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CONTRIBUTION TO THE VALORIZATION OF WHEY BY STUDYING THE INFLUENCE OF STORAGE CONDITIONS ON BOVINE WHEY MICROFLORA

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Abstract: Despite its biochemical composition that is interesting from a nutritional and technological point of view, whey is a very polluting agent because of its rejection in wastewater, hence the importance of its recovery. This study aims to optimize the conditions of its conservation for a possible use in the field of food industry. A physico-chemical and biochemical characterization of the whey samples from the ALOUANI dairy was carried out. The variation of the microbiological quality according to the two conditions of conservation (pasteurization, refrigeration) during seven days, was also carried out. The evolution of the acidifying power of whey during its storage was part of the present study. Monitoring the evolution of banal flora and contamination during storage, has shown that the rate of contamination of bacteria decreases during storage, while that of lactic acid bacteria (lactococci, lactobacilli) tends to increase. In addition, the results indicate a synergistic action of pasteurization and refrigeration to better preserve this by-product. The acidifying power of the whey has, depending on the storage conditions, a regression during the storage time. In the first two days, the ability to acidify can be qualified as good, then this power decreases considerably as the duration of storage increases.

Keywords: whey, recovery, conservation, flora ,Ghardaia

INTRODUCTION

The dairy industries are the most polluting by the rejection of significant amounts of whey, because of its richness in nutrients, such as lactose, soluble proteins, water-soluble vitamins, fat and minerals. It is therefore an excellent culture medium for microorganisms, which makes it a formidable pollution factor (ANGES, 1986). It affects the chemical and physical structures of the soil, as well as reducing aquatic life by capturing dissolved oxygen (PENASAR, 2007).

To reduce the polluting risk of whey, this by-product is used in various fields such as human food, animal feed and possibly in the field of biotechnology to produce proteins of unicellular organisms (POU), vitamins, enzymes, alcohol, organic acids (citric acid, lactic acid, ... etc.) (LIN et al, 2006). Once considered a waste, whey is now treated to recover the main constituents, including proteins. This by-product of cheese and casein production is increasingly valued.

Algeria is experiencing a notable development in the dairy sector, where the global quantity of whey, thrown daily, is 6000 liters per production unit (GANA and TOUZI, 2001).

This work is part of the whey valorization trials, as a by-product that is poorly upgraded and released to wastewater: by studying the microbiological quality of the whey samples, provided by a dairy plant located in the Ghardaia region and its acidifying power and its variations according to the storage conditions.

MATERIAL AND METHODS

Sample collection

The whey samples come from the ALOUANI dairy located in the Ghardaia Willaya. The whey collected is poured, under conditions of strict hygiene, into previously sterilized bottles. The latter are placed in a cooler containing refrigerant blocks and sent immediately to the laboratory, where the whey is refrigerated, for appropriate microbiological analysis.

Pasteurization and constitution of experimental lots

In order to purify the whey and extend its shelf life, it is generally applied a heat treatment that partially or completely destroys its microbial flora (LEVEAU, 1981).

The study of the influence of pasteurization on the microbiological quality and the acidifying power of whey allows us to better control the conditions of its conservation by maintaining its microbiological quality and its functional properties.

The whey samples that were the subject of this study undergo pasteurization at the laboratory level. The technique is summarized by TERROINE (1961). The whey samples in this study are divided into four experimental batches according to the storage conditions adopted, namely, pasteurization and storage temperature (Tab. 1).

Tab. 1.

Constitution of experimental lots

N° of lot	Pasteurization	Storage temperature	Quantity (ml)

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1	Yes	Ambient temperature	8 tubes of 20ml
2	No	4°C	8 tubes of 20ml
3	Yes	Ambient temperature	8 tubes of 20ml
4	Yes	4°C	8 tubes of 20ml

The study of the microbiological quality of whey samples, as a biotope conducive to microbial growth, by monitoring the evolution of common germs and contamination; was made using several culture media:

- PCA medium: Plate Count Agar, a standard agar for the enumeration of aerobic germs in water, milk, meat and meat products and other foodstuffs (LECOQ, 1965).
- Chapman medium: used specifically for halotolerant bacteria and staphylococci (MARCHAL et al, 1982, EL SAYED et al, 1992).
- Medium V.R.B.G: medium agar crystal violet, neutral red and bile is used for the enumeration of enterobacteria (LARPENT et al, 1997 EL SAYED et al, 1992).
- Hektoen medium: it is used especially for the enumeration of pathogenic enterobacteria (JOFFIN and LEYRAL, 2001).
- Deoxycholate-lactose medium: used for the enumeration of coliforms (LARPENT et al, 1997).
- Medium M.R.S: (from Man, Rogosa and Sharp) is used for enumeration of lactobacilli (LARPENT et al, 1997).
- M17 medium: The M17 agar is used for the enumeration of lactococci, particularly the Lactococcus lactis species in dairy products (LARPENT et al, 1997).
- Sabouraud medium: is recommended for the isolation of yeasts and molds (MARCHAL et al, 1982, EL SAYED et al, 1992).

