

## RESEARCH ARTICLE



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\* **Corresponding author.**

[fadilionne@yahoo.fr](mailto:fadilionne@yahoo.fr)

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# Elaboration of a Semi-Hard Cheese, "Gouda" Type, with Autochthonous Strains and Analysis of its Physicochemical and Sensory Composition

F Sadi<sup>1\*</sup>, N Zaouadi<sup>1</sup>, F Hallouz<sup>1</sup>, A Dilmi Bouras<sup>2</sup>, S Bensehaila<sup>1</sup>, W Mosbahi<sup>3</sup>, H Ouadjene<sup>1</sup>

<sup>1</sup> Water laboratory, rocks and plants, University Djilali Bounaama-Khemis-Miliana, Algeria

<sup>2</sup> Laboratory of local Natural Bioresources, Faculty of Science, University Hassiba Ben Bouali-Chlef, P.O Box 151, 02000 Chlef, Algeria.

<sup>3</sup> Djilali Bounaama University-Khemis-Miliana, Algeria

## Abstract

**Objectives :** The technological characteristics and flavour formation capacities of cheeses depend on the starter strains and the ripening conditions. The present study aimed at using autochthonous lactic acid bacteria strains with interesting technological and enzymatic properties, suitable as starter cultures to produce semi-hard cheese, such as Gouda, based on pasteurized cow's milk. **Methods:** This study allowed the formulation of local lactic ferments, with autochthonous strains; thanks to which we have made a semi-hard cheese, Gouda type, made from pasteurized cow's milk. Physicochemical (dry matter, fat, protein, chloride, and ash) and organoleptic (texture, color, taste, odor, aroma) analysis were carried out. **Findings:** The protein composition is very important (average value of  $24.18 \pm 0.46\text{g} / 100\text{g}$ ). The results of the sensory analysis did not show any defects in the cheese studied. The taste is sweet, characteristic; the aroma is pure, pleasant, well developed. The color is uniform yellowish, the rind is dry. **Novelty:** The use of pasteurized milk and autochthonous lactic ferments have helped maintain the typical characteristics of the cheeses.

**Keywords:** Cheese; Gouda; Lactic ferment; physicochemical analysis; Semihard paste; Sensory analysis

## 1 Introduction

Cheese making and maturation are processes involving a multitude of complex biochemical events, mainly directed by the lactic acid bacteria that make up the ferment<sup>(1)</sup>.

One of the most important challenges of the dairy industry is therefore to choose the genera, species and bacterial strains best suited to the functions imparted to them in the various technologies for processing milk by fermentation<sup>(2)</sup>.

Consumers appreciate artisanal cheeses for their typical aromas and flavors, which are generally attributed to the metabolic activity of indigenous microflora present in raw milk. Various authors have observed that the use of pasteurized milk with indigenous lactic ferments helps to obtain cheeses with similar characteristics to products made with raw milk<sup>(2)</sup>. These autochthonous strains are needed to transform curd into cheese<sup>(3)</sup> and improve the stability and sensory properties of the final products<sup>(4,5)</sup>.

Gouda cheese originated in Holland and was exported as early as 1600<sup>(6)</sup>, but similar varieties are now produced worldwide from pasteurized cow's milk, acidified by a mesophilic culture containing citrate positive bacteria<sup>(7)</sup>.

The study presented here is part of a general approach, where we have therefore tried, on the one hand, to manufacture gouda-type cheese based on pasteurized cow's milk with different ferments and to establish the links between autochthonous strains, on the other hand, to clarify the influence of leaven on the physicochemical and sensory characteristics of manufactured Gouda.

## 2 Material and Methods

### 2.1 Source of Milk

The pasteurized cow's milk was supplied by the Aribis Dairy, Ain defla, Algeria, under appropriate hygienic conditions. Before its use, the milk was subjected to a set of physicochemical analysis to evaluate its hygienic and nutritional quality according to Amariglio<sup>(8)</sup>.

