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Original Research Article

Physicochemical and microbiological assessment of baby shampoos marketed in Algeria

Boukaous Leyla [a], Benatallah Salha Amira [a,*], Seggani Moufida [b], Yousfi Billel [a], Bouziane Oumeyma [a], Betchine Kaouther Rayene [a], Boukaous Racha Bahaa [c]

[a] *Pharmaceutical Sciences Research Center (CRSP), Constantine 25000, Algeria*

[b] *Department of Microbiology, University of Frères Mentouri, Constantine 1, Algeria*

[c] *Department of Biochemistry, University of Frères Mentouri, Constantine 1, Algeria*

Abstract

Eleven baby shampoos samples were subjected to physicochemical and microbiological quality control in order to evaluate their compliance with Algerian standards established for cosmetic products intended for children under three years of age. The results revealed noticeable variations in quality among the tested products. Regarding pH, only five samples complied with the Algerian standard NA 367/1990, while six samples showed values outside the recommended range (6.5–7.5). The density values of all tested shampoos were generally within acceptable limits according to the European Pharmacopoeia, indicating satisfactory formulation uniformity. Viscosity analysis showed that most samples complied with NA 376/1990, except samples S6, S8, and S11, which presented relatively low viscosity values. Microbiological analysis revealed that eight shampoos complied with microbiological standards, whereas three samples (S3, S10, and S11) were classified as non-compliant; These products showed high counts of total aerobic mesophilic flora (TAMF), yeasts and moulds. Furthermore, the detection of pathogenic microorganisms further confirmed the unsatisfactory quality of the non-compliant samples. Overall, the results demonstrate that although most tested baby shampoos showed acceptable physicochemical and microbiological quality, a significant proportion failed to comply with official standards. The non-compliant products may present potential health risks for infants and highlight the need for stricter quality control during manufacturing, storage, and commercialization of baby cosmetic products.

Keywords:

Baby shampoos, physicochemical, microbiological quality control, Algerian standards.

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*Corresponding author

Email address: benatallahamira@yahoo.fr (Salha Amira Benatallah)

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1. Introduction

« The term cosmetic product refers to any substance or mixture intended to come into contact with various superficial parts of the human body, including the skin, hair and capillary systems, nails, lips, external genital organs, or with teeth and oral mucosa, exclusively or primarily for cleansing, perfuming, changing their appearance, protecting, maintaining them in good condition, or correcting body odors » **[1].**

The use of cosmetics is a common global practice intrinsically linked to cultural expression and individual self-confidence. Over the past 20 years, the global cosmetics market has grown by an average of 4.5% per year, generating total sales of $382.3 billion in 2010. Hair care products, including shampoos, conditioners, styling products and hair dyes, accounted for 17.3% of this market **[2]**. The manufacturer must assess the safety of all ingredients contained in the product and carry out a safety assessment of the finished product before it is marketed.

“Shampoos are formulations or preparations consisting mainly of surfactants, designed for washing the hair and scalp” **[3]**. They are among the most widely sold hygiene products in large distribution, undoubtedly because consumers feel they do not need advice to purchase this type of product, the offer is extremely varied, and the prices are suitable. The universe of shampoos seems vast given the diversity of product ranges available on the market **[4]**.

Infant care encompasses all the steps necessary for good hygiene for maintaining healthy skin, reducing the risk of skin diseases, and ensuring the baby's comfort. These steps include cleansing, bathing, hydration, and diaper care. A considerable quantity of cosmetic products, including shampoos, intended for infant care, can be found in the market (pharmacies, parapharmacies, and supermarkets). Therefore, choosing the most suitable product with the least toxic risk for babies' skin becomes challenging. Indeed, the conformity control of these products is of great importance and specific safety evaluation of products intended for children under the age of 3 is also required **[5]**.

