International Journal of Health Sciences
June 2024, Vol. 12, No. 1, pp. 10-23
ISSN: 2372-5060 (Print), 2372-5079 (Online)

Published by The Brooklyn Research and Publishing Institute
DOI: 10.15640/ijhs.v12n1a2
URL: https://doi.org/10.15640/ijhs.v12n1a2

# Powdered Flour and Follow on Formula Marketed in Algier's City- Algeria: Microbiological Quality and Chemical Stability

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**Abstract**

Powdered infant follow-up formula plays a crucial role in infant nutrition when breastfeeding is unavailable, insufficient or inadequate. Study aimed evaluated microbiological quality and physicochemical stability of ten samples of available powdered follow-on formulae, in terms of public health collected in Algiers city, Algeria, during spring season. Microbiological analysis, apart from absence of *Staphylococcus*, *Salmonella* and *Cronobacter*, revealed following results: yeasts and molds: present in 40% (4/10); Total Bacterial Count and Sulfito Reducing Clostridia in 30% (3/10), D-Streptococcus and Total Coliform Count in 20% (2/10) of samples. Total Fecal Coliforms and *Escherichia coli* present in 10% (1/10) of samples, Chemical examinations indicated samples remained stable with following mean values: Density (1.02- 103), pH (6.32- 6,92), Acidity (16.51-18.96), Viscosity (2.43- 2.89), Conductivity (1657-1827), Protein (1.45-2.33%), Fat (3.63- 5.15%), Lactose (3.55- 6.65%), Total Dissolved Extract (68.78-86.86%). Samples showed stability chemical throughout storage and, with no packaging or labeling defects noted. However, microbiological quality sample's fell below Algerian' standards. In light of these findings, further investigation is warranted through expanded sampling approach, targeting additional brands, conducting a wider array of physicochemical and toxicological tests, and examining a wider of bacterial flora/species.

Keywords: Analyse, Chemical stability, Follow-on formula, Microbiological quality

## 1. Introduction

Bovine milk and other dairy specie's milk or a combination of these milks and/or other substances that have been demonstrated to be suitable for feeding newborns are the sources of milks, Powdered Infant Formula (*PIF), follow- on formulae (*FOF) and powdered formulas used for babies (Goulet, Turck, & Vidailhet, 2012). Raw milk's shelf life is extended by being converted into powder or flour, which also enables the retention of the food's nutritional benefits and sensory qualities for extended periods of time, even at room temperature (Pal et al., 2016). By having a quantity/quality ratio, that is appropriate for the child's-age and in accordance with established criteria, this composition tends to be as similar as possible to breast milk and the demands of the infant (Kent, 2015; Kent, Fitzgerald, Hill, Stanton, & Ross, 2015). FOF and PIF are considered as prebiotic foods (Stiverson et al., 2014). In dairy technology their composition must be corrected, in proteins, amino acids, carbohydrates, lipids, vitamins, mineral salts, trace elements, certain approved food additives (Chávez-Servín, Castellote, & López-Sabater, 2008). Also, such as prebiotics: complex sugars, polydextroses, Fructo-Oligo-Saccharid (FOS), Gluco-Oligo-Saccharid (GOS) (Ben et al., 2008), lutein (antioxidant, pigment), taurine (amino- acid), essential fatty acids such as: alpha-linolenic acid and omega-3 (Goulet et al., 2012). Children who are not breastfed are significantly more overweight and obese than those who are breastfed (Scherbaum & Srour, 2016). Breastfeeding is crucial in evaluating a child's nutritional health and has a preventative influence on the onset of obesity and other nutritional problems (Dubois et al., 2022). The prevalence of exclusive breastfeeding varies from one country to another (Rodrigues et al., 2018; Wen, Baur, Rissel, Alperstein, & Simpson, 2009). Norway and Sweden have the highest breastfeeding rates globally, estimated at around 99%, while Denmark follows closely with a rate of approximately 95%. These Nordic European countries demonstrate exceptionally high rates of breastfeeding (OMS, 2005). In Algeria, estimated at around 14% with an average duration far removed from WHO recommendations. The percentage of exclusively nursing mothers generally increases as maternal education levels rise and median family income drops (Abla, Agli, & Boukazoula, 2016).