### 2.2 Lactic acid bacteria strains

The eight strains used in this study, which were isolated from cow and goat milk from local Algerian populations in the region of KhemisMiliana, Ain defla and showed good technological functionalities<sup>(9)</sup>, are: *Lb.delbrueki lactis* (V4), *Lb.plantarum* (C7); *Lb.casei* (V2); *Lc.lactissubsp lactis* (C1, V1, C11); *Lc.pseudomesenteroides* (V3); *Streptococcus thermophilus* (C12).

They are stored at -18°C in skimmed milk with 30% glycerol added. They are activated and maintained by a weekly transplanting on skimmed milk reconstituted at 10%<sup>(10)</sup>.

### 2.3 Preparation of the leaven

The starters were prepared in sterile skim milk, seeded with young cultures of the pure strains at a rate of 2%, and then incubated at 30°C for 16 hours. The ferments were chosen as follows:

**Ferment 1:** three strains of *Lactococcus lactis subsp lactis* (V1, C1, C11), and one strain of *Leuconostocsp* V3.

**Ferment 2:** three strains of *Lactococcus lactis subsp lactis* (V1, C1, C11), one strain of *Leuconostoc pseudomesenteroides* V3, one strain of *Lactobacillus delbruekii* V4, one strain of *Streptococcus thermophilus* C12 and one strain of *Lactobacillus casei* V2.

**Ferment 3:** three strains of *Lactococcus lactis subsp lactis* (V1, C1, C11), one strain of *Leuconostoc pseudomesenteroides* V3, one strain of *Lactobacillus delbruekii lactis* V4, one strain of *Streptococcus thermophilus* C12 and one strain of *Lactobacillus plantarum* C7.

**Ferment 4:** supplied by ARIBS Dairy (DI-PROX® MT 243) lyophilized lactic ferment for direct inoculation. Mixture of strains of *Lactococcus subspcremoris*, *Lactococcus lactissp lactis* var diacetylactis and *Streptococcus thermophilus*.

### 2.4 Cheese making

The cheese was made at the ARIBS dairy, Ain Defla. We followed the manufacturing steps of Gouda as described by Goudédranche et al.<sup>(7)</sup> and Hee et al.<sup>(11)</sup> The milk used was pasteurized at 85°C for 15s. It contains 11% dry matter with 3.1% fat with a titratable acidity of 16°D (degrees dornic) and a density of 1.030 recorded at 20°C.

The manufacturing and maturation operations, identical for all the tests, were carried out three times for each ferment.

The first three ferments constituted by the autochthonous bacteria (F1, F2, F3) were evaluated for their acidifying potential at 30°C for 24 hours, according to the method of Larpent<sup>(12)</sup>.

### 2.5 Physicochemical analysis

The pH (expressed in pH units), and the acidity (°D) of local ferments are determined according to the method of AOAC<sup>(13)</sup>.

Cheese samples are taken at three stages of production: after 24 hours, 6 weeks and 12 weeks.

- The dry extract (DE; expressed in g per 100 g of cheese; %) is determined according to the method of AOAC<sup>(13)</sup>.

- The fat content (fat expressed in g per 100 g of cheese; %) is measured by the acid-butyrometric method of VAN GULIK<sup>(14)</sup>. The determinations of dry extract and fat make it possible to define another parameter: the dry fat (FAT/DE) expressed in g per 100 g of dry extract (%);
- The dosage of chlorides is determined by titration using a solution of silver nitrate based on the amount of precipitated silver chloride<sup>(15)</sup>;
- The ash content is determined according to the method described by the AOAC<sup>(13)</sup>;
- The determination of nitrogenous matter is carried out according to the Kjeldahl method<sup>(13)</sup>.

## 2.6 Sensory evaluation

The cheeses were tested in triplicate by a panel of ten evaluators. A rating scale from 0 to 5 was used. The cheeses were rated on taste and paste criteria. When moving from one sample to another, the evaluators rinsed their mouths with water to erase the taste of the previous sample<sup>(16)</sup>.

