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Improving the quality of fresh artisan cheeses by encapsulating thyme essential oil with LPS activation

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Abstract
The aim of this study was to compare the effect of combining the encapsulation of essential oil with lactoperoxidase activation as a decontamination tool. Microbiological, physico-chemical (titratable acidity, pH, total dry extract, fat, fat-free dry extract, moisture and firmness) and organoleptic analyses were carried out during storage of the cheeses at +4±1ºC for 18 days, and the the shelf life was determined.
The use of encapsulation of essential oil with lactoperoxidase activation had a significant effect on microbiological quality compared with control cheeses and those activated by lactoperoxidase alone throughout the shelf life.
The combination of essential oil of thyme encapsulated with lactoperoxidase showed that on the first day, the different concentrations had a significant effect on the microbiological quality compared with the control and lactoperoxidase cheeses, but after nine days it was noted that only the HEt concentrations of 1.5 and 1ml/kg had a significant impact on the microbiological quality of the cheese compared with the other samples.
However, the activation of lactoperoxidase alone had no significant impact on the development of these germs.
With regard to sensory analysis, no descriptor had a significant effect detected by the panel throughout the shelf life.
On the other hand, these conservation combination enabled the shelf life to be extended. The control cheese had a use-by date of 3.63 days and the cheese preserved with lactoperoxidase only 3.09 days. On the other hand, the combination of thyme essential oil encapsulated with LPS increased the best-before date of the fresh cheese by 4.19, 8.01 and 10.40 days respectively for HEt concentrations of 0.5, 1 and 1.5 ml/kg with LPS activation.

Keywords: Microbiological quality, essential oil encapsulations, thyme, organoleptic quality

Introduction

Cheese is generally a nutrient-dense and well-tolerated fermented dairy product consumed worldwide. However, the health effects of cheese consumption remain a matter of controversy (Zhang *et al*., 2023) [30]
On one hand, cheese is a rich source of high-quality protein (mainly casein), lipids, minerals (e.g., calcium, phosphorus, and magnesium), and vitamins (e.g., vitamin A, K2, B2, B12, and folate), and probiotics and bioactive molecules (e.g., bioactive peptides, lactoferrin, short-chain fatty acids, and milk fat globule membrane), which may provide various health benefits (Zhang *et al.*, 2023) [30]
On the other hand, cheese has a fairly short shelf life due to its multiple varieties of micro-organisms such as yeasts, *Pseudomonas sp*, heterofermentative lactic bacteria, *Clostridia*, *Bacillus sp*, coliforms, *Klebsiella pneumoniae*, *Penicillium* and psychrophilic spoilage microflora (Zhu *et al.*, 2020) [31].
The application of heat treatments at very high temperatures can alter the composition of milk as it affects protein structures and water-soluble vitamins, resulting in a decrease in total fat and total solids and an increase in urea (Bezie, 2019) [5].
Alternative processes are therefore needed to solve this problem, mainly the use of combination technology (Peng *et al.*, 2015) [23].
At the crossroads of major economic, environmental and public health issues, food preservation techniques today deserve the mobilisation of all stakeholders to ensure that technologies evolve towards solutions that are more respectful of both the environment and the consumer (Gontard *et al.*, 2017) [10].
This makes the application of barrier technology very interesting as it can extend shelf life while preserving nutritional and sensory value. This technology works at lower temperatures and with shorter processing times, while ensuring microbiological safety (Predrag Putnik *et al.*, 2020) [24]. The combination technique involves preserving food using several agents/methods applied simultaneously or sequentially (Peleg, 2020) [22].
The combination of different technologies and/or the adjustment of a food's pH, water activity (aW), oxygen tension, etc., can be used to improve its preservation efficiency, which has been widely documented and has become common knowledge (Peleg, 2020) [22].
The latter is widely used in industrialised as well as developing countries for efficient food preservation (Peng *et al.*,2015) [23].
Combination technology is an important approach that can be used to improve quality parameters during food processing and storage. Intelligent application of barriers improves sensory characteristics, food chemical and microbiological qualities (Peng *et al.*, 2015) [23]. Moreover, more than 60 reported barriers are available, these can be used in different combinations and concentrations for a wide range of foods. This versatility makes the application of the technology possible in modern and local food processing (Peng *et al.*, 2015) [23].
A particular example is the use of lactoperoxidase, a natural enzyme considered to be an important element in the host's natural defence system against bacterial infection. Indeed, the LPO system can also be used to increase the stability of milk storage at high ambient temperatures (Zarei *et al.*, 2016) [28].

Non-thermal processes are implemented to inactivate spoilage micro-organisms and improve the nutritional, sensory and microbial characteristics of the product; a good opportunity that presents itself is the use of essential oils during cheese processing (Aprotosoaie *et al.*, 2010) [3].

The food industry uses essential oils to enhance the flavour and colour of foods. In addition, essential oils have different chemical composition profiles, enabling them to be used as natural food preservatives (Aprotosoaie *et al.*, 2010) [3].

Encapsulation is one of the techniques commonly used. It enables the volatile compounds in essential oils to be immobilised, stabilising the oil and protecting it from light, oxygen and temperature, as well as modulating its release by prolonging its kinetic profile (Kerdudo *et al.*, 2015) [12].

Consequently, this process tends to protect and preserve the biological activities of these oils. In addition, putting these virtues into action in the food product (Kerdudo *et al.*, 2015) [12].

The aim of encapsulation is to ensure the protection, compatibility and stabilisation of an active ingredient in a formulation. It can improve the presentation of a product or mask an odour or taste (Akdim, 2016) [2].

It can also modify and control the release profile of an active substance to obtain, for example, a prolonged or triggered effect (Akdim, 2016) [2].

