ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

Effect of Milk Preservation Treatments on the Ripening Quality of Saint-Paulin Cheese

Malika Mankai[1*] ; Maissa Dely[2]; Hela Dhouioui[3]; Hassouna Mnasser[4]

[1*, 2, 3, 4] Department of Food Technology, University of Carthage, Higher Institute of Food Industries of Tunisia, ESIAT, Tunisia

Corresponding Author: Malika Mankai[1*]

Publication Date: 2025/06/2

Abstract: Saint-Paulin type cheese was produced using cow's milk stored at 4 °C for 72 hours, treated with a combination of lactoperoxidase (LP) system activation and thermal processes (55 °C for 15 seconds and 72 °C for 15 seconds). This study assessed the impact of these combined treatments on the physicochemical, microbiological, and biochemical characteristics of the cheese during a 23-day ripening period. Three milk variants were used: untreated control (C0), LP-inactivated refrigerated milk (C1), and LP-activated refrigerated milk (LP). Among the samples, the LP-activated treatment showed the lowest microbial contamination. Cheese derived from milk treated at 55 °C following LP activation (P55a) exhibited similar quality to that treated at 72 °C without LP activation (P72). The LP-treated cheeses had significantly reduced levels of coliforms, yeasts, and molds ($P < 0.05$), highlighting the antimicrobial effect of the LP system. Lipolysis levels remained comparable across samples. However, proteolysis was reduced by approximately 20% in LP-activated cheeses under the same heat conditions. Proteolysis indices for P72 and P55a cheeses were notably close. These findings were corroborated through azocasein analysis, which monitored variations in soluble nitrogen and non-protein nitrogen absorbance during ripening in all four cheese types.

Keywords: *Refrigerated Milk, Semi-Hard Uncooked Cheese, Lactoperoxidase System, Antibacterial, Proteolysis.*

How To Site: Malika Mankai; Maissa Dely; Hela Dhouioui; Hassouna Mnasser (2025). Effect of Milk Preservation Treatments on the Ripening Quality of Saint-Paulin Cheese. *International Journal of Innovative Science and Research Technology*, 10(5), 4385-4409. https://doi.org/10.38124/ijisrt/25apr2212

I. INTRODUCTION

The current global cheese market is valued at ~$100 billion, and worldwide cheese consumption is expected to grow by ~13.8% between 2019 and 2029 (OECD/FAO, 2020).

Although cheese has been part of the European diet since 6000 to 8000 years ago, it is now gaining popularity in countries where it did not previously exist in countries where it was not traditionally part of the national diet, probably due to the westernization of the diet (OECD/FAO 2020).

Consumption of dairy products has grown considerably in Asia over the past two decades, with the retail value of cheese in China estimated to increase at an annual rate of 12% over the forecast period 2019-2024 (Feeney et *al*.,2021).

There are around 2,000 varieties of cheese, most prepared by coagulating milk with chymosin, with ripening times ranging from 2 weeks to 2 years (Feeney et *al*., 2021).

During ripening, numerous physico-chemical, microbiological and biochemical changes occur in cheese matrices, resulting in the development of characteristic texture, aroma and flavor.

Cheese milk is invariably subject to a number of pretreatments such as chilling and cold storage, thermisation, bactofugation and pasteurisation prior to cheesemaking to ensure the microbiological safety of cheese (Seifu et *al*. 2004a). During milk storage by refrigeration, psychrotrophs can proliferate, these bacteria hydrolyse milk proteins and lipids through secretion of a variety of heat stable extracellular proteases and lipases (Mankai et *al*.2003), which cause different defects in dairy products as various off-flavours, abnormal texture and reduced cheese yields (Zalazar et *al*. 1987). The use of such cooled milk for producing cheese results in greater losses of fat and curd fines into the whey, lower cheese yields, and difficulties in the way of draining (Raynal and Remeuf 1998).

Although, several papers have been published about the effect of the milk refrigeration at 4 °C on the physicochemical and microbiological properties of soft cheeses (Novella-Rodriguez et *al*. 2004).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

For this reason, alternative solutions to inhibit psychrotrophs have been considered e.g. use of lactic acid bacteria (LAB), activation of the lactoperoxidase (LP) system that needs the presence of two factors, hydrogen peroxide and thiocyanate to develop its antimicrobial function (Seifu et *al.* 2005). Lactoperoxidase is one of the most heat stable enzymes in milk. Its destruction has been used as an index of pasteurisation efficiency of milk. LP retains its activity during normal pasteurization of cow milk (63 C for 30 min or 72 C for15 s) but destroyed at 80 C in 4 s (Seifu et *al.*2005).

The LP system is an acceptable chemical method for raw milk preservation, especially in rural areas where refrigeration facilities are absent to farmers (Ndambi et *al.* 2008). Activation of the LP system is amongst the most cost effective approaches to extend the stability of pasteurised and raw milk (Gardea et *al.* 2002). Due to the growing interest in the use of the LP system for preservation of raw milk, LP-activated milk is used for cheese manufacture (Seifu et *al*., 2004).

The objective of the experiment was, therefore, to compare the effect of activating the LP system in cow's milk at different heat treatment 72°C/15 s and 55°C/15s on the microbiological, physicochemical and biochemical properties of Saint-Paulin cheese over a ripening period of 23 days.

II. MATERIALS AND METHODS

➢ *Cheese Manufacture*

Experimental design Saint-Paulin cheese making trials were undertaken in duplicate with four treatments: LP-inactivated cow's milk cheese refrigerated at 4 °C for 72 h and heated at 55 °C for 15s (P_{55}) and LP-activated and refrigerated at 4 °C for 72 h and heated at 55 °C for 15s (P_{55a}), and LP-inactivated milk refrigerated at 4 °C for 72 h and heated at 75 °C for 15s (P_{72}) and LP-activated and refrigerated at 4 °C for 72 h and heated at 75 °C for 15s (P_{72a}). 160 ml of cow's raw milk were collected from one farm located in the North of Tunisia for the cheese making experiment and the milk was divided equally into four aseptic containers (40 L) for P_{55}, P_{72}, P_{55a} and P_{72a} samples.

The two first samples received a refrigerated at 4 °C for 72 h before transformation to cheese. The two other sample was subjected to the refrigeration combined with the activation of the LP system by addition of sodium thiocyanate (NaSCN) as a source of thiocyanate (SCN) to a final concentration of 14 mg⁄L.

After 1 min of thorough mixing of the milk, 30 mg⁄L of sodium percarbonate was added as a source of hydrogen peroxide (H_2O_2) as recommended by the International Dairy Federation (IDF 1988). After that, the two milk samples P_{55a} and P_{72a} were kept at room temperature (ca. 25 °C) for 2 h to permit the activation of the LP system after interaction between his three components and then refrigerated at 4 °C for 72 h, before transformation to cheese.

General cheese making procedure Saint-Paulin cheeses were manufactured from cow's milk at semi-industrial plant, using the method described by Mankai et *al.* (2012). The control and treated milks were pasteurised at 72 °C or 55°C for 15 s.

After pasteurisation, when the milk temperature reached 35 °C, 5% [wet weight (w⁄w)] of calcium chloride (CaCl2) and 1% (v ⁄ v) of lactic bacteria starters (EZAL, mesophilic culture) (e.g. Lactococcus lactis spp. lactis, Srteptococcus cremoris and Streptococcus brevis) were added to each of the milk samples. After 25–30 min, when acidity of milk reached 20 °D (Dornic), 0.03% (v⁄w) of rennet was added to each milk sample. Every sample was thoroughly stirred then left undisturbed to coagulate. After 35–40 min, the coagulated milk was cut into 1 cm cubes (acidity of whey = 12 °D).

After that, a part of the whey (30% of the volume of the whey) was removed and replaced by an equal volume of water at a temperature of 32 °C, in order to remove part of lactose and thus to control lactic acid production during the manufacturing process.

After agitation, the whey was drained, the curd moulded (1 kg) and pressed until both acidity of whey and pH of cheese values reached the limits of 28 °D and 5.3, respectively. The next morning, salting was carried out by immersing the young cheese blocks in a 20% (w⁄w) brine solution for 7 h. After manufacturing, cheeses were ripened in a warm room at 13 °C and 95% relative humidity for 23 days.

These quantities were used to produce the following four products:

- P_{72}:
  Production of a control cheese made from raw milk refrigerated for 72h, not activated by the lactoperoxidase system and pasteurized at 72°C for 15s.

- P_{72a}:
  Production of a cheese activated by the lactoperoxidase system with the dose recommended by the Codex Alimentarius (cf. I-2) made from raw milk refrigerated for 72h, pasteurized at 72°C for 15s.

- P_{55}:
  Production of cheese from raw milk refrigerated for 72 hours, not activated by the lactoperoxidase system and heat-treated at 55°C for 15 seconds.

- P_{55a}:
  Production of a cheese activated by the lactoperoxidase system with the dose recommended by the Codex Alimentarius made from raw milk refrigerated for 72h, having undergone heat treatment at 55°C for 15s.

It should be noted that the 55°C/15s scale was chosen in view of the results of Parry-Hanson et *al.* (2009) showing that the average activity of lactoperoxidase in goat's milk was not affected by heat treatment at 55°C for 15s, whereas it lost 50% of its activity at heat treatment at 72°C/15s.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

➢ *Activation of the Lactoperoxidase System in Raw Milk at Reception*

In order to obtain antibacterial effects, it is possible to activate the lactoperoxidase system in raw milk, by adding sodium thiocyanate and hydrogen peroxide, in the form of sodium percarbonate, according to the method mentioned in the report of the FAO/WHO technical meeting (2005) and also in the guidelines for the preservation of raw milk by the lactoperoxidase system (CAC/GL 13-1991), using the following process:

- Add 14 mg NaSCN per liter of milk.
- Sodium percarbonate 30 mg per liter of milk is then added. The milk is then stirred for 2 to 3 minutes.

Finally, the LPS-activated milk is stored at 4°C for 72h before being used to make Saint-Paulin cheese.

➢ *Sampling of Cheese*

The whole cheese block from each batch, was aseptically grated using a sterile cheese grater. From the grated cheese, samples for microbiological analysis were aseptically transferred. The remaining samples were used for physicochemical analysis. Cheese blocks for analysis were randomly taken from each batch at 0 (coagulum), 2 (after salting), 9, 14, 19 and 23 days of ripening. All the analyses were carried in triplicate.

➢ *Physicochemical Analysis*

The Saint-Paulin cheese samples were taken periodically during ripening for analysis. The pH of cheese samples was measured with a penetration electrode as described by Seifu et *al*. (2004b). The dry matter of cheese samples was determined as described by Katsiari et *al*. (2002).

The amount of lactic acid was measured according to the method NF V04.206 (NF 1985). Cheese yield was the mathematical expression of the quantity of cheese obtained from 100 L (or 100 kg) of milk; it was measured by weighing the cheese blocks on an analytical balance and was expressed as kg dry matter per 100 litre of milk (Mankai, 2006).

Total nitrogen (TN), water-soluble nitrogen (WSN), non protein nitrogen (NPN) and phosphotungstic acid (PTA-SN) were determined by Kjeldahl method (Dimitrellou et *al*., 2010; Moatsou et *al*. 2002) at 0, 2, 9, 14, 19 and 23 days of ripening.

The protein content of cheese samples was determined by multiplying the TN by the factor 6.38 (Rouch et *al*. 2008).

The percentages of these nitrogen fractions over the TN were used as indices of proteolysis.

The method of Kuchroo and Fox (1982) was followed to fractionate cheese nitrogen into the non casein nitrogen (NCN) and NPN fractions. The nitrogen content in each fraction was determined according to the Kjeldahl method.

The NCN minus NPN gave the polypeptides previously called proteose peptone fraction (PPN) which is formed by fragments of b-casein PP8 fast (b-CN f1-28), PP8 slow (b-CN f29-105 and f29-107) and PP5 (b-CN f1-105 and 1-107), as has been pointed by McSweeney (2004).

Therefore, nitrogen quantities were determined as follows: Casein nitrogen (CN) (g ⁄ 100 g) = TN (g ⁄ 100 g))WSN (g⁄ 100 g); proteose nitrogen (PPN) (g ⁄ 100 g) = WSN (g ⁄ 100 g))NPN (g ⁄ 100 g); peptide nitrogen (PN) (g ⁄100 g) = NPN (g ⁄100 g))FN (g ⁄ 100 g). The ripening coefficient (or proteolysis level) = 100 ⋅ [WSN (g ⁄ 100 g) ⁄TN (g ⁄ 100 g)] as described by others (Hassouna et *al*. 1999; Mankai 2006).

All determinations were performed in triplicate for each lot and for each ripening time.

➢ *Electrophoresis Method*

Protein degradation in the three samples of cheeses was evaluated by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDSPAGE). The 15% polyacrylamide gel slabs were prepared and run with SDS using the continuous buffer system of Tarakci et al. (2004); Laemmli (1970) (Mini-Protean_ 3Cell with a PowerPac 300 mini protean II-BIO-RAD). Protein samples for electrophoresis were prepared by the method of Mankai et *al*. (2009); Addou et *al*. (1996). Proteins standards used in SDS-PAGE were from Biorad (France).

Saint-Paulin production diagram

Four cheese products were produced from raw milk refrigerated for 72 hours, as described in the first paragraph of this second section.

After undergoing the appropriate heat treatment, the milk was cooled to 35°C, and then 40ml of calcium chloride (CaCl2) per 100 liters was added.

It is then inoculated with mesophilic lactic ferments until it reaches an acidity of 18°D and a pH of 6.6: this is the end of the primary ripening process, which lasts around 15 hours at 10 to 12°C.

At a temperature of 32°C, the milk is inoculated by thermophilic ferments: this is the secondary ripening phase, lasting between 30 minutes and 1h30. At the end of this phase, acidity reaches 20°D and pH 6.4.

The acid coagulum is then renneted at a rate of 25-30 ml/100 liters. The rennet coagulation time is between 30-60 minutes, estimated visually.

Once the curd has hardened, the coagulum is cut into small grains in the form of corn kernels, using a curd cutter to facilitate removal of the whey.

At this point, the whey has an acidity of 12°D. Next, the whey is delactosed, i.e. 30% of the whey is drawn off, and an equivalent quantity of hot water at 32°C is added, to achieve

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

an acidity of 6°D. After stirring, the remaining whey is drained off and the curds are molded, i.e. filled into 1 kg molds.

The next step is pressing, which is carried out in two stages: for half an hour at a pressure of 1.5 bar, the cheese is turned over, then the pressure is increased to 2.5 bar. Pressing can last up to 2h30, until the acidity of the whey reaches 28°D and the pH of the cheese is 5.3. Once these two conditions have been reached, the cheese is removed from the mould and soaked in brine at a concentration of 360g Na Cl/liter of water for 7 hours, with frequent turning. The water used for brining is cooled pasteurized water.

Finally, the cheese is placed in a ripening room at a temperature of 13°C and a humidity level of 95%. The Saint-Paulin ripening process lasts 23 days, during which time the cheese balls are compulsorily turned and protected by a liquid wax surface treatment.

➢ *Microbiological Analysis*

Several selective media were performed according to the methods described by Guiraud (1998) for enumeration of dairy product organisms. Ten grams of cheese was grated and dispersed in 90 mL of sodium citrate 2% (w⁄v).

For cheese samples, appropriate dilutions $(10)^2$–$10)^7$) were carried out on Plate Count Agar (PCA, Oxoı¨d) for aerobic plate count (APC) and psychrotrophic bacteria with the plates being incubated at 30 °C for 72 h and 7°C for 10 days, respectively.

Lactic acid bacteria (LAB) were counted by using De Man Rogosa and Sharp Agar medium (MRSA, Oxoı¨d) after incubation of plates at 37 °C for 48 h.

Coliforms were enumerated using desoxycholate gelose 1%° and incubated at 30 °C for 24 h. For lipolytic and proteolytic psychrotrophs germs, Tween 80 (1%; v ⁄ v) agar (Oxoı¨d) and Standard Milk Agar (SMA, Oxoı¨d) were used, respectively, and plates were incubated at 7 °C for 10 days. Colonies surrounded by clearing zones on Tween 80 agar were considered as lipolytic (Frank 1997), while those surrounded by a white or off-white precipitate on SMA were considered as proteolytic (Litopoulou-Tzanetaki and Tzanetakis 1992).

Finally, yeasts and moulds were counted on Sabouraud after incubation at 30 °C for 3 days.

➢ *Statistical Analysis*

The data for physicochemical composition and microbiological counts were analysed using the STATGRAPHICS, Version 1.4 software (Manugistics Inc., Cambridge, MA, USA).

The mean values of each variable of cheese made from refrigerated cow's milk and preserved by the LP system, were compared with the respective mean values of the control cheeses at each analysis time. Statistical significance for differences was determined at 5% probability level.