The count of the different groups that may evolve in the samples of whey (pasteurized or not) stored at ambient temperature and at a temperature of 4 ° C for seven days, is made in triplicate, in petri dishes. We only consider dishes containing 30 to 300 colonies (GUIRAND and GALZY, 1980). Exploitation and expression of results is as follows:

We keep the boxes containing from 30 to 300colonies

The number of microorganisms per ml is calculated using the following formula:

$$\frac{\sum c}{(n1 + 0.1\,n2)d}$$

Where

c : number of colonies counted per dish

n1 : number of dishes counted in the first dilution

n 2 : number of dishes counted in the second dilution

d : dilution factor from which the first counts were obtained

Statistical analyzes

For a better interpretation of the results and to compare the results relative to the evolution of the different microbial groups, as well as that of the acidifying power, we used a statistical treatment (XLSTAT), using principal component analysis (PCA) , a multidimensional descriptive method (DUBY and ROBIN, 2006).

RESULTS AND DISCUSSION

Physicochemical and biochemical quality of the collected whey

The physicochemical and biochemical characteristics of this by-product make it possible to evaluate its state of freshness and to check the conformity of its composition in comparison with the recommended standards (Tab. 2.).

The pH value recorded is higher than that reported by SIENKIEWICZ (1990) and SOTTIEZ (1990), respectively 4.70 and 4.60. However, it is close to those cited by BOUDJEMA (2007) and BENAOUIDA (2008), that is, 5.90 and 5.89 respectively.

Tab. 2.

Physico-chemical and biochemical analyzes of whey samples collected

Parameter	Average
pH	5.20
Titratable acidity °D	53
Density	1.025
Dry matter g/l	61
Ashes g/l	7.5
Fat g/l	0.5
Lactose g/l	55
Protein g/l	10

Freshly collected whey samples have a titratable acidity of the order of 53 ° D. This value is much higher than that of fresh milk which is between 15 and 20 ° D (SAWAYA et al, 1989). Likewise, this acidic nature of whey can hinder the growth of contaminating microorganisms that require a pH close to neutrality.

The dry matter content of the whey samples studied in this work is similar to that reported by BOUDJEMA (2007) (dry extract = 79 g / l) and BENAOUIDA (2008) (dry extract = 57 g / l).). The ash content of the samples analyzed is equal to 7.5 g / l. It is slightly

lower than that of bovine milk reported by ALAIS (1984), ie 9 g / l.

The lactose content recorded in the present study is 55g / l. It is comparable to those cited by SOTTIEZ (1990) and ALAIS (1981) are respectively 65.5 g / l and 40 to 57g / l. The whey solids content is about 75% lactose, which makes it a very energetic product that can improve the nutritional quality of other food products (SOTTIEZ, 1990).

The average protein content of the analyzed whey samples is equal to 10 g / l. This value is lower than that of bovine milk, namely 35 g / l (JENNESS, 1970). It is in the range of 7 to 11 g / l reported by ALAIS (1981). The variation in protein content is probably due to the cheese-making process (ADRIAN, 1973).

Microbiological quality and storage conditions of whey

The study of the microbiological quality of the whey was carried out by counting the microbial flora of the whey and studying the evolution of the different bacterial groups (commonplace and contamination), during the storage of this by-product. Our results are shown in the following figures (Fig: 1 to Fig: 8).

The initial number of total aerobic mesophilic flora is of the order of 106 ufc / ml for unpasteurized whey samples (lot 1 and 2) and 102 ufc / ml for pasteurized whey samples (lot 3 and 4).

The evolution curves of this aerobic mesophilic flora grown on PCA medium, have the same pace. In the case of the four lots, we note a decrease in its rate up to + 0, and then an increase. This increase is more marked for lots 1 and 3, stored at room temperature. Pasteurized batches (lot 3, lot 4) are characterized by a lower initial rate of germs (of the order of 102 cfu / ml). Its evolution is comparable to that of unpasteurized batches, with a decrease in the first three days, followed by an increase until D 0+7.

