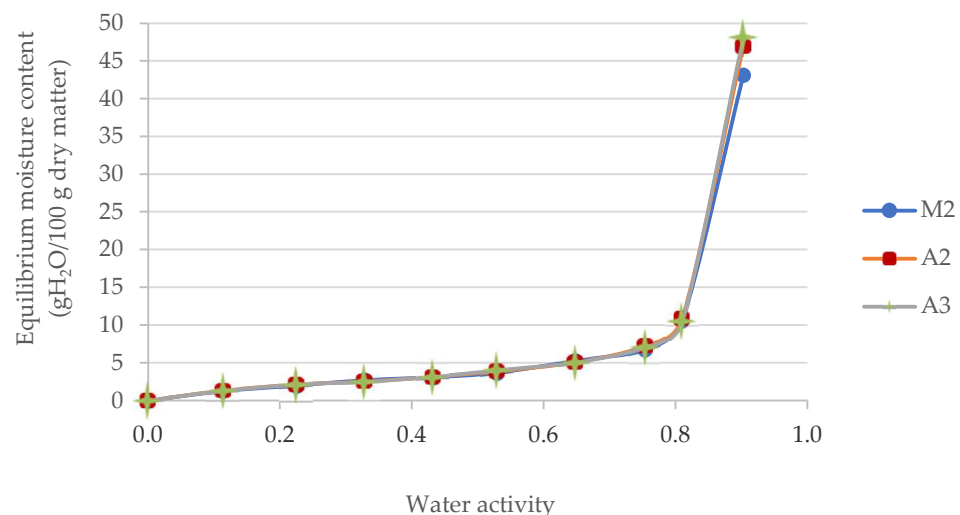


Figure 6. Sorption isotherms of mixture M2 and agglomerates A2 and A3.

Figures 5 and 6 show the influence of the agglomeration on the course of the powders' isotherms. No significant differences were found in the course and shape of the sorption isotherms for both mixtures and their agglomerates in the water activity range from 0.0 to 0.903.

The analysis of the sorption isotherms of the agglomerated products indicated a greater influence of the powders' composition than the agglomeration process itself. A

difference was observed in the amount of water absorbed by the agglomerates below and above $a_w = 0.75$, which was considered as a limit value. Kowalska et al. [35] determined the isotherms and kinetics of the water sorption of powder mixtures, glucose, whey proteins, maltose syrup, and soy isolate. They stated that mixing components with different sorption properties resulted in uniform values of the equilibrium water content that was obtained. A large amount of protein in the mixtures increased the water sorption capacity; however, the high carbohydrate content resulted in its lowering. Foster et al. [36] determined the sorption isotherms, among others, for powdered cream with fat contents of 56 and 72% at various temperatures (4, 20, 37, and 50 °C). It was concluded that the content of the milk fat did not influence the course of the powdered cream's isotherms.

3.4. Fat Content in Infant Formulas

The fat content was determined for the powdered infant formulas (Table 4). The fat content in the agglomerates reached a level similar to the fat content in the mixtures from which the agglomerates were obtained (Table 4).

Table 4. Fat contents in mixtures and agglomerates of powdered infant formulas.

Type of Mixture	Fat Content (%)	Agglomerate	Fat Content (%)
M1	19.8 ± 0.1^a	A1(M1)	20.3 ± 0.2^a
M2	14.7 ± 0.1^b	A2(M2)	14.6 ± 0.2^b
M3	14.2 ± 0.1^b	A3(M2)	14.7 ± 0.1^b

Values represent means $\pm$ standard deviations. Data denoted by the same lowercase letters are not statistically different ($\alpha = 0.05$) in terms of different rows ($p < 0.05$) by Tukey's test.

Insignificant differences were observed for the fat contents in the mixtures and their agglomerates. The A1 agglomerate was characterised by a slightly higher fat content than the M1 mixture. There were also insignificant differences in the fat contents in the agglomerates (A2 and A3). These agglomerates were obtained from the M2 mixture. In the analysed samples, statistically insignificant differences were observed in the fat content in the mixtures in relation to the fat content in the agglomerates obtained from these mixtures (Table 4).

The agglomeration process did not significantly effect changes in the fat content of the powders. There were no influences of the type and concentration of the wetting liquids (water, lactose, and maltodextrin) on the fat content. Murrieta-Pazos et al. [37] found no effect of agglomeration on the fat contents in whole and skimmed milk powder. They studied fat extracted from powdered milk and milk agglomerate. The total fat, free and enclosed in natural shells (encapsulated), contained in milk powder was determined. A layer of proteins, lactose, and fat is formed on the surface of spray-dried milk particles. The fat is surrounded by lactose and protein particles and forms "islands" on the surface [38]. There is more surface fat in whole powdered milk than in skimmed milk [37].

3.5. Influence of Storage on Content of Fatty Acids Extracted from Infant Formulas

The fatty acid composition was determined in the studied mixtures and agglomerates. The presence of fatty acids derived from milk powder, such as oleic (C18:1c) and linoleic (C18:2c), was observed in the powdered infant formulas.

Figures 7–9 present the contents of fatty acids in the studied infant formulas (mixture), fresh and after six months of storage. Table 5 shows the percentage differences in the contents of fatty acids in the powdered infant formulas due to the storage time.

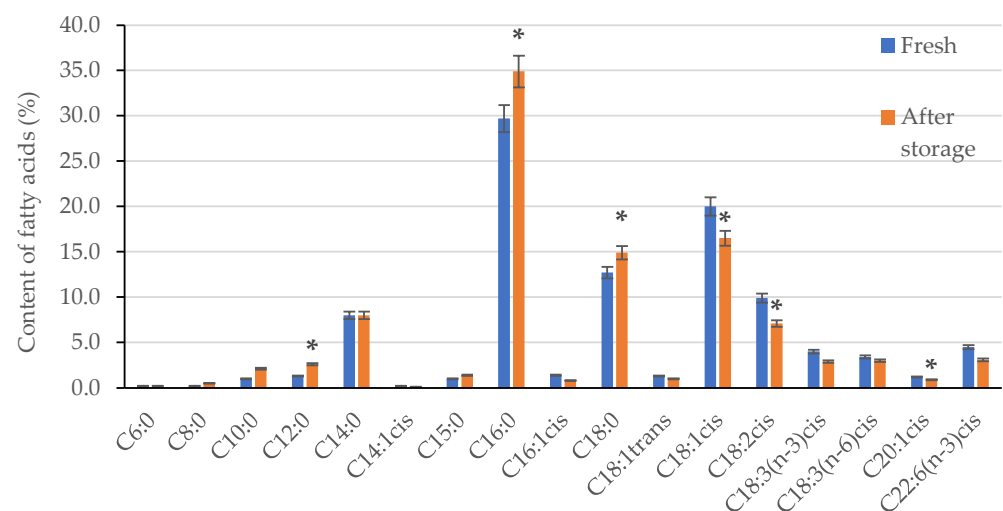


Figure 7. Fatty acid compositions in fat extracted from fresh and stored powdered infant formulas M1 (mixture). Data marked with asterisk (*) are statistically different ($\alpha = 0.05$) in terms of storage.