➢ *Determination of Fatty Acid Composition by Gas Chromatography*

- *Chromatographic Conditions*

Determination of lipid composition was carried out on an Agilent chromatography apparatus (Agilent 6890N) equipped with an INNOWA X polar capillary column (length 30 m, diameter 0.25 µm), a Flame Ionisation Detector (FID) and a plitless injector. The temperature program was set at 275°C. A volume of 10 µL was injected in split mode, with the injector temperature set at 220°C.

Chromatographic profiles were integrated and quantified by a computer controlling the GC system and acquiring the signal using Chem Data Station software (Agilent Technologie). Methylated fatty acid peaks were identified by comparing their retention times with those of standard methylated fatty acids (Supelco, PUFA3) injected under the same conditions. The quantity of each GA was determined as a percentage of its peak area relative to the sum of the total areas of the fatty acids present.

The averages of the sums of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were also calculated.

The ratio, ∑w-3/∑w-6 was calculated to assess the degree of degradation of fatty acids most susceptible to oxidation.

➢ *Determining Cheese Yield*

Cheese yield is generally expressed in Kg of cheese obtained from 100litres of milk, using the following equation:

Yield (Kg/100L)= (Weight of cheese obtained)/ (number of liters processed)×100

In order to monitor the performance of Saint-Paulin, cheeses from all four productions were weighed at the same stages of ripening (Choisy et *al.*, 1997).

➢ *Sensory Analysis of Cheese*

Organoleptic characteristics are an important criterion of food acceptability to consumers. The cheese's appearance, color, smell, consistency and flavor stimulate the senses of sight, touch and taste, provoking more or less intense reactions of acceptance or rejection. A descriptive test was used to assess the organoleptic characteristics of the four Saint-Paulin uncooked pressed cheeses produced for this study.

➢ *Descriptive Test with Five-Category Scales*

This is a full sensory description of the sample, with tasters rating intensity on a number of sample characteristics.

They give a total sensory description of the sample in terms of appearance (color), odor, flavor, texture, aftertaste and preference, noting the intensity of each of these characteristics. There are several descriptive tests whose sensory profile is quoted using category scales, the total

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

number of categories being five, and giving them descriptive names.

➢ *Analysis Description*

Principal component analyses (PCA) were used to process the sensory results.

Preference mapping superimposed on a level map was carried out in order to study tasters' preferences.

The data were processed using XLSTAT 2012 software.

III. RESULTS AND DISCUSSION

➢ *Milk for Cheese Production*

Table 1 Evolution in Sample Parameters for Raw, Chilled and Treated Milk (Chilled and LPS-Activated)

	L0	Lr	Lt
Total aerobic mesophilic flora Log(UFC/ml)	6,8±0,36[a]	7,9±0,35[b]	6,03±0,25[c]
mesophilic lactic flora Log(UFC/ml)	3,88±0,20[a]	4,22±0,50[a]	3,16±0,76[b]
Total psychrotrophic flora Log(UFC/ml)	6,11±0,54[a]	6,97±0,90[a]	5,2±0,56[b]
Yeasts and molds Log(UFC/ml)	4,57±0,50[a]	4,78±0,50[a]	3,34±0,67[b]
pH	6,8±0,02[a]	6,71±0,06[a]	6,75±0,05[a]
Acidity (°D)	16,33±0,61[a]	17,43±0,81[a]	16,87±0,47[a]
Dry extract (g/l)	95,13±5,20[a]	100±6,24[b]	115±8,19[c]

Mean Values in the Same Row with Different Exponents are Significantly Different (p<0.05); ES=Total Dry Extract; L0=Raw Milk, Lr=Chilled Milk; Lt=LPS-Activated Milk.

➢ *Microbiological Characterization of Milk Samples*

Comparisons of total aerobic mesophilic flora numbering results show that the three samples considered are significantly different (p<0.05).

Sample L0 of raw milk contained a load of around 6.31.106 CFU/ml of total aerobic mesophilic flora (TAMF) on receipt (**table 1**).

This may be attributable to poor hygiene conditions during milking, storage and collection. Furthermore, refrigeration of raw milk did not appear to slow down the proliferation of initial contamination germs: after 72 hours of refrigeration, the average value for total aerobic mesophilic flora (TAMF) reached 7.94.107 UFC/ml. There was also a reduction of 0.8 decimal units in the lactoperoxidase-activated (Lt) refrigerated milk sample, compared with the L0 milk sample.

Mesophilic lactic flora (MLF) changes insignificantly (p<0.05) after refrigeration. Its level rose from 7.59.103 to 1.66.104 CFU/ml after 72h storage at 4°C. On the other hand, this flora was significantly inhibited (p>0.05) after activation of the LP system, for the lowest load of 1.26.103 CFU/ml.

For total psychrotrophic flora (TPF), the highest concentration was detected in the Lr refrigerated milk sample (9.33.106UFC/ml). After activation of the LP system, this concentration decreased significantly (p<0.05) to 1.58.105 CFU/ml, a reduction of 1.77 decimal units. These results confirm the inhibitory effect of lactoperoxidase on these gram-negative psychrotrophic bacteria.

On receipt, the milk contained relatively high levels of 3.72.104 CFU/ml total coliforms (TC), a contaminant flora indicative of the bacteriological quality of raw milk. It should also be noted that there was a small, non-significant increase (p>0.05) after refrigeration (6.03.104 CFU/ml). These coliforms were significantly (p<0.05) inhibited following LPS activation, with a reduction of almost 30% recorded. This confirms the bactericidal effect of this system on gram-negative bacteria.

As far as yeasts and moulds (LM) are concerned, a value of 2.82.103 CFU/ml was recorded for the L0 raw milk sample. The yeast and mold content decreased slightly with refrigeration, but dropped remarkably with the activation of the LP system. As a result, their proliferation is curbed both by refrigeration and by the inhibitory action of the lactoperoxidase system.

Finally, it should be noted that the results obtained here coincide with those reported by Boulares et *al*, (2010).

➢ *Physico-Chemical Parameters of Milk Samples*

As can be seen from this **table 1**, after 72 h of refrigeration, there was a slight decrease in pH which was not significant (P> 0.05). In fact, the pH value rose from 6.8 to 6.71 pH units after 72 h storage at 4°C. There was also a slight decrease of 5% for the LPS-activated milk sample, compared with the pH of raw milk.

As for Dornic acidity, there was a slight non-significant increase (P> 0.05) after 72 h of refrigeration. This acidification is attributed, on the one hand, to the action of extracellular lipases from psychrotrophic bacteria, leading to hydrolysis of triglycerides into free fatty acids and glycerol and, on the other, to the fermentative activity of certain strains of psychrotrophic microflora (Bornert, 2000).

There was also a significant increase (p<0.05) in the total dry extract of the LPS-activated milk sample compared with the raw milk sample.

In conclusion, activation of the lactoperoxidase system in refrigerated cow's milk enabled good hygienic quality to be

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

maintained for a period of 72 h, which may be of industrial interest.

➢ *Evolution and assessment of different treatments of Saint-Paulin type uncooked pressed cheese during the ripening phase*

- *Microbiological Analysis of Saint-Paulin-type Uncooked Pressed Cheeses During Ripening*

The total aerobic mesophilic flora (TAMF) cultivated on PCA medium shows an increasing and similar evolution during ripening of the four cheeses produced, independently of milk treatment (**figure 1**)

The results showed an average increase in total aerobic mesophilic flora of 2.77±0.86 log units between the LPS-activated milk and the curds from the different cheese productions. This increase was interpreted by de Souza et *al.* (2003) as a normal phenomenon in cheese-making, resulting on the one hand from microbial multiplication during curdling and on the other from the physical retention of these microorganisms in the curd during unlactosage.

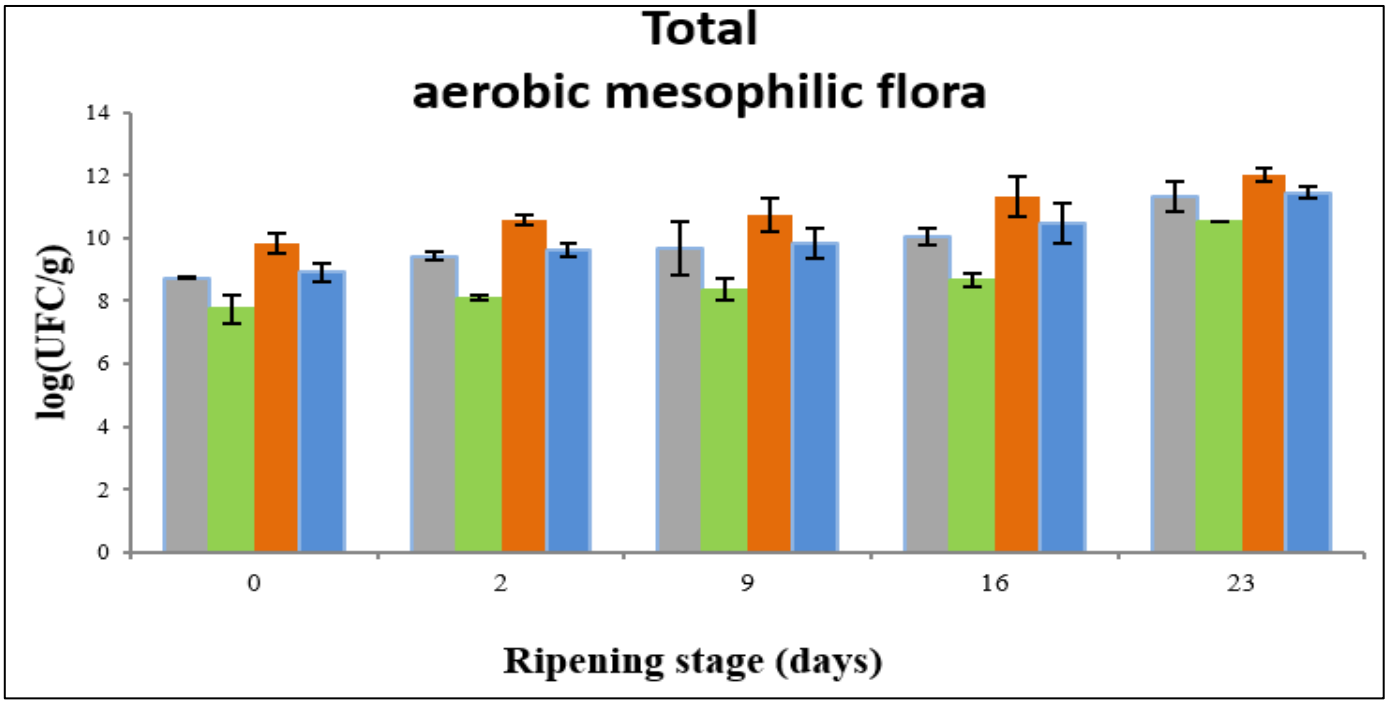

Fig 1 Changes in the Total Aerobic Mesophilic Flora During Ripening of Saint-Paulin Uncooked Pressed Cheeses

——— : P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

At the start of ripening (D=0), total germ levels were around 5.37.108; 5.49.107; 7.08.109 and 8.13.108 CFU/g in curds from P_{72}, P_{72a}, P_{55} and P_{55a} respectively. This load increased significantly ($p<0.05$) during maturation, reaching levels of 2.14.1011; 3.38.1010; 1.1012 and 2.82.1011 CFU/g on day D+23 for P_{72}, P_{72a}, P_{55} and P_{55a} respectively.

It seems clear from these results that the four batches of cheese studied are characterized by a non-negligible total flora, particularly for the P_{55} production sample. This flora is significantly ($p<0.05$) lower for the P_{72a} sample, whose lactoperoxidase system was activated, as well as for the P_{72} sample.

On the other hand, during ripening, there were significant differences between P_{72} and P_{72a}, as well as between P_{55} and P_{55a}. This result is not consistent with that found by Atamer et *al* (1999), who found no significant difference in the evolution of FAMT between Käser cheese made from cow's milk preserved by the LP system and the control cheese.

➢ *Mesophilic Lactic Flora*

Mesophilic lactic flora is relatively abundant in all four cheese products. This flora is the dominant microflora in cheeses at all stages of ripening, and its predominance can be explained by the use of lactic ferments as starters (**figure 2**)

In cheese curds from P_{72}, P_{72a}, P_{55} and P_{55a} productions, they represent 95.65%, 96.77%, 98.17% and 95.96% respectively of the total aerobic mesophilic flora.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

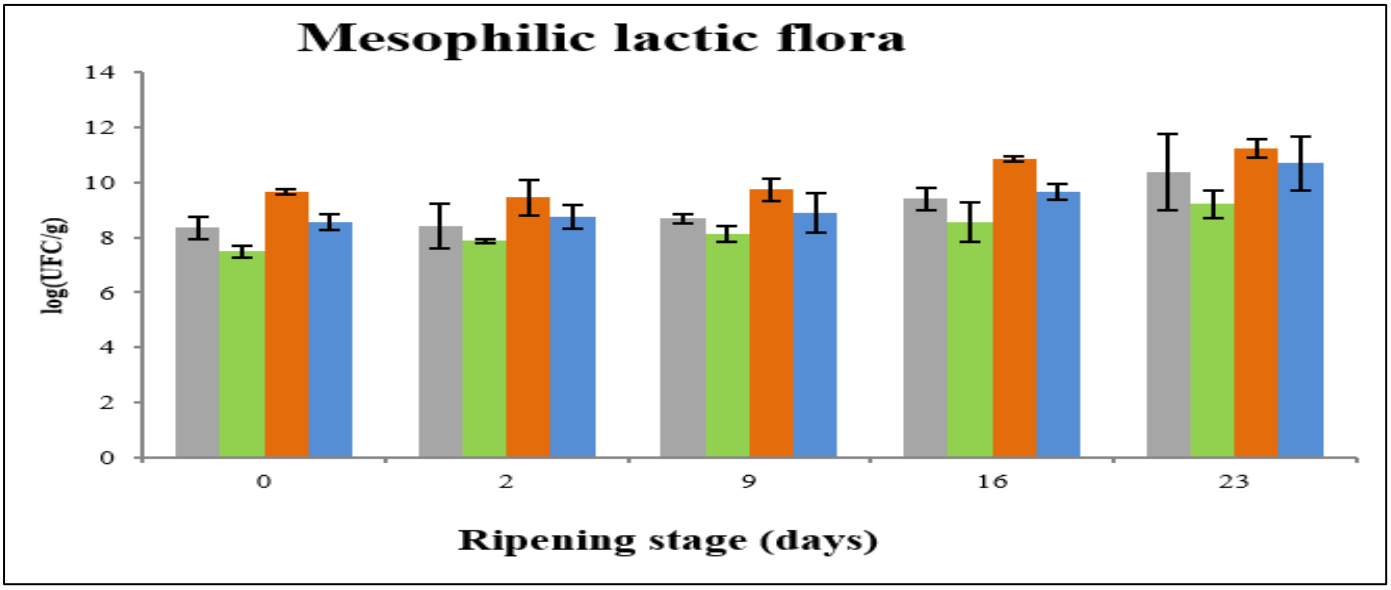

Fig 2 Changes in Mesophilic Lactic Flora during Ripening of Saint-Paulin Uncooked Pressed Cheeses

——: P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s, ...P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

At the end of ripening, the levels of these germs change significantly ($p<0.05$) but remain high, representing 96.62%; 87.56%; 96.56% and 93.45% of the total microbial population of cheeses from P_{72}; P_{72a}; P_{55} and P_{55a} respectively.

The predominance of mesophilic lactic flora has also been observed during the ripening of many cheeses; for further reading on this subject, see Fox and McSweeney (2004).

We also note that the evolution of these bacteria remains less dense for cheeses from LPS-activated milks. In fact, the rates of increase were 81.23% and 79.9% for cheeses from P_{72a} and P_{55a} respectively, compared with 86.1% for cheeses from P_{55}, confirming the effect of the lactoperoxidase system on these bacteria (Boulares et *al.*, 2010). However, Seifu et *al* (2004) reported that the count of this flora in LPS-activated goat's milk cheese was also similar to that in control cheese.

➢ *Yeasts and Molds*

The fungal flora count in the four cheese samples studied shows that this flora represents around half of the total flora of the cheese. In fact, it represents 55% of the total mesophilic flora in curds from P_{72} and P_{55a} productions, compared with 51% and 53% for curds from P_{72a} and P_{55} productions (**figure 3**).