## 2.7 Statistical analysis

The interpretation of the results obtained for the physicochemical and hedonic analyzes of the formulated cheeses is based on a statistical analysis using the statistical software XL-STAT Version 2014.5.03 (<https://www.xlstat.com>). The statistical analysis consists of a Multivariate Analysis of Variance with one factor (MANOVA) at the 5% level, a correlation using the Pearson correlation coefficient to detect a link between the various physicochemical parameters studied at the 5% threshold and finally, a Principal Component Analysis (PCA) at the 5% threshold.

# 3 Results and Discussion

## 3.1 Acidifying power of local ferments

The results found reveal that the three ferments F1, F2 and F3 have very important acidifying activities which results in the production of lactic acid during the 24 hours of incubation whose values reach  $61 \pm 0.04^\circ\text{D}$ ,  $75 \pm 0.31^\circ\text{D}$  and  $85 \pm 0.02^\circ\text{D}$  respectively [Table 1]. Likewise, it appears that the production of acid is accompanied by a drop in pH whose values recorded after the same incubation phase are of the order of  $4.64 \pm 0.20$ ;  $4.5 \pm 0.12$  and  $4.35 \pm 0.05$  [Table 1]. The main role of starter bacteria in cheese making is to provide coagulant activity and rapid acidification<sup>(1)</sup>. These same authors found pH values between 5.27 to 6.15 during 6h and 4.28 to 5.27 at 48h on a set of *Lactococcus* strains.

**Table 1. Evolution of acidity and pH of the three local ferments**

Time	F1	F2	F3
6 hours			
Acidity ( $D^\circ$ )	$43 \pm 0.09$	$50 \pm 0.15$	$55 \pm 0.12$
pH	$5.33 \pm 0.32$	$5.21 \pm 0.05$	$5.1 \pm 0.02$
24 hours			
Acidity ( $D^\circ$ )	$61 \pm 0.04$	$75 \pm 0.31$	$85 \pm 0.02$
pH	$4.64 \pm 0.20$	$4.5 \pm 0.12$	$4.35 \pm 0.05$

Means  $\pm$  standard deviation (SD) of three separate determinations

## 3.2 Finished product

The prepared cheeses are cylindrical in shape, flat, 12 cm in diameter and  $4.5 \pm 0.10$  cm thick, with convex sides and a net weight of  $520 \pm 25$ g [Figure 1]. Their characteristics comply with the General Standard for Cheese<sup>(17)</sup>: firm / semi-hard ripened cheese (Gouda).

The distribution of holes is reasonably even throughout the interior of the cheese, but with the acceptable presence of some openings and cracks. The shape is flattened cylindrical with convex sides. According to Huc<sup>(18)</sup> the presence of bubbles or "eyes" is an important quality parameter for uncooked pressed cheeses. Their characteristics (number, size, and appearance of the eyes) are directly involved in the legal denomination of certain cheeses, as well as composition parameters such as water or fat content. Thus, the openings contribute 60% of the cheese classification score. Guggisberg et al.<sup>(19)</sup> showed that the use of an *Lactobacillus casei* strain leads to an increase in the number and volume of eyes in a Tilsit cheese devoid of propionic fermentation, via the complete degradation of citrate.



Fig 1. Processed Cheese Samples

### 3.3 Physicochemical characteristics

Cheese composition has also been identified in the literature as an important influencing parameter on the rheology of cheeses<sup>(20)</sup>. It is generally associated with proteolysis; the salt content often linked to the water content, as well as the fat content is the factor that have the most impact on the rheological properties of cheeses.