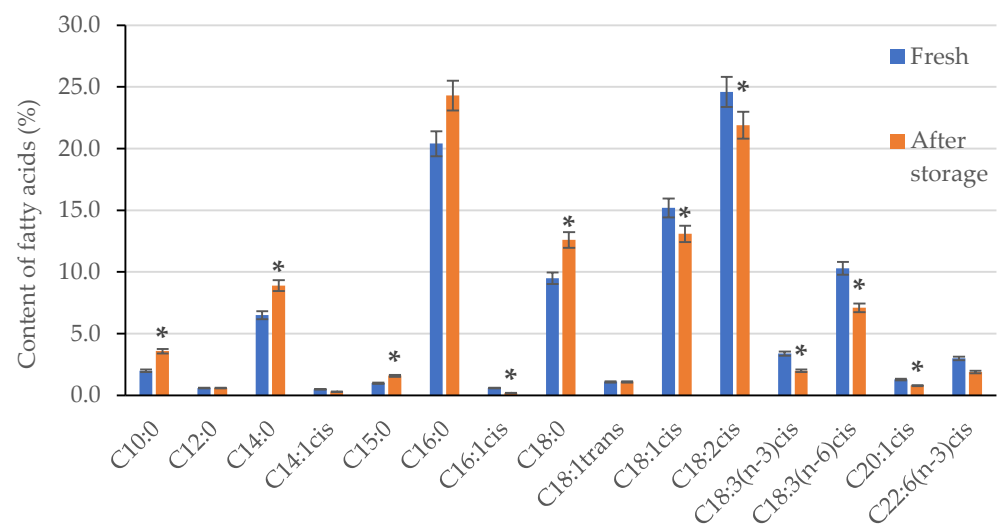


Figure 8. Fatty acid compositions in fat extracted from fresh and stored powdered infant formulas M2 (mixture). Data marked with asterisk (*) are statistically different ($\alpha = 0.05$) in terms of storage.

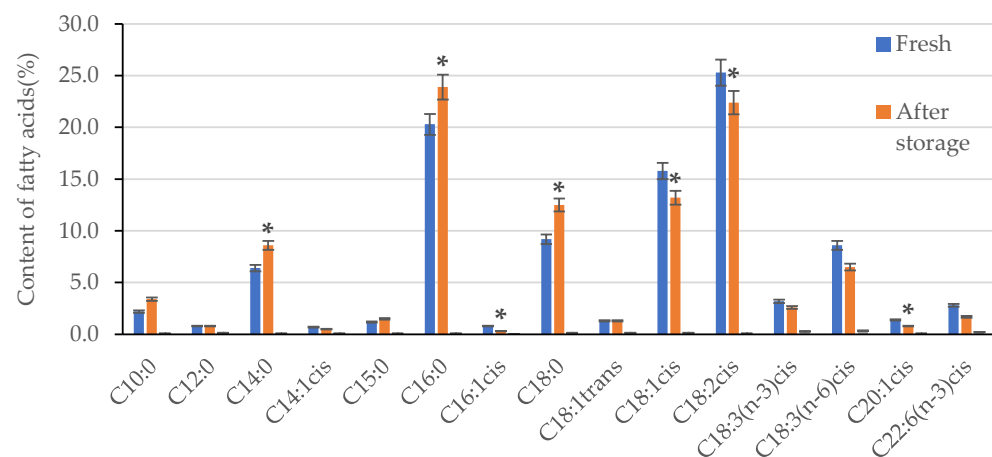


Figure 9. Fatty acid compositions in fat extracted from fresh and stored powdered infant formulas M3 (mixture). Data marked with asterisk (*) are statistically different ($\alpha = 0.05$) in terms of storage.

Table 5. Percentage differences between the contents of fatty acids in the mixtures at the beginning and after the 6-month storage period.

Fatty Acid	The Change (Beginning-6 Months of Storage) (%)		
	M1	M2	M3
C6:0 Caproic acid	0.00	-	-
C8:0 Caprylic acid	−150.00	-	-
C10:0 Capric acid	−110.00	−80.00	−54.55
C12:0 Lauric acid	−100.00	0.00	0.00
C14:0 Myristic acid	0.00	−36.92	−34.38
C14:1 Cis-9-tetradecenoic acid	50.00	40.00	28.57
C15:0 Pentadecanoic acid	−40.00	−60.00	−25.00
C16:0 Palmitic acid	−17.51	−19.12	−17.73
C16:1 Cis-9-hexadecenoic acid	42.86	66.67	62.50
C18:0 Stearic acid	−17.32	−32.63	−35.87
C18:1t Vaccenic acid	23.08	0.00	0.00
C18:1 Oleic acid	17.5	13.82	16.46
C18:2 Linoleic acid	28.28	10.98	11.46
C18:3n-6 γ -Linolenic acid	11.76	31.07	24.42
C18:3n-3 α -Linolenic acid	27.5	41.18	18.75
C20:1 Gondoic acid	25.00	38.46	42.86
C22:6n-3 Docosahexaenoic acid	31.11	36.67	39.29

Particular attention was paid to those acids that were added to the Ropufa ‘10’ n-3 and Ropufa ‘10’ n-6 preparations. The main ingredients of the Ropufa ‘10’ n-3 preparation were α -linolenic acid (C18:3 n-3) and docosahexaenoic acid (C22:6 n-3). In the Ropufa ‘10’ n-6 preparation, the main ingredient was γ -linolenic acid (C18:3 n-6). In all the analysed samples, after a 6-month storage period, the share of all the unsaturated fatty acids decreased.

