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Microbial Quality, Chemical and Sensory Stability assessment of first-age powdered infant formula

Abdelmalek Meribai[1], Ahmed Bahloul[2,3], Abdelouahab Diafat[4], Hamdi Bendif[5]

1. Faculty of Nature and Life Sciences, El Bachir El Ibrahimi University, (34000) Bordj Bou Arreridj, Algeria. Laboratory of Characterization and Valorization of Natural Products, Tel: + 213 554635232. abdelmalek.meribai@univ-bba.dz.
2. Faculty of Science and Technology, Mohamed El Bachir El Ibrahimi University, (34000) Bordj Bou Arréridj, Algeria. Laboratory of Electrochemistry and Environment (LEE).
3. Department of Chemistry, Faculty of Sciences, Mohamed Boudiaf University, (28000) M'sila, Algeria. ahmbahloul@yahoo.fr
4. Department of Biological Sciences, Faculty of Nature and Life Sciences, El Bachir El Ibrahimi University, (34000) Bordj Bou Arreridj, Algeria. Laboratory of Characterization and Valorization of Natural Products. abdelouahab.diafat@univ-bba.dz
5. Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, Algeria. Laboratory of ethnobotany and natural substances, ENS Kouba Algiers, Algeria. hamdi.bendif@univ-msila.dz

Correspondence:

Abdelmalek Meribai: Laboratory of Characterization and Valorization of Natural Products (LCVRN), Faculty of Nature and Life Sciences, El Bachir El Ibrahimi University, (34000) Bordj Bou Arreridj, Algeria. Tel: + 213 554635232/ abdelmalek.meribai@univ-bba.dz. Phone number +213554635232.

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Abstract

Background: Powdered infant formulas (PIF) play role in infant nutrition when breastfeeding is not available. However, both dairy companies and consumers encounter challenges related to PIF physicochemical stability, microbiological quality throughout storage. Methods: Study aimed to evaluate physicochemical stability and microbiological quality of eleven PIF samples, collected in Algiers city, Algeria, during spring season (February- April) of 2023. Results: Chemical tests showed stable samples, with following values: Density (1.024±0.005), pH (6.655±0.176), Acidity (17.628±0.3514), Viscosity (2.574±0.2577), Conductivity (1805.72±68.397µS/cm), Total Dissolved Solids (85±8.9493 mg/L), FAT (3.96±0.4664g/100ml), Protein (1.66±0.2865g/100ml), Lactose (1.5509±0.10446 g/100ml). Microbiological analysis revealed following flora: yeasts and molds present in 36.36%, total bacterial count 27.27%, total coliforms 9.09%, total fecal coliforms 9.09%, *Escherichia coli* 9.09% and *D-Streptococcus* 18.18%. Conclusion: Samples seem chemically stable throughout storage, with no flaws in labeling or packing. Consequently, in line with Algerian standards. These samples, however, are not up to Algerian microbiological standards. In perspectives, It is preferable to

conduct thorough studies using larger sample sizes in order to understand the sources of contamination.
Keywords: Algeria, Bacterial Load, Food contamination, Infant Formula

Abbreviation

Acidity (°D): in Dornic degrees
AFNOR: Association Française de Normalisation
AFSSA: Agence Française de Sécurité Sanitaire des Aliments
AOAC: Association of Official Analytical Chemists
Cd/Conductivity: (µS/cm) in micro-siemens/centimeter
CFU: Colony Forming Unit
Density: (g/cm^3): gram/cubic centimeter
EFSA: European Food Safety Authority
FAO: Food Agriculture Organization
FOF: follow- on formulae
FOS: Fructo-Oligo-Saccharid
GOS: Gluco-Oligo-Saccharid
IDF: International Dairy Federation
ISO: International Organization for Standardization
MENA: Middle East and North Africa
MICS: Multiple Indicator Cluster Survey
TSW: tryptone salt water
MPN/ml: Most Probable Numbers (MPN) per milliliter.
PIF: Powdered Infant Formula
R-V B: Rappaport Vassiliadis Broth
SFB: *Salmonella* Fecal Broth
Spp: sub-species
TDE: Total Dry Extract
TSA: Trypton Sels Agar
TVCs: Total Number of Live (mesophiles)
UNFPA: United Nations Fund for Population Activities
UNICEF: United Nations International Childrens Fund
V/Viscosity (mPa.s): in milli-Pascal/second
WHO: World Health Organizaton