The functional properties of cheeses are controlled by the chemical composition, including the moisture content. After 24 H, the average dry matter values recorded were of the order of  $50.56 \pm 1.39\%$ ,  $52.07 \pm 0.47\%$ ,  $50.38 \pm 0.4\%$  and  $53.45 \pm 1.01\%$  for the four cheeses manufactured respectively, these values increase after 6 weeks of ripening to reach  $58.93 \pm 1.77\%$ ,  $58.03 \pm 0.39\%$ ,  $59.08 \pm 0.67\%$  and  $58.85 \pm 0.86\%$  respectively. After 12 weeks, a loss of water was recorded and the dry matter values reached  $70 \pm 0.57\%$ ,  $67.77 \pm 0.46\%$ ,  $65.12 \pm 0.13\%$  and  $67.34 \pm 0.65\%$  respectively [Table 2]. In addition, a significant difference ( $p \leq 0.05$ ) was noted for the four cheeses during their ripening. According to Renda et al.<sup>(21)</sup> the moisture content in cheeses is affected by various factors such as temperature, cooking time and salt content. Salt plays, directly or indirectly, several important roles. During production, it promotes drainage under the effect of osmotic pressure, reduces the moisture content of the curd and lowers water activity ( $A_w$ )<sup>(22)</sup>. While the FAT/ DE levels for the finished product after 12 weeks are in the order of  $29.67 \pm 1.24\%$ ,  $28.28 \pm 1.21\%$ ,  $30.7 \pm 0.8\%$  and  $32.41 \pm 1.57\%$  for the four cheeses respectively. Analysis of variance ( $p \leq 0.05$ ) reveals the existence of a significant difference for cheeses formulated during maturation. According to the food codex classification<sup>(23)</sup>, the cheese we produced falls into the group of semi-hard cheeses, type "gouda" with a FAT/ DS content of 25-40%. More precisely, the minimum content of milk fat in the dry extract (m / m) is fixed at 30% by CODEX STAN 266<sup>(24)</sup>. The relatively low content recorded for the F1 and F2 cheeses we prepared may be due mainly to the initial fat content of the milk used, and which has not undergone standardization and/or the presence of significant lipolytic metabolism of the strains used. According to Sundaram Gunasekaran and Mehmet<sup>(25)</sup> the functionality of low-fat cheese may improve due to the additional moisture present. Therefore, increasing the moisture content is generally recommended to improve the quality of low-fat cheeses. However, it should be kept in mind that Heat treatment not only influences the microbiological, biochemical, physicochemical, and functional characteristics of cheese, but also, in turn, significantly affects the sensory properties of these products<sup>(26)</sup>. Rezaei et al.<sup>(27)</sup> showed that the pasteurization process, ripening time and temperature influenced the chemical, physicochemical and sensory properties of traditional Motal cheeses.

Table 2. Physicochemical and sensory analysis of the four cheeses

Time	Parameter	F1	F2	F3	F4
24h	FAT/DE DE	$51.45 \pm 2.10$ $50.56 \pm 1.39$	$47.05 \pm 1.26$ $52.07 \pm 0.47$	$53.59 \pm 2.25$ $50.38 \pm 0.4$	$54.58 \pm 1.05$ $53.45 \pm 1.07$
6 weeks	FAT/DE DE	$40.16 \pm 0.67$ $58.93 \pm 1.77$	$38.17 \pm 0.65$ $58.03 \pm 0.39$	$37.79 \pm 1.03$ $59.08 \pm 0.67$	$41.04 \pm 2.17$ $58.85 \pm 0.86$
12 weeks	FAT/DE DE	$29.67 \pm 1.24$ $28.28 \pm 1.21$	$30.7 \pm 0.8$ $32.41 \pm 1.57$	$29.67 \pm 1.24$ $28.28 \pm 1.21$	$30.7 \pm 0.8$ $32.41 \pm 1.57$
	Ash	$1.28 \pm 0.03$ $1.64 \pm 0.01$	$1.51 \pm 0.02$ $1.32 \pm 0.04$	$1.6 \pm 0.02$ $2.45 \pm 0.10$	$1.72 \pm 0.03$ $2.55 \pm 0.04$
	Protein	$22.73 \pm 0.34$ $3.33 \pm 0.57$	$21.75 \pm 0.93$ $3.66 \pm 0.57$	$25.47 \pm 0.53$ $3.66 \pm 0.28$	$26.95 \pm 0.06$ $3.66 \pm 0.28$
	Texture	$3.16 \pm 0.28$ $3.66 \pm 0.28$	$2.83 \pm 0.76$ $3.5 \pm 0.5$	$3.33 \pm 0.76$ $3.66 \pm 0.28$	$3.33 \pm 0.28$ $3.5 \pm 0.35$
	Flavor	$3.5 \pm 0.5$ $3.5 \pm 0.5$	$3.16 \pm 0.57$ $3.16 \pm 0.57$	$3.16 \pm 0.28$ $3.33 \pm 0.57$	$3.83 \pm 0.28$ $3.83 \pm 0.28$
	Color				
	Odor				
	Aroma				