McKenna et al. [39] studied whole milk powder particles (instant) using confocal and transmission electron microscopies. They found that during drying, protein particles (casein and lactoglobulin) are partially denatured and produce “hairy ball” structures. These structures form bridges with the fat surface, which leads to the fat being surrounded by proteins and forming a cluster. The protein coat (cluster) protects fats against the influence of unfavourable external conditions. Lactose (in its amorphous state) protects polyunsaturated fatty acids against environmental influences. Vega et al. [40] studied the effects of sugars (lactose and trehalose) used to microencapsulate milk fat emulsions stabilised with sodium caseinate and whey proteins. They found that the addition of a small amount of lactose combined with proteins creates a protective layer on the particle surface. This layer protects the fat on the surface of the powder particles. Vega and Roos [41] stated that sugars act as a filler in the formation of powder particles. Lactose, in its amorphous form, seals the clusters it produces with milk proteins during spray-drying [39,42,43]. The structures created in this way protect fats against the influence of unfavourable external factors causing oxidation. The contents of saturated fatty acids (Figures 7–9) in the analysed mixtures of powdered infant formulas ranged from approximately 40 to 55%. The presence of saturated fatty acids is a natural phenomenon: they come from milk fat. However, the total content of polyunsaturated fatty acids was the highest for the M2 mixture, 41.2%. The lowest content of monounsaturated fatty acids was also observed in the M2 mixture, approximately 19% (Figure 8).

After a six-month storage period, a decrease in the content of unsaturated fatty acids was observed for all the mixtures. At the same time, the content of saturated fatty acids increased (Figures 7–9). Lipid oxidation leads to the formation of many undesirable chemical compounds (e.g., aldehydes, ketones, esters, and free fatty acids). Unsaturated fatty acids are characterised by low oxidative stability due to the presence of multiple bonds. The oxidative stability of fatty acids depends on the number of these bonds in the molecule [42,43].

Table 5 presents the percentage differences between the contents of fatty acids in the fresh and six-month-stored mixtures of the infant formulas. The values presented in Table 5 were calculated according to Equation (1) in Section 2.5.

Lactose is an additive that protects polyunsaturated fatty acids against the harmful effects of the environment. In the M1 mixture, the main ingredient was whole milk powder.

Mixtures M2 and M3 were characterised by a similar composition (Table 1). The addition of the n-6 preparation to the mixtures was very large and amounted to 34%, while the addition of the n-3 preparation was only 6.5%. Owing to the high intake of n-6 fatty acids, special attention was paid for examining their resistance to oxidation. Fatty acids from the n-6 series, mainly linoleic acid (C18:2c), are commonly present in foods containing even small amounts of fat [44,45]. The loss of the C18:3 n-6 acid content in the M3 mixture amounted to 24.4% and was lower than the loss of that in M2 by 6.7 percentage points (Table 5). A smaller decrease in the C18:3 n-3 acid content was also observed for the M3 mixture than for the M2 mixture by 22.4 percentage points. However, the loss of C22:6 n-3 acid for the M3 mixture was 39.3%. The influences of milk proteins (casein and whey proteins) on the protection of polyunsaturated fatty acids cannot be clearly determined.

Figures 10–12 present the contents of fatty acids in the studied infant formulas (agglomerates), fresh and after six months of storage. Table 6 shows the percentage differences in the contents of fatty acids in agglomerated infant formulas due to the storage time.

Vignolles et al. [44] and Murrieta-Pazos et al. [37] found that the structure and porosity of particles of bulk materials provide a greater opportunity for solvents to penetrate particles during the extraction of fat from powders. The agglomeration process changes the structure and porosity of the particles. During this process, the amount of fat on the surface of the particles also changes. The higher porosity of the agglomerate particles allowed for better penetration of solvent particles and improved extraction of fatty acids.

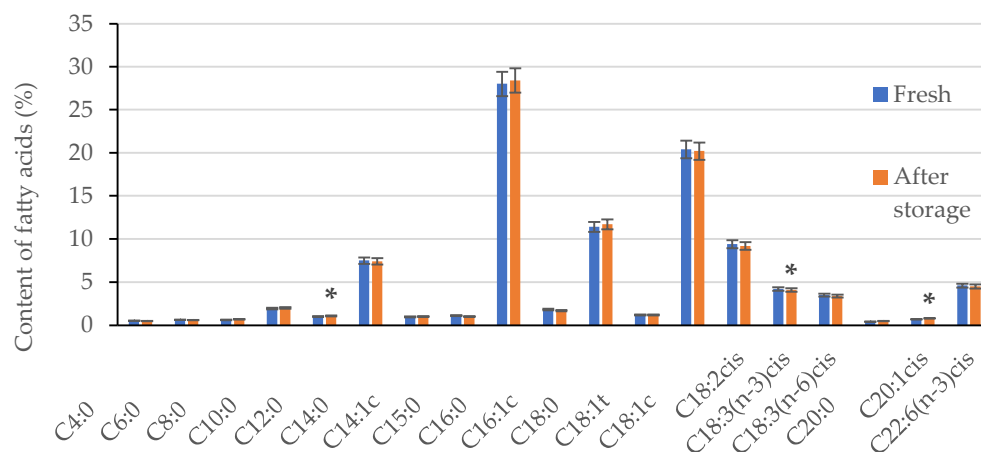


Figure 10. Fatty acid composition in agglomerate of infant formula A1. Data marked with asterisk (*) are statistically different ($\alpha = 0.05$) in terms of storage.