Materials and methods

Harvesting and drying

The freshly harvested plant material (thyme) is cleaned of weeds, dried in the shade in an airy, dry place away from light and pollution, then cut into very fine pieces.

Extraction

Steam stripping

This is one of the official methods for obtaining EO. In this extraction system, the plant material is subjected to the action of a stream of steam without prior maceration. The vapours, saturated with volatile compounds, are condensed and then decanted into the essencier, before being separated into an aqueous phase (HA) and an organic phase (EO) (Nadjib *et al.*, 2019) [21].

The absence of direct contact between the water and the plant material, and then between the water and the aromatic molecules, avoids certain hydrolysis or degradation phenomena that could harm the quality of the oil. In addition, the fragrance of the EO obtained is more delicate and distillation, which is regular and faster, means that the top notes are rich in esters (Nadjib *et al.*, 2019) [21].

Moreover, according to the AFNOR standard (1986), the essential oil yield is defined as the ratio between the mass of the essential oil obtained and the mass of the plant material used. The yield expressed as a percentage is calculated by the following formula

$$Y(\%) = (W1 / W2) \times 100$$

Y: yield of essential oil ;
W1: weight of essential oil obtained in g;
W2: weight of thyme in g

Cheese manufacture

The cheese was produced from raw cow's milk. Subsequently, the standardized milk was pasteurized at 85°C/10 min, cooled to 32 C, and inoculated by adding 0.125 g/l of mesophilic starter culture (Barukcic *et al.*, 2020).

Fermentation (acid coagulation) of the milk was carried out at 30 C for about 16 h until curd was formed and pH of about 4.6 was reached (Barukcic *et al.*, 2020).

At this point, the fermentation process was interrupted by rapid cooling. The resulting curd was cut by a sterile knife and heated to a maximum of 40°C while continuing agitation to allow separation of the whey. Transferred to molds in cold storage (15-16°C) and drained (Barukcic *et al.*, 2020).

The cheese yield is calculated according to the following formula:

$$Y\% = \frac{Wc}{w} * 100$$

Y: means the cheese yield in %.
Wc: means the weight of cheese obtained in kilograms.
W: means the weight of the milk in kilograms.

LPS activation

The sample was subjected to refrigeration combined with 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 (Boulares *et al.*, 2011) [7].

After 1 min of thorough mixing of the milk, 30 mg ⁄L of sodium percarbonate ($2Na_2CO_3 3H_2O$) was added as a source of hydrogen peroxide (H_2O_2) as recommended by the International Dairy Federation (IDF1988).

Encapsulation of essential oils

Encapsulation is a potentially beneficial procedure for the protection and proper preservation of essential oils against degradation processes (oxidation and hydrolysis), as well as for stabilizing the release of high-value compounds extracted from fruits, vegetables, and waste products (Le Priol, 2021) [14].

The sodium alginate gel is poured very gradually to form the essential oil bead, and each bead remains in the calcium chloride solution for about 30min (Le Priol, 2021) [14]. After the recovery of the EO capsules, dry them by absorbent paper, store the capsules in cold (4°C) in sterile petri dishes (Le Priol, 2021) [14].

The samples were distributed as follows

Samples	Control	LPS	LPS+ 0,5 ml/kg	LPS+ 1 ml/kg	LPS+ 1,5 ml/kg

All the tests are spread over three periods: t_0, t_9 and t_{18}.

Physicochemical analysis

pH

The pH is measured with a pH meter (KDD002) to 0.01 units of accuracy.

For cheese, the electrode of the pH meter is placed directly in the cheese (Fedala *et al.*, 2020) [9]

Titratable acidity

This is an acid-base titration, lactic acid is neutralized by a solution of sodium hydroxide NaOH in the presence of phenolphthalein as a colored indicator ((Fedala *et al.*, 2020) [9].

For milk the acidity is expressed in degree Dornic (°D). The titration is performed by an alkaline solution (NaOH,

N/9) in the presence of phenolphthalein at 1% (w/v), (1 mL of NaOH (N/9) corresponds to 0.01 g of lactic acid per cent) (Fedala *et al.*, 2020) [9].

Determination of fat

The method is based on the acid-butyrometric method of Van Gulik. It is based on the dissociation of cheese proteins by the addition of sulfuric acid and separation of the fat by centrifugation in a Van Gulik butyrometer.

In a Van – Gulik butyrometer, put 3g of cheese, add sulfuric acid so that it covers the cheese mass, making the proteins dissociate in a water bath. After complete dissociation, fill the graduated rod with sulfuric acid and add 1 ml of isoamyl alcohol (Fedala *et al.*, 2020) [9]. The separation of fat is done by centrifugation (Fedala *et al.*, 2020) [9].

The fat expressed in g/100g of cheese is obtained by direct reading on the butyrometer scale (Fedala *et al.*, 2020) [9]

Determination of total dry extract

The total dry extract (TDE) is determined using an oven set at 103±2°C. The test samples are measured to the nearest 1 mg and the dry matter is expressed as a percentage by weight by the remainder after drying (Fedala *et al.*, 2020) [9]. Two grams of cheese are weighed after homogenization of the cheese paste, and the dry matter is expressed as weight percentage by the remainder after drying (Fedala *et al.*, 2020) [9].

$$TDE = E2 - MC / E1 \times 100$$

TDE: total dry extract
E1: weight in grams of the capsule and the test sample.
E2: weight in grams of the capsule and residue after drying and cooling.
Mc: weight in grams of the empty capsule.