F1, F2, F3, F4: average of the 3 trials of cheeses made respectively with the ferments F1, F2, F3 and F4; DE: dry extract (%); FAT: fat (%); Ash (%); Protein (%); Texture, Flavor, Color, Odor, Aroma (note/5)

### 3.4 Chlorides, ash, and protein from matured cheeses after 12 weeks

Chloride levels are significantly different ( $p \leq 0.05$ ) in the four cheeses. It is higher in cheeses F3 and F4 ( $1.6 \pm 0.02$  and  $1.72 \pm 0.03\%$ ) than those of F1 and F2 ( $1.28 \pm 0.03$  and  $1.51 \pm 0.02\%$ ). Salazar et al. <sup>(28)</sup> found in their study that the salt contents of Gouda cheese samples ranged from 1.21 to 2.39%. Ismail et al. <sup>(29)</sup> reported fluctuations in salt during ripening of Urfa, Domiati and Urfa cheeses. This fact could be related to the increase of dry matter throughout the ripening process.

We also found that the ash content in processed cheeses is significantly different ( $p \leq 0.05$ ) and estimated at  $1.64 \pm 0.01\%$ ,  $1.32 \pm 0.04\%$ ,  $2.45 \pm 0.10\%$  and  $2.55 \pm 0.04\%$  for F1, F2, F3, and F4 respectively. Ashes are mainly composed of minerals from dairy raw materials; we find calcium, phosphorus, potassium, chlorine, sodium, magnesium, and table salt. Varunsatian et al. <sup>(30)</sup> reported that the latter have a non-negligible role in the gelation of caseins which depends on the ionic strength and the nature of the ions; at equal ionic strength, the  $\text{Na}^+$  ions would prevent the aggregation of proteins heated to more than  $70^\circ\text{C}$ , while the  $\text{Ca}^{+2}$  or  $\text{Mg}^{+2}$  ions would promote it. This effect of calcium would nevertheless be dependent on its concentration, a certain amount of calcium being required for inter-protein cross-linking <sup>(20)</sup>. According to these authors, calcium at high concentrations inhibits gel formation by promoting protein-protein interactions leading to aggregation, which will negatively influence the viscosity of the cheese.

Experimental values show significant protein levels in the finished product:  $22.73 \pm 0.34\%$ ,  $21.75 \pm 0.93\%$ ,  $25.47 \pm 0.53\%$  and  $26.95 \pm 0.06\%$  for F1, F2, F3 and F4 respectively. The analysis of variance reveals the existence of a significant difference ( $p \leq 0.05$ ) between the different cheeses manufactured. According to Felfoul et al. <sup>(31)</sup> the protein level for Gouda, after 60 days of ripening, is  $27.10 \pm 0.93\%$ . Proteolysis plays a key role in the development of typical sensory characteristics of a cheese <sup>(32,33)</sup> with the production of different amino acids that are precursors of specific sensory active metabolites

### 3.5 Sensory quality

In the cheese industry, cheese quality is largely determined by sensory perception, which is a complex process. It is influenced by several factors such as the content of aromatic compounds, texture, and appearance <sup>(16)</sup>. The results of the tasting tests of the four cheeses are represented in the form of a spider star Figures 1 and 2. The paste of the cheese we made is firm with a slightly salty taste and is not acidic. The slightly bitter taste of Cheese (F2) was appreciated by the tasters. This is probably due to excessive proteolysis by *Lactobacillus casei* V2, which was found to be the most proteolytic in the study by Sadi et al. <sup>(9)</sup> and / or ripening conditions. We recorded a very pleasant smell and a hard crust with a dark yellow color. By comparison, the tasters confirm that the cheeses produced belong to the "Gouda" type. The analysis of variance reveals that the difference is not significant ( $p > 0.05$ ) for the formulated cheeses, between the attributes (texture, flavor, color, odor, and aroma).