1. Introduction

For newborns and young infants, human milk is the ideal food that best meets their nutritional needs. In fact, human milk is the best and most natural food for infants, especially during the first months of life (OMS 2005; Agostoni et al. 2014). Nordic European countries have the highest global breastfeeding rates, with approximately 99% in Norway and Sweden, 95% in Denmark (OMS 2005). In Algeria, Ministry of Health, in collaboration with United Nations agencies; such as United Nations Population Fund (UNFPA), United Nations International Children's Fund (UNICEF), and the World Health Organization (WHO), conducted a series of survey cycles known as the Multiple Indicator Cluster Survey (MICS). Additionally, MICS 6 was carried out in 2018, targeting a sample of 31000 families (OMS 2005; MICS 2006). MICS provides up-to-date, internationally comparable information on the situation of children and women, addressing issues related to child nutrition, including anthropometric measurements, as well as child development and protection. In Algeria, additional objectives have been incorporated, focusing on chronic diseases, disability, childhood accidents, and general mortality and birth rates (MICS 2006). Overall, the duration of breastfeeding is essentially determined by the resumption of labor by mother and dietary supplements including artificial milk. Furthermore, lack and insufficiency of milk is the main reason for women not to breastfeed. The percentage of exclusively nursing mothers generally increases as maternal education levels rise and median family income drops. The prevalence of exclusive breastfeeding is around 14 % with an average duration that is very far from the WHO recommendations (OMS 2005; Abla et al. 2016). Infant formula is still a great substitute for breast milk and serves a crucial function in a baby's nutrition. Infant milk formulae considered to be prebiotic food, must be corrected consist of proteins,

carbohydrates, lipids, vitamins, minerals, trace elements, certain food additives approved, such as prebiotics; Dietary fiber, complex-sugars, polydexteroses, Fructo Oligo-Saccharides (F.O.S), Gluco-Oligo-Saccharides (G.O.S), lutein (antioxidant pigment), taurine (amino-acid), essential fatty acids such as: alpha-linolenic acid, omega-3 (Agostoni et al. 2014; Kent et al. 2015; Pal et al. 2016). The high number of colony forming units (CFU) of bacteria lowers the infant milk's hygienic value; it appears as a sign of poor manufacture, packaging, and possible fecal contamination (Buchanan and Oni 2012; Cho et al. 2019). A number of foodborne illnesses had milk and different types of Powdered Infant Formula (PIF) as potential carriers worldwide (Aman et al. 2016; Cho et al. 2019). Due to their complex biochemical composition, richness in vitamins, proteins, lipids, sugars, their neutral pH, makes them very vulnerable to microbial enzyms attacks (Buchanan and Oni 2012). Similarly their richness in oxidable compounds: vitamins, fats, prebiotics can be transformed under the influence of light, oxygen and temperature of preservation, hence the interest of exploring their stability by different physicochemical tests (Aman et al. 2016; Lang et al. 2016). In Algeria, powdered milk (PIF) in general, including follow-on formulas (FOF) and baby meal formulas, is exclusively imported. Algeria ranks as third-largest importer of milk powder. With a population of over 43 millions and a high fertility rate estimated at three predefined children per woman. Algerian PIF market is ranked as fifth in the Middle East and North Africa (MENA) areas for infant food. Anticipated annual demand for Algeria is greater than three hundred million milk liters. According to Unpublished data from Algerian Ministry of Commerce indicates that Algeria imports more 13800 tons of infant powdred milk annually. While PIF is available in highly innovative, waterproof, and sanitary packaging, maintaining the stability of these products during storage, shipping, and marketing poses a challenge for dairy technology firms and food quality control and hygiene agencies. Study aims to investigate the physicochemical stability of first-age PIF by conducting various physicochemical tests (density, pH, titratable acidity, viscosity, levels of protein and lactose …). Additionally, the microbiological quality of infant milk powder and PIF, will be assessed through research involving colony-forming unit (UFC) counting on classical culture media, evaluation of total aerobic flora, total and thermotolerant coliforms,…. search for pathogenic and/or toxinogenic microbial species (*Escherichia coli, Salmonella, Listeria, Cronobacter, Bacillus cereus and Staphylococcus aureus)* for eleven first-age PIF samples (different brands) collected from various officins in Algiers city- Algeria, during the spring (February–April) of 2023. Results will be compared to the both national (Algerian) standards as well as the standards found in the scientific literature (MAC 1998; Agostoni et al. 2014).

2. Material and methods

2.1.Sampling

Eleven PIF samples of different brands, from different batches, were collected from pharmacies in Algiers city- Algeria, during spring season. Sampling lasted from February 20th to April 24th, 2023. Samples were selected based on their market availability and use frequency. All collected samples were suitable for children aged 0 to 6 months (First-age PIF). Before analysis, samples were stored at room temperature. Composition of all formulas was meticulously collected from the information on the sample's packaging, including verification of manufacturing and expiration dates. During the analysis, the samples were stored in a dryer to maintain hermetic environment. All the products used for chemical and microbiological analysis in this study were of high-quality reagents, the product origin and characteristics of all studied samples are indicated in Table 1.