Determination of the dry defatted extract

It is the result of the difference between the total dry extract and the fat content and
determined as follows:

$$ESD = TDE - MG$$

TDE: total dry extract
ESD: dry extract defatted
MG: Fatty matter

Measurement of moisture

The moisture is calculated by the following formula:

$$Moisture(\%) = 100 - TDE$$

Rheological study

Hardness (N), of cheese was determined by texturometer analyzer type TVT 6700. Cheese samples were cut into 5 * 5 cm cubes and stored at a room temperature before measurements (Barukcic *et al.*, 2020).

Then the prepared samples were subjected to double compression at a traverse speed of 1 mm/s and a penetration distance of 40 mm up and down with 10 s between the two cycles (Barukcic *et al.*,2020)

Sensory analysis

Once the production is finished, a hedonic test is carried out for the two cheeses. The purpose of this test is to compare the overall hedonic appreciation of the different cheeses by focusing on the individual feelings related to the pleasure or displeasure caused by the food (Fedala *et al.*, 2020) [9].

Consumer acceptance was determined using a 9-point scale. The results of the sensory analysis are represented by a radar graph. It allows to highlight the data around a reference value (Fedala *et al.*, 2020) [9].

Microbiological analysis

The cheese was aseptically removed from the package and the surface was cut with a sterile knife. The cheese slices were weighed into a sterile beaker with 100 ml of sterile distilled water. The cheese was grated and homogenized thoroughly (Fedala *et al.*, 2020) [9]

Serial dilutions of the homogenates were placed on appropriate media in Petri dishes and analyzed immediately (Fedala *et al.*, 2020) [9]. The numbers of total coliforms, total aerobic mesophilic flora, yeasts and molds were monitored on consecutive days during storage (Fedala *et al*,. 2020) [9]

The media and incubation conditions used were as follows:

Coliforms: spread on violet red bile agar (VRBA), with a double cover layer of the same medium, incubated at 30° C for 24 to 48 hours (Fedala *et al*,. 2020) [9]

Total aerobic mesophilic flora: spread on PCA (Plate Count Agar) and incubation is done at 30°C for 48 hours (Fedala *et al*,. 2020) [9]

Yeasts and molds: spread on Sabouraud dextrose agar, incubated at 25° C for 5 to 7 days (Fedala *et al*,. 2020) [9]

The determination of shelf life

The general equation that describes the loss of quality of a food is applicable for any factor A is as follows:

$$r = \frac{d[A]}{dt} = K[A]^n$$

r: rate of the degradation reaction = rate of formation of A ;
K: reaction rate constant or apparent rate ;
A: concentration of the factor to be followed;
n: order of the degradation reaction.

Statistical analysis

All experiments were repeated at least three times. The results were subjected to a one-factor analysis of variance with a significance level of 95% using Excel for the radar presented for the sensory analysis and by SPSS software using the one-factor ANOVA test.

Results and disscusion

Thyme essential oil extraction yield

According to the formula defined by the AFNOR standard (1986), we obtained a yield in OEt: Y= 1.96%.

Sahraoui *et al*, (2016) [25] have shown that the yield of essential oil obtained by steam stripping varies between 1.50% and 2.96%.

This variability in essential oil, both in terms of composition and yield between these plants, can be explained by various factors of intrinsic origin, specific to the plant's genetic or extrinsic, linked to the plant's growth and development conditions (Sahraoui *et al.*, 2016) [25].

We would also stress the importance of choosing the thyme harvesting period in order to obtain the quality and quantity of oil. Yields generally differ from one period to another (Sahraoui *et al.*, 2016) [25].

Cheese yield

Cheese yield is of great interest in the cheese industry because it reflects the overall quantitative distribution of milk constituents during draining (Vignola *et al.*, 2002) [27]. It requires a high level of dry extract and more specifically protein (casein) and high fat concentrations (Vignola *et al.*, 2002) [27].

According to our results, we obtained a Cheese yield Y= 10.7%.

Physicochemical analyses

Effect of Hurdle technology on pH

No significant difference ($P > 0.05$) in pH was observed between the control and the LPS-activated cheese. Seifu *et al*, (2004) [26] stated that activation of cheese by LPS had no effect on pH. Similarly, no significant difference in pH was observed between the controls and the cheeses activated by LPS throughout the ripening period (Boulares *et al.*, 2011) [7].

Table 1: Effect of thyme essential oil encapsulation with LPS activation on fresh cheese physicochemical parameters