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Additionally microbiological quality analyzes ensure a safe product free of bacteriological dangers (Kent, 2015) and/or toxicological hazards (Cho et al., 2019; Keck, 2009). A species/group of microorganisms or a by product 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 (AFNOR, 1999; 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 (Kurtz & Thomopoulos, 2021). According to some authors (Buchanan & Oni, 2012; Ji, Wong, Cai, & Liu, 2014; 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. Numerous research conducted in numerous nations (nations importing/consuming PIF milk/FOF formulae) have showed high levels of bacterial contamination brought on by diverse microbial groups/species: In Abidjan- Ivory coast (Kouadio et al., 2012), in Libya (Shadlia-Matug, Aidoo, Candlish, & Elgerbi, 2008), in Negeria (Falegan & Oluwaniyi, 2015), in Egypt (Tahoun & Abdelfatah, 2015; Aman, Abbas, & Elkassas, 2016), in Iraq (Abdelreda & Ajmi, 2016), in Turkey (Sezer, Vatansever, & BİIge, 2015), in Pakistan (Rajput et al., 2009), in Indonesia (Estuningsih et al., 2006), in Iran (Mardaneh & Dallal, 2013), in China (Ji et al., 2014; Liu et al., 2012). Milk and various Powdered Infant Formula PIF and FOF, were potential vectors for a number of food illnesses around the world (Buchanan & Oni, 2012; Hamrin & Hoeft, 2012). *Bacillus* spp endospores and particularly strains within species like *Bacillus cereus* were heavily involved: in India (Bedi, Sharma, Gill, Aulakh, & Sharma, 2005), in Egypt (Sadek, Fathi, & Salem, 2006; Sadek, Refaat, El-Shakour, Mehanna, & Hassan, 2015), in Brazilia (Rezende-Lago, Rossi Jr, Vidal-Martins, & Amaral, 2007), in south Korea (Hwang, Lee, & Park, 2008), in Australia (Eglezos, 2010) , in Ireland (Haughton, Garvey, & Rowan, 2010), in Italy (Di Pinto et al., 2013); in Malysia (Lesley, Ernie, Kasing, & Son, 2017; Sani, Hartantyo, & Forsythe, 2013). Furthermore, according to (Becker, Schaller, von Wiese, & Terplan, 1994) a rate of 54% of infant powdred milk and powdered formulas were contaminated with *Bacillus cereus* sporas on a staff of 261 samples manufactured in Seventeen countries worldwide. According to several authors, aerobic *Bacillus* endosporas may have avoided any industrial procedures and/or heat/radiation treatment intended to eradicate them (Rossi, Aguilar, Silva, & Vidal, 2018; Scurrah, Robertson, Craven, Pearce, & Szabo, 2006; Stoeckel, Lücking, Ehling-Schulz, Atamer, & Hinrichs, 2016; Stoeckel, Westermann, Atamer, & Hinrichs, 2013). In addition, *Cronobacter* spp. species are cause of infections associated with (fecal) contaminations of FOF and several infant powdred preparations (Henry & Fouladkhah, 2019; Proudy, 2009). These species are universally involved in several infants outbreak: In France 2004 (Alerte, 2005), in India 2007 (Ray et al., 2007), in Spain 2007 (Conde, Legorburu, Urcelay, Zárate, & Zugazabeitia, 2007); in Mexico 2015 (Jackson, Flores, Fernandez-Escartin, & Forsythe, 2015) , in USA 2017 (Bowen et al., 2017), in Australia 2018 (McMullan et al., 2018). In milk and powder formulae, particularly those created for premature and hypotrophic infants, *Cronobacter* species are the most closely detected in dairy technology (Pal et al., 2016). On the other hand, certain baby's powdred formulas/meals in thick powder may present risks, depending on the ingredients and additives that make up them, the way they are incorporated in the manufacturing process (Hein et al., 2009; Proudy, 2009). In addition, *Salmonella* spp species, are coliforms, intestinal pathogens (DuPont, 2007) naturally present in the digestive tract of humans/animals, this constitutes their reservoir; they can, as a result of fecal contaminations, survive in the environment and food for several months/years (Korsak, Clinquart, & Daube, 2004). FOF's intricate biochemical makeup, abundance of vitamins, proteins, lipids, and sugars, as well as their neutral pH, render them highly susceptible to microbial enzymes attacks. Likewise, their high concentration of oxidizable substances such as vitamins, fats, proteins and prebiotics can undergo transformation when exposed to light, oxygen, and high temperature during preservation. Therefore, it's important to investigate their stability through various physicochemical tests. Additionally, dairy technology companies and food quality control and hygiene agencies face a challenge in ensuring follow-on formula (FOF) and baby milk powder stability throughout storage, transportation and marketing, despite the availability of advanced and leak-proof packaging that prioritizes innovation and sanitation. In Algeria, all types of powdered infant milk, including infants follow-on formulas and baby powdered meals, formulas, are exclusively imported. Algeria holds the position of the third-largest importer of milk powder globally. The country has a population of over 43 million people and a high fertility rate estimated at an average of three children per woman (Abla et al., 2016). In terms of infant nutrition, the Algerian infant milk powder market is estimated to be the fifth largest in the Middle East and North Africa (MENA) region. The projected annual demand for milk in Algeria exceeds three hundred million liters. Study aims to evaluate the microbiological quality of flour and powdered infant formula (FOF), through research/enumeration, on bacteriological media, in colony-forming units (in CFU), based on conventional methods (ISO), carried on classical culture media, including evaluation of total aerobic floras, total thermotolerant coliforms floras, D-Streptococus group etc... search for pathogenic and/or toxigenic microbial species (*Escherichia coli*, *Salmonella* spp, *Listeria* spp, *Cronobacter* spp, *Bacllus cereus* and *Staphylococcus aureus*) (AFNOR, 1999).