2.2.Physicochemical analyses

The table 2 shown a comprehensive overview of the physicochemical analysis conducted on the eleven PIF samples. The pH is important for ensuring the product's suitability for consumption and its compatibility with a baby's digestive system. The acidity a method used

to determine the concentration of acids in a solution. Viscosity measures the thickness or resistance to flow of the formula at 20°C. Understanding viscosity is important as it can affect how easily the formula can be prepared and consumed by infants. The conductivity measurement assesses the ability of PIF samples to conduct an electrical current. It can provide information about the ionic content of samples. The proteins content is obtained in three steps; this likely refers to a comprehensive protein analysis involving three steps. The FAT/Gerber method is a common technique for determining the fat content of milk. Lactose is the primary carbohydrate in milk. Determining lactose content is crucial for assessing the nutritional composition of the formula.

2.3.Microbiological analyzes

The samples were transferred to laboratory in accordance with appropriate sampling practices. Carrying out decimal dilutions: From each sample, sterile take (surface and depth) of 25 g of milk powder in 225ml of Tryptone Salts Water diluent. This constitutes the first 1/10 dilution. After homogenization (Vortex) carrying out series of dilutions (by sterile transfers of 1ml into 9 ml tryptone salt water until dilution 10^{-6}. The research/enumeration of microbial: flora/species was carried out using routine (classic) methods recommended by AFNOR guidelines/standard (AFNOR 2002). The outcomes (Absence/Presence) given in CFU/g, were contrasted with Algerian standards and those reported in the scientific literature. Table 3 provides a comprehensive overview of the microbiological analysis conducted. Each parameter or microorganism is assessed for its presence and count in the samples. The Total Bacterial Count (TBC) measures the total number of bacteria present in the samples, including both beneficial and harmful bacteria. Total Coliform Count identifies the presence and quantity of coliform bacteria, which can indicate contamination. *E. coli*, which is a specific type of coliform bacteria, is counted separately as it can be an indicator of fecal contamination and potential health risks. *Streptococcus*-D to quantify the presence of *Streptococcus*- D flora, which can be relevant to assessing the microbiological quality of the samples. *Staphylococcus* bacteria are identified and counted to determine their presence and potential health risks. *Staphylococcus aureus* is a specific strain of *Staphylococcus* group, identified and quantified using the NF-ISO 6888 method. *Clostridia Sulphito-Reducer* test focuses on identifying and quantifying *Clostridia bacteria*, particularly those capable of reducing sulfite compounds. *Bacillus cereus* wich indicate the presence and potential growth of *Bacillus cereus*, a bacterium associated with food poisoning, are assessed. *Listeria monocytogenes* is to identify the presence of *Listeria monocytogenes*, a pathogenic bacterium that can cause foodborne illness. *Salmonella spp* is to refer the various *Salmonella* species, and this test identifies their presence, which is crucial for food safety. The *Cronobacter spp* is identified and quantified using ISO-TS 22964, which is essential for infant formula safety. The US-FDA 2002 refers to compliance with U.S. Food and Drug Administration (FDA) standards and regulations, ensuring the safety of the PIF samples.

2.4.Statistical analyzes

By statistical analysis (Principal Component Analysis) we verified the presence of possible correlation between sample's chemical composition and rates contamination by microbial floras (Figure 2).

3.Results

The results of the chemical analyzes are illustrated in Table 4. The results of the microbiological analyzes are presented in Figures 1 and 2.

Table 1: Origin and characteristics of PIF samples

S	Weight (G)	manufacture date	Expiration date	Sampling Date	Packaging defects	Visual Defects

S_1	750	February 10, 2023	January 30, 2024	February 20, 2023	Waterproof	Abs
S_2	400	January 10, 2023	January 9, 2024	March 11, 2023	Waterproof	Abs
S_3	400	February 15, 2023	January 30, 2024	March 22, 2023	Waterproof	Abs
S_4	800	March 10, 2020	February 1, 2024	March 25, 2023	Waterproof	Abs
S_5	400	February 1, 2023	January 30, 2024	March 20, 2023	Waterproof	Abs
S_6	400	March 1, 2023	February 1, 2024	April 19, 2023	Waterproof	Abs
S_7	400	March 15, 2023	February 28, 2024	April 24, 2023	Waterproof	Abs
S_8	700	February 2, 2023	February 28, 2024	February 25, 2023	Waterproof	Abs
S_9	400	March 1, 2023	January 2, 2024	March 20, 2023	Waterproof	Abs
S_{10}	400	March 15, 2023	January 10, 2024	March 25, 2023	Waterproof	Abs
S_{11}	400	February 10, 2023	January 30, 2024	March 5, 2023	Waterproof	Abs

Abs: Absence, S: sample, Weight (G): weight in grams

Table 2: Physicochemical analyses

Test	Device (brand)	Result (Unit)	Method used by:
Stability (Test) at 20°C		No unity	
Density at 20°C	Densitometer: LAUDA-model TD_1	No unity	Vierling (2003) Method
pH at 20°C	pH-meter: Inolab-pH730	No unity	Kristensen et al. (1991)
Acidity at 20°C	milk: Lactate neutralized by NaOH solution (0.1N)+color indicator	Dornic D (°D)	By Method used by Soceanu et al. (2015)
Viscosity at 20°C	Rheology Viscosimeter: (Rion-ViscotesterVT-03F-Germany).	Millipascal/Second	Gasmalla et al. (2013) Method
Conductivity at 20°C	Conductmeter-Inolab-cond-730-(Germany)	Microsiemens/centimeter	Mabrook & Petty, 2003 Method