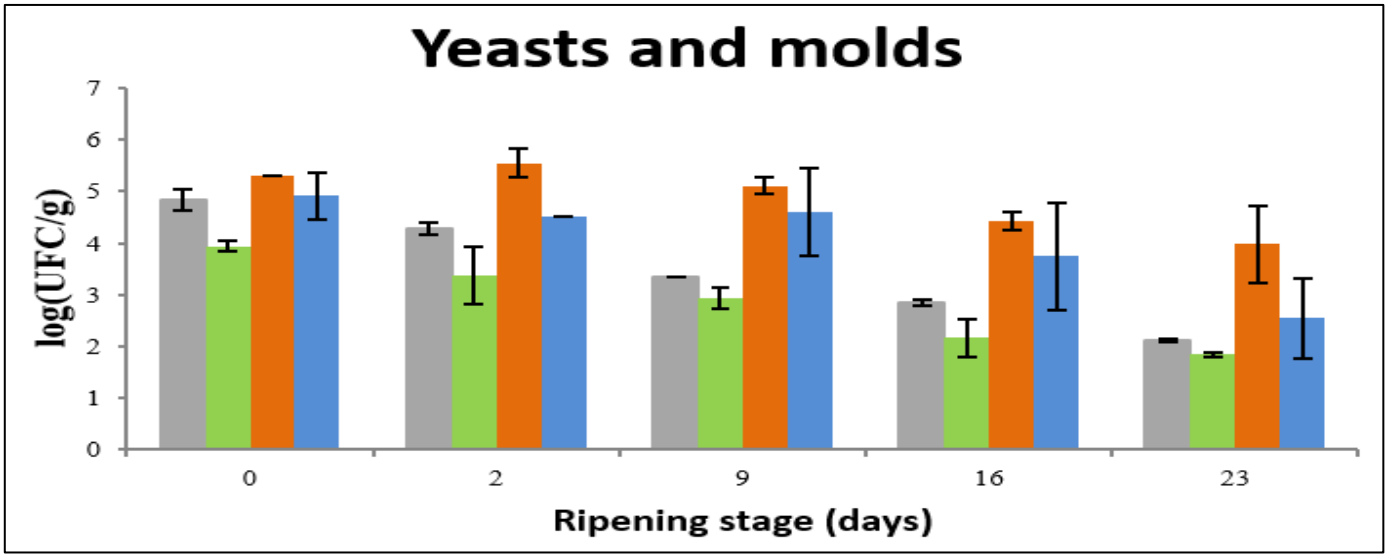

Fig 3 Changes in Yeasts and Molds During Ripening of Saint-Paulin Uncooked Pressed Cheeses

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

——— : P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

Despite the predominance of lactic acid bacteria during the ripening period, this fungal flora plays an important role in proteolysis, lipolysis and deacidification of the cheese mass (Welthagen et *al*., 1998).

It should also be noted that the development of yeasts and molds (LM) is intense in the first days of ripening for all four samples. In fact, levels of 6.92.104; 8.71.103; 2.105 and 8.13.104 CFU/g were recorded for P_{72}, P_{72a}, P_{55} and P_{55a} respectively, with low standard deviations.

Wethagen et *al*. (1999) explained this proliferation by the fact that young cheese constitutes a favorable environment for the development of yeasts and molds (relatively low pH, variable dissolved oxygen content, water activity and favorable external contamination).

Subsequently, after this period, this content decreased significantly ($p<0.05$) in samples P_{72} and P_{72a}, reaching levels of 1.32.102 and 6.92.101 CFU/g respectively at D+23, but not significantly for productions P_{55} and P_{55a}, where levels of 9.55.103 and 3.54.102 CFU/g were recorded respectively.

We also note that during ripening, the lowest levels were recorded for P_{72a} cheese, while the highest levels were recorded for P_{55} cheese. Interestingly, during ripening, no significant difference was observed between the P_{55a} and P_{72} samples, confirming the inhibitory effect of the lactoperoxidase system against yeasts and moulds.

➢ *Total Psychrotrophic Flora*

Diagrams showing the evolution of total psychrotrophic flora (TPF) show that the levels of this microflora decrease over the ripening period.

These levels range from 3.47.108; 4.47.108; 2.75.109 and 5.37.108 CFU/g at the start of ripening (D=0) to 4.37.106; 4.47.105; 2.24.107 and 5.89.106 CFU/g at the end of ripening (D+23) for cheeses from P_{72}; P_{72a}; P_{55} and P_{55a} respectively.

It is also important to note that this reduction remains significant for samples P_{72}; P_{72a} and P_{55a}, has the highest levels of this contaminant flora, with a non-significant reduction of 18.33%.

The dominance of psychrotrophic flora was thus observed in these samples, particularly in cheeses made from milk chilled for 72 hours at 4°C and not activated with LPS, such as cheeses from P55 production, confirming the effect of chilling on the evolution of this contaminating microflora.

Consequently, these results show that LP activation, whether combined with pasteurization or "low" heat treatment, had a bacteriostatic effect against psychrotrophic flora in the cheeses studied (Mankai et *al*., 2003).

On the other hand, the (FPP) and (FPL) diagrams in **figure 4** and **figure 5** show the evolution of the levels of proteolytic and lipolytic psychrotrophic flora over the refining period.

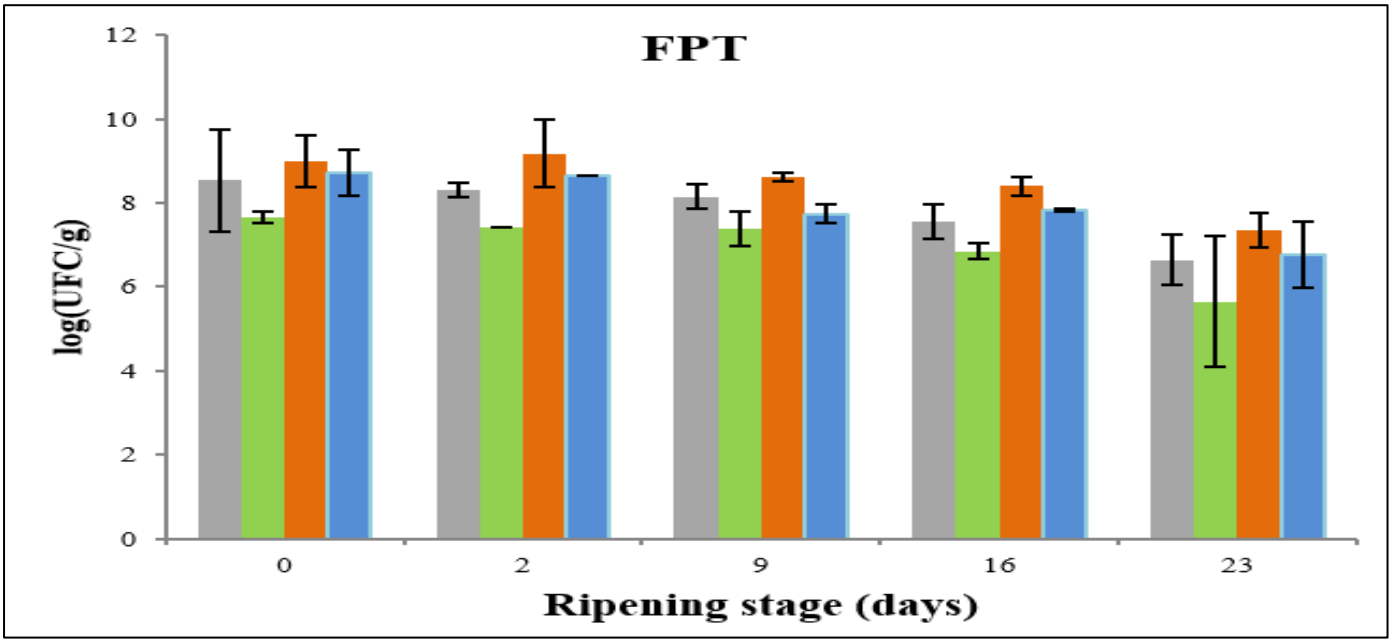

Fig 4 Changes in Total Psychrotrophic flora During Ripening of Saint-Paulin Uncooked Pressed Cheeses

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

——— : P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

The first flora results from extracellular proteases and represents the vast majority of psychrotrophic bacteria. The diagram (FPP) shows that these follow the same evolution regardless of the sample studied. In fact, the levels of this flora decrease, albeit insignificantly, during ripening, with reduction rates of 24.28%, 31.69%, 20.69% and 27.19% respectively for cheeses from P_{72}, P_{72a}, P_{55} and P_{55a} productions.

As for psychrotrophic lipolytic bacteria, resulting from the action of lipases which break down fat into free fatty acids, they present lower levels. Their values decrease, albeit not significantly, during ripening, with a reduction rate of around 30% for cheeses from P_{72a} production, due to the activation of the lactoperoxidase system, which inhibits these germs, and a reduction of 25% observed for cheese samples from P_{72} and P_{55a} productions.

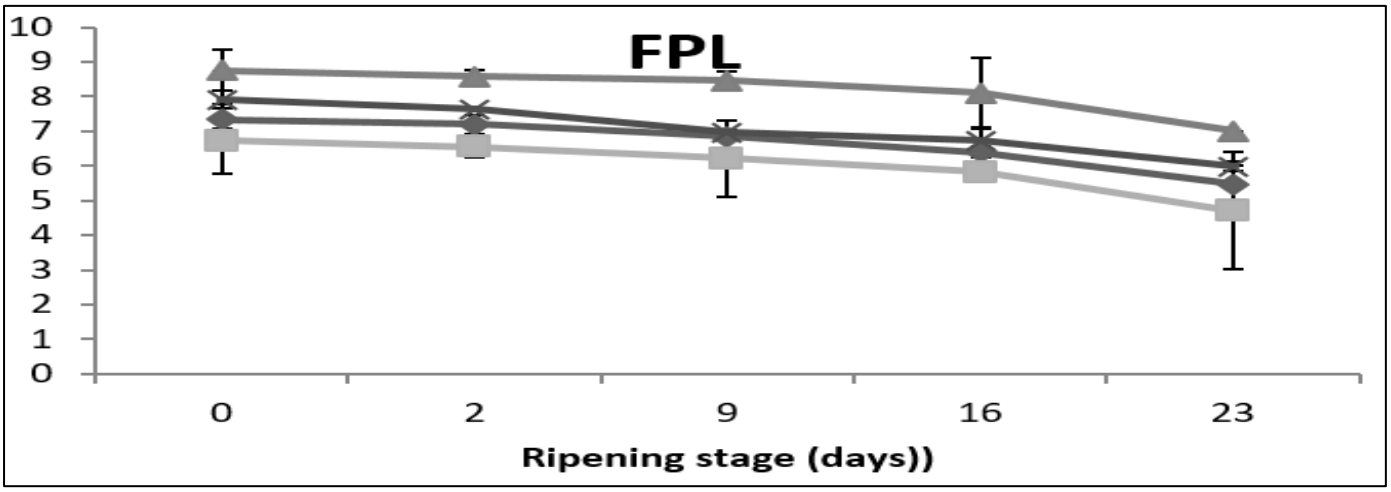

Fig 5 Changes in Psychrotrophic Bacteria During Ripening of Saint-Paulin Uncooked Pressed Cheeses

——— : P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

➢ *Total Coliforms*

Total coliforms are a flora indicative of the bacteriological quality of the milk used to manufacture the cheese, and of hygiene conditions during ripening.

Our monitoring shows that this flora decreases for the different types of cheese studied, with high levels of 1.51.106, 2.24.105, 4.47.106 and 2.04.106 CFU/g recorded for cheeses from P_{72}, P_{72a}, P_{55} and P_{55a} respectively at D=0.

These levels dropped steadily and significantly for all four cheese productions over the 23-day ripening period, with the lowest value seen in cheeses whose LPS system had been activated, or more precisely cheeses from the P_{72a} production (5.3.101 CFU/g) with a reduction rate of around 99%. Next came P_{72} and P_{55a} with a higher load (3.09.102 and 103 CFU/g), followed by P_{55} cheeses (1.58.104 CFU/g), proving that this enzyme possesses a very significant antibacterial power which helps to improve the microbiological quality of cheese.

In the same context, studies carried out on cheeses made from goat's milk preserved by activation of the LP system suggest that activation of the LP system in goat's milk prior to cheese manufacture could be of practical importance, especially for small-scale cheese producers who, in most cases, produce cheese from unpasteurized milk (Seifu et *al*., 2004).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

➢ *Summary of the Main Results of the Microbiological Analysis of the Various Cheese Samples*

Looking at the results of the microbiological analysis of the four cheese samples presented above, we note that the general appearance of these diagrams differs according to the flora studied. However, the evolution of each flora is similar for all four cheese samples during the different stages of ripening.

Comparisons of the four cheese samples over the 23-day ripening period show that the P_{72a} production of cheese made from LPS-activated milks pasteurized at 72°C/15s has the lowest microbial loads of the samples considered. On the other hand, the highest microbial loads were recorded for the P_{55} production of cheese made from milk chilled to 4°C/72h, not activated with LPS and heat-treated at 55°C/15s, demonstrating the obvious importance of pasteurization.

Furthermore, the results obtained for P55a production appear to be comparable to those for P_{72} production. This highlights the beneficial effect of this enzyme with low heat treatments (55°C/15s).

Hence the decision to add a statistical study comparing the P_{72}, P_{72a}, P_{55} and P_{55a} cheese productions and highlighting the common aspects that would justify the efficacy of the treatments tested here, or on the contrary, their inaction. In fact, we thought it would be interesting to analyze the Mahalanobis distances between the four productions at D+23 **(figure 6).**

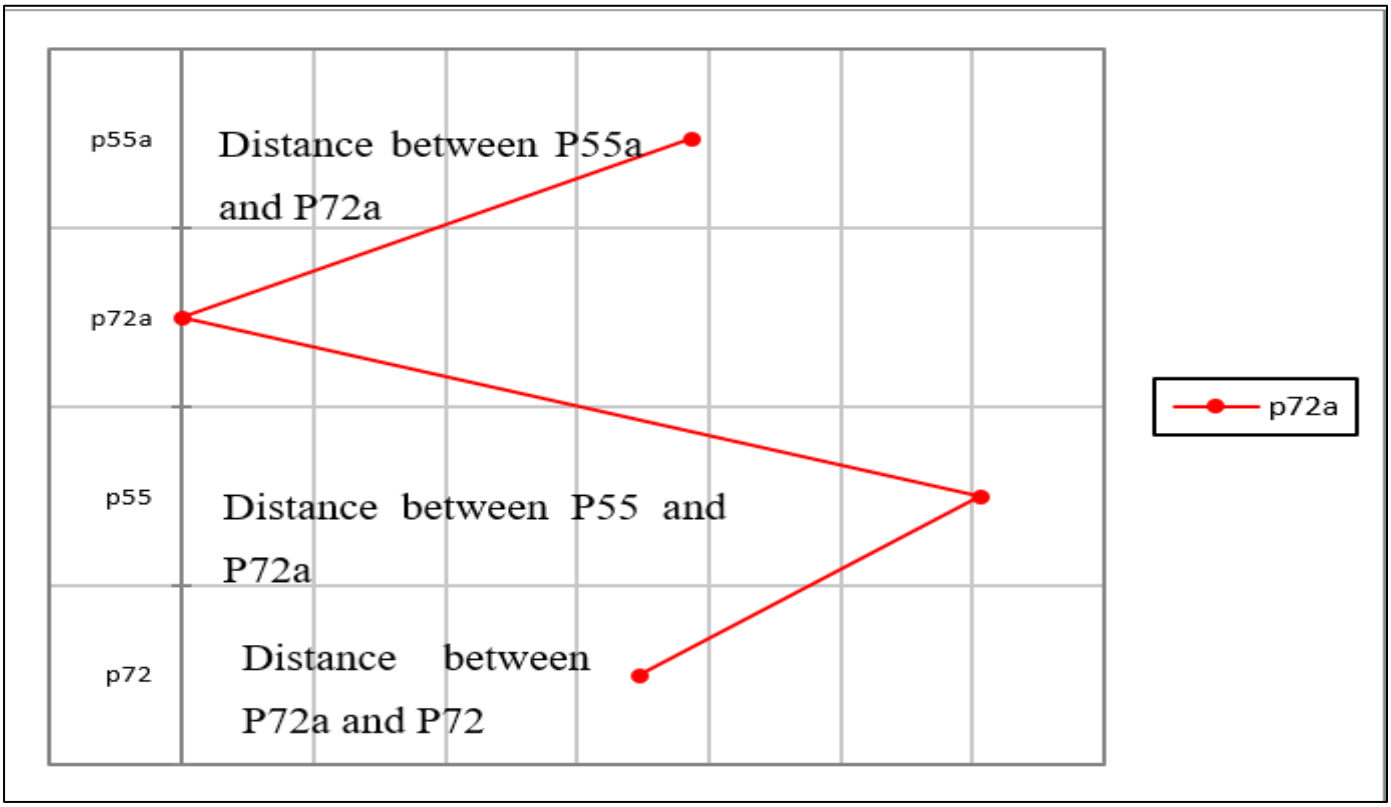

Fig 6 Semi Graphical Representation of Mahalanobis Distances between Samples of Saint -Paulin type Uncooked Pressed Cheese at D=0

The statistical results in **Figure 6** clearly confirm our initial findings. Indeed, the distance between the two samples P_{72a} and P_{55a} is slightly greater than that between P_{72a} and P_{72}. Whereas the distance between P_{55} and P_{72a} is the greatest (i.e. just under double the distance between P_{72a} and P_{72}). In other words, this comparison combined with the microbiological results highlights the beneficial effect of activating the lactoperoxidase system in pasteurized milk on the microbiological quality of cheese, results confirmed by several studies (Boulares et *al*., 2011).