The physico-chemical stability of the samples will be evaluated using different tests: (Stability, density, pH, titratable acidity, viscosity at 20°C, conductivity, protein rate, fat rate, lactose rate and Total Dry Extract levels, etc.) on ten samples of flour and powder preparations for infants (different brands) collected from various pharmacies during the spring period, in Algiers city- Algeria. A statistical analysis by Principal Component Analysis (ACP) will be carried out to search for possible correlations between the physicochemical parameters and the flora rates of microbiological contamination. The obtained results will be compared to both the current national Algerian standards (M. A. C. M. A. d. C. M.A.C., 2012; M. A. d. C. M.A.C., 2017.) and the standards documented in scientific literature ((EFSA), 2014; AFNOR, 1999).

## 2. Material And Methods

### 2.1. Samples collection

Twelve FOF's samples, of imported origin, of different brands, from different batches, were collected from pharmacies in Algiers city- Algeria, during the spring season. The samples were selected based on their availability on the market and their high use frequency. Before analysis, the samples were stored at room temperature. All FOF sample's can be used, after recombination, as complementary foods for children. Prior to examination, the specimens were kept at room temperature. Every formula's composition (for the 10 FOF's samples) was carefully gathered from the sample packaging's details, including the confirmation of the manufacturing and expiration dates. Samples were kept airtight during analysis by being kept in a dryer. All of the items utilized in this study's microbiological and chemical analyses were premium reagents.

### 2.2. Microbiological analyzes

Following proper collection procedures, all samples were moved to the laboratory while keeping the cold chain intact. Performing decimals dilutions: Take a sterile sample from each sample and dilute 25 g of milk powder (both surface and depth) in 225 ml of Tryptone Salts Water. This is the initial 1/10 dilution. Following homogenization (in Vortex), dilutions are made in steps of 10-6, using sterile transfers of 1 ml into 9 ml of tryptone salt water (TSW). The usual (traditional) procedures advised by AFNOR guidelines/standards were used to conduct the research and count the species of microorganisms (AFNOR, 1999) Table 1- The results (Presence/Absence) expressed in CFU/g were compared to those described in the scientific literature ((EFSA), 2014; AFNOR, 1999) and Algerian standard (M. A. d. C. M.A.C., 2017.).

Table 1: Microbiological analyzes (AFNOR, 1999).

| Groups/species | Medium | Additive | Brand/ Meker | Incubation | Code |
|---|---|---|---|---|---|
| Mold and Yeast count (MYC) ISO 21527 | Sabouraud Agar | // // | IPA-Algeria | 25°C/5 days | NF V 08- 051 |
| TBC (MPN) ISO 13559/IDF 153 | PCA Agar | // // | 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 | // // |
| *Streptococcus* D | Eva Litsky (Broth) | MPN. Confirmation | Pronadisa | 37 °C/24H | //.. // |
| *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 | | | Merck/Pro nadisa | 37 °C/ 42°C 3 to 5 days | NF V 08- 055 (Barre et al., 2020) |
| *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 | (ISO. ISO/TS 22964. de Normalisation, 2017) (US FDA Food, 2002) |

2.3. Physicochemical analyses

Stability at 20°C: Stability test provides information on how well a product will hold its chemical and physical's characteristics over time and in particular situations. Density: For quality control, the density at 20°C is crucial because it can reveal compositional variations in the FOF formulas. pH: In order to be sure that a product is safe to eat/drink and won't harm a baby's digestive system, its pH level is crucial. Acidity: One technique to ascertain the amount of acids in a solution is to measure its acidity. It might be applied here to evaluate the PIF samples' degree of acidity. Viscosity: Viscosity measures the thickness or resistance to flow of the FOF formula at 20°C after recombination. It is important to understand viscosity because it can affect the ease with which FOF can be prepared and consumed by infants. Conductivity: The PIF samples's ability to conduct an electrical current is evaluated by the conductivity measurement. It can reveal information about the sample's general composition and sels and ionic content. Proteins content: Proteins rate is obtained in three steps: probably alludes to a thorough protein analysis that takes three steps. Another word for nitrogen is azote, and it's used here to refer to the amount of protein. Fat: One popular way for figuring out how much fat is in milk and other dairy products is the FAT/Gerber method. Most typically, a chemical procedure is used to isolate and quantify the fat. Total Dry Extract (TDE): The solid fraction of mixture that remains after all moisture has been removed is known as the entire dry extract. The solids concentration is shown in the powdered formula. Lactose: The main carbohydrate in FOF and pouderd infants formulas is lactose. Finding out how much lactose is in the formula is essential to evaluating its nutritional value (Table 2).