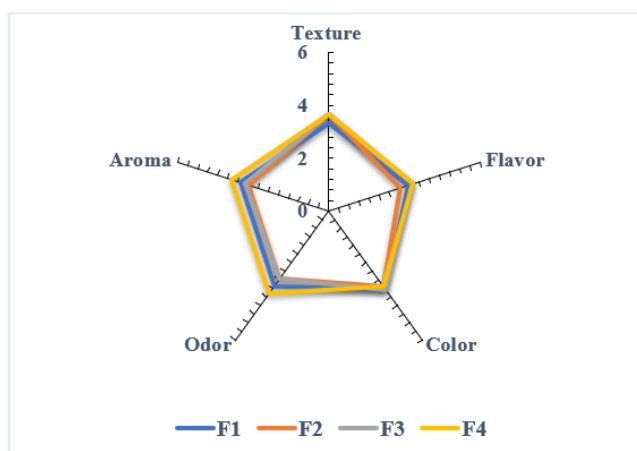


Fig 2. Sensory profile of manufactured cheeses

Gunasekaran and Mehmet <sup>(25)</sup> indicate that cheeses are often very salty, in order to prolong their shelf life, so the rind of the cheese plays a protective role that explains its hardness. The formation of aroma in cheese is also influenced by the type of milk (raw vs. pasteurized) and the maturation period <sup>(34)</sup>.

Several studies investigating the incorporation of lactic acid bacteria in spun pastes have reported a higher level of sensory perception of bitterness compared with conventional cheeses <sup>(33,35)</sup>. Lee et al. <sup>(1)</sup> found malty, cabbage, alcoholic, fruity, sweet,

buttery, and sulphurous flavors for the miniature Gouda cheeses that tested. Gonzalez et al.<sup>(36)</sup> found similar quality, flavor and intensity values between experimental and controlled cheeses and demonstrated that the correct technological characterization of the autochthonous strains allows the selection of strains with the desired properties in the lactic ferments for use in industrial productions, maintaining some characteristics typical of traditional cheeses. The results found by<sup>(37,38)</sup> showed that technological characteristics and flavor-forming capabilities depended on strain. The flavor of cheese varies according to the type of cheese, and even the same type of cheese varies in flavor depending on the starter bacteria and the ripening conditions<sup>(39)</sup>. In general, Gouda cheese is ripened for a period ranging from more than two months to several years<sup>(39)</sup>. Long-term maturation of cheese in the laboratory has limitations; therefore, it is necessary to shorten the maturation period. Starter bacteria affect aroma formation at least 2-3 weeks after cheese production. Miniature cheeses need about 28 days to mature<sup>(40)</sup>. Long-term experiments on large-scale production of Gouda cheese are needed to evaluate actual aroma formation and aroma characteristics<sup>(38)</sup>.

#### 4 Statistical analysis

The correlation test between the different physico-chemical and sensory parameters was studied. A Principal Component Analysis (PCA) is performed considering the different physicochemical and sensory parameters studied and the cheeses in question. The PCA shows that 82.63% of the total variance is represented on axes 1 and 2, with 52.35% for axis 1 and 30.28% for axis 2 Figure 3.

The first PCA square characterizes the variables studied [Figure 3], so a negative correlation was noted for Dry matter, Chlorides, proteins, Ash, Texture and Flavor as well as for Texture, Odor, Color and Aroma. Two main positive correlations have been noticed; the first one gathers Fat, Odor, Aroma, Flavor, Ash, and Proteins, the second one gathers Proteins, Aroma and Flavor. Other positive correlations were also noted; Odor and Aroma, Ash, Aroma, Texture, odor and Flavor. Also note the presence of weak significant positive correlations: between Ash and Color, Chlorides, Flavor, Odor and Aroma, Dry matter and Fat, Fat and Chlorides).