Furthermore, the equivalent distances between P_{72a}; P_{72} and P_{72a}; P_{55a} lead to the conclusion that lactoperoxidase activation in cow's milk treated at 55°C/15s not only improved the microbiological quality of the cheese, but also gave results equivalent to those of cheese made from pasteurized milk (72°C/15s).

According to Parry-Hanson et *al* (2009), this can be explained by the fact that the activity of this enzyme was not affected by heat treatment at 55°C/15s, whereas heat treatment at 72°C/15s reduced it by 50%. For this reason, the application of the LP system prior to heating ensures a complementary, even synergistic combination.

➢ *Changes in Physico-Chemical Parameters of Saint-Paulin Type Uncooked Pressed Cheese Samples During Ripening*

Average values for physico-chemical parameters (pH, dry matter, fat/dry matter, fat content and protein content) are shown in figure below.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

➢ *PH*

It's important to note at the outset that pH is linked to cheese quality: a pH of 5.0 corresponds to good-quality cheese, while a pH above 5.2 means that cheese deteriorates more quickly than cheese with a lower pH. This is due to its important influence on the development and selection of microbial flora, and consequently on the overall progress of ripening (Alais, 1994 cited by Serhan 2008).

Observation of the experimental values in **Figure 7** shows that the evolution of pH during refining is approximately identical for the four samples studied (P72, P72a, P55 and P55a). In fact, there was a significant decrease ($p<0.05$) in pH from values of 5.31; 5.41; 5.61 and 5.72 at D=0 to values of 4.51; 4.61; 5.19 and 5.17 at D+23 of refining for P72, P72a, P55 and P55a respectively.

Usually, low pH values at D=0 are linked to the high production of lactic acid from residual lactose during fermentation. This decrease is obviously linked to the degradation of the acid, but also to the production of alkaline compounds generated during protein degradation which takes place during ripening. It was also noted that pH values showed no significant differences between the four cases, a result affirmed by Seifu et *al*. (2004) and Boulares et *al*. (2011).

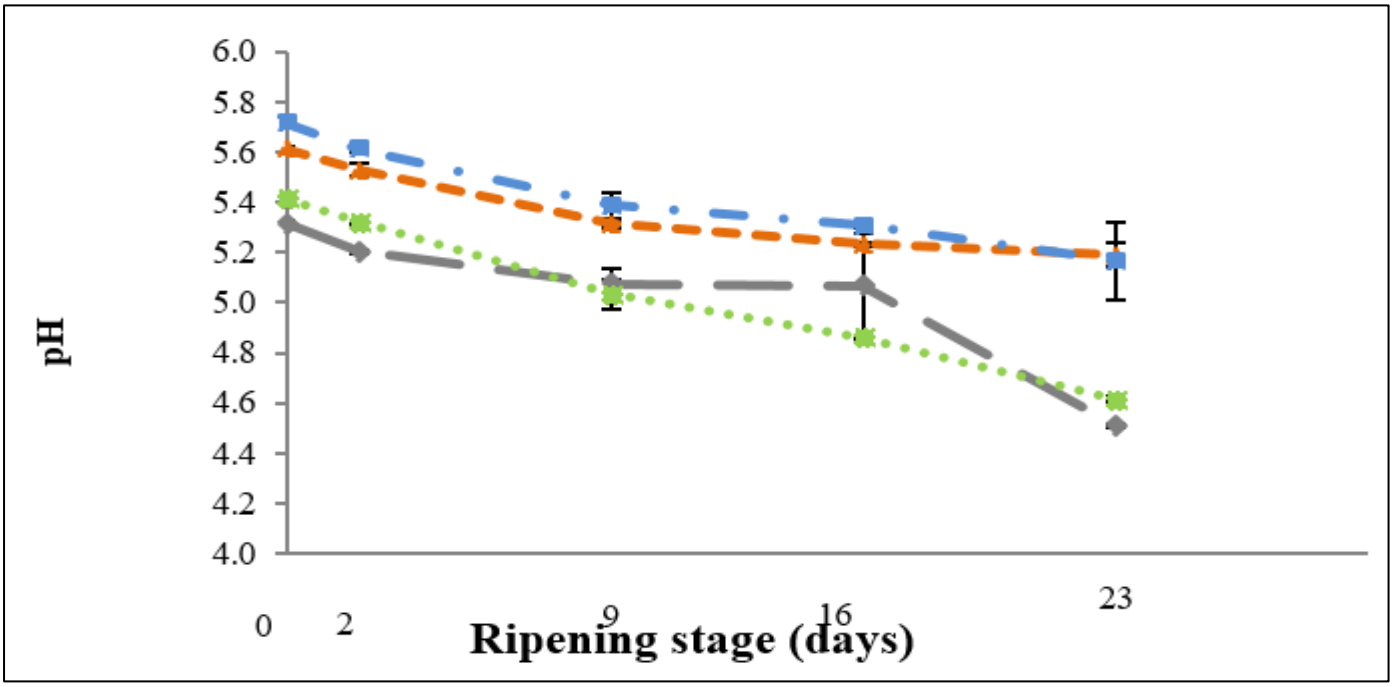

Fig 8 Changes in pH During Ripening of Saint-Paulin Uncooked Pressed Cheeses

P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s, ….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

➢ *Total Dry Extract (%)*

During ripening, total dry matter content increases progressively and significantly ($p<0.05$), with small standard deviations, for all four samples studied. The increase in total dry matter essentially corresponds to the exchange of volatile products (moisture, ammonia, volatile fatty acids) between the cheese and its environment (Hassouna et *al*., 1996), as well as to the increased water absorption capacity of casein micelles when changes in their size and characteristics are established due to the loss of calcium phosphate in colloidal form when the pH reaches values between 5.5 and 5.0.

Water content plays an important role in cheese ripening, conditioning the microbial and enzymatic activities involved in proteolysis (Hassouna and Guizani, 1995), which in part determines the texture of the finished product.

The lowest total solids contents were recorded both for cheese made from chilled, activated and pasteurized milk (P_{72a}) and for cheese made from chilled, activated and thermized milk (P_{55a}). In addition, the dry matter of cheese not activated by the lactoperoxidase system is higher than that of activated cheese.

This drop in total dry extract is certainly due to the increase in the number of psychrotrophic bacteria during refrigeration of raw milk (Mankaï et *al*., 2003) and to the action of active proteases, which are responsible for the formation of soluble casein fragments that pass into the whey.

Finally, we note that for all samples, the dry matter content at the end of ripening complies with the standard set by FAO/WHO (2002), which requires a minimum dry matter content of around 57% for Saint-Paulin-type cheese (**figure 8**)

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

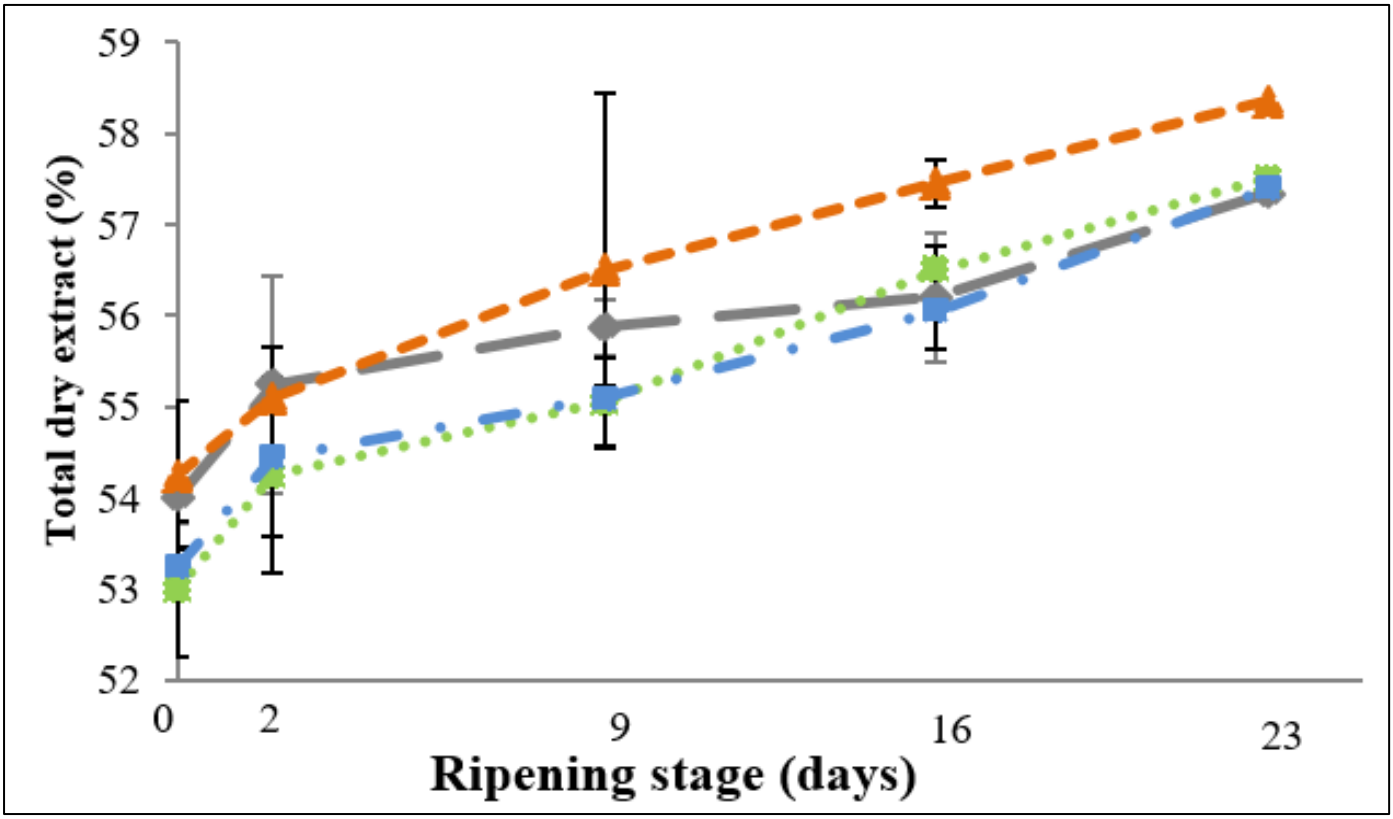

Fig 8 Changes in Total Dry Extract During Ripening of Saint-Paulin Uncooked Pressed Cheeses

P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s, ….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

➢ *Fat Content (%)*

Fat content changes very little during ripening ($p>0.05$). The small variation between the four cheese samples is due to the characteristics of the raw materials. The relative constancy of this quality criterion is attributed to overall lipolysis, which only affects around 1 to 10% of the total fat content of pressed uncooked cheeses (Choisy et *al.*, 1997).

For this composition parameter during ripening, the average fat content values measured are around 26%; 26.22%; 27.21% and 27.4% for P_{72}, P_{72a}, P_{55} and P_{55a} respectively.

The fat/dry ratio decreases insignificantly during ripening of the cheeses studied. The average fat/dry quantities recorded are around 46.66; 47.47; 48.32 and 49.60 respectively for P_{72}, P_{72a}, P_{55} and P_{55a} (**figure 9**).

However, in the case of cheese samples P_{72}, P_{72a} and P_{55}, this ratio (fat/dry) is significantly lower than the minimum required for Saint Paulin of around 49%. This can also be explained by the increased dry extract at the cheese rind.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

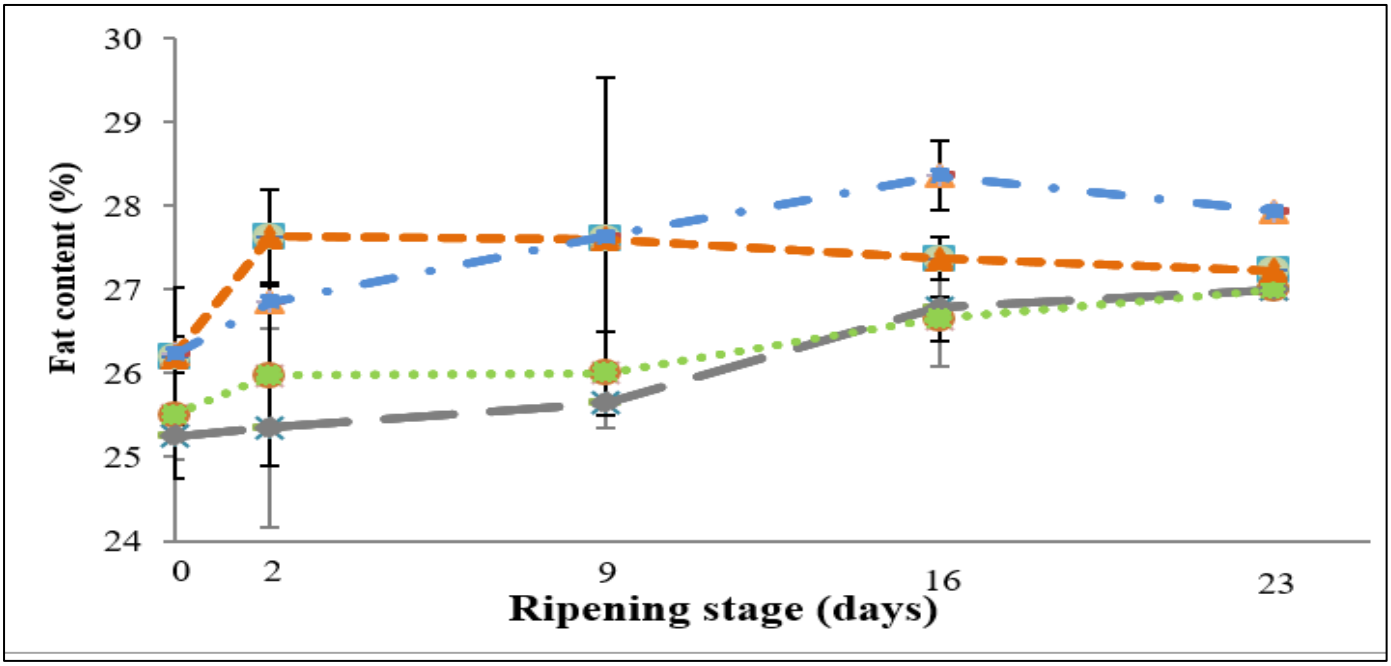

Fig 9 Changes in fat Content During Ripening of Saint-Paulin Uncooked Pressed Cheeses

P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

➢ *Protein Levels*

Protein levels (total nitrogen x 6.38) changed significantly ($p>0.05$) during ripening of the four cheeses studied. Protein levels also differed significantly between the four batches (**figure 10**).

At the start of ripening, the curds from P_{55} and P_{55a} had the highest protein levels (around 16g/100g of cheese), which proves that heat treatment at 55°C/15s did not affect the protein content of these samples. As for the P_{72} and P_{72a} productions, the protein levels are respectively of the order of 13 and 15/100g of cheese. At the end of the ripening period, these levels reached 21.88, 23.35, 17.99 and 21.69 g/100g of cheese respectively for batches P_{72}, P_{72a}, P_{55} and P_{55a}.