	pH	Acidity (°D)	Fat (g/kg)	Total dry extract (g/kg)	Defatted dry extract (g/kg)	Moisture (%)
Control	t_0 : 4,58±0,01	t_0 : 14,53±0,06	t_0 : 30,50±0,26	t_0 : 35,73±0,21	t_0 : 5,23±0,23	t_0 : 64,27±0,21
	t_9 : 4,34±0,04	t_9 : 16,43±0,06	t_9 : 29,60±0,17	t_9 : 34,83±0,06	t_9 : 5,23±0,15	t_9 : 65,17±0,06
	t_{18} : 4,25±0,02	t_{18} : 17,17±0,12	t_{18} : 28,70±0,36	t_{18} : 34,20±0,17	t_{18} : 5,60±0,53	t_{18} : 65,90±0,00
LPS	t_0 : 4,56±0,01	t_0 : 14,77±0,06	t_0 : 30,80±0,1	t_0 : 35,17±0,25	t_0 : 4,33±0,25	t_0 : 64,83±0,25
	t_9 : 4,32±0,01	t_9 : 16,73±0,06	t_9 : 30,23±0,42	t_9 : 34,43±0,06	t_9 : 4,27±0,25	t_9 : 65,50±0,17
	t_{18} : 4,22±0,01	t_{18} : 17,57±0,12	t_{18} : 29,17±0,12	t_{18} : 33,90±0,1	t_{18} : 4,73±0,06	t_{18} : 66,10±0,1
LPS + 0,5ml/kg	t_0 : 4,53±0,01	t_0 : 15,53±0,12	t_0 : 31,27±0,21	t_0 : 36,10±0,2	t_0 4,83±0,15	t_0 : 63,90±0,2
	t_9 : 4,51±0,01	t_9 : 15,33±0,06	t_9 : 30,70±0,26	t_9 : 35,90±0,1	t_9 : 5,20±0,36	t_9 : 64,10±0,1
	t_{18} : 4,48±0,01	t_{18} : 15,67±0,12	t_{18} : 30,20±0,17	t_{18} : 35,37±0,38	t_{18} : 5,17±0,47	t_{18} : 64,63±0,38
LPS + 1 ml/kg	t_0 : 4,51±0,01	t_0 : 15,77±0,06	t_0 : 31,57±0,15	t_0 : 36,70±0,17	t_0 : 5,13±0,12	t_0 : 63,30±0,17
	t_9 : 4,50±0,01	t_9 : 15,73±0,06	t_9 : 30,77±0,49	t_9 : 35,93±0,06	t_9 : 5,17±0,47	t_9 : 64,07±0,06
	t_{18} : 4,47±0,01	t_{18} : 15,97±0,12	t_{18} : 29,87±0,21	t_{18} : 35,13±0,06	t_{18} : 5,27±0,23	t_{18} : 64,87±0,06
LPS + 1,5ml/kg	t_0 : 4,48±0,01	t_0 : 15,97±0,12	t_0 : 31,90±0,2	t_0 : 37,10±0,1	t_0 : 5,53±0,38	t_0 : 62,57±0,21
	t_9 : 4,46±0,01	t_9 : 15,93±0,15	t_9 : 31,60±0,1	t_9 : 36,77±0,15	t_9 : 5,17±0,06	t_9 : 63,23±0,15
	t_{18} : 4,45±0,01	t_{18} : 16,33±0,12	t_{18} : 31,03±0,38	t_{18} : 36,30±0,17	t_{18} : 5,27±0,47	t_{18} : 63,70±0,17

Table 1 shows the effect of the pH of the cheese at t_0, t_9 and t_{18} of storage at 4±1°C after a combination of treatment with the incorporation of the encapsulated essential oil at different concentrations with 0.5, 1 and 1.5 ml /kg and the activation of LPS compared with a control and a sample activated only with LPS.

In fact, the average pH of the control was 4.58 ± 0.01 at t_0 and decreased over time to reach a value of 4.25 ± 0.01 at the end of storage.

The pH values were found to be proportional to the high concentrations of essential oil added to the cheeses, decreases from 4.54 ± 0.01 to 4.51 ± 0.01 and 4.48 ± 0.01 for the doses of 0.5, 1 and 1.5ml/kg added respectively to the products at t_0.

Following the addition of the essential oils combined with LPS, we recorded a significant decrease in pH compared with the control and those activated by LPS can be attributed to the inhibition of the microbial flora and in particular the lactic acid bacteria responsible for the acidification of the cheese.

On the other hand, at t_9 and t_{18}, the different combinations of essential oil and LPS had no significant effect on the pH of the cheese, which resulted in pH stability.

Messouadi *et al*, (2019) found that the combination of LPS and essential oil preserved the pH of raw cow's milk during storage.

This allows the use of this combination technique to be prioritised since the decrease in pH in foods could adversely affect sensory quality, resulting from metabolic degradation by microorganisms, particularly in carbohydrate-rich foods, which can be used by microorganisms to produce acids (Cheon *et al.*, 2016).

Effect of Hurdle technology on acidity

The acidity developed in cheese results from the transformation of lactose into lactic acid. It is measured by titration (Gadi *et al.*, 2020). The low titratable acidity values

recorded in our results reflect weak lactic fermentation in the cheese samples, which depends on the casein, mineral salt and ion content. It also depends on the hygienic conditions during milking, the total microbial flora and its metabolic activity and the handling of the milk (Gadi *et al*., 2020).

Table 1 shows the effect of cheese acidity at t_0, t_9 and t_{18} of storage at 4±1°C after a combination of treatment with the incorporation of encapsulated essential oil at different concentrations of 0.5, 1 and 1.5 ml/kg and LPS activation compared with a control and a sample activated only with LPS.

Also, these values increase significantly with the doses of Thyme incorporated into the products by encapsulation compared to that of the control and that activated only by LPS.

These values increase from 14.53 to 14.77 to 15.53 to 15.77 and to 15.97°D on average for Thyme concentrations varying from 0, LPS, 0.5; 1; 1.5ml/kg respectively, in the cheeses.

Lactic acid bacteria ferment lactose and acidify milk through the massive production of lactic acid (Leksir, 2018). The growth of lactic acid bacteria in the milk, then in the curd, leads to the consumption of lactose and the excretion of lactic acid, lowering the pH. This acidifying function of lactic acid bacteria is decisive in the cheese-making process (Leksir, 2018).

The increase in titratable acidity is mainly due to proteolysis and the decarboxylation of amino acids released by microorganisms accumulated during product preservation (Leksir, 2018).

Usually, pH and acidity are inversely proportional. It is suggested that this proportionality is due to the activity of the lactic flora, which is fairly high, or that the environment does not allow the development of lactic acid degradation flora in large numbers (Leksir, 2018).

On the other hand, any increase in acidity favours the solubilisation of minerals and the destabilisation of casein micelles, leading to excessive losses in the whey during processing, which has a direct influence on the quality of our cheeses (Leksir, 2018).

Effect of Hurdle technology on total dry extract

The level of dry extract varies from one type of cheese to another. This difference is due to the use of salt and the draining time. The cheese's high dry matter content gives it a relatively firm consistency.