Good Manufacturing Practices (GMP) are the set of guidelines designed to minimize risks during product manufacturing, including microbiological analysis. The microbiological purity of cosmetics ensures user safety while preventing physicochemical changes in a formulation, as well as skin infections and diseases. Thus, microbiological control is essential in cosmetics manufacturing, as it ensures that the product stays within safe limits for an appropriate duration without posing a health risk to humans due to microbial contamination **[6]**.

Nowadays, the market for children’s cosmetics is booming, and parents are keen to buy products tailored to their children’s specific needs. Products intended for children under three years of age are not simply standard cosmetics, as an infant’s skin exhibits numerous physiological and functional immaturities that make it more delicate. The thinness of the epidermis and the more alkaline skin pH represent significant structural differences compared to adult skin, and must be taken into account **[7]**. With this in mind, 11 samples of baby shampoos underwent microbiological and physicochemical testing to verify their quality and the extent to which the manufacturer complies with national and international standards.

2. Materials and Methods

Eleven baby shampoos samples were subjected to microbiological and physicochemical analyses to assess their compliance with manufacturing standards.

The practical phase was conducted in two laboratories: the Physico-chemical Analysis Laboratory of Pharmaceutical Sciences Research Center (CRSP). Constantine, and the Joud-Lab Quality and Compliance Laboratory in Bkira, Constantine.

2.1. Physicochemical analysis

2.1.1. pH measurement

The pH of the samples will be determined in accordance with the requirements of standard NA 367/1990.

2.1.2. Density measurement

Density is measured using the standard formula:

Density = Density of shampoo / density of water

2.1.3. Viscosity measurement

Viscosity was determined using a VISCO ATAGO viscometer according to Algerian Standard 376/1990. The measurements were carried out at an ambient temperature of 25 °C using spindle A3 at a rotational speed of 250 rpm

2.2. Microbiological analysis

2.2.1. Preparation of the stock solution of the sample to be analysed : (ISO/TR 19838 : 2016)

Place 10 g of the product to be analysed (shampoo) in a sterile flask, add 90 ml of TAT diluent (Tryptone-azolectin-Tween) or peptone water; Tween 20 serves to neutralise the preservatives. The dilution (10^{-1}) thus obtained is thoroughly homogenised and left for 30 minutes at room temperature. This is followed by the preparation of decimal dilutions.

2.2.2. Determination of Total Mesophilic Aerobic Flora : NA ISO 21149 (NA 8287)

Dispense 1 ml of the stock solution and the prepared dilutions into Petri dishes containing 10 to 15 ml of TSA (Tryptone-Casein-Soy) medium which or Eugon LT100 has been previously melted and cooled. Inoculation is carried out into the medium in triplicate After
the medium has solidified, the dishes are incubated at 32.5 ± 2.5 °C for 24 to 72 hours in a thermostatic incubator. A control dish containing no test product is included.
The colonies appear as lenticular shapes having grown within the medium ; they are counted and multiplied by the corresponding dilution factor.

$$N = \Sigma c / dV$$

- **Σc** : the sum of all colonies counted across all selected plates.
- **V** : the volume of inoculum applied to each plate (1 ml).
- **d** : the dilution

2.2.3. Detection of yeasts and moulds NA ISO 16212

A pour plate inoculation is carried out in the same manner as the previous analysis on Sabouraud medium, intended for the isolation of yeasts and molds. The plates are incubated at a temperature of 22 to 25°C for 3–5 days. A control not containing the product to be analyzed is included.

2.2.4. Detection of pathogenic microorganisms

- **Detection of *Pseudomonas aeruginosa* : NA ISO 22717**

Using a sterile Pasteur pipette, streak an aliquot of the stock solution (SS) onto the surface of Cetrimide agar. Incubation is carried out at 32°C ± 2.5°C for 48 hours. The presence of characteristic colonies is then checked: yellow-green pigment (pyocyanin).

- **Detection of *Staphylococcus aureus* : NA ISO 22718 (NA 14809)**

Using a sterile Pasteur pipette, streak an aliquot of the stock solution (SS) onto the surface of Chapman agar. Incubate at 32.5°C ± 2.5°C for 48 hours. Check for the presence of characteristic colonies: yellow colonies.