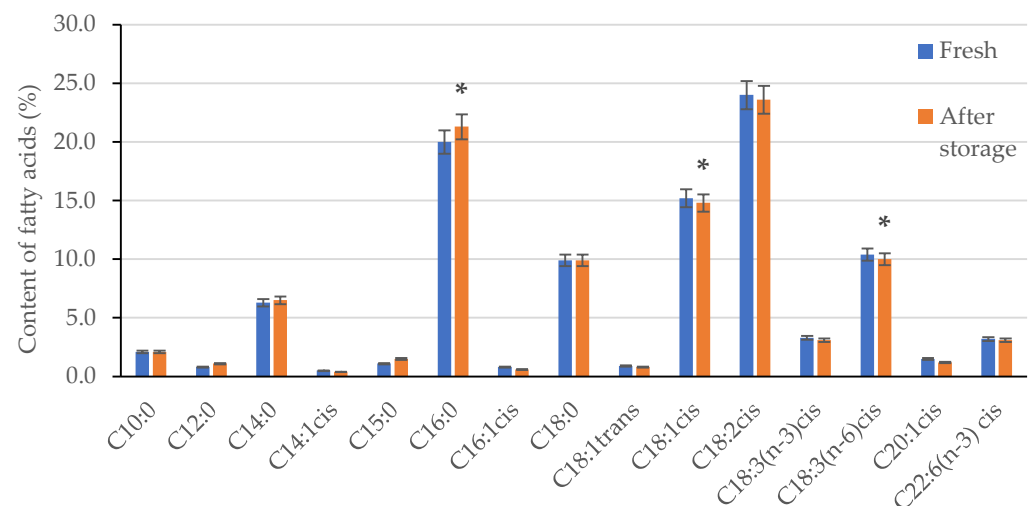


Figure 11. Fatty acid composition in agglomerate of infant formula A2. Data marked with asterisk (*) are statistically different ($\alpha = 0.05$) in terms of storage.

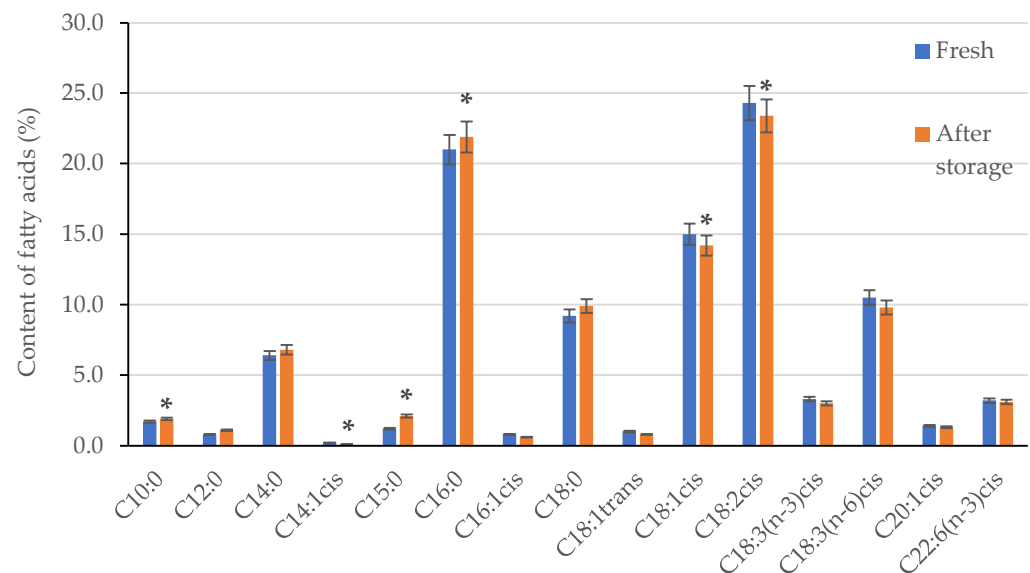


Figure 12. Fatty acid composition in agglomerate of infant formula A3. Data marked with asterisk (*) are statistically different ($\alpha = 0.05$) in terms of storage.

Table 6 presents the percentage differences between the contents of fatty acids in the fresh and six-month-stored agglomerates of the infant formulas. The values presented in Table 6 were calculated according to Equation (1) in Section 2.5.

Storing the agglomerates (for 6 months) resulted in a decrease in the contents of C18:3 fatty acids from the n-3 and n-6 groups (Table 6). Agglomerate A1 was characterised by higher contents of fatty acids from n-3 and n-6 preparations before and after storage compared to mixture M1 (Figures 7 and 10, respectively). The content of the C18:3 (n-6) fatty acid in the M2 mixture from which agglomerates A2 and A3 were obtained was 10.3%. The contents of all the added fatty acids in the agglomerates obtained from the M2 mixture were higher than those of the added fatty acids in the mixture. The six-month storage period of the agglomerates influenced the contents of all the added fatty acids (Figures 8, 11 and 12).

Table 6. Percentage differences between the contents of fatty acids in the agglomerates in the beginning and after the 6-month storage period.

Fatty Acid	The Change (Beginning—6 Months of Storage) (%)		
	A1	A2	A3
	M1	M2	M2
C4:0 Butyric acid	0.00	-	-
C6:0	0.00	-	-
C8:0	−13.40	-	-
C10:0	−2.74	0.00	−11.76
C12:0	−10.00	−37.50	−37.50
C14:0	1.33	−3.17	−6.25
C14:1c	0.00	20.00	50.00
C15:0	9.84	−36.36	−75.00
C16:0	−1.43	−6.50	−4.29
C16:1c	7.67	25.00	25.00
C18:0	−2.63	0.00	−7.61
C18:1t	0.00	11.11	20.00
C18:1c	0.98	2.63	5.33
C18:2c	2.13	1.66	3.7
C18:3n-6	2.38	3.85	6.67
C18:3n-3	2.86	6.06	9.09
C20:1c	−16.69	20.00	7.14
C22:6n-3	2.17	3.13	3.13

The agglomeration process prevents undesirable factors (oxygen and moisture) from accessing the fat inside the powder particles. The agglomeration process protects polyunsaturated fatty acids against environmental factors and helps to maintain the stability of fatty acids.

Figures 11 and 12 show the contents of monounsaturated and saturated fatty acids in the agglomerates obtained from the mixture M2 before and after the six-month storage period. Table 6 shows the relative differences in the fatty acid contents before and after storage. A higher content of saturated acids was found in the A2 and A3 agglomerates compared with that in the M2 mixture (Figures 8, 11 and 12).