Proteins (Azote total): Test in 3 steps:	Mineralization: BuchI Digestion Unit Mineralizer K- 424. 2. Distillation: BuchI Distillation Unit K-350 Distiller Titration	AOAC Official Method 991.20 (2006b)	AOAC (1997) Method- Methode by AOAC (2006).
FAT/Gerber Method	ISO 19662	Routine method	Method Gerber- I.D.F, 2006 Method.
Lactose (Rate)	Lactose estimation in recombined PIF. trichloroacetic acid (TCA) used as precipitating agent	(λ: 520 nm)	(Abu-Lehia (1987) (1987);(Alfaris et al. (2022))
TDE: Total Dry Extract	105°C/ water Elimination by heating (105°C)	Distiller (Mammert)	Martins et al. (2018) Method by Gasmalla et al. (2013)

3.1.Microbiological analyzes

Groups/species	**Medium**	**Additive**	**Brand/ Meker**	**Incubation**	**Code**
Mold and Yeast count (MYC) ISO 21527	Sabouraud Agar	N.D	IPA-Algeria	25°C/5 days	NF V 08- 051
TBC (MPN) ISO 13559/IDF 153	PCA Agar	N.D	Pronadisa	30°C/3 days	NF V 08- 051
TCC (MPN) ISO 4831	BGLBB (Broth)	Bille Green Brillant Presumption	Pronadisa	37°C/24H	NF- EN 12824 1998
Coliforms Thermotolerant (MPN) ISO 4831	BGLBB (Broth)	Confirmation	Pronadisa	44°C/24H	NF- EN 12824 1998
***E. Coli* Count ISO 11866 -2/ IDF 170- 2**	Mac Konkey Agar	Confirmation Mc Kenzy- Test	Pronadisa	44°C/24H	NF V08-053
***Streptococcus* D (MPN)**	Rothe (Broth)	MPN. Presumption	Pronadisa	37°C/24H	N.D
***Streptococcus* D**	Eva Litsky (Broth)	MPN. Confirmation	Pronadisa	37 °C/24H	N.D
Staphylococcus	Giolliti - Cantoni	MPN presumption	Pronadisa	37 °C/24H	NF V08 - 052
***Staphylococcus aureus:* NF** ISO 6888	Baird ParkerAgar	Confirmation	Pronadisa	37 °C/24H	NF V 08- 052
Clostridia Sulphito Reducers (ISO15213)	Liver-Meat Agar	Na-sulfite Iron allun	Merck	37 °C/72H	NF V 08- 056

Bacillus cereus	Mossel Agar	Egg-Yolk	Merck	37 °C/24H	NF ISO 7932
Listeria monocytogenes **NF EN ISO11290-1**	N.D	N.D	Merck/ Pronadisa	37 °C/ 42°C 3 to 5 days	NF V 08- 055
Salmonella spp **NF-ISO 6579**	02. differential Broth	02. differential medias	IPA/ Pronadisa	37 °C/42°C	NF V 08-052 NF-EN- 12824 1998
Cronobacter spp **ISO/ TS 22964. US- FDA 2002**	several broths	several media/steps	Merck+ Pronadisa	5 to 7 days 5 days	US- FDA 2002. Method + ISO/ TS 22964.

Table 3: Microbiological analyzes

PCA: Plate Count Agar, BGBLB: Brilliant Green Bile Lactose Broth, **IPA**: Institut Pasteur d'Algérie, **M Y C**: Mold and Yeast count, **MPN**: Most Propable Number, **ND**: Not determined, **TBC**: Total Bacterial Count, **TCC**: Total Coliform Count, **TFCC**: Total Fecal Coliform Count.

Table 4: Results of chemical analyzes

S	T/A	Stability	Density g/cm³	pH	Acidity °D	Viscosity mPa.s	Cd (µs/c)	TDE (%)	FAT (%)	Prt g/100ml	
S1	Normal	+	1.03	6.74	18.12	2.51	1823	87	3.91	1.78	1
S2	Normal	+	1.03	6.72	17.21	2.8	1786	69	3.62	2.11	1
S3	Normal	+	1.02	6.35	17.03	2.72	1793	91	3.42	2.06	1
S4	Normal	+	1.02	6.61	17.5	2.63	1649	73	3.23	1.73	1
S5	Normal	+	1.03	6.81	17.64	2.81	1742	82	3.72	1.58	1
S6	Normal	+	1.02	6.32	18	2.62	1912	94	5.15	1.62	1
S7	Normal	+	1.03	6.71	17.67	2.75	1827	85	3.81	1.48	1
S8	Normal	+	1.02	6.89	17.52	2.84	1833	94	3.92	1.45	1
S9	Normal	+	1.02	6.75	17.33	2.07	1797	76	4.21	1.33	1
S10	Normal	+	1.02	6.63	17.08	2.22	1822	85	4.63	1.35	1
(M) Md (x)	N.D	N.D	1.02	6.711	17.64	2.63	1823	87	3.91.	1.62	1. 1
SD (σ)	N.D	N.D	0.005	0.176	0.3514	0.2577	68.397	8.9493	0.464	0.2865	0.1