- **Detection of *Escherichia coli* : NA ISO 21150 (NA 14808)**

Stripe an aliquot of the solution (SM) onto the surface of Hektoen agar. Incubation was carried out at 32.5°C ± 2.5°C for 48 hours. Check for the presence of characteristic colonies: red-orange colonies.

- **Detection of *Candida albicans*: NA ISO 18416**

Streak an aliquot of the solution (SM) onto the surface of OGA (Oxytetracycline-Glucose-Agar) medium Incubate for 3 to 5 days at 25 °C. Check for the presence of characteristic colonies: convex and creamy, white to beige in colour.

Table 1 : Microbiological criteria for cosmetics and personal care products (Products intended for children under three (3) years of age, for use around the eyes and on mucous membranes) **(according to the Official Journal Of The Algerian Republic No. 16, 2020)**

Types of microorganisms	**Microbiological limits (CFU [1]/g or CFU/ml) [2]**	
	m [3]	**M [4]**
Total mesophilic aerobic microorganisms	$\leq 10^2$	$\leq 2 \times 10^2$
Yeasts and moulds	$\leq 10^2$	
Escherichia coli	Absent in 1 g or 1 ml	
Pseudomonas aeruginosa	Absent in 1 g or 1 ml	
Staphyloccoccus aureus	Absent in 1 g or 1 ml	
Candida albicans	Absent in 1 g or 1 ml	

[1] **CFU**: Colony-forming unit
[2] **CFU/g** : Colony-forming unit for solid products.
CFU/ml : Colony-forming unit for liquid products.
[3] **'m'**: number of bacteria present in one gram or one millilitre of the product analyzed, corresponding to the value below which the quality of the product is considered satisfactory;
[4] **'M'**: number of bacteria present in one gram or one millilitre of the product analyzed, which corresponds to the value above which the quality of the product is considered unsatisfactory.

3.Results and Discussion

3.1. Physico-chemical analysis

The values presented in the following table are the mean values obtained from three replicates

Table 2 : Results of the physicochemical analyses of the shampoos tested

	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**Standard**
pH	6.55	**5.72**	**5.67**	7.47	**4.45**	6.70	6.85	**7.90**	**5.23**	7.29	**4.98**	**NA 367 pH : (6.5-7.5)**
Density	1.017	1.023	1.030	1.029	1.030	1.004	1.032	1.002	1.030	1.021	1.013	**NFT 20-053 1.010-1.030**
Viscosity (mPas)	2298.9	2111.4	1660.1	1916	1201.4	**754.6**	1005.7	**754.6**	2437.0	1289.2	**436.8**	**NA 376/1990 (10000 ≥)**

3.1.1. pH measurement

Among the eleven shampoos tested, five complied with the Algerian standard NA 367/1990, while six samples did not meet the required specifications, exhibiting pH values either below or above the acceptable range established by the standard, which is between 6.5 and 7.5.

This parameter is particularly important in baby shampoos because the scalp and eyes of infants are highly sensitive. A pH close to neutrality helps reduce ocular irritation and preserves the integrity of the hair cuticle. Similar observations were reported by Ashok and Mali (2011) **[8]**, who stated that inappropriate pH values may lead to hair damage and scalp irritation, particularly in baby care products. According to Deffaugt-Sanchez (2012) **[9]**, baby shampoos should ideally have a pH close to that of tears to prevent discomfort during use.

3.1.2. Density measurement

The density values obtained in this study were generally within acceptable limits, indicating satisfactory formulation homogeneity, which agrees with Barel et al. (2009) **[10]**.

Density reflects the uniformity of the formulation as well as the balance between the raw materials used in the shampoo. Garg et al. (2010) **[11]**. indicated that density variations may reflect poor dispersion of ingredients or instability during storage.