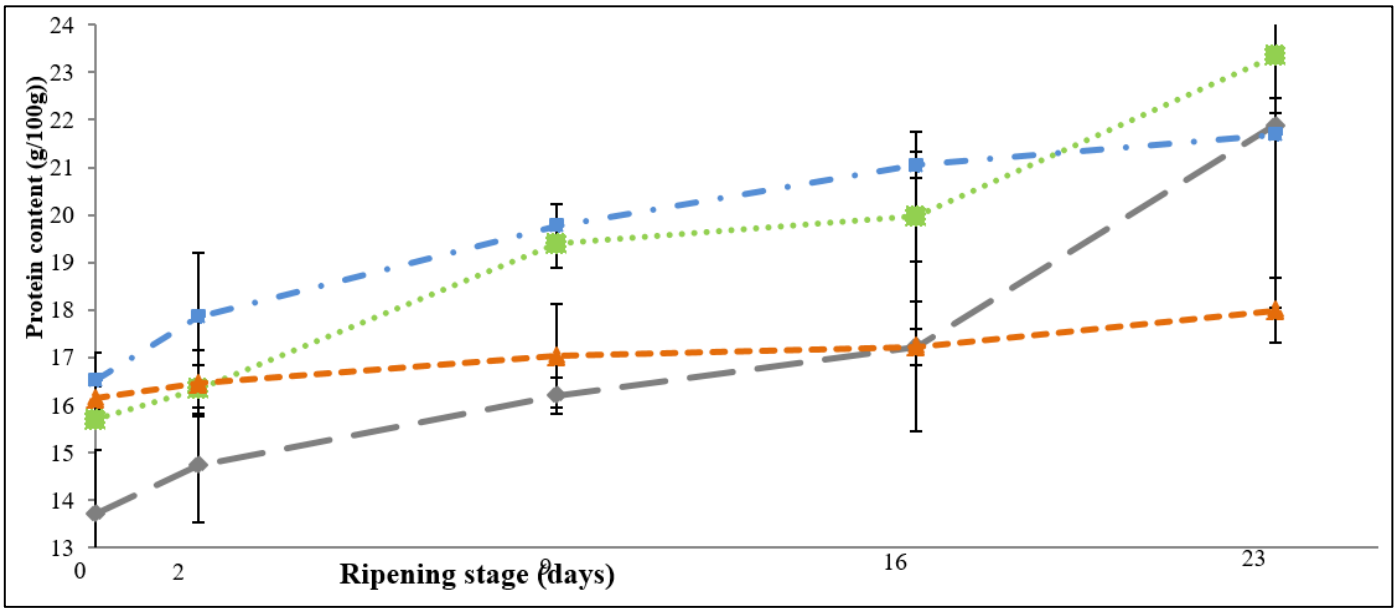

Fig 10 Changes in Protein Content During Ripening of Saint-Paulin Uncooked Pressed Cheeses

P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

➢ *Fractional Precipitation Method : Secondary Proteolysis*

Analysis of the data on the evolution of the different forms of nitrogen in samples P_{72}; P_{72a}; P_{55} and P_{55a} over 23 days of ripening, shown in **Table 2**, highlights the significant effect of LP system activation in the chilled milk and the stage of ripening on the intensity and nature of proteolysis.

Examination of the variations in casein nitrogen and proteose nitrogen for the four samples reveals that the proportion of soluble nitrogen at pH=4.6 varies significantly according to the stage of ripening and milk treatment (**figure 11, figure 12 and figure13**).

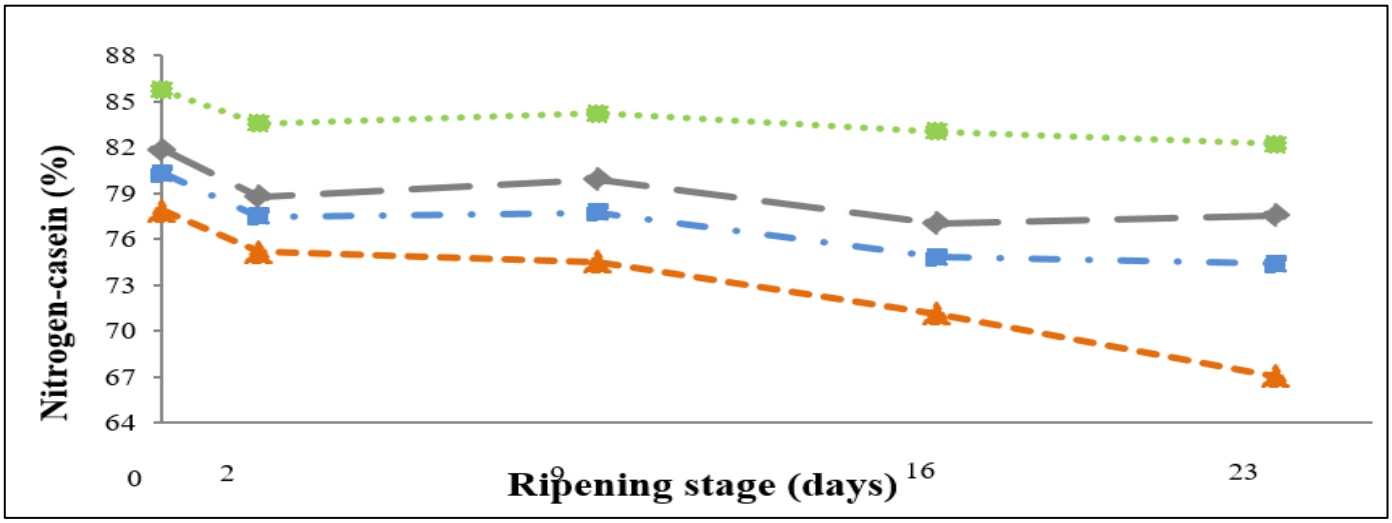

Fig 11 Changes in Casein Nitrogen During Ripening of Saint-Paulin Uncooked Pressed Cheeses

——, P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

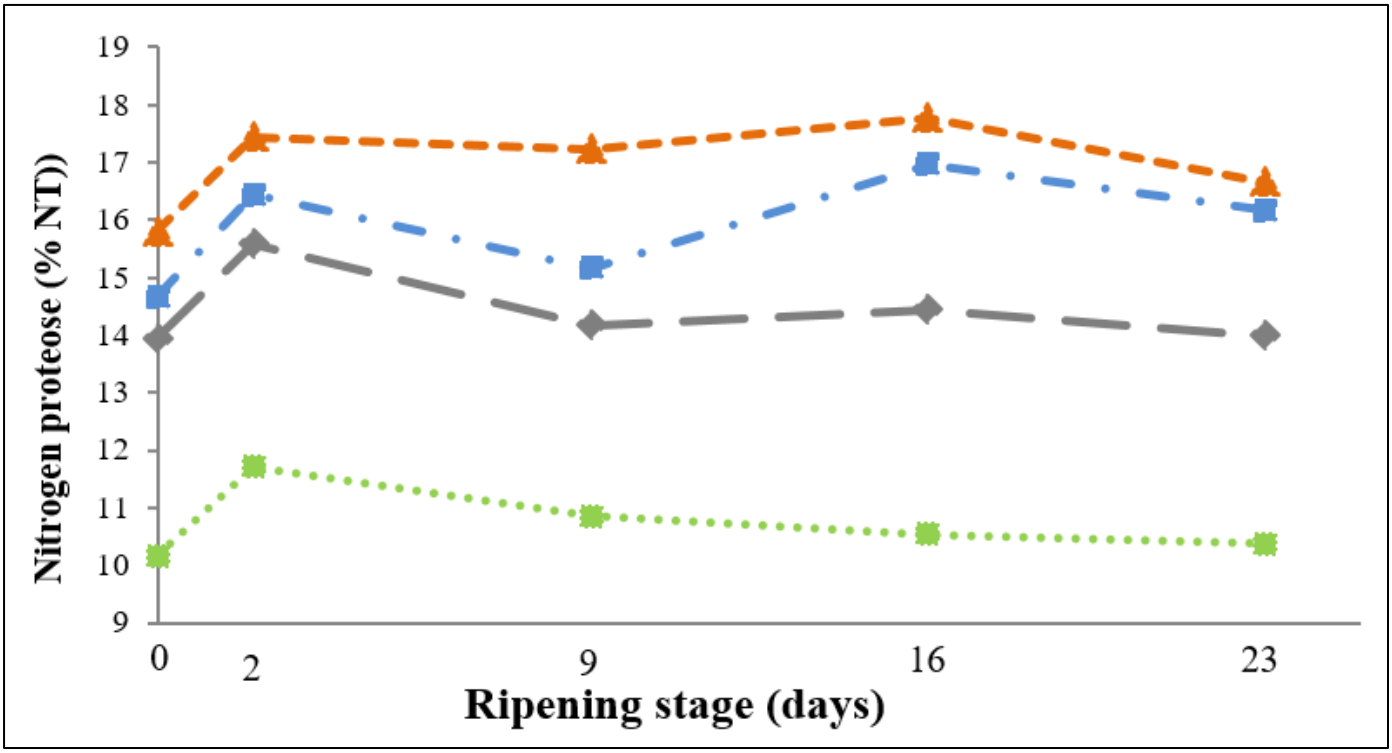

Fig 12 Changes in Proteose Nitrogen During Ripening of Saint-Paulin Uncooked Pressed Cheeses

——, P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s,….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

Traditionally, in cheeses, the development of proteolysis is followed by an increase in the percentage ratio of water-soluble nitrogen (WSN) to total nitrogen (TN). In fact, soluble nitrogen provides information on proteinase activity and characterizes refining in width (degradation of caseins towards water-soluble fragments), while non-protein nitrogen is linked to peptidase activity and characterizes refining in depth (degradation of soluble nitrogen towards smaller fragments) (Serhan, 2008). We also note that the fraction of soluble nitrogen at pH 4.6 in total nitrogen is often used as an indicator of cheese ripening (Gorostiza et *al*., 2004).

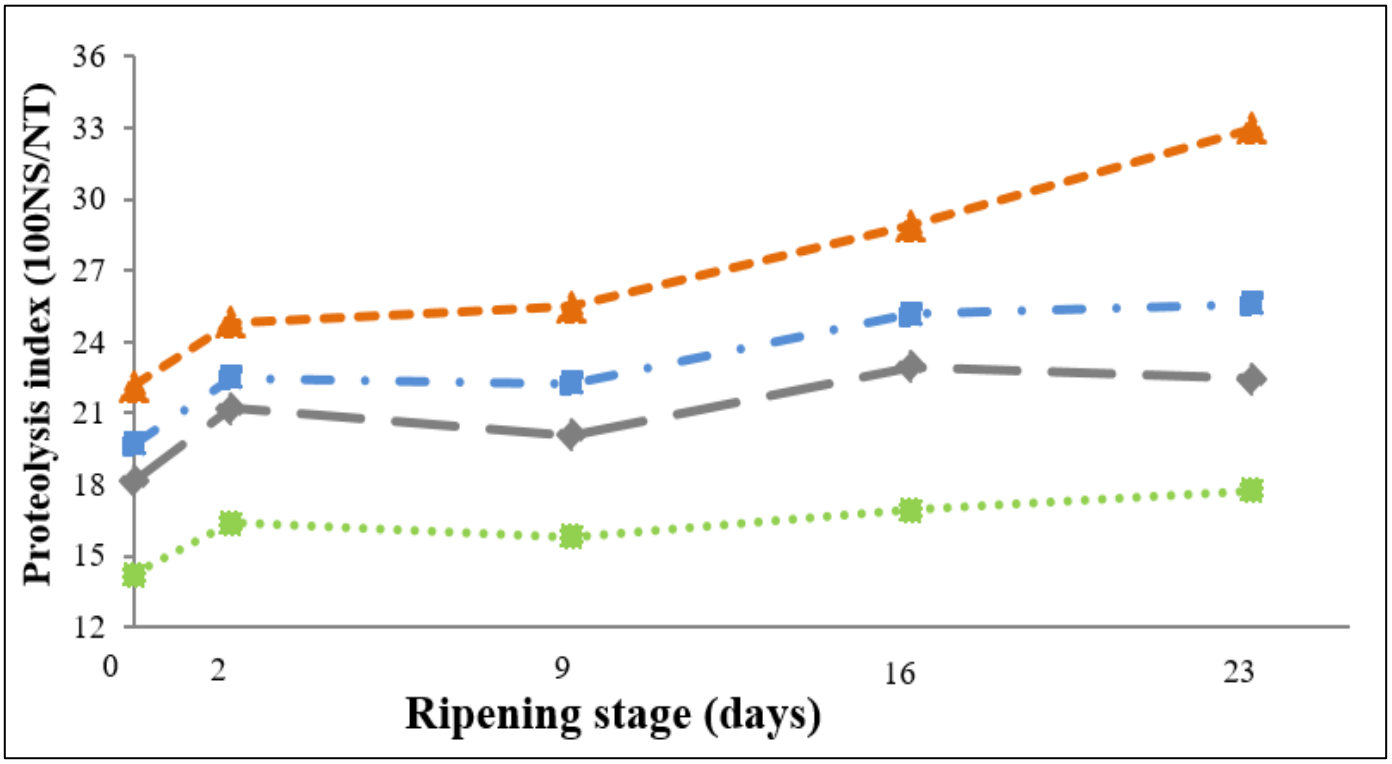

Fig 13 Changes in Total Nitrogen During Ripening of Saint-Paulin Uncooked Pressed Cheeses

P_{72} : cheese with milk chilled to 4°C/72h, non-LPS-activated, pasteurized to 72°C/15s, ….P_{72a}: cheese with milk chilled to 4°C/72h, LPS-activated, pasteurized to 72°C/15s, ---P_{55}: cheese with milk chilled to 4°C/72h, non-LPS-activated, heat-treated to 55°C/15s, -.-.- P_{55a} : cheese with LP-activated milk chilled to 4°C/72h, heat-treated to 55°C/15s.

It should first be noted that the ripening period appears to be an important factor affecting cheese proteolysis indices. Indeed, at the start of ripening (D=0), samples P_{72}; P_{72a}; P_{55} and P55a showed a degree of proteolysis equal to 18.14%; 14.23%; 22.13% and 19.69% respectively. This amount of soluble nitrogen results essentially from the combined effect of the proteolytic action of rennet (Gorostiza et *al*., 2004) and the activity of plasmin, whose activity remains present in cheese obtained from pasteurized milk due to its thermoresistance (Choisy et *al*., 1997).

During ripening and up to D+23 (end of ripening), the proteolysis index increased significantly ($p<0.05$) in the four samples studied, particularly for P_{55} cheese. Significant increases of 19.2%, 19.89%, 32.88% and 23.05% were noted for P_{72}, P_{72a}, P_{55} and P_{55a} samples respectively.

It is important to note that this proteolysis index is lowest for cheese from P_{72a} production (with an average of 16.22% over the five samples taken over 23 days), and highest for cheese from P_{55} production (with an average of 26, 86% on the five samples taken over 23 days), while samples from productions P_{72} and P_{55a} were comparable, although deference was significant, with respective averages of 20.97 and 23.04% on the five samples considered during ripening.

The results of this index for sample P_{72} confirm those obtained by Mankai et *al*. (2012) on Gouda-type cheeses made from cow's milk refrigerated at 4°C/72h.

Those of sample P_{72a} are comparable to a cheese made from cow's milk refrigerated at 4°C/24h in the same study. This proves the preponderant effect of LPS activation in moderating proteolysis from the psychrotrophic flora of chilled milk. The proteolysis index of P55a cheese was slightly higher than that recorded for cheese refrigerated for 72h, although there was a clear improvement over the P_{55} sample.

As a result, activation of the lactoperoxidase system in processed milk seems to have a considerable effect on cheese proteolysis, due to its effect against the psychrotrophic flora of milk and therefore on the production of extracellular proteases capable of degrading milk caseins.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

Table 2 Changes in Nitrogen Fractions During Ripening of Saint-Paulin Pressed Uncooked Cheese Samples

FormsNitrogen (g/100g)	**Samples**		**Ripening stages (days)**			
		0	2	9	16	23
NT	P_{72}	2,15± 0,21	2,31 ± 0,19	2,54 ± 0,06	2,7± 0,28	3,43± 0,04
	P_{72a}	2,46± 0,014	2,56±0,08	3,04±0,28	3,13±0,61	3,66±0,14
	P_{55}	2,53±0,04	2,58±0,05	2,67±0,13	2,7±0,09	2,82±0,18
	P_{55a}	2,59±0,09	2,8±0	3,1±0,07	3,3±0,23	3,4±0,11
NS	P_{72}	0,39±0,01	0,49±0,14	0,51±0,18	0,62±0,04	0,77±0
	P_{72a}	0,35±0,21	0,42±0,28	0,48±0,01	0,53±0,17	0,65±0,13
	P_{55}	0,56±0,1	0,64±0,07	0,68±0,03	0,78±0,16	0,93±0,01
	P_{55a}	0,51±0,04	0,63±0,11	0,69±0,03	0,83±0,14	0,87±0,06
NNP	P_{72}	0,09±0	0,13±0,06	0,15±0,02	0,23±0,05	0,29±0,08
	P_{72a}	0,1±0,01	0,12±0,05	0,15±0,016	0,2±0,09	0,27±0,1
	P_{55}	0,16±0,03	0,19±0,01	0,22±0,04	0,3±0,005	0,46±0,007
	P_{55a}	0,13±0,08	0,17±0,03	0,22±0	0,27±0,06	0,32±0,03

NT = Total Nitrogen, NS= Soluble Nitrogen, NNP= Non Protein Nitrogen, Level of Significance <5%.

On the other hand, the non-protein nitrogen fraction increased steadily over the 23 days of ripening, but not significantly, while the protein nitrogen fraction (%NS) decreased significantly ($p<0.05$) as a function of ripening stage.