Table 2: Physicochemical analyses

| Test | Principe | Result/ Expression | Reference |
|---|---|---|---|
| Stability (Test) at 20°C | | // // | method used by: (Thieulin & Vuillaume, 1967). |
| Density at 20°C | Densitometer: LAUDA-model $TD_1$ | // // | Method by (Vierling, 2003) |
| pH at 20°C | pH-meter: Inolab-pH730 | // // | method by (Kristensen, Salomon, & Kokholm, 1991). |
| Acidity at 20°C | Test milk freshness/ milk: Lactate neutralized by NaOH solution (0.1N)+color indicator | Dornic D (in °D) | Method used by (Soceanu, Popescu, & Dobrinas, 2015). |
| Viscosity at 20°C | Rheology Viscosimeter: Rion-ViscotesterVT-03F(Germany). | Milli-pascal/second | Method by (Bylund, 2003; Gasmalla, Khadir, Musa, Aboshora, & Zhao, 2013) |
| Conductivity at 20°C | Conduct- meter- Inolab-cond-730-(Germany). | microsiemens/centimeter | method used by (Horwitz, 1975) |
| Proteins (Azote total): Test in 3 steps: | 1Mineralization: BuchI Digestion Unit Mineralizer K-424. 2. Distillation: BuchI Distillation Unit K-350 Distiller 3.Titration | // AOAC Official Method 991.20 (2006b) | method used by (AOAC, 1970). |
| FAT/Gerber Method (%) | ISO 19662 | | Method used by (Alfaris, Alothman, Aldayel, Wabaidur, & Altamimi, 2022; IDF FIL & Federation, 2006). |
| Lactose (Rate %) | | | Method used by (Abu-Lehia, 1987; Alfaris et al., 2022). |
| TDE: Total Dry Extract % | 105°C/ water Elimination by heating (105°C) until a stable sample obtained | Distillateur/// Mammert | Method used by (Martins, Ferreira, & Carvalho, 2018) |

## 3. Statistical Analyses

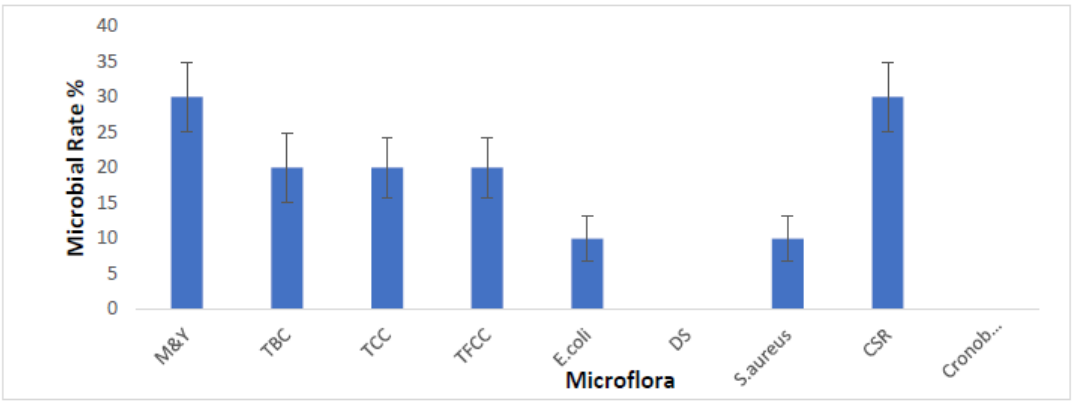
A statistical study or study of possible correlation, using the Principal Component Analysis (ACP) method to explore possible correlations between chemical tests (the results relating to the different physico-chemical analyzes in this study: Stability, density, pH , titratable acidity, viscosity at 20°C, conductivity, protein rate, fat rate, lactose rate and Total Dry Extract levels) and the rates of contamination of the samples by microbial flora or the rates of presence of microbial flora/species in the samples FOF (Figure 3 and 4.)