3.1.3. Viscosity measurement

As shown in Table 2, the viscosity values are almost all in line with the NA 376 standard, with the exception of three samples (**S6, S8, S11**), which have low values.

Barel et al. (2009) **[10]**, Handbook of Cosmetic Science and Technology, stated that low viscosity may indicate poor formulation stability. Low viscosity values, as observed in samples S6, S8 and S11, may indicate instability of the formulation, which agrees with the findings of Al Quadeib et al. (2018) **[12]**.

3.2. Microbiological analysis

The microbiological analysis of the eleven shampoo samples revealed that eight complied with the established standards, whereas three failed to meet the required microbiological criteria.

Table 3 : Results of microbiological analyses of the baby shampoos tested

	S1			**S2**			**S3**			**S4**			**S5**			**S6**		
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**
TAMF	5 UFC/ml	Abs	Abs	15 UFC/ml	Abs	Abs	**$4.5.10^2$ UFC/ml**	**$4.35.10^2$ UFC/ml**	**$4.1.10^2$ UFC/ml**	5 UFC/ml	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
TYMC	Abs	Abs	Abs	Abs	Abs	Abs	**$3.1.10^2$ UFC/ml**	**$2.85.10^2$ UFC/ml**	**$2.6.10^2$ UFC/ml**	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
E. coli	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs

P. aeruginosa	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
S. aureus	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
C. albicans	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
	Compliant			**Compliant**			**Non-compliant**			**Compliant**			**Compliant**			**Compliant**			

	S7			**S8**			**S9**			**S10**			**S11**			**Standard UFC/ml or UFC/g**	
	10^{-1}	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-1}**	**10^{-2}**	**10^{-3}**		
TAMF	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	**$4.3.10^2$UFC/ml**	**4.10^2 UFC/ml**	**$3.9.10^2$ UFC/ml**	**$4.15.10^2$ UFC/ml**	**4.10^2 UFC/ml**	**$3.95.10^2$ UFC/ml**	**m** ≤ 10^2	**M** ≤ 2.10^2
TYMC	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	**$3.1.10^2$UFC/ml**	**$2.9.10^2$ UFC/ml**	**2.10^2 UFC/ml**	Abs	Abs	Abs	**≤10^2**	
E. coli	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			Abs	Abs	Abs	**Absence**	
P.aeruginosa	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			***Presence***			**Absence**	
S.aureus	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			Abs	Abs	Abs	**Absence**	
C. albicans	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	***Presence***			***Presence***			**Absence**	
	Compliant			**Compliant**			**Compliant**			**Non-Compliant**			**Non-Compliant**				

'm': number of bacteria present in one gram or one millilitre of the product analyzed, corresponding to the value below which the quality of the product is considered satisfactory;

'M': number of bacteria present in one gram or one millilitre of the product analyzed, which corresponds to the value above which the quality of the product is considered unsatisfactor.

3.2.1. Determination of Total Mesophilic Aerobic Flora

The results of the count of aerobic mesophilic microorganisms at 30°C for the shampoos tested, as shown in Table 3, and in comparison with the NA ISO 21149 standard published in the Official Journal of the Algerian Republic, regarding the category of cosmetic products used for children under 3 years of age, which recommends a threshold of 100 CFU/ml or/g for FTAM, show:

A slight level of contamination was detected in three shampoos (**S1, S2, S4**), which is below the standard specified in the standard, whilst there was a total absence of bacteria in the products (**S5, S6, S7, S8, S9**), indicating that these tested products comply with the standard.

A culture of abundant total flora was found in the shampoos (**S3, S10, S11**). The values are significantly higher and exceed the average specified by the standard, which is $M \leq 2 \times 10^2$ bacteria per gram of product, even at lower dilutions, indicating the product's non-compliance.

The high microbial load observed in samples S3, S10 and S11 may be related to inadequate preservation systems, as previously reported by Campana et al. (2006) **[13]**.

In microbiology, an abundant total flora is an indication of the presence of pathogenic bacteria, which is verified by the following tests.