S: Samples, Av: Average, Md (x): Middle value, SD (σ): Standard deviation, TDE: Total Dry Extract (%), V/Viscosity (mPa.s): in milliPascal/second, N.D: Not determined, (//): sign signifies the same thing (EDEM).

n , where:

x- Variant;
n- Number of variants (sample size).
Standard deviation (σ):

$$(\sigma)=\sqrt{\frac{\Sigma(M-X)2}{n-1}},$$

Error arithmetic mean (m):

$$m = \sqrt{\frac{\sigma}{n}}$$

Criterion validity of Student to compare the two samples (t):

$t = \frac{M1-M2}{\sqrt{m1^2+m2^2}}$, Where:

M1- the arithmetic mean of a variation series, M2- the arithmetic mean of the number of other variations,

m1 and m2- there may be errors of arithmetic means of variation series. According to the Student table to have significantly differences P. Differences were considered significant at P ≤ 0.05.

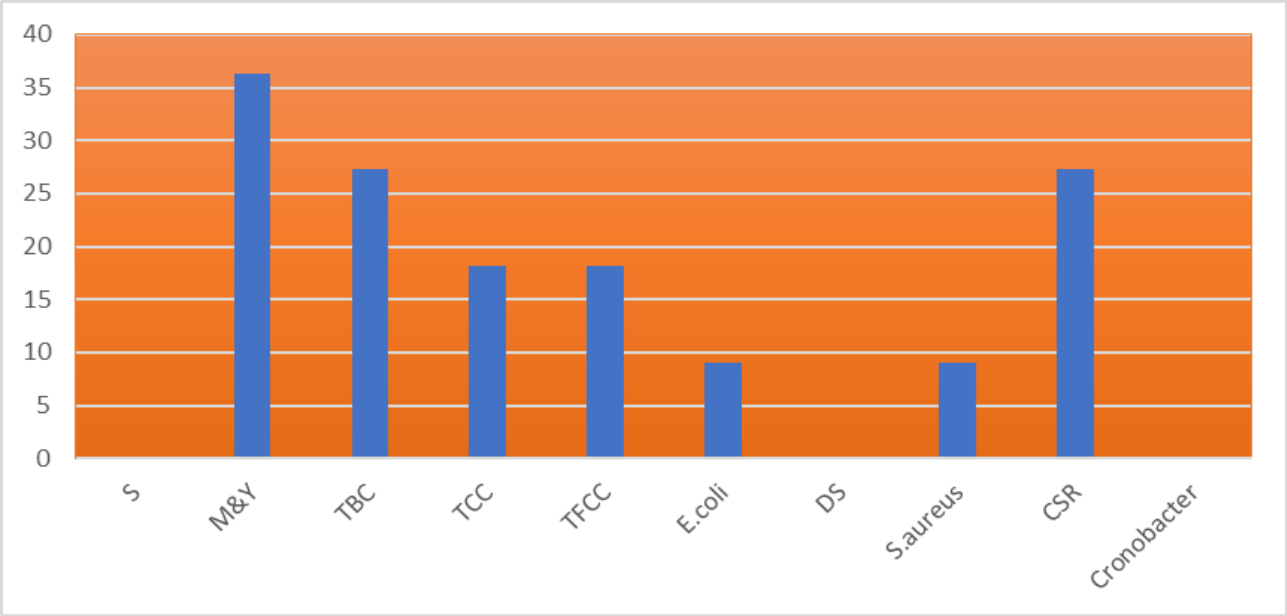

Figure 1: Illustrative histogram of PIF contaminating flora

M & Y: Mold and Yeast, TBC: Total Bacterial Count, TCC: Total Coliform Count, TFCC: Total Fecal Coliform Count, D- S: D- Streptococcus, CSR: Clostridia sulphito-reducer.