## 4. Result at and Discussion

Table 3: Results of microbiological analyses

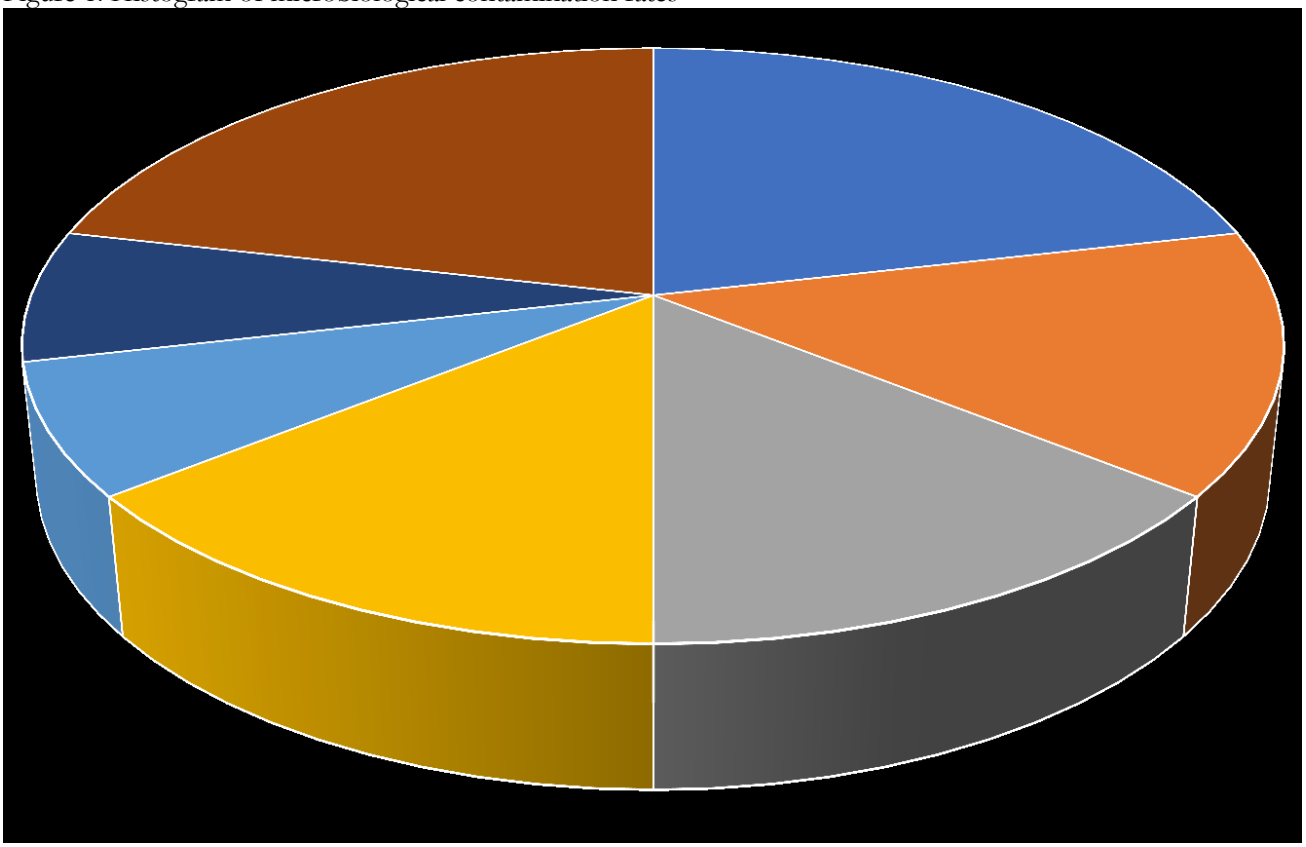
| S | M &Y | TBC (UFC/g) | TCC (UFC/g) | TFCC (UFC/g) | *E. coli* | D S (UFC/g) | *S.aureus* (UFC/g) | CSR (UFC/g) | Cronobacter (UFC/g) |
|---|---|---|---|---|---|---|---|---|---|
| E1 | - | - | - | - | - | - | - | - | - |
| E2 | - | - | - | - | - | - | - | - | - |
| E3 | ++ | 4.1 x$10^3$ | ++ | + | + | - | + | + | - |
| E4 | - | - | - | - | - | - | - | - | - |
| E5 | - | - | - | - | - | - | - | - | - |
| E6 | - | - | - | - | - | - | - | - | - |
| E7 | ++ | 1.8 x $10^3$ | ++ | + | - | - | - | ++ | - |
| E8 | ++ | -- | - | - | - | - | - | ++ | - |
| E9 | - | - | - | - | - | - | - | - | - |
| E10 | ++ | 2 x $10^3$ | - | - | - | - | - | - | - |
| Rate | 4/10 | 3/10 | 2/10 | 1/10 | 1/10 | 2/10 | 00/10 | 3/10 | 00/10 |
| % | 40% | 30% | 20% | 10% | 10% | 20% | 00% | 30% | 00% |

ACP Analysis results




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 1: Histogram of microbiological contamination rates



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: Representative circles of microbiological contamination rates

| E | Stability | Density ($g/cm^3$) | pH | Acidity (°D) | Viscosity (m Pa.s) | Cd (µs/cm) | Prt | FAT (% ) | Lac (% ) | TDE (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| E1 | ++ | 1,02 | 6,76 | 17,97 | 2,43 | 1827 | 2,25 | 3,92 | 6.65 | 78,80 |
| E2 | ++ | 1,02 | 6,92 | 18,71 | 2,82 | 1788 | 1,83 | 4,43 | 3,62 | 68,78 |
| E3 | ++ | 1,02 | 6,35 | 16,51 | 2,54 | 1794 | 1,54 | 4,35 | 4,15 | 70,34 |
| E4 | ++ | 1,03 | 6,32 | 16,63 | 2,61 | 1647 | 1,35 | 3,63 | 5,25 | 80,26 |
| E5 | ++ | 1,03 | 6,81 | 17,15 | 2,89 | 1810 | 2,11 | 5,15 | 3,55 | 86,86 |
| E6 | ++ | 1,03 | 6,32 | 18,92 | 2,79 | 1741 | 2,00 | 3,65 | 4,27 | 84,38 |
| E7 | ++ | 1,03 | 6,73 | 18,45 | 2,63 | 1780 | 1,48 | 4,62 | 3,95 | 91,27 |
| E8 | ++ | 1,02 | 6,93 | 18,83 | 2,76 | 1787 | 2,06 | 3,65 | 5,64 | 80,15 |
| E9 | ++ | 1,03 | 6,34 | 17,82 | 2,47 | 1826 | 1,45 | 3,81 | 6,57 | 76,45, |
| E10 | ++ | 1,02 | 6,75 | 18,96 | 2,81 | 1785 | 2,33 | 4,02 | 4,46 | 81,25 |
| VMax | | 1.03 | 6,92 | 18,96 | 2,89 | 1827 | 2,33 | 5,15 | 6,65 | 86,86 |
| VMin | | 1.02 | 6,32 | 16,51 | 2,43 | 1647 | 1,45 | 3,63 | 3,55 | 68,78 |