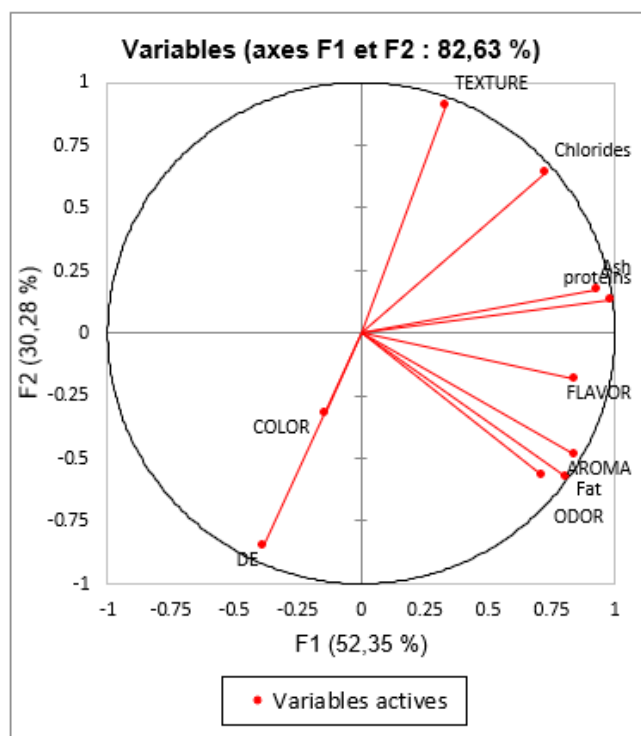


Fig 3. PCA; square of the correlations between the studied parameters

On the PCA square characterizing the observations Figure 4, we can draw the following remarks:

- F4 cheese distinguishes itself from other cheeses by its aroma, Odor and Flavor, but also by its high fat content;

- F3 cheese is characterized by its high content of Proteins, Ash, and chlorides, and the better texture compared to other cheeses;
- F1 cheese is characterized by a considerable Dry matter content and preferred Color;
- The F2 cheese is very different from the F4 cheese; poor in terms of nutrition (it contains the lowest levels of protein, fat, and ash than the other tests), and the least appreciated during sensory analysis.

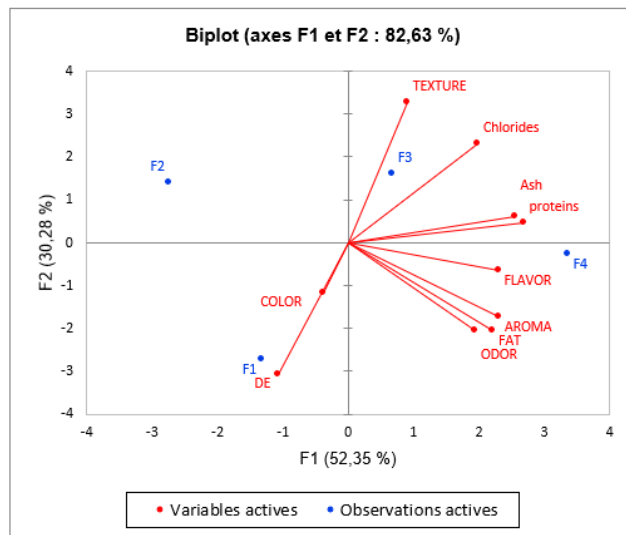


Fig 4. PCA; square of observations

## 5 Conclusions

We have succeeded in making a semi-hard "gouda" type cheese from non-standardized heat-treated cow's milk, with ferments of appreciable organoleptic qualities and a satisfactory nutritional quality. All the indigenous strains used have good functional properties and can be promising cultures on the industrial level. After 12 weeks of ripening, we recorded a humidity of 31% and a fat content on dry matter of 30% on average. On the other hand, the prepared "Gouda" type cheeses have a very high protein content of  $24.77 \pm 0.43$ , which gives them a high nutritional value.

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