The results relating to the evolution of lactococci and lactobacilli, respectively grown on M 17 and MRS medium, show a decrease in number of lactococci, the initial number of lactococci of unpasteurized whey (lot 1 and 2) being of the order of 103ufc / ml. Their evolution is similar to that developing in pasteurized whey, with a faster increase when stored at room temperature (Lot 1) (J0 + 7: 106 cfu / ml).

Lactic bacteria in the form of lactococci and lactobacilli, and during storage of whey, evolve gradually increasing with a difference in the initial rate related to the action of pasteurization. Lactic bacteria are consistently destroyed above 72 ° C for 15 minutes during pasteurization (LARPENT, 1997).

The ability of lactic acid bacteria to develop at acidic pH and to produce simultaneously active substances (lactic acid, acetic acid, hydrogen peroxide and bacteriocins ...), explains their bacteriostatic or bactericidal role vis-à-vis the harmful species, responsible defects in fermented foods (Pseudomonas, Achromobacter, Flavobacterium, etc.) or presenting risks to public health (Salmonella, Clostridia, Staphylococcus, etc.) (CHAMBA et al, 1989, KLAENHAMMER et al, 1994).

The initial number of halotolerant germs, pasteurized whey (lot 3 and 4), is of the order of (105 ufc / ml). Its evolution is characterized by an increase in the first three days, followed by a decrease until D0+7. This progression is more rapid in the case of lot 4. Samples of unpasteurized whey (lot 1 and 2), characterized by an initial rate of higher halotolerant bacteria and of the order of 104 ufc / ml, show a progressive decrease in function of time. Storage at room temperature (Lot 1) seems to accelerate this decrease.

The rate of evolution of coliforms is the same in all four lots. The pasteurized whey (lot 3 and 4), which has an initial level of 102 cfu / ml, shows a considerable decrease in the first four days, then this decrease became light until day 7. These results provide information on the importance of pasteurization and refrigeration during the whey storage process. Indeed, as in the case of bovine milk, whey, loaded with coliforms, is likely to cause gastrointestinal infections in subjects, which can hinder its use without pasteurization treatment (TRYSTRAM, 1991).

It is noted that the different lots have the same pace of evolution of enterobacteria. Unpasteurized whey (lot 1 and 2), with an initial level of about 106 cfu / ml, is characterized by a decrease in enterobacteria. This regression is faster in the case of lot 1 (J0 + 7: 10 cfu / ml) than in the case of lot 2 (J0 + 7: 102 cfu / ml). Pasteurization seems to reduce the initial number of Enterobacteriaceae, which goes to 102ufc / ml in the case of Lot 3 and 4.

The level of fungi (yeasts and molds) present in the analyzed whey samples appears to decrease during storage. The conditions of conservation (pasteurization, refrigeration) have a considerable influence on the initial rate of fungi and the speed of its growth.

The initial rate of fungi of unpasteurized whey (lot 1 and 2) is of the order of 105 ufc / ml. Its evolution is characterized by a rapid decrease in the first five days, then, this decrease is less accentuated over time, with a higher fall in the case of lot 1.