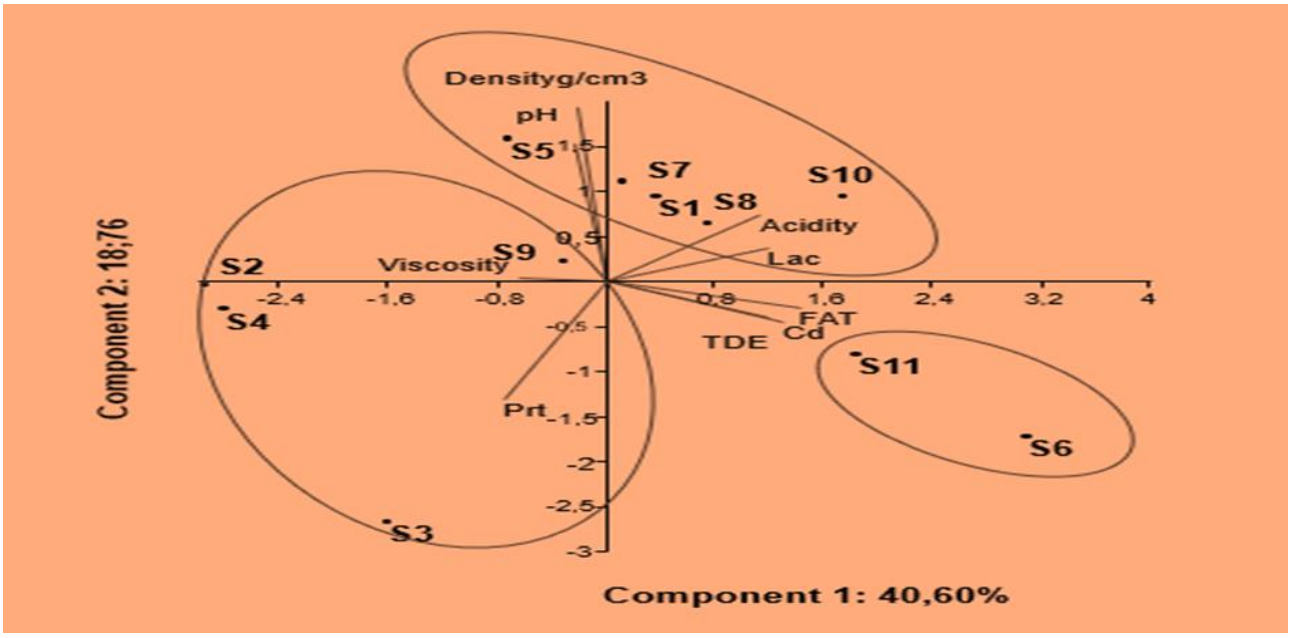

Figure 2: Correlation between PIF composition and microbiological contamination
(S: Sample Acidity: titrable acidity, Lac:Lactose Prt: Protin, TDE:Total Dry Extract)

4.Discussion

4.1.Physicochemical analyses

The results of physicochemical tests of the eleven PIF samples (Table 4) suggest that these samples meet Algerian standards. The stability of all eleven PIF samples remained stable at 20°C without any coagulation, which indicates that they meet Algerian standards for stability (MAC 2012). The range of density values for all samples falls within the acceptable limits, with a maximum of 1.05 and a minimum of 1.02 and an average of 1.030. The middle pH value for the samples was 6.655, indicating a slightly acidic to neutral pH level. While the specific Algerian standard for pH is not mentioned, this result is within the typical range for infant formula products. Titrable acidity level for the samples was 17.62, which should be evaluated against specific Algerian standards to determine compliance. The middle viscosity value is 2.57 mPa.S, which can affect the ease of preparation and consumption. Again, compliance with Algerian standards would be necessary to make a conclusive assessment (Table 4). The middle conductivity value was 1805.72, which may be influenced by the concentration of dissolved elements and non-fat solids. The TDE value is 85%, which is an indicator of the solid content in the samples. Compliance with Algerian standards would be necessary for a complete evaluation. The protein rate is 1.66 g/100 ml of milk, indicating the protein content in the samples. Specific standards would be needed to assess compliance. The lactose content was 1.55g/100 ml of milk, which is an important component of infant formula. Compliance with standards is essential. The results suggest that samples have properties that are generally in line with Algerian standards, particularly regarding stability, density, and pH. Additionally, the findings regarding density contrast with those observed for milk powder sold in Algeria, highlighting potential differences in composition between PIF and other milk products on the market (Table 4). The freshness indicator treatable acidity in (°D) is commonly used as a measure of freshness for milk. It indicates how acidic the milk is, with higher values suggesting greater acidity, which can be indicative of milk starting to sour. The assessment of Freshness, the results of the acidity measurements suggest that the samples being evaluated were fresh. In other words, they did not exhibit signs of spoilage or significant sourness. The comparison with other studies by regarding pH and treatable acidity in this study differ from those reported by a previous study (Soceanu et al. 2015).

Notably, the acidity levels in the recombinant PIF milk samples were higher in this study compared to the previous one. However, the results were similar to another study, which noted a higher acidic pH in newborn milk powder compared to raw milk. In summary, the results suggest that the recombinant PIF milk samples assessed in this study were fresh and did not exhibit significant sourness or spoilage. The use of (°D) provides a quantitative measure of milk acidity, which is important for assessing milk quality and freshness.

The average viscosity measurements for our samples exhibited different values, ranging from the highest value (2.84 mPa.S for S8) to the lowest value (2.07 mPa.s for S9).