Fatty acids constituted the interior of the microcapsules made of maltodextrin. These preparations were added to mixtures and agglomerates. The presence of free fat on the surface of the particles is an undesirable effect and reduces the durability of the microcapsules [41,45,46]. The fat contained in the powder particles includes the following fractions: free surface fat, free internal fat, and encapsulated fat [37]. Kim et al. [47] investigated the influence of a six-month storage (under normal conditions) on the fat distributions in skimmed and whole milk and cream powders. They showed that during such a short storage period, lactose (in an amorphous state) did not start the crystallisation process and that the composition of the powder surface did not change significantly. However, the encapsulated fat began to migrate towards the surface of the sheaths. Agglomeration may slow down the processes for fats moving to the surface of powder particles.

Murrieta-Pazos et al. [37] determined the distribution of fats on the surface of milk powder particles. Agglomeration, during which films of moisturising solutions are formed on the surface of powder particles, enables the protection of surface fats. Tables 5 and 6 present the changes in the fatty acid contents of mixtures and agglomerates. A significant

reduction in the value of these changes was observed for agglomerates compared to mixtures. The fats contained in the powder particles moved towards the surface faster and easier than proteins or lactose. The lactose contained in powdered milk is in an amorphous state and constitutes a “seal” for protein capsules in which milk fat is enclosed [39]. During storage under conditions of increased humidity, the crystallisation of amorphous sugars may occur, which increases the percentage of free fat. During crystallisation, sugar crystals with sharp edges are formed, damaging the fat coatings and causing compressive stresses, which allow the fat to squeeze out to the surface [7,48]. Lactose, as one of the natural components of milk, is probably the sugar that is the most often used to produce the matrix in the spray-drying of milk powders [24,30].

3.6. Differential Scanning Calorimetry—Phase Transitions and Crystallisation Curves

Figure 13 shows the DSC curves for mixtures M1, M2, and M3 and agglomerates A1, A2, and A3. In the temperature range from 3 to 39 °C, characteristic endothermic peaks indicating fat melting were observed for the M1 mixture. This preparation contained whole milk powder (Table 1). In the M1 mixture, the fat content was 19.7% (Table 4). No peaks indicating the presence of added n-3 and n-6 fatty acid preparations were observed.

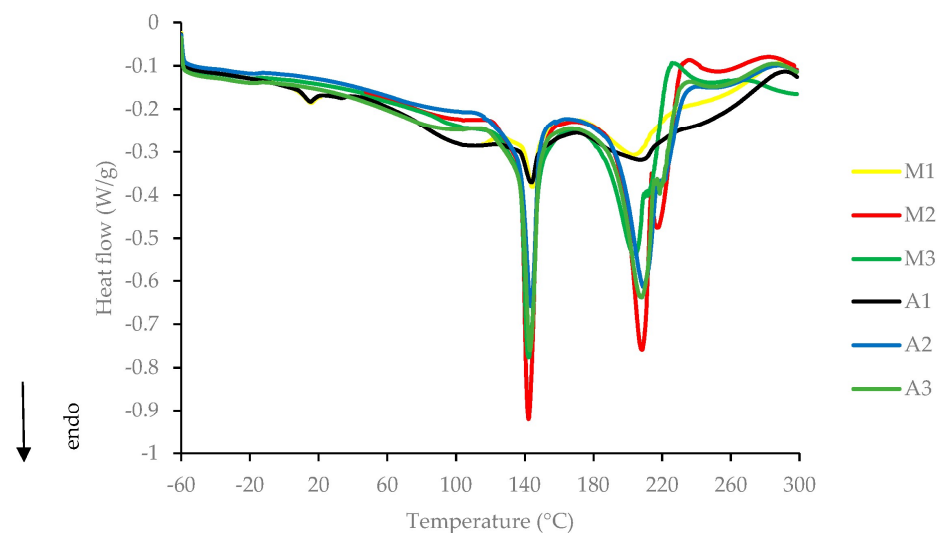


Figure 13. DSC curves of powdered infant formula mixtures M1, M2, and M3 and agglomerates A1, A2, and A3.

Rahman et al. [49] determined DSC curves for freeze-dried skimmed and whole camel’s milk powders. In the DSC diagram of the whole milk powder, endothermic softening peaks of the milk fat in the temperature range from 5 to 50 °C were observed. All the diagrams were characterised by phase transitions that can be observed for milk proteins. Whey proteins were present in all the mixtures (Table 1). The amount of the whey protein addition (in M1, M2, and M3) had no effect on the DSC curves.

Lactose was an ingredient in all the powdered mixtures of the infant formulas. Crystalline lactose was added to all the mixtures (Table 1). The DSC curves of these mixtures were characterised by distinct lactose-melting peaks [50] (Figure 13). The first sharp endothermic peak, the maximum of which was at approximately 143.24 °C, was the peak corresponding to the loss of crystalline water by the lactose. Mixture M1 contained 10% crystalline lactose (Table 1). The first endothermic peak corresponding to the transformation of the lactose was clearly smaller for the M1 mixture than for the M2 and M3 mixtures (Figure 13). Endothermic peaks corresponding to the transformation of the α -lactose were observed for all the powdered mixtures at temperatures around 207.11 °C. Phase transitions were very pronounced for mixtures M2 and M3, while the M1 mixture was characterised by a weak peak for the phase transition. The M2 mixture was characterised by a third peak

attributed to the melting of β -lactose at a temperature of approximately 215.69 °C. For the remaining mixtures, no phase transformation of this form of lactose was found (Figure 13).

Chiou et al. [51] studied the influences of spray-drying parameters on the thermokinetic properties of lactose. They showed that the size of the exothermic peak depended on the amorphous lactose content. Szepes et al. [52] and Gombas et al. [50] tested lactose in various forms and found that the transformations of the α - and β -lactose forms corresponded to temperatures of 213 and 224 °C, respectively. The temperature values obtained in the cases of the powdered mixtures M1, M2, and M3 were at a similar level. After exceeding a temperature of 250.47 °C, the DSC curves were very similar and were characterised by exothermic peaks. At such high temperatures, the material already decomposed. Exothermic peaks were the peaks corresponding to these transformations.

The individual ingredients in powdered mixtures of infant formulas undergo phase changes, just like pure substances. The different shapes and courses of the peaks were the result of mutual interactions between the components (Figure 13). In the case of the melting-temperature peaks of the pure crystalline lactose, maximum temperatures different than those for the peaks of the lactose transformation were observed in the DSC curves of mixtures M2 and M3.