**Maximum Value (Max V)-, Minimum Value (Min V)-, Average (Av)-, Median (M)-, Standard deviation (Sd)**
**Acidity (°D):** in Dornic degrees, **Av (M):** Average, **Cd/Conductivity:** (µs/cm) in microsiemens/centimeter, **Density** ($g/cm^3$): Gram/cubic centimeter, **Max V:** Maximum value, **Md (x):** Middle value, **Min V:** Minimum value, **SD (σ):** Standard deviation, **TDE:** Total Dry Extract (%), **V/Viscosity (mPa.s):** in milliPascal/second, **E: Sample**

Figure 4: Pie Chart representation of physicochemical tests

Study of the correlation between the physicochemical and microbiological tests of the different FOF samples

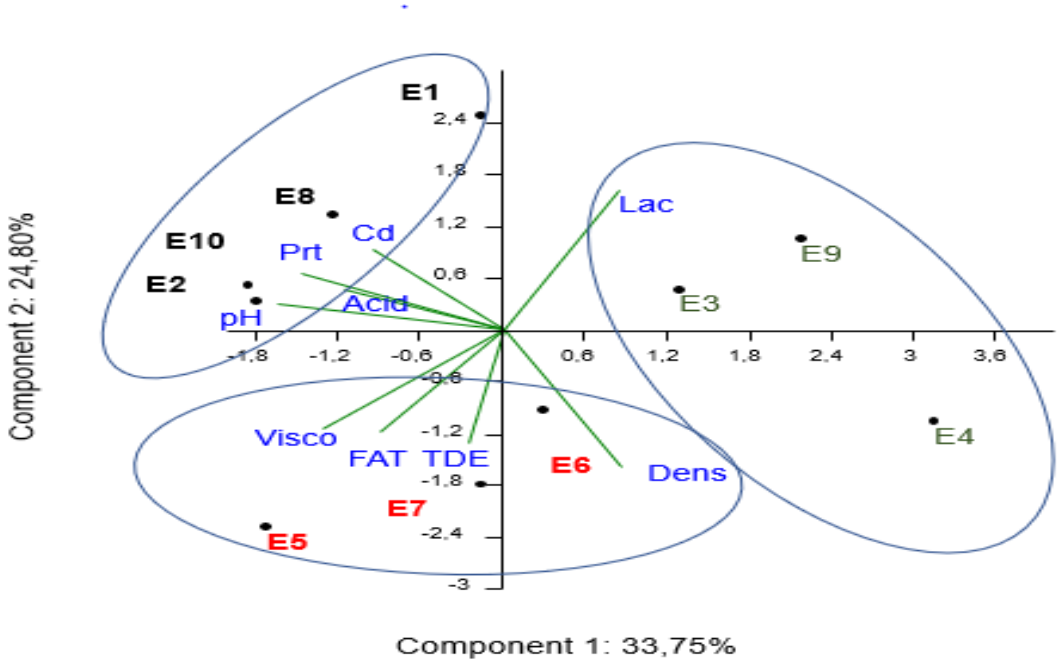



Figure 3: Groups obtained after ACP analysis

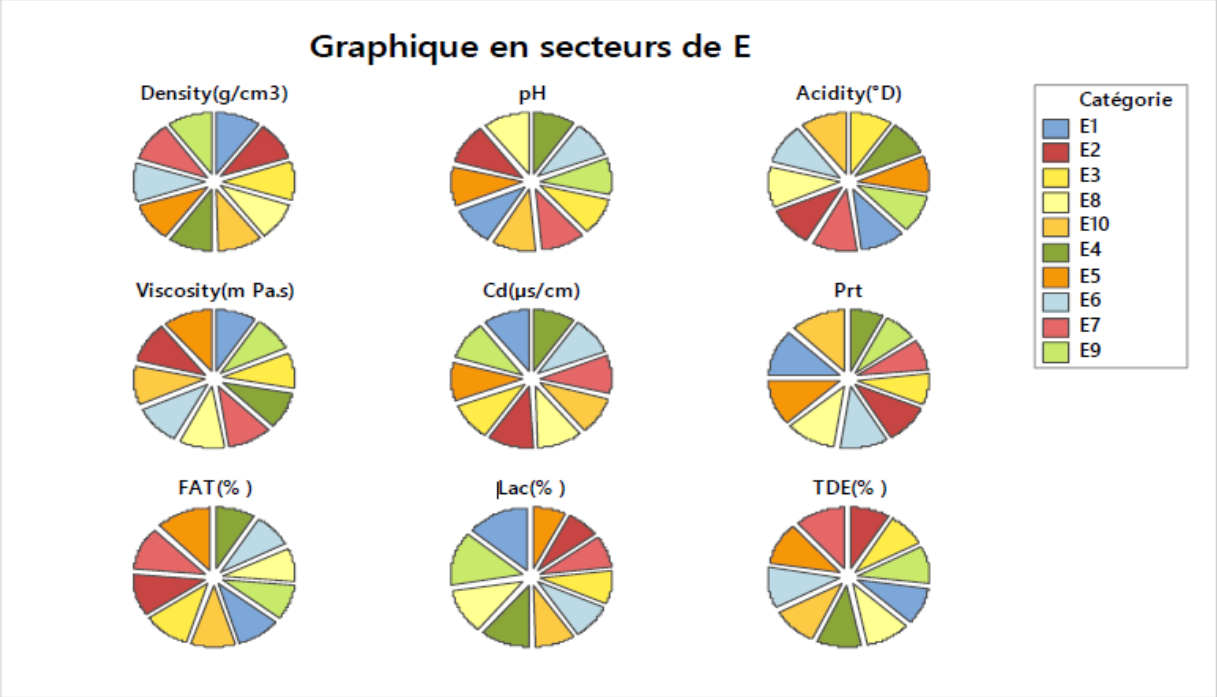



Figure 4: Pie Chart representation of physicochemical tests

Discussion: results relating to microbiological analyzes (Figure 1 and 2; Table 3): First of all, the samples as a whole have a common appearance. We made sure that every sample was free of flaws in terms of consistency, color, look, odor, swelling, or packing. Practices pertaining to baby care, powdered milk preparations, and utensils carry significant risks of toxin and pathogenic species transfer that are often overlooked ((EFSA), 2014; AFSSA), 2005). Microbiological tests are widely used to verify the effectiveness of efforts to guarantee the microbiological safety and quality of a variety of foods including our FOF samples (Falegan & Oluwaniyi, 2015; Shadlia-Matug et al., 2008; Tahoun & Abdelfatah, 2015). According to our findings, the FOF milk samples were much below Algerian standards (M. A. C. M. A. d. C. M.A.C., 2012; M. A. d. C. M.A.C., 2017.). All of the samples presented various contaminations: 40% of samples are contaminated by yeasts and molds: (samples E3+ E7+ E8+ E10). While 30% of samples are contaminated by total aerobic flora (E3+ E7+ E10). Finally, 30% are contaminated for spore-forming bacteria (E3+E7+E8) (Table 3). It should be remembered that in the context, the experimental conditions of our study, it's difficult to identify the origin of contaminations. When considering the conditions of storage, preparation, consumption habits, and the intrinsic properties of the food, microbiological examinations can be used to ascertain whether FOF samples pose health risks to the newborn (AFSSA), 2005; Cho et al., 2019; Haughton et al., 2010; Sani et al., 2013). The microbiological analyses in this investigation involved counting the microbial flora on classic selective bacteriological medium (Buchanan & Oni, 2012) and showing recent and ancient fecal and/or other contaminations (eukaryotic