The overall average viscosity across all samples was 2.574 mPa.s, which was higher than obtained in literature (Bylund 2003; Soceanu et al. 2015). It's important to note that milk's viscosity, a property primarily influenced by the emulsified and dissolved colloidal particles in recombined milk, presents a particularly complex aspect of rheology. Moreover, the viscosity of milk is subject to multiple factors, including technological variables and external conditions like ambient temperature and atmospheric pressure. Given the observed correlation between rheological characteristics and consumers' perceptions of milk quality in the market, viscosity emerges as a critical determinant of milk quality (Bylund 2003). In the conductivity measurements, the two samples that exhibited extreme conductivity values were (Sample 6) and (Sample 11), recording the highest scores at 1912 µS/cm and 1868 µS/cm, respectively, indicative of their higher ion content. Conversely, the sample with the lowest conductivity was (S5), with a minimum value of 1649 µS/cm. The average conductivity across all samples was 1805.72 µS/cm. It's important to note that factors such as temperature, pH, and milk composition, particularly the fat content, exert a significant influence on the electrical conductivity of milk (AOAC 1997). As ions are present in milk and reflect the concentration of these elements, any alteration in ion concentration within milk will consequently lead to a change in its conductivity (Daunoras and Knyš 2006; Binnur and Serap 2016). Significant variations in conductivity values were observed across all samples. Furthermore, the analysis of total dry extract (TDE) content in the milk powder samples revealed that our results fell below the national standards, which are set at a range of 110 to 112 g/l. As a result, our samples did not conform to these standards. In analytical chemistry and food science, the words "*dry matter*" and "*total dry extract*" are used to compare a substance's solid composition. Despite having a similar notion, there is a small distinction between the two: Dry matter is the quantity of solid stuff left in a substance after all the water has been taken out. It represents the total weight of solids, excluding water content, in a certain sample. In order to represent the percentage of solids in the sample, dry matter is frequently stated as a percentage. Total Dry Extract (TDE) that is frequently used in reference to several beverages like milk, wine or beer (Table 4). It describes the total volume of solid material left over after the liquid in the beverage has evaporated (Mabrook and Petty 2003). For PIF and FOF milk, the use of a default conversion factor of 6.25 to calculate protein content from total nitrogen content is proposed, regardless of the protein source (Agostoni et al. 2014). The EFSA panel concluded in 2014, that regardless of the protein source, PIF and FOF must contain conditionally essential amino acids in amounts at least equal to the reference protein (breast milk) (Agostoni et al. 2014).

4.2.Microbiological analyzes

In this study, microbiological analyses involved counting microbial flora that indicate both recent and old fecal contaminations (eucaryotes flora, TBC, total and fecal coliforms, endosporas and pathogenic/toxinogen species) (AFNOR 2002; Buchanan and Oni 2012). The results (Figure 1 and 2) were then compared to Algerian microbiological standards (MAC 1998). In this investigation, we identified a gap in the Algerian microbiological standards for powdred milk and PIF markted in Algeria. Several research/authors recommend the need to establish an universel standard regulation for preparations; formula, milk flour and other

foods intended for infants (PIF and FOF) (Kent et al. 2015; Pal et al. 2016). Infant care practices, as well as those relating to milk powder preparations, to utensils, are high risks of contamination and transmission of toxins/pathogenic species too underestimated (AFNOR 2002; Buchanan and Oni 2012; Garbaj et al. 2023). In order to confirm the efforts success to guarantee the quality and safety microbiological of a wide range of foods, microbiological testing are frequently used (AFNOR 2002; AFSSA 2005; Buchanan and Oni 2012; Kouadio et al. 2012; Cho et al. 2019). Using microbiological investigations, it's possible to determine whether PIF samples poses hazards to the infant's health while taking into account the circumstances of storage, preparation, consumption patterns, and inherent product qualities (AFNOR 2002; Buchanan and Oni 2012; Cho et al. 2019). A species/group of microorganisms or a byproduct of microbial metabolism known as a microorganism index can be used to determine whether a food has been exposed to conditions that increase the risk of pathogen contamination or whether it has been stored in an environment that would promote the growth of pathogens (Cho et al. 2019). Numerous samples in this investigation showed contaminations at different rates: Yeasts and moulds contaminated 25% of the samples (samples: S3+ S7+ S10). Conversely total aerobic flora contaminates 19% of the samples (S3+ S7+ S10). D- Streptococcus present at 13% of the samples (S2+ S10). Lastly, 12% of samples contaminates by Total Coliform Count (S3+ S7). Likewise, we recorded the presence of fecal coliforms and E. coli in 6% of the samples. The existence of microbiological groups revealing old and current fecal contamination seems to be linked to inappropriate manufacturing and conservation practices.

Some authors (Buchanan and Oni 2012; Lang et al. 2016) believe that *thermotolerant* coliforms are indicators of the adequacy of the cold chain and the degree of sanitation after processing rather than reliable indicators of faecal contamination in refrigerated cooked foods. However, faecal contamination index microorganisms are rarely reliable, particularly when levels of the index organism are low and the pathogen is sporadically present at low levels (AFNOR 2002; Lang et al. 2016).