Mixtures M2 and M3 were characterised by similar chemical compositions (Table 1). Lactose influenced the DSC diagrams of mixtures M2 and M3. The shape of the DSC curves of the mixtures was determined by the shape of the diagrams for the main components of the mixtures.

In the M1 mixture, the main ingredient was whole milk powder. Lactose and maltodextrin constituted approximately 30% of the composition (Table 1). The shape of the DSC curve indicated the presence of all these components; owing to their different contents, the peaks attributed to the transformation of the components were characterised by different shapes (Figure 13).

Agglomerate A1 was obtained using lactose as a wetting liquid (Table 2) from mixture M1. Mixture M1 contained whole milk powder (Figure 13). The DSC diagram of the agglomerate A1 obtained from this mixture showed phase transitions characteristic for milk fat. These transformations were observed in the temperature range from 2 to 39 °C.

Lactose was added to the M1 mixture (Table 1). In the DSC diagrams of M1 and A1, sharp endothermic peaks were observed at a maximum temperature of approximately 145.35 °C, indicating the loss of water by the lactose. The presence of weaker endothermic peaks at a temperature of approximately 204.05 °C, characteristic of the melting of α -lactose, was found. There were no influences of the type of wetting liquid on the course and shape of the DSC curve of the agglomerates.

Figure 13 presents the influences of agglomeration on the DSC curves of the M2 powdered mixture infant formula and the agglomerates, A2 and A3, obtained from that mixture.

The type of wetting liquid did not significantly influence the course of the DSC curves. The shape and course of the curves for agglomerates A2 and A3 were very similar to those of the curve for the M2 mixture (Figure 13). The main ingredient of the mixture and agglomerates was crystalline lactose (Tables 1 and 2). The first distinct and sharp endothermic peak was attributed to the loss of water by the lactose. It was observed in all the DSC diagrams (Figure 13). The second endothermic peak present in all the curves corresponded to the melting of α -lactose. The third endothermic peak observed in the DSC curves was typical for the melting of β -lactose [50].

Szulc et al. [24] determined the DSC curves of infant formula powders with different compositions. Based on the DSC diagrams, the differences in the compositions of the tested powders were observed.

Figure 14 presents the DSC cooling curves of the powdered mixtures of the infant formulas and the agglomerates, A1, A2, and A3, obtained from them. The DSC diagrams of all the mixtures with added fatty acid preparations showed the presence of three small exothermic peaks in the temperature range from −16 to −46 °C, with the maximum temperatures at the crystallisation temperatures of the fatty acids from the n-3 and n-

6 groups. A distinct peak characteristic of milk fat was observed for the M1 mixture (Figure 14). The DSC curves of the M2 and M3 mixtures were characterised by very slight peaks at temperatures of -23.13 and -40.42 °C, respectively.

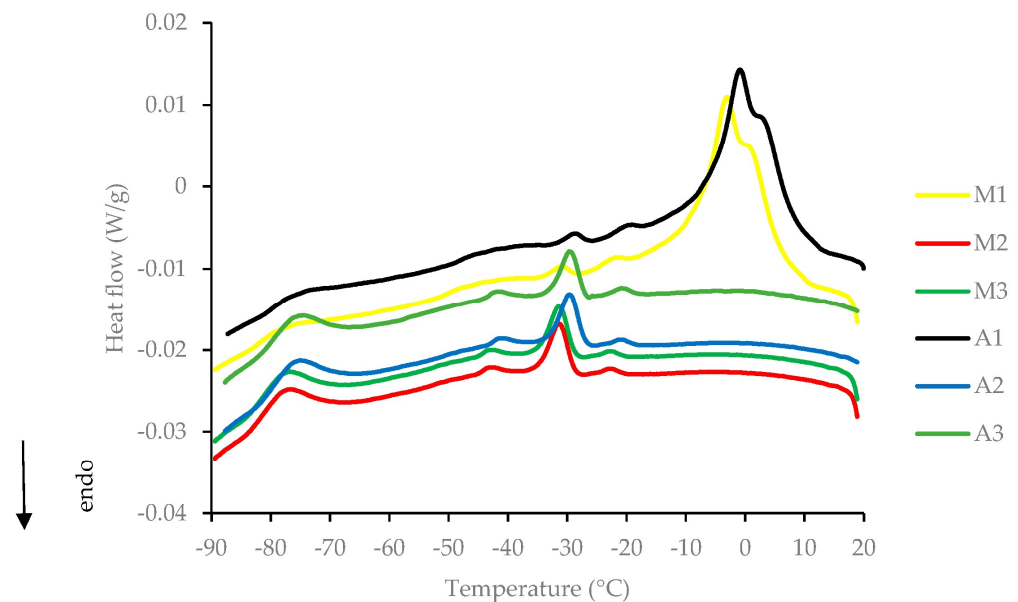


Figure 14. DSC cooling curves of powdered infant formula mixtures M1, M2, and M3 and agglomerates A1, A2, and A3.

In the case of the M2 sample, an distinct exothermic peak at a temperature of -2.52 °C was observed in the cooling curve. Distinct exothermic peaks were observed in the DSC cooling diagrams of the M2 and M3 mixtures, with a maximum temperature of -31.12 °C. This was due to a significant (34%) share of n-6 fatty acids in these infant formulas. The DSC curves of the M2 and M3 mixtures were characterised by a weak exothermic peak at a temperature of approximately -76.54 °C. In the DSC cooling curves, the observed peaks indicated the presence and amount of all the fatty acids that were added or were present in the compositions of the powdered infant formulas.

Based on the course and shape of the DSC cooling diagrams, it can be concluded that the M1 mixture and the A1 agglomerate obtained from it included whole milk powder (Tables 1 and 2). The course and shape of the exothermic peak indicated the crystallisation of the milk fat. The weak exothermic peaks observed in the DSC cooling curves indicated the presence of added fatty acids from n-3 and n-6 preparations (Figure 14).

The DSC cooling curves for agglomerates A2 and A3 (Figure 14) had a course similar to that of the DSC cooling curve for the M2 mixture. It was observed that the heat flow through the powders was at different levels. Different levels of heat flow were observed for the A2 agglomerate and for the A3 agglomerate.