3.2.2. Detection of yeasts and moulds

The testing of eleven shampoos, based on the enumeration of yeasts and moulds, in relation to the category of cosmetic products intended for children under 3 years of age which recommends a limit of 100 CFU/ml or/g for yeasts and moulds reveals :

- A complete absence of fungal flora in eight of the shampoos tested (S1, S2, S4, S5, S6, S7, S8, S9) indicates a satisfactory result.
- Abundant total microbial growth was observed in the shampoos (S3, S10, S11), exceeding the specified limit, which indicates an unsatisfactory result; therefore, the products do not comply.

Testing for yeast and mould is essential for assessing the microbiological stability of cosmetic products.

The fungal contamination observed in samples S3, S10 and S11 may be explained by insufficient preservative efficacy and poor storage conditions, as reported by Lundov et al. (2009) **[14]**.

3.2.3. Detection of pathogenic microorganisms

▪ **Detection of *Pseudomonas aeruginosa***

As shown in the table, a positive culture for *Pseudomonas aeruginosa* was observed in the shampoos **(S3, S10, S11)** on Cetrimide medium. In contrast, a negative result was reported for the remaining shampoos tested.

The presence of *Pseudomonas* sp. may indicate bacterial contamination during the manufacture, storage or use of the product; this bacterium can cause skin irritation, redness, itching, rashes or infections **[15]**.

▪ **Detection of *Staphylococcus aureus***

A negative culture for *Staphylococcus aureus* was observed on Chapman medium for all the shampoos tested, with the exception of product (**S10).** The detection of *Staphylococcus aureus* in sample S10 indicates poor hygienic practices during production and represents a potential health risk for infants.

Siegert (2011) **[16]** reported that *Staphylococcus aureus* may cause skin infections, folliculitis, and irritation, particularly in infants and immunocompromised individuals.

▪ **Detection of *Escherichia coli***

A positive result was observed for two products (**S3** and **S10**), indicating that these samples do not comply with the standards specified in the Official Journal. In contrast, no *Escherichia coli* contamination was found in the remaining samples tested.

The presence of *Escherichia coli* in baby shampoo is completely unacceptable, as it indicates faecal contamination. This may result from poor hygiene during the manufacturing process, cross-contamination with other raw materials, or a failure in quality control procedures, and it can pose serious health risks to

babies, who have less developed immune systems and more sensitive skin. Exposure to *E. coli* can lead to skin infections, irritation, allergic reactions and gastrointestinal disorders **[17]**.

▪ **Detection of *Candida albicans***

Candida albicans was identified in samples S3, S10, and S11, while absent in the remaining products. This fungal contamination may lead to scalp irritation, itching, redness, and fungal infections, especially in children with delicate skin. Arenas (2001) **[18]**. Reported that *Candida albicans* contamination in cosmetic products is often associated with inadequate storage conditions and insufficient preservative systems.

4 Conclusion

In conclusion, this study demonstrated that the majority of the baby shampoos tested showed acceptable physicochemical and microbiological quality and complied with the Algerian standards established for cosmetic products intended for children under three years of age. However, some samples exhibited significant non-conformities, particularly in terms of pH, viscosity, total aerobic mesophilic flora, yeasts and moulds, as well as the presence of pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. These contaminations may pose potential health risks to infants due to their sensitive skin and immature immune systems. Therefore, strict quality control during manufacturing, storage, and commercialization is essential to ensure product safety and protect consumer health. Continuous monitoring and compliance with regulatory standards remain necessary to guarantee the microbiological and physicochemical safety of baby cosmetic products.

Author Contributions

Idea and conceptualize article writing are done by L.Boukaous, A.S.Benatallah. participated in the theoretical background and discussion, experimental analysis is done by M.Seggani ; Yousfi Billlel O.Bouziane and R.Betchine ; RB.Boukaous contributed to the theoretical framework and reviewed the text.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

No ethical approval was required.

Data Availability Statement

All data analyzed during this study are included in this published article

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