It is important to keep in mind that pinpointing the source of contaminations is challenging given the circumstances and experimental setup of our investigation.

Microbiological analyses can help determine whether samples pose risks to the newborn's health by taking into account factors such as food preparation, storage, consumption patterns, and inherent food qualities (AFSSA 2005; Haughton et al. 2010; Sani et al. 2013; Cho et al. 2019).

Furthermore, the discovery of spores on the meat-liver bacteriological medium in 19% of our samples supports the possibility of previous contaminations, which may have contaminated raw materials, industrial circuits, packing, the air, or production surfaces.

The complex meat-liver medium, which was enhanced with iron Alun and Na Sulfite, was used to spores count. Following a thermal shock that involves heating the Bath Marie for ten minutes at 80ºC in order to recover all types of vegetation and/or resistance form. Anaerobiosis incubation was verified for 72 hours at 30ºC, with readings taken every 24 hours. According to Rajkovic et al. (2008) the bacteriological technique ISO 7932 and ISO 21871- that were employed for the presumed enumeration of Bacillus cereus species, in this instance have very little analytical power to differentiate between toxic and non-toxic Bacillus cereus strains.The purpose of the 37 ºC incubation temperature is to restore all types of mesophilic and thermophilic resistance strains (Garbaj et al. 2023).

Scientific data report several food poisonings, where milk and powdered infant formula were vectors, Bacillus species in particular certain strains of the B. cereus species were strongly involved: In Germany (Wiebe 1999), in India (Bedi et al. 2005), in Egypt (Sadek et al. 2006), in Brazil Rezende-Lago et al. (2007) in Korea (Hwang et al. 2008), in Australia (Eglezos et al. 2010; Thompson 2010), in Ireland (Haughton et al. 2010), in Italy (Di Pinto et al. 2013) and

in Malysia (Tong 2015). Bacillus cereus is the main pathogen/toxinogen species in the dairy sector, followed by Clostridium tyrobutyricum species (Heyndrickx 2011); Aman et al., 2016).However; the ability of Bacillus cereus spores, to survive in milk powder and dairy products with low water activity appears strains dependent (Lv et al. 2021; Romero-Rodríguez et al. 2023).

However, this survival is influenced by temperature (Igura et al. 2003), changes pH (Sala et al. 1995) and the presence of competitive flora (Peter and Nicola King ; Valero et al. 2007). According to Becker et al. (1994) a rate of 54% of infant milk and formula was contaminated by B. cereus out of a total of 261 samples manufactured in 17 countries. Furthermore, in the opinion of certain authors, the aerobic spores of Bacillus spp. can escape any industrial process aimed at thermal/hygienic treatment (Scurrah et al. 2006; Stoeckel et al. 2013; Rossi et al. 2018). Numerous research conducted in numerous nations (nations importing/consuming PIF milk/FOF formula) have showed high levels of bacterial contamination brought on by diverse microbial groups/species, which partially agrees with our findings Figure 1and 2: In Abidjan-Ivory coast (Kouadio et al. 2012), in Libya (Shadlia-Matug et al. 2008), in Nigeria (Falegan and Oluwaniyi 2015), in Egypt (Tahoun and Abdelfatah 2015; Aman et al. 2016), in Iraq (Abdelreda and Ajmi 2016), in Turkey (Sezer et al. 2015), in Pakistan (Rajput et al. 2009), in Sudan (Hussein 2007), in Indonesia (Estuningsih et al. 2006), in Iran (Mardaneh and Dallal 2013), in Malysia (Sani et al. 2013), in Korea (Hwang et al. 2008). Under conditions/limitations of this study, it is difficult to determine the origin of the contamination flora. However, the presence of indicator flora such as Total Bacterial Count, Total Fecal Coliform Count, D-Streptococcus, Clostridia sulphitoreducers, E. coli indicates poor manufacturing, hygiene, conservation, distribution practices.

5.Conclusion

Study aimed to evaluate the chemical and microbiological stability of various brands of first-age powdered infant formula marketed in Algiers, Algeria. PIF samples met Algerian Standards, demonstrated chemical stability, and were free of flaws in the packing and labeling. Nonetheless, it seems that the samples' microbiological purity is much below Algerian standard. Statistical study revealed relationships between PIF biochemical composition and bacterial flora contamination. Still, this study provided a significant step toward improving bacteriological quality and chemical stability of powdered infant formula sold in Algeria. From this standpoint, it would be ideal to extend the research using bigger PIF sample sizes, a variety of brands, and a greater variety of tests.

P.S. To our knowledge, this is the first report contributing to the evaluation of the chemical stability and microbiological quality of the first-age powdered infant formula marketed in Algeria.

Conflict of interest: The authors hereby declare that they have no known conflict of interests could have appeared to influence the work reported in this paper. The authors have no competing interests to declare.

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