On the other hand, total nitrogen (TN) levels also increased in a similar, albeit non-significant, way for all four cheese samples. Mean levels of 2.63, 2.97, 2.66 and 3.04g/100g of cheese were recorded for cheeses from P_{72}, P_{72a}, P_{55} and P_{55a} respectively, corresponding to mean total nitrogen values of 16.75, 18.95, 16.97 and 19.38g/100g of cheese respectively.

It's also worth noting that at maturity, most of the total nitrogen in P_{72a}, P_{72} and P_{55a} cheeses is presented by undegraded casein, i.e. 82.24%, 77.55% and 74.41% of total nitrogen respectively, compared with 67.02% of total nitrogen for P_{55} cheese, showing that total proteolysis remains relatively low throughout the ripening phase, affecting only around 14.43 ; 10.73 and 15.88% respectively for P_{72}, P_{72a} and P_{55a} of the average amount of total casein nitrogen, compared with 17% for P_{55}.

This content is the result of enzymatic degradation by the psychrotrophic enzymes favored during cold storage, which are highly thermostable. These enzymes, which can withstand pasteurization (72°C/15 s) and even UHT treatment (138°C/2 s or 149°C/10 s), are responsible for the release of amino acids, biogenic amines, nitrogenous sulfur compounds and low-molecular-weight peptides, giving cheeses their characteristic texture and aroma (Hassouna et *al*., 1999). As a result, the casein fraction is preserved with the application of the lactoperoxidase system.

- *Study of Primary Proteolysis by Acrylamide Gel Electrophoresis (SDS -PAGE)*

Primary proteolysis in cheese corresponds to changes in casein-(α_{s1}, α_{s2}), β and *γ*. Qualitative evaluation by monitoring the electrophoretic behavior of these cheese caseins enables us to track their enzymatic degradation during the various stages of ripening, and the formation of peptide fragments of varying sizes.

SDS-PAGE acrylamide gel electrophoresis is particularly well-suited to casein separation. Indeed, the sensitivity and resolving power of this technique have earned it a reputation as a powerful tool for assessing casein proteolysis. This is made possible by the action of the dissociating agents used, whose role is to break hydrogen bonds (in the case of urea) and disulfide bridges (in the case of β-mercaptoethanol), enabling protein entities to migrate in their simplest form, depending on molecular weight and charge.

Literature references mention that rennet is the first agent of proteolysis in cheese. It preferentially attacks αS1 casein by fragmenting the (Phe23-Phe24) bond, then hydrolyzes β casein.

The latter, however, is more sensitive to the action of plasmin, which releases the caseins and the corresponding proteose-peptone fragments. After this primary hydrolysis, which generally produces high molecular weight peptides, it's the turn of bacterial proteases to trigger secondary proteolysis (Ouali, 2003).

In this study, the evolution of primary proteolysis, using the SDS-PAGE electrophoretic method, was applied to casein fractions extracted from samples of young cheeses from productions P_{72}, P_{72a} and P_{55a} after brining (D+2) and at the end of ripening (D+23). Standards with well-defined molecular weights (SDS-PAGE-Molecular Weight Standards, Broad Range, Bio-Rad) are deposited on the left-hand side of the gel. The molecular weights of the standards used were: ovalbumin (46 kDa), carbonic anhydrase (31 kDa), trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa).

The electrophoretic profiles obtained **(figure 14)** showed different bands corresponding to native caseins (very intense characteristic bands on the figure), and the fragments resulting from their hydrolysis (less intense bands on the figure).

The major caseins in the three cheese samples migrate in ascending order of mobility; the band with the highest

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

mobility was casein-αs, while casein-β moves with lower mobility (b-CN < a-CN).

Comparison of the electrophoretic profiles shows that the variation in electrophoretic band intensities for samples P_{72}; P_{72a} and P_{55a} remains low due to the relatively low level of proteolysis, as previously explained.

The effect of ripening on casein degradation is not perceptible for cheeses P_{72} and P_{72a}. However, for sample P_{55a}, a slight degradation of the β-casein and κ-paracasein fractions is observed: the bands corresponding to these caseins decrease slightly in intensity on the electrophoretic profile after 23 days of ripening.

Bands 1, 4 and 5 representing casein samples from cheeses P_{72}, P_{55a} and P_{72a} respectively at D+2 show equivalent intensities.

Band 3, corresponding to the casein sample from cheese P_{55a} at D+23, is slightly less dark than bands 2 and 6 representing the casein samples from cheeses P_{72} and P_{72a} at D+23, while remaining close.

These findings imply that the level of proteolysis is fairly comparable for the two samples P_{72a} and P_{55a}, although it is slightly higher in the case of sample P_{72}.

This suggests that activation of the lactoperoxidase system in chilled raw milk reduces the likelihood of proliferation of psychrotrophic flora, bacteria that secrete thermoresistant proteases capable of preferentially degrading κ-paracasein, β-casein and to a lesser extent αs1-casein (Mankaï et *al*., 2009).

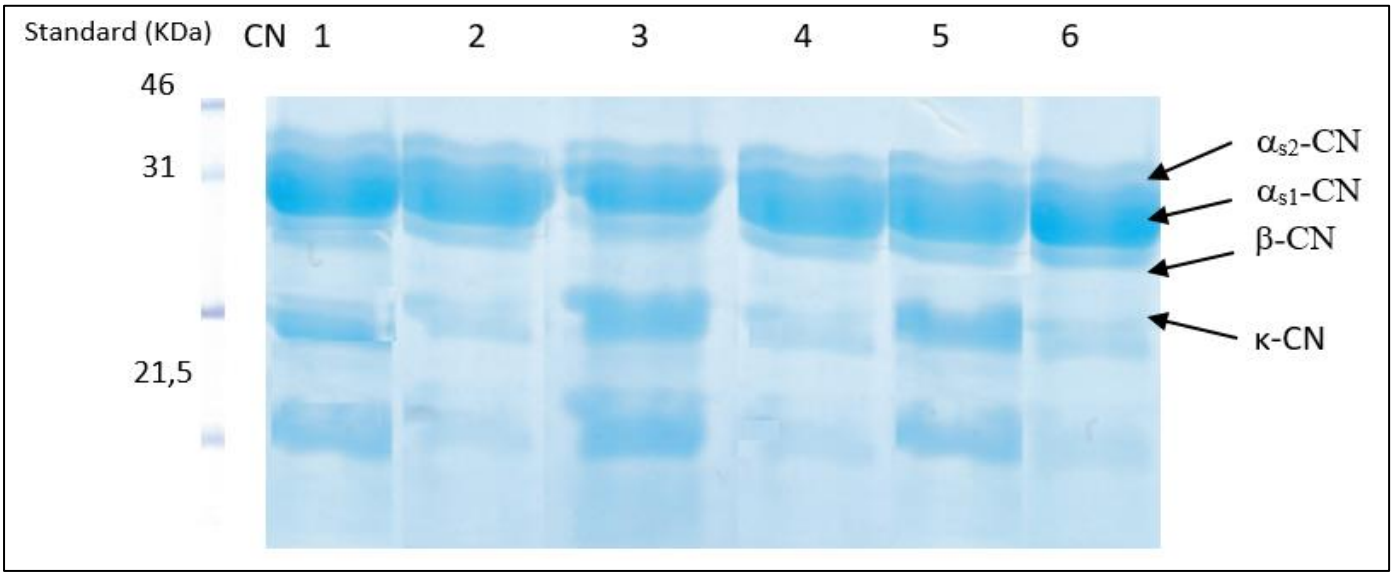

Fig 14 Evolution of the Electrophoretic Profile Obtained on Acrylamide Gel in the Presence of SDS (SDS-PAGE) During the Ripening of Saint-Paulin Pressed Uncooked Cheese Samples.

1: sample of P_{72} cheese at stage D+2;

2: sample of P_{72} cheese at stage D+23;

3: P_{55a} cheese sample at D+23 stage;

4: P_{55a} cheese sample at D+2 stage;

5: P_{72a} cheese sample at D+2 stage;

6: P_{72a} cheese sample at D+23 stage.

- *Measurement of Proteolytic Activity using the Azocasein Method*

The Azocasein method measures the absorbance of the enzymatic activity of proteases found in the medium degrading an artificial substrate, azocasein.

The results of the variation in the optical density of soluble nitrogen and non-protein nitrogen over the ripening period of the four cheese samples studied are shown in **Table 3**.

These results show that the internal composition of protease enzymes in soluble nitrogen varies but does not change significantly, depending on both the stage of ripening and the type of treatment: activation of the LPS system and heat treatment.

The smallest increase in proteolytic activity was recorded in the cheese sample whose lactoperoxidase system had been activated. Indeed, at the start of ripening, the curd had optical densities of around 0.491, 0.312, 0.534 and 0.46, with low standard deviations for cheeses P_{72}, P_{72a}, P_{55} and P_{55a} respectively.

At the end of ripening, proteolytic activity increased non-significantly ($p<0.05$) for all four cheese samples, while remaining higher for the cheese sample from P55 production.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

The curds obtained from chilled milk and processed at 55°C/15s contain more proteolytic enzymes than other curds. This proteolytic activity can be assumed to be due to the action of proteases produced by psychrotrophic bacteria, which are thermoresistant and subsequently resist low heat treatment.

These results also confirm that the immediate consequence of activating the lactoperoxidase system is a reduction in the development of gram-negative psychrotrophic bacteria favored by refrigeration, and therefore in the quantity of enzymes they secrete.

Similarly, proteolytic activity in non-protein nitrogen (see **table 4**) follows the same pattern as that in soluble nitrogen. An increase in proteolytic activity is recorded during ripening, with cheese from milk not activated with LPS showing the highest proteolytic activity. Indeed, optical density increases from 0.143, 0.133, 0.169 and 0.157 to 0.164, 0.154, 0.186 and 0.177 respectively for cheeses P_{72}, P_{72a}, P_{55} and P_{55a} after 23 days of ripening. This confirms once again that the lactoperoxidase system has a powerful effect in inhibiting the psychrotrophic bacteria responsible for protease secretion at both 72°C and 55°C.

Table 3 Changes in Proteolytic Activity in Soluble Nitrogen During Ripening of Saint-Paulin Pressed Uncooked Cheeses

DO NS at 345nm	**Ripening stages (days)**		
	0	**9**	**23**
P_{72}	0,491±0,005	0,525±0,02	0,51±0,035
P_{72a}	0,312±0,003	0,462±0,01	0,453±0,02
P_{55}	0,534±0	0,613±0,04	0,582±0,01
P_{55a}	0,46±0,001	0,554±0	0,573±0,01

NS= Soluble Nitrogen; Level of Significance <5%.

Table 4 Changes in Proteolytic Activity in Non-Protein Nitrogen During Ripening of Saint-Paulin Pressed Uncooked Cheeses

DO NNP at 345 nm	**Ripening stages (days)**		
	0	**9**	23
P_{72}	0,143±0	0,156±0,003	0,164±0,01
P_{72a}	0,133±0,001	0,147±0,001	0,154±0,005
P_{55}	0,169±0,001	0,181±0,002	0,186±0,03
P_{55a}	0,157±0,002	0,163±0,004	0,177±0,001

NNP= Non Protein Nitrogen; Level of Significance <5%.

- *Changes in Total Fatty Acid Content During Cheese Ripening*

Lipolysis is an important biochemical event occurring during cheese ripening and has been studied quite extensively in varieties such as blue and hard Italian cheeses, where lipolysis reaches high levels and is a major pathway for flavor production. However, in the case of cheeses such as cheddar and gouda, in which the level of lipolysis during ripening is moderate, the contribution of lipolysis end products to the quality and flavor of these cheeses has received relatively little attention (McSweeney et *al*., 2000).

The aim of this section is to examine the evolution of fatty acids in cheese samples P_{72}; P_{72a} and P_{55a} during ripening, in order to highlight the effect of activation of the lactoperoxidase system and heat treatment on lipolysis of the fat in these cheeses (**table 5**).

Traditionally, discussion of cheese lipolysis is based on the measurement of free fatty acid concentrations (which are an indication of the degree of lipolysis) at a given period during ripening. In the case of our study, free fatty acids were not separated from triacylglycerols, and fatty acid methyl esters (FAME) are therefore an indication of the mixture formed by free fatty acids and acylglycerols (Serhan, 2008).

Nine fatty acids were identified in the total lipids of cheese samples P_{72}, P_{72a} and P_{55a} at the start (D+2) and end (D+23) of ripening.

Saturated fatty acids make up the highest proportion of total fatty acids in the three cheeses studied, both at the start and end of ripening. They are represented by myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0) and stearic acid (C18:0), followed by monounsaturated fatty acids, the majority of which are oleic acid (C18:1), with palmitoleic acid (C16:1) present in smaller proportions.

Polyunsaturated fatty acids represent the lowest proportion (around 2.5% of total fatty acids) for the three cheeses studied. These are mainly fatty acids from the w3 and w6 series. These are (C18:2 w6), (C18:3 w3) and (C18:4 w3).

Figure 15 (a and b) shows the chromatographic profile of total fatty acids in P_{55a} cheese at D+2 and D+23.

Analysis of the acidic composition of the cheeses analyzed revealed that total fatty acid content (saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA)) showed no significant differences between the P_{72}, P_{72a} and P_{55a} cheese samples at D+2 and D+23. It can also be seen that over the ripening period the greatest (but statistically insignificant ($p>0.05$)) decrease in total fatty acid content was recorded for P_{72} cheese (from 95.8% on D+2 to 94.98% on D+23), followed by P_{55a} cheese (from 95.97% on D+2 to 95.18% on D+23) and the smallest decrease was for P_{72a} cheese (from 95.41% on D+2 to 94.95% on D+23).

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

The proportion of saturated fatty acids was similar for both P_{72} and P_{72a} samples, while the P55a cheese sample contained an average of 57.7% SFA during ripening.

In terms of MUFAs, the P_{55a} cheese sample contained the highest content: an average of 34.072%, compared with 28.348% for the P_{72} batch and 28.903% for P_{72a}.

The difference mainly concerns the C18:1w9 fatty acid, present in higher proportions in the P55a sample.

Variation in the proportion of polyunsaturated fatty acids is a marker of lipid oxidation (Gray et *al.*, 1992). The PUFA content of P_{55a} cheese varies from 3.815% on D+2 to 3.79% on D+23, i.e. a non-significant reduction of 2.5%, compared with 3.659% on D+2 to 3.347% on D+23 for P_{72}, i.e. a non-significant reduction of 0.314, and 3.512% on D+2 to 3.416 on D+23, i.e. a reduction of 0.096 for P_{72a} cheese. These variations concern the C18:4w3 fatty acid present in greater proportion in the P_{55a} sample.

In addition, the ratio ∑w-3/∑w-6 was calculated to estimate the degree of degradation of the fatty acids most susceptible to oxidation, as well as the nutritional value of the lipid fraction in cheeses. Under our conditions, this ratio is slightly but not significantly ($p>0.05$) higher for P_{55a} cheese. Lipid degradation in cheese during ripening is catalyzed by the milk's indigenous lipoprotein lipase LPL and by microbial lipases.

Many authors have reported that activation of the LP system in cow's milk inhibited lipoprotein lipase activity and reduced free fatty acid levels in milk. Thus, less lipolysis was observed in LPS-activated milk-based Gouda cheeses. These results are in line with those of Seifu et *al* (2004).