The DSC cooling curves were characterised by three exothermic peaks in the temperature range from -22 to -42 °C. The peaks corresponding to the crystallisation of fatty acids from the n-3 group were much less pronounced than the peaks of the fatty acids from the n-6 group. The addition of the n-6 fatty acid preparation to the M2 mixture and the agglomerates obtained from it was approximately 34% (Tables 1 and 2), as indicated by the intensity of the exothermic peaks at a temperature of -31.78 °C (Figure 14).

There were no impacts of agglomeration and the use of various wetting liquids on the shape and course of the DSC cooling curves of the agglomerates formed from appropriate mixtures.

Ostrowska-Ligeza et al. [22] studied mixtures and powdered infant formulas. They determined DSC cooling curves for mixtures, agglomerates, and coated agglomerates. They indicated that the cooling curves allowed the detection of the presence of added fats.

Figures 15 and 16 present the influences of storage on the thermal properties of mixtures and agglomerates of powdered infant formulas. After a period of six months of storage, the DSC diagrams of the samples were characterised by changes in the shape and course.

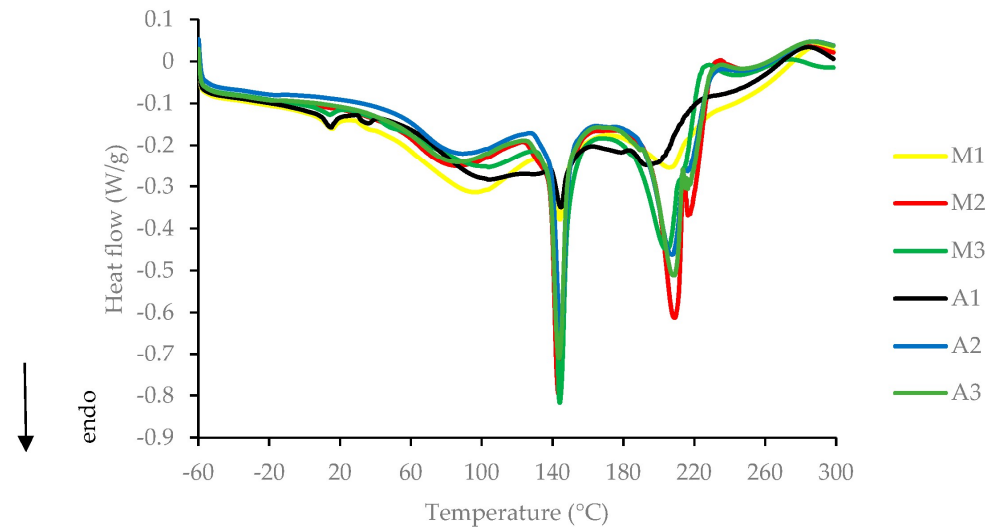


Figure 15. DSC curves of powdered infant formula mixtures M1, M2, and M3 and agglomerates A1, A2, and A3 after six months of storage.

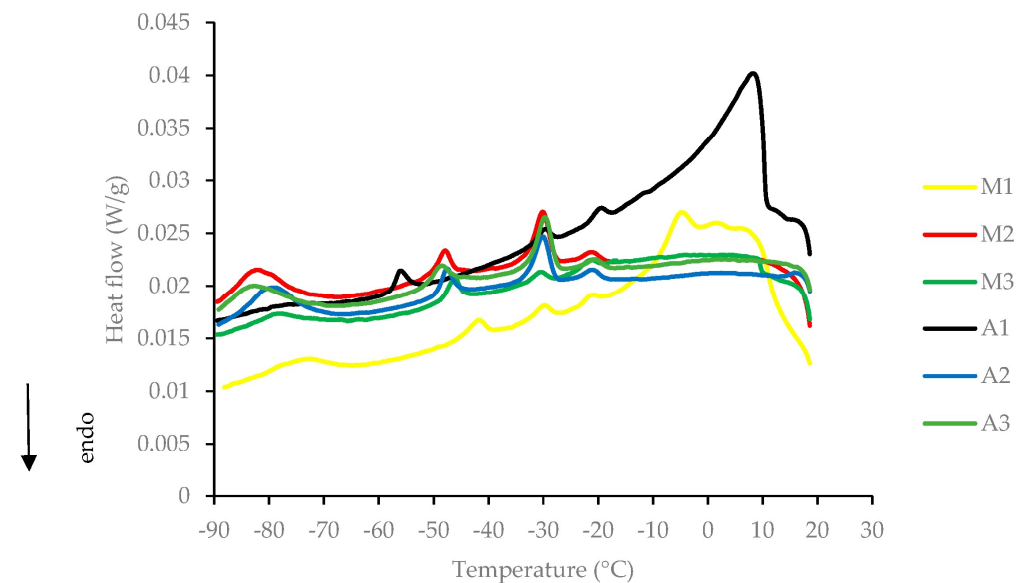


Figure 16. DSC cooling curves of powdered infant formula mixtures M1, M2, and M3 and agglomerates A1, A2, and A3 after six months of storage.

Figure 15 presents the DSC curves of the mixtures M1, M2, and M3 and agglomerates A1, A2, and A3 obtained from them after storage. Based on the observation of the DSC curve of the stored M1 mixture, it can be concluded that the temperature range (7–45 °C) of the peaks corresponding to the presence of fats did not change compared to the DSC curve of the fresh M1 mixture (Figure 13). A large endothermic peak was observed in the temperature range from 65 to 125 °C. The occurrence of this peak indicated water sorption by the powder. The state of the water in food and biopolymers was examined by evaporating it from a sample placed in a DTA (differential thermal analyser) or DSC [53]. In the initial studies, the endothermic surface area was taken as a measure of the amount

of free water in the material, while differences in the transformation temperature and changes in the heat of evaporation were considered as indicators of the bonding strength of water with the material [53–55]. DSC allows us to determine the thermal energy needed to evaporate water. Haque et al. [56] studied the influences of the relaxation kinetics and ageing of whey protein concentrate on the solubility of these proteins. They observed that as the water activity of the protein concentrate increased, weak endothermic peaks appeared in the DSC curve.

The main component of the M2 and M3 mixtures (Table 1) was lactose, and it presented a large impact on the DSC diagrams. Very weak peaks were observed in the DSC diagrams of the stored M2 and M3 mixtures in the temperature range 35–110 °C (Figure 15). This transformation was the result of an increase in the water content in the tested sample.