Table 1 illustrates the variation in the total dry extract content of cheese treated with different concentrations of essential oil combined with LPS as well as cheeses (control and only LPS-activated) during 18 days of refrigerated storage at 4 ± 1°C and tested on days 0, 9 and 18.

Throughout the storage period, there was a significant increase in the total dry extract of the control and LPS-activated cheeses compared to the cheeses that had undergone the hurdle treatment.

This result is in agreement with that of Messaour (2018) [19]. The total dry extract is around 43.26% for the 0.1%, 45.94% for the 0.5% and 48.86% for the 1%, this small difference is probably due to the addition of the essential oil.

It can be seen that the loss of total dry extract increases over time. In fact, the control cheeses had an average total dry extract of 35.73 g/kg, falling to 34.2 g/kg after 18 days at 4±1°C.

Similarly, for the cheese that had only undergone LPS n activation, the average dry extract decreased from 35.17 g/kg at t_0 to 33.9 g/kg at t_{18}.

On the other hand, the total dry extract increased slightly for the cheeses treated with the hurdle technique compared with the controls and those activated by LPS.

In fact, the decrease in total dry extract over time is due to the liquefaction of the product Messaour (2018) [19].

Effect of Hurdle technology on fat content

Fat content and dry matter are very important in cheese production because milk lipids are characterised by the presence of relatively short-chain fatty acids which can be absorbed by a simpler mechanism than long-chain fatty acids. Milk fat plays an essential role in the development of both the taste and texture of cheese (Vignola, 2002) [27].

Cheese texture depends on its fat content. In fact, the water content and the proportions of long polysaturated fatty acids in the milk determine the texture of the dough: extra hard, semi-soft, soft, and so on. For example, with more than 60% fat and less than 51% water, you get an extra-hard cheese. Too high a fat content can lead to problems with draining and coagulation (Vignola, 2002) [27].

Table 1 shows that the control cheese samples had a fat content of 30.5 g/kg and that activated by LPS 30.8 g/kg, while the cheese treated with the combination of activated LPS essential oil had an average of 31.27 g/kg, 31.57 g/kg and 32.03 g/kg respectively.

This technique had a significant effect on fat content, with a significant increase in the cheese with the highest concentration combined with LPS compared with all the other samples at t_0, t_9 and t_{18}.

This was approved by Messouadi *et al*, (2019) showing that the activation of the LPS system combined with the addition of juniper and Phoenicia essential oils have a significant effect on the variation of the fat content of cow's milk.

On the other hand, LPS activation had no significant effect on the variation in fat content compared with the controls.

Similarly, the other two concentrations 0.5 and 1ml/kg combined with LPS have no significant effect compared with the controls and those activated by LPS throughout the shelf life.

According to Bellivier *et al*, (2000) [6], the lipid content of milk intended for cheese production largely determines the fat content of the finished product.

In fact, the fat content of cheese depends solely on the nature and initial composition of the milk used to make it.

According to Morgan el *al* (2001) [20], cheese fats are a good source of energy and are generally easy to digest (88% to 94%).

Effect of Hurdle technology on defatted dry extract

Table 1 shows the variation in fat-free dry extract of treated and untreated cheeses stored at 4±1°C. It can be seen that the defatted dry extract values increased proportionately, rising from 4.83, 5.13 and 5.53 g/kg at t_0 and remaining non-significant.

On the other hand, we note that all the samples that underwent the hurdle combination had a significant effect compared to the cheeses activated only by LPS at t_9, and we note that the combination with the 1.5ml/kg concentration with LPS had a significant effect compared to the control cheeses.

This result is in agreement with that of Messouadi *et al*, (2019) showing that the activation of the LPS system combined with the addition of the essential oils of juniper oxycèdre and Phénicie has a significant effect on the variation of the defatted dry extract of cow's milk.

Effect of Hurdle technology on humidity

For the moisture parameter, if the values are outside the standards, there is a risk of having a fairly hard dough (if the moisture content is less than 58%), or a fairly moist, and therefore fragile, dough if the moisture content% is greater than 60%. The TSE content is linked to the moisture content%, so outside the range given in table (40-42), there is a risk of varying the moisture content% and therefore ultimately contributing to the hardness or brittleness of the dough.

Table 1 shows that the moisture content of cheese samples with encapsulated thyme essetille oil was significantly lower than that of control samples and LPS-activated cheeses at t0. Indeed for the concentration of 1.5ml/kg combined with LPS, there was a significant difference compared to all other samples, as well as for the cheese with encapsulated essential oil concentration 1.5ml/kg with LPS, there was a significant effect of moisture compared to control cheeses and those only with LPS throughout the shelf life. The difference in moisture content may be due to the water composition of the milk.

Rheological study

Texture is an essential factor in consumer acceptance of a product. The term 'firmness' is commonly used to describe a parameter assessed by means of empirical mechanical tests and considered as an attribute that must be maintained during storage and processing.

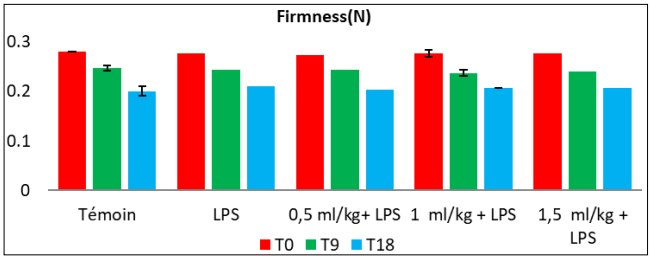

Fig 1: Effect of encapsulated thyme essential oil incorporation on fresh cheese firmness (Control ; LPS ; 0.5ml/kg +LPS ; 1 ml/kg +LPS ; 1.5ml/kg+LPS)

Figure 1 shows the variation in the firmness of treated and untreated cheeses stored at 4±1°C. It can be seen that the average firmness value of the cheeses decreases directly following over time.