Table 5 Changes in Fatty Acid Content (%) During Ripening of Saint-Paulin Pressed Uncooked Cheese Samples

	P72		**P55a**		**P72a**	
	2	**23**	**2**	**23**	**2**	**23**
C14:0	15,029±0,283[a]	14,825±0,5[a]	12,065±0,168[b]	11,726±0,178[b]	15,348±0,17[a]	14,874±0,436[a]
C15:0	1,451±0,119[a]	1,363±0,169[a]	1,3375±0,006[a]	1,338±0,097[a]	1,429±0,014[a]	1,415±0,096[a]
C16:0	39,133±0,412[a]	37,907±1,194[b]	34,62±0,576[c]	35,255±1,121[c]	38,9435±0,633[a]	38,081±0,959[b]
C16:1 w7	2,998±0,511[a]	3,087±0,308[a]	3,2005±0,008[a]	2,929±0,093[a]	3,333±0,164[a]	2,955±0,12[a]
C18:0	8,18±0,225[a]	7,917±0,424[b]	9,451±0,093[a]	9,612±0,173[a]	7,415±0,144[b]	8,118±0,141[b]
C18:1 w9	25,35±1,266[a]	26,532±2,509[b]	31,482±0,893[c]	30,534±1,631[d]	25,4255±0,165[a]	26,092±1,877[b]
C18:2 w6	2,497±0,076[a]	2,3445±0,101[a]	2,3485±0,036[a]	2,367±0,085[a]	2,5215±0,006[a]	2,37±0,067[a]
C18:3 w3	0,5675±0,001[a]	0,4845±0,016[a]	0,7815±0,006[a]	0,755±0,037[a]	0,4795±0,002[a]	0,516±0,028[a]
C18:4 w3	0,5945±0,002[a]	0,5175±0,021[a]	0,6845±0,062[a]	0,668±0,052[a]	0,511±0,058[a]	0,53±0,028[a]
Total	95,8±2,04[a]	94,976±0,392[b]	95,969±0,03[a]	95,181±0,338[a]	95,406±0,622[a]	94,95±0,355[b]
∑ SFA	63,793±0,351[a]	62,01±2,287[b]	57,4725±0,842[c]	57,9295±1,212[c]	63,136±0,672[a]	62,488±1,632[b]
∑ MUFA	28,348±1,776[a]	29,619±2,817[b]	34,682±0,901[c]	33,462±1,724[d]	28,759±0,001[a]	29,047±1,998[b]
∑LCP	3,659±0,077[a]	3,3465±0,138[a]	3,8145±0,091[a]	3,7895±0,173[a]	3,512±0,049[a]	3,416±0,011[a]
∑LCP w-3	1,162±0,001[a]	1,002±0,037[a]	1,466±0,055[a]	1,4225±0,088[a]	0,991±0,056[a]	1,046±0,057[a]
∑LCP w-6	2,497±0,076[a]	2,3445±0,101[a]	2,3485±0,036[a]	2,367±0,085[a]	2,522±0,006[a]	2,37±0,067[a]
∑w-3/∑w-6	0,466±0,013[a]	0,427±0,003[a]	0,624±0,014[a]	0,601±0,016[a]	0,399±0,023[a]	0,442±0,036[a]
LCP/ SFA	0,056±0,22[a]	0,053±0,06[a]	0,065±0,108[a]	0,064±0,14[a]	0,056±0,07[a]	0,053±0,007[a]

[a, b, c, d,] mean values in the same row with different exponents are significantly different ($p>0.05$) ; C14:0 : Myristic acid; C15:0 : Pentadecanoic acid; C16:0 : Palmitic acid; C16:1 : Palmitoleic acid; C18:0 : Stearic acid; C18:1 w9 : Oleic acid; C18:2 w6 : Linoleic acid; C18:3 w3 : A-linolenic acid; C18:4 w3 : Stearidonic acid.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

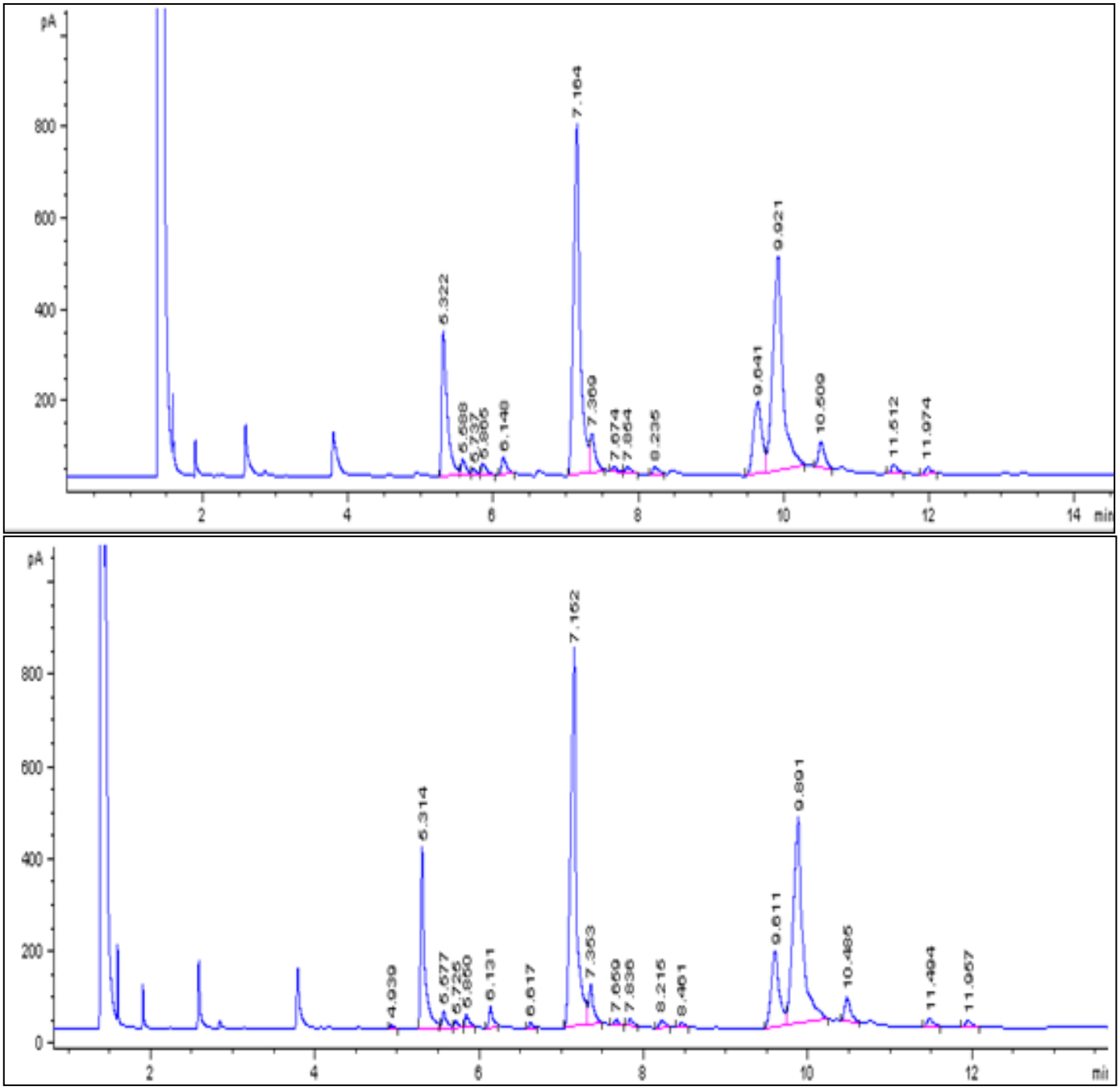

Fig 15 Chromatographic profile of Total Fatty Acids in a Sample of Saint-Paulin Uncooked Pressed Cheese Activated with LPS and Treated at 55°C/15s on Day 2 (D+2) (a) and at the End (D+23) of Ripening (b).

➢ *Statistical Study of Results*

The aim of this section is to analyze the relationships between the variables (proteolysis index, PUFA content and quantitative variations in the microbial population) and to identify the proximities between the cheese samples.

The first two factors of the PCA return around 98.48% of the total inertia, which is very satisfactory for interpreting the results. The first F1 axis accounts for most of the variability in the data, with 93.39% of information (**figure 16**).

Note also that the variables (the red dots on the figure) are well represented on the F1 axis, being very close to the edge of the correlation circle and to this axis. The individuals, here representing the cheese samples (blue dots on the figure) are also well placed on the F1 axis. As a result, the proximity in space between the samples reflects their real similarity (i.e. real proximity) in terms of the values taken by the variables.

It seems quite clear that, in this plane, cheese P_{55a} is quite close to cheese P_{72} and different from the others.

Consequently, we conclude that, from the point of view of proteolysis index, PUFA content and quantitative variations in the microbial population, P_{55a} and P_{72} cheeses are really close. This confirms the various findings made previously: lactoperoxidase activation implemented in chilled

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

milk treated at 55°C/15s differentiated, on the one hand, the microbiological quality, degree of proteolysis and lipolysis from those of P_{55} cheese, and gave, on the other hand, results equivalent to those of P_{72} cheese.

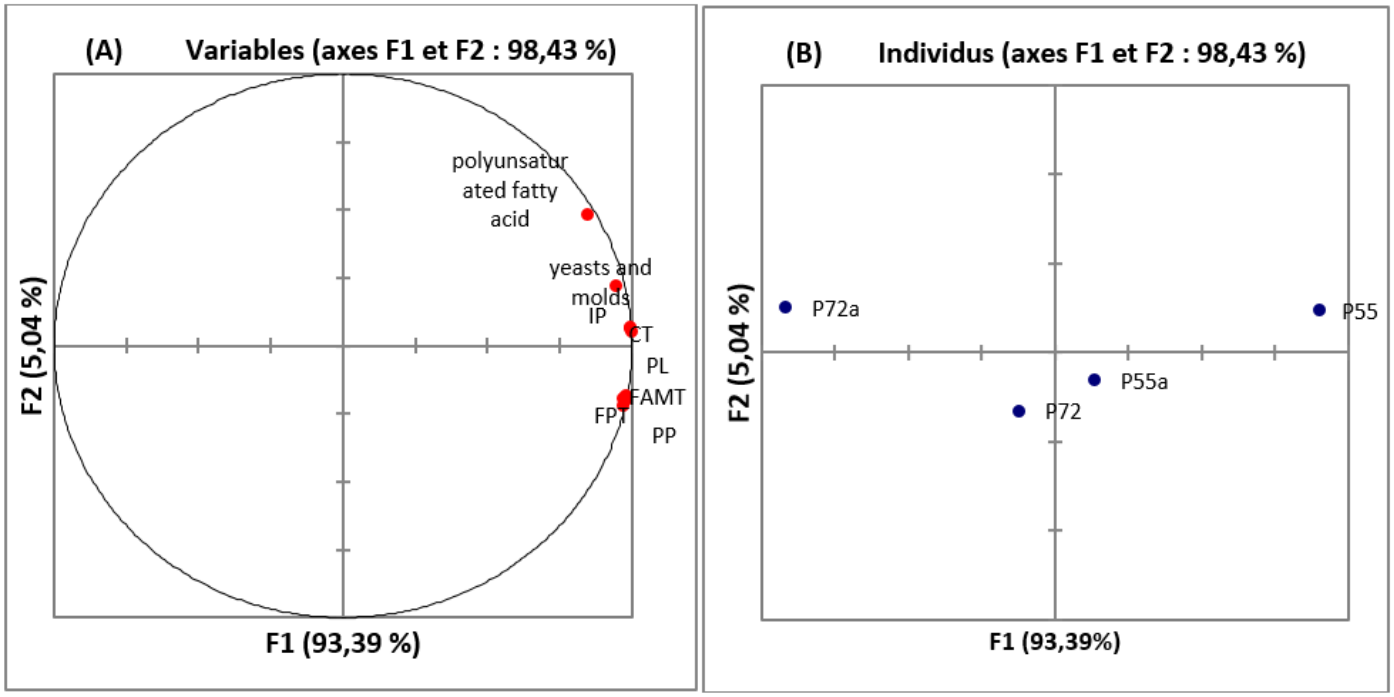

Fig 16 PCA Representation of Individuals (A) and Variables (B) for Saint-Paulin Pressed Uncooked Cheese Samples

➢ *Cheese Yields*

Cheese yield is the mathematical expression of the quantity of cheese obtained from a given quantity of milk.

The drop in cheese yield is a problem correlated with a number of factors, cooling being one of them. Indeed, keeping milk cold destabilizes its components by solubilizing calcium and proteins, which can then be evacuated into the whey, leading to a reduction in the micelle's mineral load and a sharp increase in soluble casein, the intensity of which increases with the duration of refrigeration. The development of psychotropic bacteria with proteolytic activity also irreversibly degrades proteins (Mankai et *al.*, 2003). Examination of data relating to changes in cheese yield as a function of activation of the lactoperoxidase system and heat treatment **(Table 6)**, reveals a gain of around 1kg in cheese yield per 100 liters of milk, with LPS activation in cheeses treated at 72°C/15s. Cheese made from milk treated at 55°C/15s after LPS activation showed a significantly higher gain of 2.41 Kg.

We can thus conclude that activation of the LPS system, which inhibits the proliferation of psychrotrophs responsible for casein degradation through the action of their proteases, improved cheese yield.

On the other hand, the increase in yield was more marked at 55°C, which is in line with the results found in the previous sections of increases in dry extract and the proportion of soluble nitrogen predominating.

Samples P_{55a} and P_{72}, which showed clear similarities in proteolysis index and proportion of soluble nitrogen, as well as in the evolution of dry extract over the ripening period, had the same cheese yields.

Table 6 Variation in Cheese Yield During Ripening of Saint-Paulin Uncooked Pressed Cheeses Studied

	Cheese samples			
	P_{72}	**P_{72a}**	**P_{55}**	**P_{55a}**
Cheese Yield (Kg/100l)	9,53	10,61	6,82	9,23

➢ *Results of Sensory Analysis of Cheeses*

In this section, we will use the results of the descriptive test with five-category scales to highlight the direct and indirect effects of heat treatment and activation of the lactoperoxidase system on the organoleptic quality of the cheeses studied.

Appreciation of the organoleptic properties of cheeses. The results of the assessment of organoleptic properties, which play a key role in the perception of cheeses, were evaluated by a panel of 30 people trained in accordance with Afnor standards.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

➢ *Taster Preference Analysis: Preference Mapping*

Figure 17 shows the plan determined by principal components 1 and 2, with the respective positioning of the four cheeses from productions P_{72}, P_{55}, P_{55a} and P_{72a}, and the six descriptive variables.

This plan contains 85.27% of the total information, which is quite satisfactory for interpreting the results. Principal Component 1 (the horizontal axis CP1) is determined towards its positive end (right-hand side of the graph) by the variables: acid taste, bitter taste and texture to the touch. CP1 is determined towards its negative end (left of graph) by the mouth texture variable, the opposite of the previous variables.

Principal Component 2 (the CP2 vertical axis) is determined towards its positive end (top of graph) by the aftertaste variable and towards its negative end (bottom of graph) by the yellow color intensity variable.

The map obtained, which is of fairly good quality since it represents 85.27% of the variability, shows that the cheeses were perceived by the tasters as quite different.

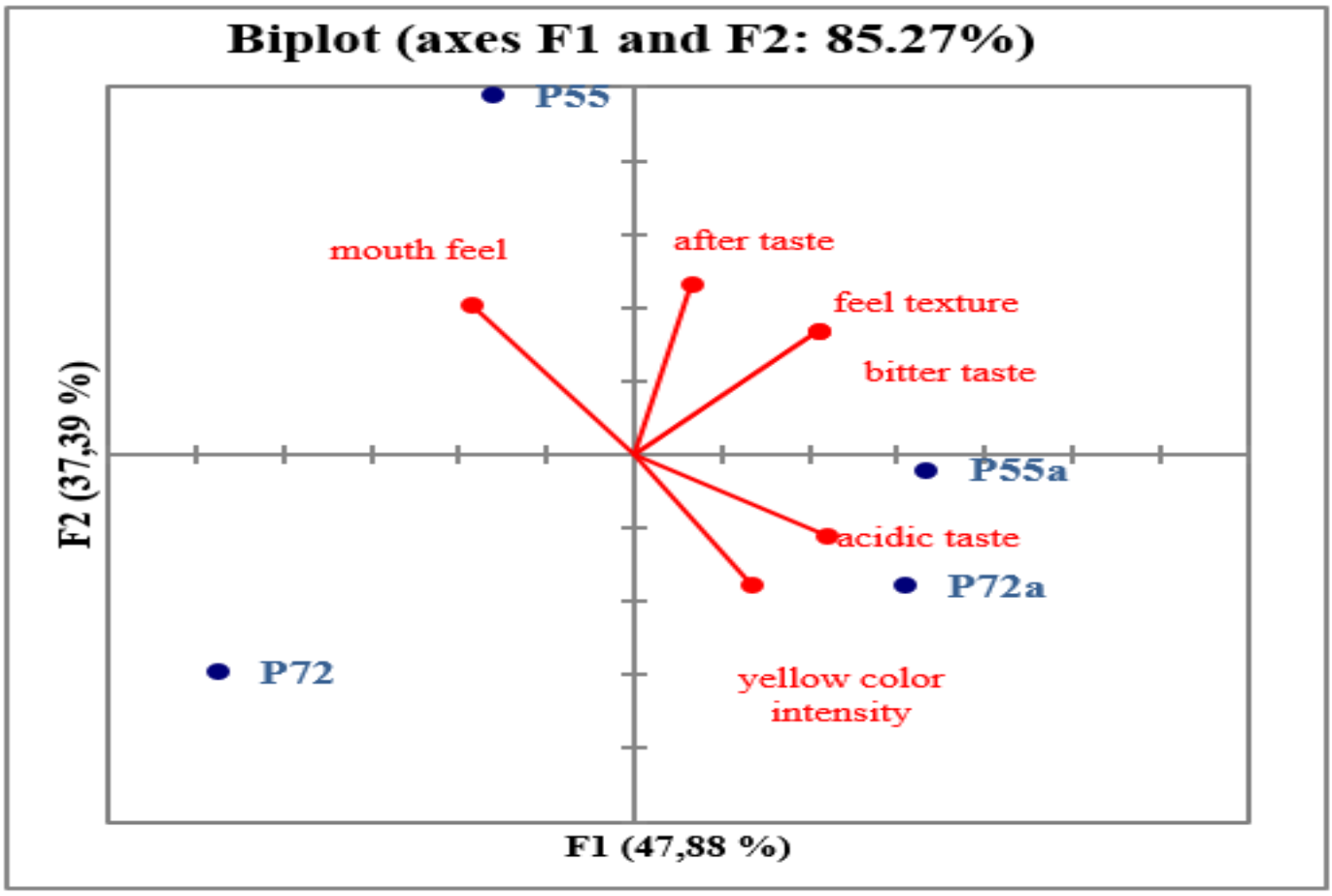

Fig 17 First factorial design of the Principal Component Analysis (PCA) of Saint-Paulin uncooked pressed cheeses.