flora, TBC, total and fecal coliforms, endospores, and pathogenic/toxigenic species) Next, the outcomes on Table 3- were contrasted with Algerian (Standard) criteria (M. A. C. M. A. d. C. M.A.C., 2012). During this study, we found a gap in the microbiological standard for powdered milk in Algeria, specifically for FOF that is sold in the country. The enumeration of presumptive *Bacillus cereus* species/ spores was carried out following the (routine) method recommended by the standard NF-EN ISO 7932 (2005). According to (Rajkovic et al., 2008) aerobic spores of *Bacillus cereus* species can survive in milk powder and infant formulas, reconstitution of milk gives these forms of resistance optimal conditions for germination and proliferation (Ronimus, Rueckert, & Morgan, 2006). On the other hand, *Bacillus cereus* appears to be the main pathogen in the dairy industries, followed by Clostridium tyrobutyricum species (Heyndrickx, 2011; Aman et al., 2016). The ability of *Bacillus cereus* spores to survive in milk powder and dairy products (low water activity foods) appears to be strain dependent, however, survival is influenced by a number of parameters e.g. temperature: (Igura, Kamimura, Islam, Shimoda, & Hayakawa, 2003), variations in pH (Sala, Ibarz, Palop, Raso, & Condon, 1995) and the presence of competitive flora (Cressey, King, & Soboleva, 2016; Valero, Fernandez, & Salmeron, 2003; Valero, Hernandez-Herrero, & Giner, 2007). According to (Rajkovic et al., 2008) even the two methods used for the presumptive enumeration of *Bacillus cereus* species in this case: The standard method-NF-ISO 7932 and the standard NF- ISO- 21871, have analytical power very limited, to distinguish between strains within the species.

In this Density (g/cm3) pH Acidity(°D) Viscosity(m Pa.s) Cd (µs/cm) PrtFAT (% )Lac(% ) TDE (% ) E9E1E2E3E8E10E4E5E6E7 Catégorie Graphique en secteurs de E regard, it should be noted that the differentiation between B. cereus strains sensu stricto, potential agents of food poisoning (responsible for diarrheal and hemolytic syndrome (Ombui, Schmieger, Kagiko, & Arimi, 1997; Wijnands, Dufrenne, & van Leusden, 2002; Wijnands, Dufrenne, Zwietering, & Van Leusden, 2006) and other non-toxigenic B. cereus strins, other species of the genus *Bacillus* is a subject of great controversy (Rossi et al., 2018). Faecal contamination of FOF pouwder preparations can occur during manufacturing, reconstitution, or storage of the product (Proudy, 2009). More than 90% of faecal *Cronobacter* spp infections have been epidemiologically linked to powdered infant formula (PIF) (Kalyantanda, Shumyak, & Archibald, 2015; Kalyantanda et al., 2015).