In fact, the firmness values of control cheeses fell from 0.28 to 0.2 N after 18 days of refrigerated storage.

For cheeses activated only by LPS, firmness fell from 0.277 to 0.21 N after 18 days of refrigerated storage.

The incorporation of the essential oil with LPS had no significant effect on firmness compared with the controls and those activated by LPS.

Microbiological quality

The results of the evolution of the microbial flora of the cheeses at (t_0) and after 9 and 18 days of storage at 4±1°C.

The germs sought and counted in our work are considered to be indicators of the overall quality of the finished product and reflect compliance or non-compliance with good hygiene practices (Gadi *et al.*, 2020).

Total coliforms

Figure 2 shows a total absence of total coliforms directly after treatment (t_0) with all doses combined with LPS. Even with LPS activation alone, there was a reduction in proliferation compared with the control.

In fact, at t_0, there was a significant decrease in the evolution of total coliforms with all the concentrions of essential oils combined with LPS compared with the control cheeses and those activated by LPS. On the other hand, LPS activation significantly reduced the evolution of total coliforms.

On the other hand, at t_9, there was a significant decrease in the evolution of total coliforms in the cheese with the highest concentration of essential oils (1.5ml/kg) combined with LPS compared with the control and activated by LPS only, and the other two samples.

There was also a significant decrease in the evolution of total coliforms in the cheese with an essential oil concentration of 1ml/kg combined with LPS compared to the control. The same results were obtained at t_{18} as at t_9.

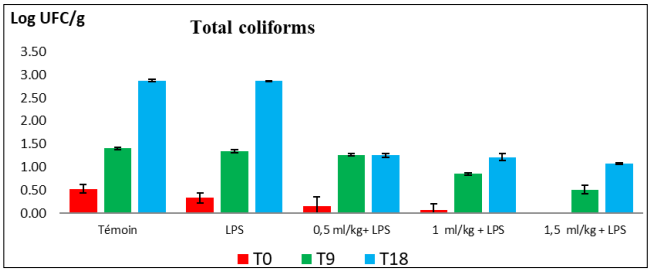

Fig 2: Effect of encapsulated thyme essential oil incorporation on total coliforms in fresh cheese (Control; LPS; 0.5ml/kg +LPS; 1 ml/kg +LPS; 1.5ml/kg+LPS)

Mesophilic aerobes

The number of total aerobic mesophilic flora in cheese can cause premature swelling and the production of enterotoxins in cheese (Seifu *et al.*, 2004) [26].

Figure 2 shows the effect of the incorporation of encapsulated thyme essential oil combined with LPS on the total mesophilic aerobes of fresh cheese compared with control cheese and cheese activated only by LPS refrigerated at 4 ± 1°C and tested on different days 0, 9 and 18.

The control cheese initially had 3.0 log CFU/g, while the LPS-only activated cheese showed a slight decrease to 2.93 log CFU/g.

The reduction in this flora in cheeses made from milk preserved by activation of the LPS system suggests that activation of this system in the milk before cheese manufacture could be of great practical importance (Seifu *et al.*, 2004) [26].

Moreover, Seifu *et al*, (2004) [26] found that preservation of cheese milk by the LPS system can be used to improve the microbiological quality and flavour of cheese.

At t_9 and t_{18}, there was a significant difference in all the samples. Moreover, the two other combinations resulted in a significant reduction in mesophilic aerobes at t_0.

There was a total absence of mesophilic aerobes for the LPS combination with the highest dose of 1.5ml/kg, which may be due to the high concentration of thyme essential oil applied.

The combination of LPS with the highest dose (1.5ml/kg) at t_0 resulted in a significant decrease compared with the control cheeses and those activated by LPS and those treated with a dose of 0.5ml/kg combined with LPS.

During the 9th day of storage, we recorded a sudden increase in the concentration of aerobic mesophilic bacteria in the cheese preserved by the addition of 1.5ml/kg of HEt. This can probably be explained by the fact that a chemical reaction between the proteins and the functional groups of the essential oils reduced the availability of the active molecules (Malecky *et al.*, 2007) [15].

From the 18th day, an increase in the number of mesophilic aerobes was observed in all cheeses. This increase was significantly less for cheeses activated by LPS combined with thyme essential oil. We conclude that incorporating 1.5ml/kg of thyme essential oil with LPS into the cheese has a significant effect on the development of mesophilic aerobes.

Our result is similar to that of Guiraud (2003) [11] who demonstrated that the incorporation of thyme essential oil at different concentrations (1 and 1.5ml/kg) showed that only at the highest concentration 1.5 ml/kg was there a significant effect on the inhibition of the development of mesophilic aerobes.

This highlights the importance of encapsulating thyme essential oil and combining it with LPS, since a significant reduction in the development of mesophilic aerobes was observed at different essential oil concentrations (0.5, 1 and 1.5 ml/kg) with LPS.

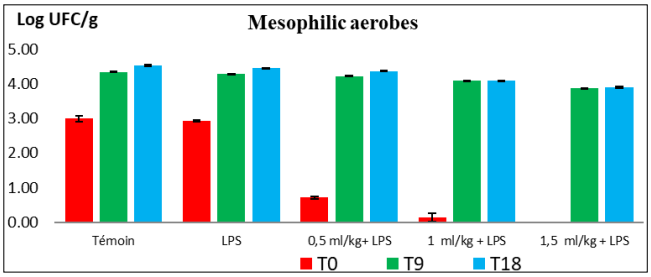

Fig 3: Effect of encapsulated thyme essential oil incorporation on total mesophilic aerobes of fresh cheese (Control; LPS; 0.5ml/kg +LPS; 1 ml/kg +LPS; 1.5ml/kg+LPS)

Yeasts and moulds

Yeasts and moulds in cheese are considered to be spoilage organisms leading to flavour and texture deterioration, including softening and discolouration (Aly *et al.*, 2012).