The DSC diagram of the stored A1 agglomerate obtained from the M1 mixture was characterised by a weak endothermic peak in the temperature range 40–125 °C, indicating the transformation of the evaporated water (Figure 15).

The DSC curves of the stored agglomerates A2 and A3 obtained from the M2 mixture (Figure 15) did not present significant differences from the DSC curves of the fresh powders (Figure 13). The DSC diagrams of the powders after storage were characterised by a weak endothermic peak in the temperature range 44–120 °C, which corresponded to the evaporation of water.

The water content in the tested samples of the powdered infant formulas increased during the storage period, which can be determined based on the observation of the DSC diagrams of the stored powders. Thomas et al. [57] indicated that the high lactose content improved the stability of the mixtures. Lactose, which was partially in an amorphous form, prevented fat oxidation and protein degradation. Rao et al. [58] studied the effects of the storage conditions (time, water activity, and temperature) on egg white powder. During storage, changes in the protein structure, the loss of free amino groups, and the initiation of the non-enzymatic browning reaction (Maillard reaction) were observed.

Figure 16 presents the DSC cooling curves of the M1, M2, and M3 mixtures and agglomerates A1, A2, and A3 obtained from them. In the DSC diagram of mixture M1, containing whole milk powder, broad exothermic peaks and several single peaks were observed in the temperature range from 11 to −15 °C. The DSC cooling curve of the M1 mixture showed the presence of three small peaks. During storage, milk fat moved from the centre of the milk powder particle to its surface [47]. On the surface of the particle, fats have increased contact with factors causing their oxidation (air, water, temperature, and light). Based on the shapes of the milk-fat peaks in the DSC curves, it can be concluded that the milk fat has undergone transitions compared to the milk fat in the fresh mixtures (Figure 14). Kim et al. [47] studied milk powders with different fat contents. After a six-month storage period, they indicated that the fat content had not changed significantly. However, the melting characteristics of the milk fat changed after storage.

Fatty acid preparations n-3 and n-6 were added to all the mixtures. The DSC curves of these mixtures were characterised by three exothermic crystallisation peaks of n-3 and n-6 fatty acids (Figure 16). The maximum temperature range for the first small, weak exothermic peaks was from −18 to −22 °C. The temperature was not significantly different from that of the temperature for the fresh mixtures (Figures 14 and 16). The presence of this type of peak indicated the crystallisation of α -linolenic fatty acid from the n-3 group. The temperature of the second weak exothermic peak ranged from −29 to −31 °C. This peak concerned the presence of γ -linolenic acid from the n-6 group.

The peak characterised by a temperature of −29.47 °C in the DSC diagram of the M2 mixture was the most distinct. Even though the addition of n-6 acid preparations was also high in the M3 mixture, the peak was not as distinct (Figure 16). The greatest differentiation in the maximum temperature was observed for the third peak, which indicated the presence of docosahexaenoic acid from the n-3 group.

For the M1 mixture, this temperature was approximately −41.86 °C, and for the M2 and M3 mixtures, it was approximately −47.24 °C (Figure 16). Docosahexaenoic acid has

six double bonds in its molecule; the period of storage and environmental factors could reduce the number of bonds and change the properties of this acid [59]. In the DSC cooling diagram of the M2 mixture, a small exothermic peak was observed at a temperature of approximately -81.09°C (Figure 16).

The DSC cooling diagram of the A1 agglomerate was characterised by a sharp, distinct, exothermic peak at a temperature of about 9.28°C and three small peaks.

The DSC cooling curve of the M1 mixture was characterised by a broad, exothermic peak with three single peaks and an additional three small peaks (Figure 16). The greatest differences were observed for the third small peak; its temperature in the DSC curve of the M1 mixture was 41.78°C , while that in the DSC curve of agglomerate A1 was 56.24°C . The third small peak did not exist in fresh powders' DSC curves (Figure 14).

The DSC cooling curves of the M2 mixture and agglomerates were characterised by a similar shape and course. The temperature ranges from the first to the fourth peaks were, respectively, from -18 to -22°C , from -26 to -35°C , from -44 to -51°C , and from -72 to -86°C (Figure 16). The peaks with the highest intensity indicated the presence of docosahexaenoic acid from the n-6 group. The addition of the n-6 fatty acid preparation was very large in the M2 mixture (Table 1). The distinctness of the fatty acid peaks from the n-3 group in stored agglomerates increased compared to that of the peaks from the fresh agglomerates (Figure 14). This was caused by the migration of fats to the surface of the agglomerate particles during storage. There were no influences of the type of wetting liquid on the shape and course of the DSC cooling curves of the stored powders.

Masum et al. [8] studied the influences of the storage conditions on the physico-chemical properties of infant milk formula powders prepared with various lactose-to-maltodextrin ratios by spray-drying. The powders were stored for 180 days at 22 and 40°C and relative humidities of 11, 23, and 54%. The surface fat content and degrees of aggregation and caking increased during storage. An increase in the amount of surface fat was accompanied by decreases in the surface protein and carbohydrate contents.

4. Conclusions

The isotherms of the agglomerates presented shapes and courses similar to those of the isotherms of the mixtures from which they were obtained, so it can be concluded that the agglomeration process did not have a significant impact on the course of the isotherms. The agglomeration process influenced the protection of the stability of fatty acids during the storage process in the analysed powdered infant formulas. Based on the obtained results, it can be stated that the agglomeration process of the powdered infant formula limited the adverse impacts of the external environment on the essential unsaturated fatty acids. This is most likely due to the protective effect of the amorphous form of the lactose, which is able to produce protective structures on the surface of milk powder particles. Lactose combined with whey proteins seals the structures of milk powder particles, limiting the effects of, for example, oxygen and water. The composition of the fatty acids changed during the storage process. The shapes and courses of the DSC curves registered for the mixtures and the agglomerates obtained from them did not show any significant differences, so it can be summarised that agglomeration did not significantly affect the thermal properties of the powdered infant formulas. Differences were not observed for either the heating or cooling DSC curves. It is worth emphasising that using the DSC method, it is possible to determine whether the fat was a natural component of the powder or it was added in the form of fatty acid preparations.

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