The figure also shows that P_{55a} and P_{72a} cheeses are perceived as acidic and characterized by an intense yellow color. However, the P_{55} cheese stands out for its mouthfeel and aftertaste.

Finally, it would be interesting to relate tasters' preferences to the sensory characteristics of the cheeses and visualize their relationships.

To do this, we used preference mapping superimposed on a level map. Indeed, **Figure 18** below reflects the mathematical correlations between variables (in this case, tasters): the closer two variables are on the plane, the more they vary in the same direction (in other words, taster preferences are convergent), and conversely, the further apart they are on the plane, the more they evolve differently (in this case, taster preferences are divergent). The proximity on the graph of a taster point (the red dots in the figure) to a cheese point (the blue dots in the figure) indicates that this taster appreciated this cheese.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

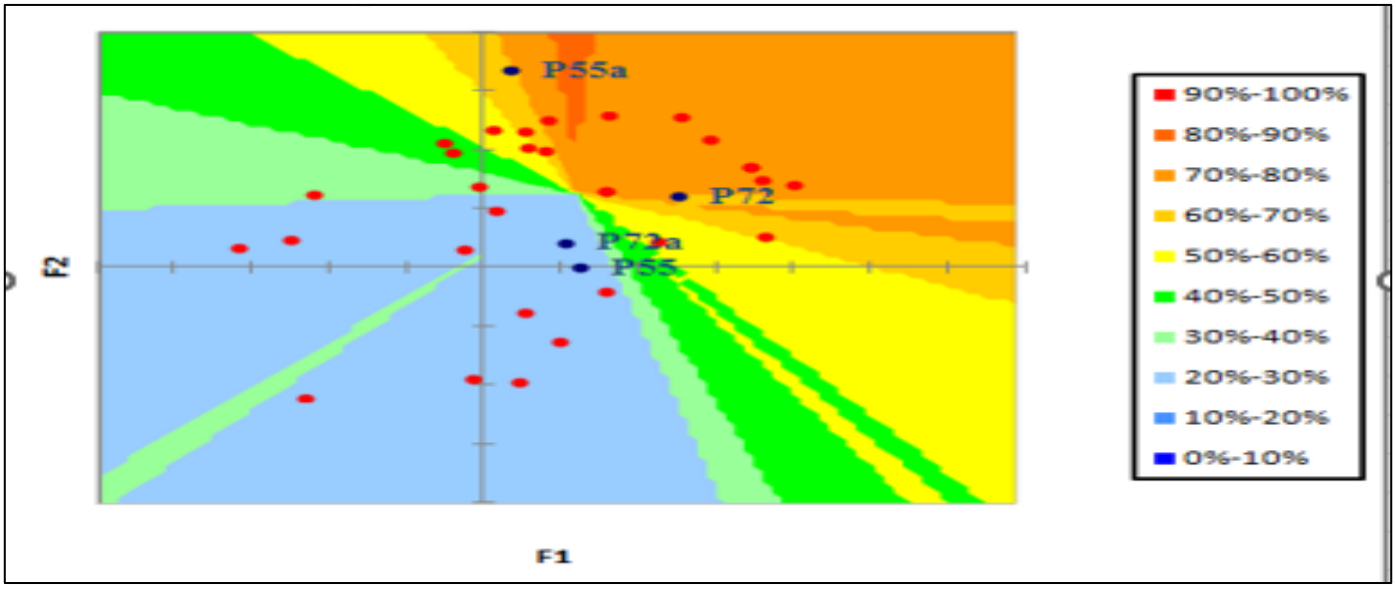

Fig 18 Preference Mapping and Contour Lines. Plan Determined by Principal Components 1 and 2

Largely attributable to the higher degrees of proteolysis in these cheeses compared to P72a and lower degrees compared to P_{55}. As a result, proteolysis contributed to the production of precursor amino acids for a multitude of catabolic reactions, resulting in a wide variety of volatile compounds (alcohols, aldehydes, acids) (Fox et *al*., 2004), thus contributing to the construction of the organoleptic properties of these cheeses.

Nevertheless, proteolysis indirectly affects texture through an increase in pH as a result of NH3 production following amino acid catabolism.

In addition, lipolysis, even if it does not occur to a significant extent, plays a part in shaping the flavours and organoleptic characteristics of these cheeses.

On the subject of the contribution of LPS activation to the organoleptic quality of milk and dairy products, results diverge. Ramet. (2004) found that activation of the LP system in raw milk did not alter its sensory properties compared with control milk. Seifu et *al*. (2005) claimed that the flavor of fermented milk and goat's cheese could be enhanced by the action of the LP system.

However, Ozer et *al*. (2003) reported some limited effects of LP system activation on the gel-like texture of yoghurt.

IV. CONCLUSIONS

This study was conducted to evaluate the effect of lactoperoxidase activation combined with sub-pasteurization treatment (55°C/15s) in cow's milk refrigerated at 4°C/72h on cheese quality.

The microbiological, physico-chemical, biochemical and sensory characteristics of a Saint-Paulin type uncooked pressed cheese were established. In parallel with this objective, the assessment focused on the ripening state of a Saint-Paulin made from pasteurized cow's milk during its ripening phase (23 days).

It was also carried out, on the one hand, by analyzing the physico-chemical parameters of the cheese through the various stages of ripening of the samples considered, and on the other hand, by qualitatively and quantitatively evaluating the proteolytic and lipolytic activity taking place during this key stage.

The work presented here therefore involved five main stages: 1) four productions of Saint-Paulin-type uncooked pressed cheeses made from chilled, non-LPS-activated and pasteurized cow's milk (P_{72}); chilled, LPS-activated and pasteurized (P_{72a}); refrigerated, non-LPS-activated and treated at 55°C/15s (P_{55}) and finally refrigerated, LPS-activated and treated at 55°C/15s (P_{55a}), 2) physico-chemical, proteolysis and lipolysis characterization, 3) monitoring of the microbiological quality of these products over the 23-day ripening period, 4) sensory evaluation, 5) assessment of the contributions of the treatments tested here, by comparing them with each other.

The analyses carried out on the initial product (cow's milk) highlighted the effect of activating the lactoperoxidase system in improving or preserving the qualities of cold-stored milk. Indeed, we have shown that, thanks to the activation of the lactoperoxidase system, moderate cooling (4°C) enables cow's milk to be stored and preserved in good hygienic condition for an industrially interesting period (72h).

With regard to monitoring during ripening, the results of microbiological and physico-chemical analyses depend on the duration of ripening and the treatment considered. Generally speaking, the results improved for LPS-activated samples, and were very similar for P_{55a} and P_{72} cheeses.

The biochemical study of ripening showed that the proteolytic activity of P_{72}, P_{72a} and P_{55a} cheeses, measured by the Kjeldhal method, the azocasein method and by

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

examination of the electrophoretic behavior of caseins in SDS-PAGE, was moderate compared with that of P55 cheese. It resulted in a non-significant increase in soluble nitrogen (SN) and non-protein nitrogen (NP) values during ripening. An increase in the proteolysis index was also discerned throughout the ripening phase, while remaining very similar for samples P_{72} and P_{55a}.

Monitoring of fatty acid lipolysis, measured by gas chromatography, revealed no significant differences between samples P_{72}, P_{72a} and P_{55a}. In fact, the differentiation in polyunsaturated fatty acid content between the various production batches was minor. The ratio ∑w-3/∑w-6, calculated to estimate the degree of degradation of the fatty acids most susceptible to oxidation, evolved fairly uniformly over the course of refining.

As for cheese yield, the LPS system remedied the drop due to refrigeration by the action of casein-degrading proteases, and revealed a minimization of losses for the P_{55a} sample. Finally, sensory evaluation showed a preference for P_{55a} and P_{72} cheeses.

The importance of this study lies in the fact that we were able to demonstrate, through a number of diagnostics and analyses, the synergistic effect of a “low-cost” heat treatment with activation of the lactoperoxidase system, giving results comparable to those found with ordinary pasteurization treatment. Hence the industrial interest in this combination.

As a follow-up to this work, the evaluation of this study can be further detailed by rheological examination and evolution of volatile compounds, and also by studying its effect on particular strains such as, for example, *E.coli* or *Listeria monocytogenes*.

ACKNOWLEDGMENT

We acknowledge that an earlier version of this manuscript was presented as a conference abstract at the IV. INTERNATIONAL AGRICULTURAL BIOLOGICAL & LIFE SCIENCE CONFERENCE AGBIOL 2022. The abstract can be found in the proceedings available at

[https://agbiol.congress.gen.tr/files/site/16/files/AGBIOL%202022%20FULL%20PROCEEDINGS%20BOOK.pdf].

➢ *Compliance with Ethical Standards*

- *Conflict of Interest*

The authors declare that they have no competing interests.

- *Funding*

The authors declare that there are no financial or non-financial interests that could be perceived as influencing the results or interpretations of this manuscript.

➢ *Author information*

- *Authors and Affiliations*

[1] Department of Food Technology, University of Carthage, Higher Institute of Food Industries of Tunisia, ESIAT,58, Street Alain Savary, 1003, Tunis, Tunisia

- *Contributions*

Malika Mankai wrote the main manuscript text of this document ; Maissa Dely contributed to the execution of certain experiments ; Hela Dhouioui contributed to the execution of certain experiments and the interpretation of the obtained results and Mnasser Hassouna reviewed the manuscript. All authors read and approved the final version of the manuscript.

- *Data Availability*

The datasets of the present study are available online from the corresponding author upon reasonable request.

REFERENCES

[1]. Abdou S., Dawood A., El-Nagar G.F. (1996) : utilization of H_2O_2 and the LP system treated milk in the manufacture of Domiati cheese. Egyptian Journal of Dairy Science.,24(1),127-140.

[2]. Atamer M., Koçak C., Çımer A., Odabası S., Tamuçay B., Yamaner N. (1999) : Some quality characteristics of Kaşar cheese manufactured from milk preserved by activation of lactoperoxidase/thiocyanate/hydrogen peroxide (LP) system. Milchwissenschaft,54(10), 553-556.

[3]. Bornert. G. (2000) : Importance des bactéries psychrotrophes en hygiène des denrées alimentaires, Service Central d'Études et de Réalisations du Commissariat de l'Armée de Terre, B.P. 309, 00447 Armées, Revue Méd. Vét., 151, 11, 1003-1010.

[4]. Boulares M., Mankai M., Hassouna M. (2010). Effect of thiocyanate and hydrogen peroxide on the keeping quality of ovine, bovine and caprine raw milk. International Journal of Dairy Technology 64(1),52 – 56.

[5]. Choisy C., Desmazeaud M., Gripon J.C., Lamberet G. , Lenoir J. (1997). La biochimie de l'affinage. Le fromage : De la science à l'assurance-qualité, 3ème éd., Edited by A.Eck and J.C.Gillis. Technique and documentation, Lavoisier, (Paris),87-160.

[6]. Feeney E.L., Lamichhane P. and Sheean J. (2021) : The cheese matrix: Understanding the impact of cheese structure on aspects of cardiovascular health – A food science and a human nutrition perspective .International Journal of Dairy Technology., 74(4).

[7]. Fox P.F. and McSweeney P.L.H. (2004). Cheese: Chemistry, Physics and Microbiology, chapter : cheese an overview,1-19,Eds : Fox P.F., McSweeney P.L.H., Cogan T.M. and Guinee T.P., Elsevier Academic Press, San Diego,564p.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/25apr2212

[8]. Gorostiza A., Cichoscki A.J., Valduga A.T., Valduga E., Bernardo A., Fresno J.M. (2004) : Changes in soluble nitrogenous compounds, caseins and free amino acids during ripening of artisanal prato cheese; a Brazilian semi-hard cows variety. J. Food Chemistry.,85(3), 407-414.

[9]. Gray J.I. and Monahan F.J. (1992) : Measurement of lipid oxidation in meat and meat products. Trends in Food Science and Technology., 3, 315-319.

[10]. Hassouna M., Mankaï M., Baccar M., Guizani N. (1999) : Evolution de la maturation d'un fromage industriel tunisien a pate pressée non cuite: caractéristiques microbiologiques, physico-chimiques et biochimiques. Rev.Ind. alim. Agri,116,33-43.

[11]. Mankaï M., Ben Moussa O., Barbana C., Dhouib I., Hassouna M., Alvarenga N.B. and Canada J. Study of antibacterial activity of lactococcus lactis against spoilage psychrotrophic bacteria isolated from refrigerated raw milk. J. Culture Collections, 6, 42-51.

[12]. Mankai M., Boulares M., Ben Moussa O., Karoui R. and Hassouna M. (2012) : The effect of refrigerated storage of raw milk on the physicochemical and microbiological quality of Tunisian semihard Gouda-type cheese during ripening. International Journal of Dairy Technology., 65(2),250-259.

[13]. Mankai M., Hassouna M. and Boudabous A. (2003) : Influence de la durée de réfrigération sur la microflore psychrotrophe, la protéolyse et la composition chimique et minérale du lait cru de collecte tunisien. Ind.Alim. et Agri.,120 (12),12-17.

[14]. Mouna B., Mankai M. and Hassouna M (2010) : Effect of activating lactoperoxidase system in cheese milk on the quality of Saint-Paulin cheese. Dairy technology.,64 (1), 75-83.

[15]. MC Sweeney P. L.H. and Sousa M.J. (2000) : Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Lait, 80, 293–324.

[16]. OECD/FAO (2020) OECD-FAO Agricultural Outlook 2020–2029. Paris/ FAO, Rome: OECD Publishing.

[17]. Ozer B., Grandison A., Robinson R. and Atamer M. (2003) : Effects of lactoperoxidase and hydrogen peroxide on rheological properties of yoghurt. J. Dairy Res., 70(2), 227-32.

[18]. Parry-Hanson A., Jooste P.J. and Buys E.M. (2009) : The influence of lactoperoxidase, heat and low pH on survival of acid-adapted and non-adapted *Escherichia coli* O157:H7 in goat milk. International Dairy Journal., 19(6–7), 417-421.

[19]. Ramet J.P. (2004): Influence of sodium thiocyanate and percarbonate on the freezing point and on the sensory properties of milk. Ecole Nationale Superieur Agronomie et des Industries Agro limentaire (ENSAIA), Nancy, France. Technical paper presented at the Fifth Global Lactoperoxidase Experts Meeting. Capetown, South Africa, Nov.

[20]. Rouch D.A., Roginski H., Britz M.L., Roupas P. (2008) : Determination of a nitrogen conversion factor for protein content in Cheddar cheese. International Dairy Journal., 18 (2), 216-220.

[21]. Seifu E., Donkin E.F. and Buys E.M.(2004) : Application of the lactoperoxidase system to improve the quality of goat milk cheese. International Dairy Journal, 14,581–589.

[22]. Seifu E., Donkin E.F. and Buys E.M.(2005) : Significance of the lactoperoxidase system in the dairy industry and its potential applications: A review. Trends in Food Science & Technology., 16(4),137-154.

[23]. Serhan M. (2008) : Valorisation durable des laits de chèvre de la région du Nord Liban. Transformation en fromage "Darfiyeh" et établissement de caractéristiques physico-chimiques et microbiologiques en vue de la création d'une appellation d'origine. Thèse de doctorat de Institut National Polytechnique de Lorraine, 199p.

[24]. Souza C.F.V., Dalla Rosa T. and Ayub M.A.Z. (2003) : Changes in the microbiological and physicochemical characteristics of Serrano cheese during manufacture and ripening. Braz. J. Microbiol., 34 (3),260-266.

[25]. Welthagen J.J. and Viljoen B.C. (1998) : Yeast profile in Gouda cheese during processing and ripening. Int J Food Microbiol., 41(3),185-194.

[26]. Welthagen J.J. and Viljoen B.C. (1998) : The isolation and identification of yeasts obtained during the manufacture and ripening of Cheddar cheese. Food Microbiology., 16(1), 63-73.

[27]. Zalazar C.A., Meinardi C.A., Reinheimer J. A. and Bernal S. (1987). Effect of cold storage of raw milk on the making of semi-hard cheese. Scienza e Tecnica Lattiero-Casearia,. 38(5), 415-431.