For yeasts and moulds, the trend was similar to that for mesophilic aerobes.

There was a significant reduction in the microbial load following the combination of encapsulated essential oil at different concentrations with LPS at t_0.

In fact, the lowest concentration (0.5ml/kg) and the average concentration (1ml/kg) combined with LPS showed average yeast and mould values of 1.8 and 1.37 log CFU/g respectively, compared with the control, which was initially at 2.34 log CFU/g and 2.31 log CFU/g for those only activated by LPS. The levels were even lower with the OEt concentration of 1.5 ml/kg combined with LPS (0.85 log CFU/g).

However, no significant difference was recorded in the evolution of yeasts and moulds in the LPS-activated cheese compared with the control.

In fact, the addition of thyme essential oil to fresh cheese had a significant effect on yeasts and moulds for the two doses incorporated (1 and 1.5ml/kg) with LPS, with a more effective effect for the 1.5ml/kg dose at t_0 and t_{18}.

Our results are in agreement with Guiraud (2003) [11] who showed that the incorporation of thyme essential oil at 1ml /kg and 1.5 ml/kg have a significant effect on the development of yeasts and moulds.

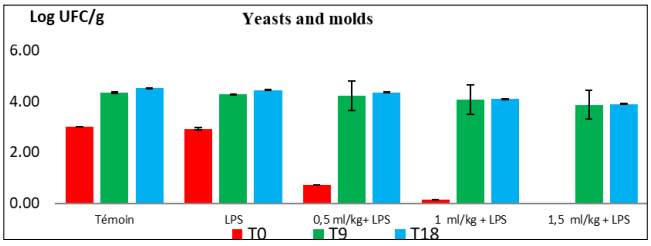

Fig 4: Effect of the incorporation of encapsulated thyme essential oil on yeasts and molds in fresh cheese (Control; LPS; 0.5ml/kg +LPS; 1 ml/kg +LPS; 1.5ml/kg+LPS)

Sensory analysis

The sensory analysis, hedonic test, was carried out directly after treatment at t_0, t_9 and t_{18} days of storage at 4°C on control cheeses, activated by LPS and cheeses with different concentrations of encapsulated essential oils (0.5, 1 and 1.5ml/kg) combined with LPS. We chose to carry out these analyses throughout the 18-day storage period on the basis of the cheese quality assessed.

The characterisation of the sensory properties of the cheeses was analysed using the radar diagram constructed with the scores obtained for the different parameters evaluated by the panellists.

On the basis of the deterioration index reported, if the overall acceptability score was less than 5, the product would be considered a putrid food (Lainez *et al.*, 2008) [13].

The highest score for aroma was given to cheeses with an essential oil concentration of 0.5 ml/kg with LPS, with an average of 5.58 out of 9 at t0, which decreased over time to an average of 5.25 at t_9 and an average of 4.33 at t_{18}. On the other hand, the lowest score was awarded to the cheese with the highest concentration of essential oil in combination with LPS, which totalled 4.75 out of 9 and fell over time to 3.75 at t_{18}.

For the other descriptors, such as salty taste and acid taste, the highest scores were attributed to the cheeses with the lowest concentration of essential oil combined with LPS, which scored 5, 33 and 5.25 out of 9 respectively at t_0 and decreased over time to values of 4.58 and 3.58 over 18 days of storage.

For the other descriptors, neither colour nor odour had a significant effect on all the cheese samples. On the other hand, time had a significant effect throughout the shelf life.

For the descriptor "texture", the score attributed by the panel with all the concentrations combined with LPS did not have a significant effect ($p>0.05$) compared with the control and the cheese activated only by LPS. This is in agreement with our result found in the paragraph studying the rheology of the cheeses, in which we found that the incorporation of essential oils with LPS had no significant effect on the texture of the cheeses.

However, the panel found that the texture deteriorated significantly throughout the shelf life. Similarly for overall acceptability, there was no significant difference between all the samples. However, shelf life had a significant effect on overall acceptability at t_{18}, whereas there was no significant difference between t_0 and t_9. On the other hand, overall acceptability was strongly correlated with colour, odour and texture ($p<0.01$).

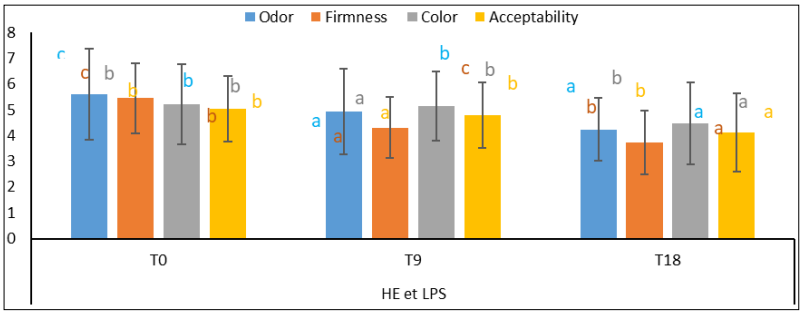

Fig 5: Effect of incorporating encapsulated thyme essential oil on the organoleptic properties of fresh cheese

Effect of hurdle technologies on the evolution of the the shelf life of cheese

Estimation of the use-by date using the accelerated aging test

The spoilage kinetics of a foodstuff is a representation of the deterioration of a parameter A as a function of time and temperature. These kinetics are generally of order zero or of order one.