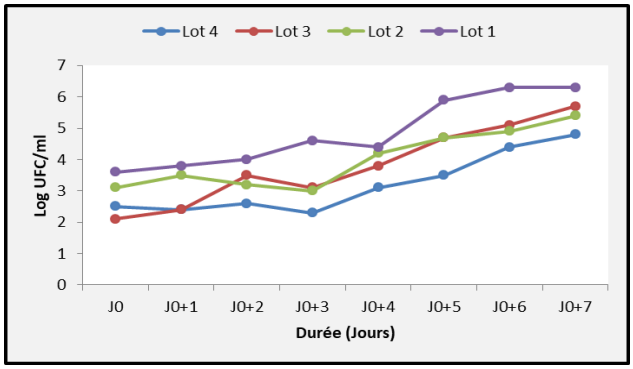

Fig. 1. Evolution of lactococci

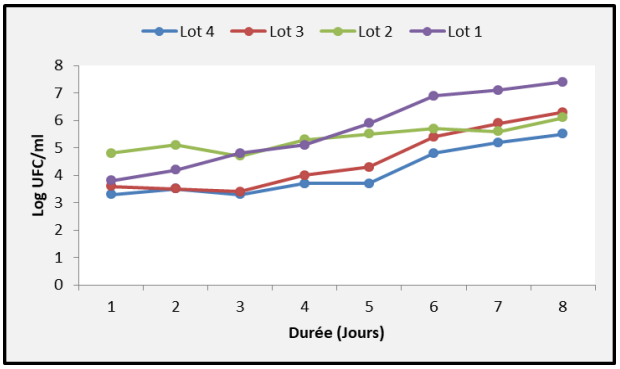

Fig. 2. Evolution of lactobacilli

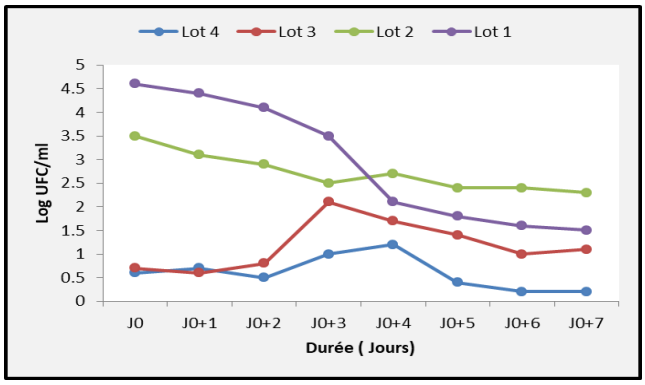

Fig. 3. Evolution of halotolerant bacteria

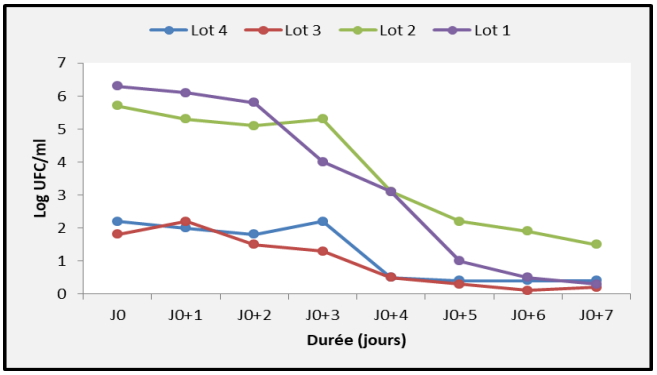

Fig. 4. Evolution of coliforms

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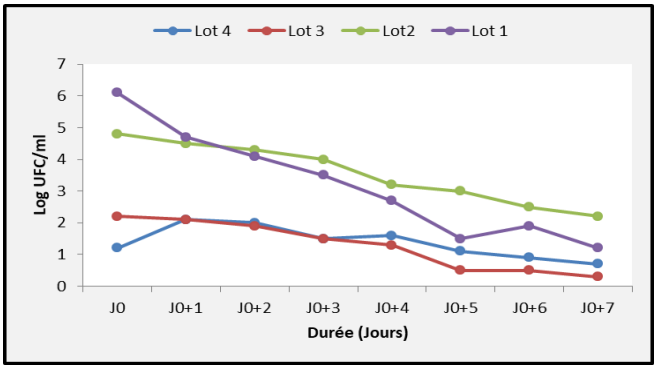