The choice of the standard method (ISO/TS 22964:2017) for searching for Enterobacter spp. was its IDF recommendation. Its disadvantage was its slowness, involving two-step enrichment followed by isolation (Fox & Jordan, 2008). Furthermore, Kandhai et al. (2004) developed a routine method based on the screening of *Cronobacter* spp. strains with constitutive α-glycosidase activity and pigmentation yellow when cultured on TSA agar. For this purpose, selective agars (ESIA, DFI, and Compass) were compared by Derzelle et al. (2007) and estimated to be equivalent in terms of sensitivity for the detection/enumeration of *Cronobacter* spp. Many methods have been designed for the recovery/enumeration of *Cronobacter* spp. phenotypes in powdered milk (Chon, Seo, Oh, Jeong, & Song, 2023; Osaili et al., 2010) (Figure 1 and 2).

Discussion: results relating to physicochemical analyzes: All of FOF samples remained stable at 20°C without coagulating, proving that they satisfy Algerian stability standard. All of the samples' density values, with an average of 1.025, a minimum of 1.02, and a maximum of 1.03, are within allowable bounds (Thieulin & Vuillaume, 1967). Viscosity values fluctuated between 2.43 and 2,89 mPa.s, which may affect the ease of preparation and consumption of FOF. Here again, compliance with Algerian standards would be necessary to make a conclusive assessment (M. A. C. M. A. d. C. M.A.C., 2012). The range of conductivity values in the FOF was 1827 and 1647, which could be impacted by the amount of dissolved solids and continuous non-fat solids present When assessing the composition of FOF samples, these factors are crucial. The TDE values, which represents the samples' solid composition, ranged from: 68.78 to 86.86. The terms "dry matter" and "total solids" are used in analytical chemistry and food science to compare a substance's solid makeup. Furthermore, our results were below the national standards for the total dry extract (TDE) content of the powder preparation samples, meaning that our samples did not fulfill these standards (M. A. C. M. A. d. C. M.A.C., 2012). Adherence to Algerian criteria would be required for a thorough evaluation (Table 4). The protein level was between 1.45 and 2.33and g/100ml of FOF samples, reflecting the protein content of the samples. To evaluate whether samples adhere to standards, appropriate biochemical procedures and particular standards would be required. The samples had lactose levels between and 5.612 g/100 ml, which is a crucial ingredient in baby powdered formula. A number of standard analytic methods are suggested for figuring out lactose. All samples showed slight differences in conductivity ratings (Table 4). Any variation in the ion content of the samples will consequently cause a change in its conductivity since the ions found in the FOFs reflect the concentration of these elements (Binnur & Serap, 2016; Daunoras, 2008; Demirci, 2011). According to the results, our FOF samples have characteristics that are typically in line with Algerian norms, especially when it comes to pH, density, and stability. For every metric, it is crucial to verify conformity with certain Algerian standards. Furthermore, the density data show a divergence from those seen in powdered milk sold in Algeria, suggesting that FOF, PIF and other dairy products on the market may differ in composition. Additional studies focusing on sizable sample sizes and standard compliance are necessary to guarantee the caliber and security of these baby-oriented products. Statistical analyzes in principal components (PCA), applied to all the samples (from E1 to E10) and the results of the physicochemical tests gave three groups distributed as follows: Group 1: contains samples E2, E8, E10 correlated to the following tests: Acidity (°D), pH, Conductivity and protein levels. Group 2: Contains samples E5, E6, E7 correlation with the following tests: Viscosity, FAT and TDE. Group 3: contains samples E3, E4, E9 with lactose levels

## 4. Conclusion

Powdered infants' milk in general and follow- on formulae (FOF) play a crucial role in infant nutrition when breastfeeding is not available. However, both dairy companies and consumers encounter challenges related to the physicochemical stability, as well as the microbiological quality of FOF throughout storage and distribution. In this stydy Microbiological analysis, showed apart from absence of *Staphylococcus*, *Salmonella* and *Cronobacter*, revealed following results: yeasts and molds: present in 40% (4/10); Total Bacterial Count and Sulfito Reducing Clostridia in 30% (3/10), D-Streptococcus and Total Coliform Count in (2/10) of samples. Total Fecal Coliforms and *Escherichia coli* present in 10% (1/10) of samples. However chemical tests indicated samples remained stable with following mean values:

Density (1.02- 103), pH (6.32- 6,92), Acidity (16.51-18.96), Viscosity (2.43- 2.89), Conductivity (1657-1827), Protein (1.45-2.33%), Fat (3.63- 5.15%), Lactose (3.55- 6.65%), Total Dissolved Extract (68.78- 86.86%). Samples showed stability chemical throughout storage and, with no packaging or labeling defects noted the microbiological examination revealed a notable absence of *Staphylococcus*, *Salmonella*, and *Cronobacter* species. In light of current conclusive results, it would be desirable for future studies to expand the study sample size, include a wider range of infant milk brands, categories and ages, and administer a greater number of tests. This would provide a more in-depth understanding of the quality of infant powdered milk marketed Algerian market.

P.S. To our knowledge, this is the first report contributing to the evaluation of the microbiological quality of powdered infant formula marketed in Algeria. Future research should include more in-depth and well-controlled studies to comprehensively assess the bacteriological quality, risks associated with powdered flours for infants of different ages.

**Acknowledgments**

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (DGRSDT, MESRS), 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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