Fig. 5. Evolution of Enterobacteriaceae

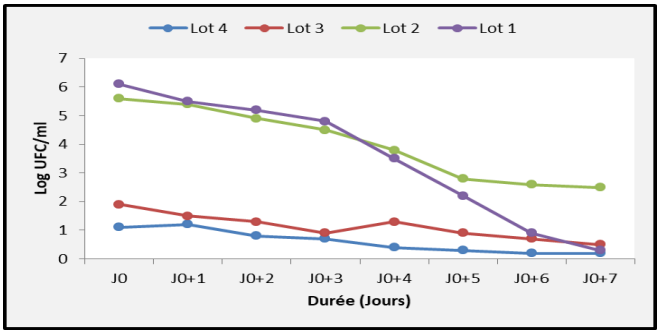

Fig. 6. Evolution of pathogenic enterobacteria

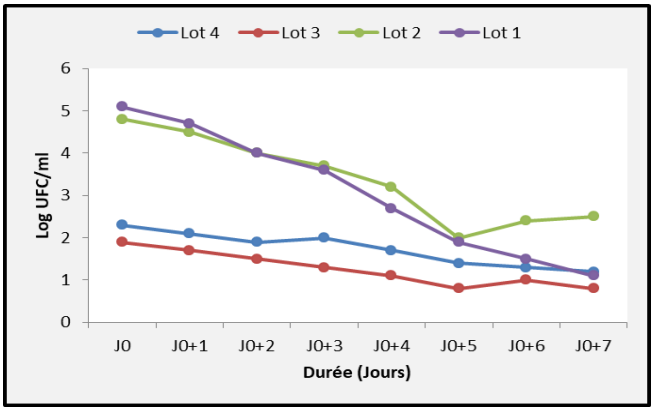

Fig. 7. Evolution of fungus

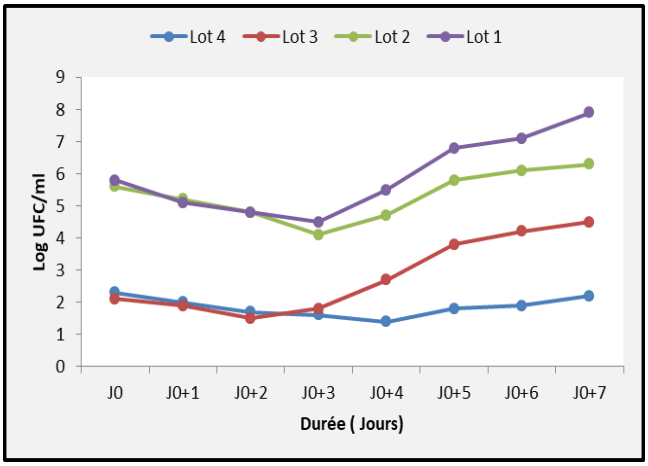

Fig. 8. Evolution of FMAT

In general, the flora of contamination studied in the present study, essentially represented by coliforms, enterobacteria, pathogenic enterobacteria, and halotolerant bacteria, is characterized by a common developmental aspect:

Pasteurization at 63 ° C for 20 minutes decreases the initial number of germs of contamination. It seems that at this temperature, a considerable rate of bacteria is destroyed.

Refrigeration at 4 ° C has slowed the growth of this flora by reducing its development during conservation.

Principal Components Analysis of the Results

The principal components analysis (PCA), allows to know from the correlation circles, the most contributive variables (Fig.9). These are near the circles; those with a small contribution are close to the center.

Overall, we note that the variables considered in this study are grouped according to their evolution into three. Halotolerants, Enterobacteriaceae, Pathogenic Enterobacteriaceae, Coliforms and Fungi represent the first group, acidifying power and pH are the second group, while total aerobic mesophilic flora, lactococci, lactobacilli and titratable acidity. constitute the third group.

It seems that each group can present, at the same time, a contribution to the explanation of the variability on the axis 1 and 2, this is due to the distribution of the variables with respect to the axes.

From Figures 10 and 11, respectively representing the projection of the individuals on the two axes 1 and 2, and the graphical representation of the variables and the individuals, one notices the existence of four groups of the individuals. Each lot (1, 2, 3 and 4) is a separate group. This grouping of individuals shows the importance of the action of pasteurization and refrigeration during whey preservation.

In sum, the principal component analysis allowed us to have a global vision on the relationships existing between the parameters studied during the present study (evolution of the different microbial groups), with the individuals (all the lots). These results show a negative correlation of titratable acidity, lactic acid bacteria with all germs of contamination. Indeed, each group of individuals (lots) is characterized by parameters specific to it, for example, the evolution of microbial groups, during storage of whey, follows a specific pace for each experimental batch.

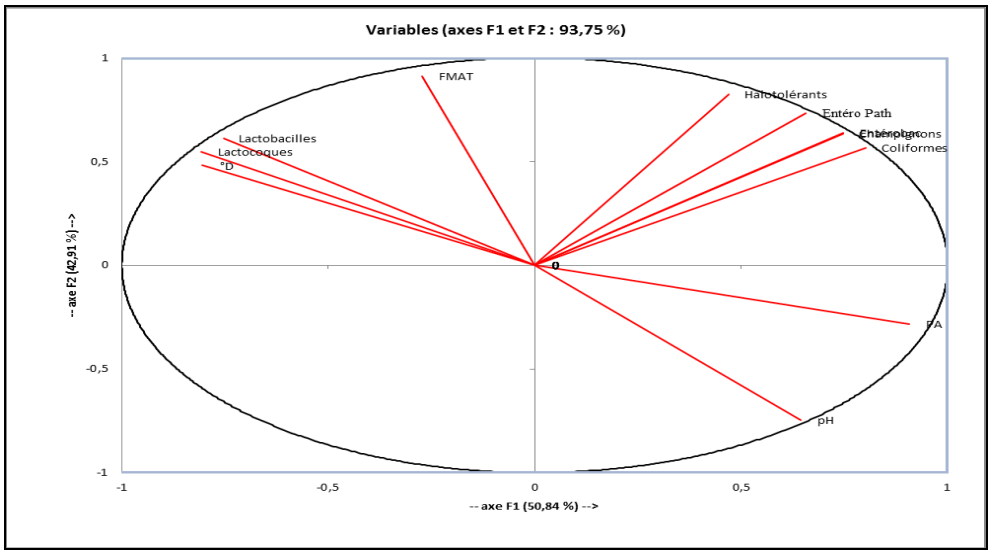

Fig. 9. Projection of variables

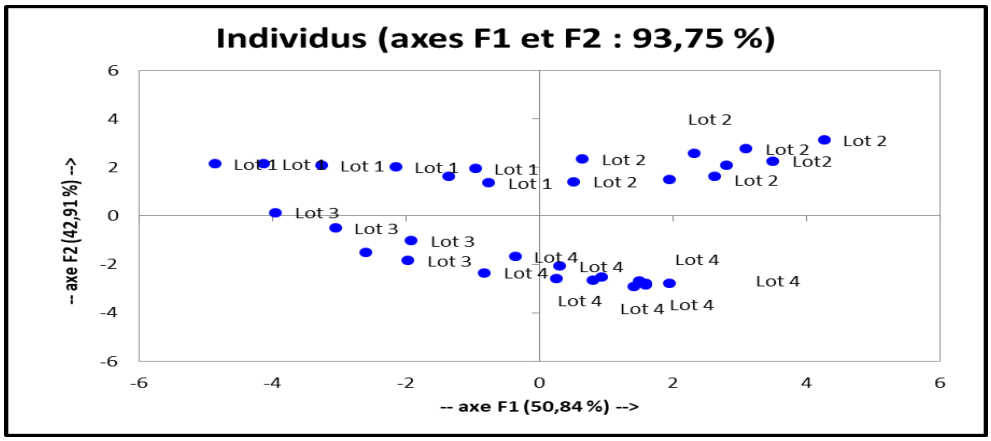

Fig. 10. Projection of individuals

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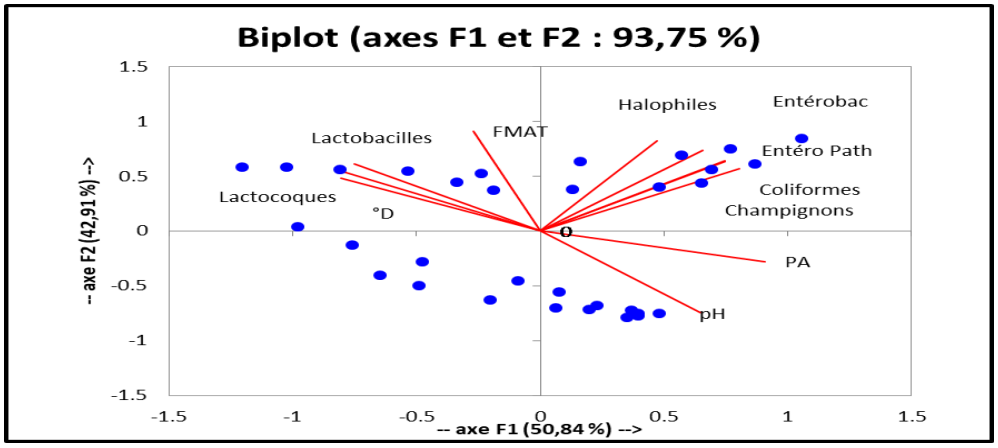

Fig. 11. Projection of variables and individuals

CONCLUSION

The whey samples analyzed are characterized by a rather important biochemical composition with a lactose level of 55 g / l, a protein composition of 10 g / l and a fat content of 0.5 g / l.

Monitoring the evolution of banal flora and contamination during storage of whey by modifying the storage conditions has highlighted the self-purifying aspect of this by-product. This microbiological study has shown that the rate of contamination bacteria (halotolerants, coliforms and enterobacteria) and fungi decreases during storage, while that of the original flora (lactococci, lactobacilli) tends to increase.

The study of the action of pasteurization and refrigeration, shows the importance of these two processes during the storage of whey. Pasteurization (63 ° C for 20 minutes) reduced the initial number of germs of contamination by 10 ° C and refrigeration (4 ° C) has an influence on the growth of microorganisms, decreasing their speed of development.

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