The determination of the order of the spoilage reaction is obtained by comparing the coefficient of determination R2 of the linear regression of the three kinetic models related to a quality criterion A by drawing the graphs

Order zero: A = f(t)

Order 1: Ln (A) = f(t)

Order 2: (1/H) = f(t)

Determination of the use-by date by monitoring the evolution of yeasts and molds,indeed the microbial limit of acceptability was estimated by fitting the experimental data to the Gompertz equation modified by Corbo (Zantar *et al.*, 2013) [29]

A concentration ≥ 105 CFU / g of yeasts and molds marks the end of the useful life of fresh cheese. This level of contamination corresponds to the appearance of defects, abnormal colors and odors (Zantar *et al.*, 2013) [29]

We observed that both combinations prolonged the shelf life of fresh cheese compared with control cheeses and those activated only by LPS.

In contrast, Zantar *et al,* (2013) [29] showed that the addition of bulk thyme (0.5 or 1 ml/kg) to fresh goat cheese did not have a significant effect on extending the shelf life of fresh cheese. On the other hand, we recorded an increase in the use-by date of 4.19, 8.01 and 10.40 days for the different concentrations of thyme encapsulated with LPS at 0.5, 1 and 1.5 ml/kg respectively. This highlighted the importance of thyme essential oil encapsulation and the synergistic effect with the activation of the LPS system in the raw milk.

Conclusion

Today, one of the main challenges facing industry and the economy is to meet the needs of a growing world population while preserving the environment.

The aim of the agri-food industries is to introduce a rational use of renewable raw materials that exploit the complementary nature of the food and non-food sectors. This study was targeting the industrial market in order to encourage it and to replace the conventional techniques with more ecological, innovative and sustainable ones.

We were able to demonstrate that the storage combination had no significant effect on texture throughout the duration of refrigerated storage. On the other hand, these combination had a significant impactt on all the physico-chemical parameters (pH, titratable acidity, total dry extract, fat, defatted dry extract and moisture) throughout the shelf life.

On the other hand, the panel did not detect any significant effect on the descriptors (colour, odour, texture and overall acceptability) for the two combinations throughout the shelf life.

In addition, the determination of the shelf life showed that the use of these combination resulted in its extension of by 3.63 days, compared with 3.09 days for the LPS cheese alone. On the other hand, the combination of thyme essential oil encapsulated with LPS gave different results depending on the concentration. The dose of 0.5 ml/kg with activation of the lactoperoxidase system resulted in a shelf life of 4.19 days, compared with 8.01 and 10.40 days respectively for 1 and 1.5 ml/kg with LPS.

References

1. Agherghour N, Khemis L. Essai de fabrication d'un camembert au thym et étude de son effet sur la durée de vie du produit, 2015.
2. Akdim L. Comparaison de méthodes d'absorption et d'encapsulation de l'huile essentielle de Copaifera Officinalis L. en vue d'une application en cosmétique, 2016.
3. Aprotosoaie AC, Spac AD, Hancianu M, Miron A, Tanasescu VF, Dorneanu V, *et al.* The chemical profile of essential oils obtained from fennel fruits (Foeniculum vulgare Mill.). FARMACIA,2010:58(1):46-54.
4. Barukcˇic I, Mario S, Marasovic´ I, Katarina LJ, Kata G, Rajka B. Evaluation of quality parameters and shelf life of fresh cheese packed under modified atmosphere. Food Sci Technol, 2020.
5. Bezie A. The Effect of Different Heat Treatment on the Nutritional Value of Milk and Milk Products and Shelf-Life of Milk Products. A Review. Dairy and Veterinary Science,2019:11:2196-2573.
6. Bellivier AC, Gaborit P. Lipolyse naturelle du lait de chèvre et qualité organoleptique des fromages. Renc Rech Rumin,2000:7:315-319.
7. Boulares Mouna, Mankai Melika, Hassouna Mnasser. Effect of activating lactoperoxidase system in cheese

milk on the quality of Saint-Paulin cheese. International Journal of Dairy Technology, 2011, 64.
8. Emma L, Feeney, Anne P, Nugent, Breige Mc Nulty, Janette Walton, *et al.* An overview of the contribution of dairy and cheese intakes to nutrient intakes in the Irish diet: results from the National Adult Nutrition Survey. British Journal of Nutrition,2016:115:709–717.
9. Fedala N, Mokhtari M, Mekimene L. Contribution à la valorisation des dattes (DEGLET-NOUR) dans la fabrication du fromage de chèvre. Revue Agrobiologia,2020:10:1918-1928.
10. Gontard N, Guillard V, Gaucel S, Guillaume C. L'emballage alimentaire et l'innovation écologique dans toutes leurs dimensions. Innovations Agronomiques,2017:58:1-9.
11. Guiraud JP. Microbiologie alimentaire. Ed. Paris : DUNOD,2003:651:653.
12. Kerdudo A, Antipolis NS, Directeur C. Optimisation de la conservation des cosmetiques : impact de la formulation, recherche de nouveaux conservateurs naturels, encapsulation, 2015.
13. Lainez E, Vergara F, Bárcenas ME. Quality and microbial stability of partially baked bread during refrigerated storage (2008). Food Engineering,2015:89:414–418.
14. Le Priol L, Étude de la co-encapsulation d'huiles alimentaires oxydables et d'antioxydants phénoliques, 2021.
15. Malecky M. Metabolisme des terpenoides chez les caprins. Thèse de doctorat. Institut des Sciences et Industries du Vivant et de l'Environnement (AgroParisTech). Paris, 2007, 206.
16. McSweeney PLH. Biochemistry of Cheese Ripening: Introduction and Overview. In: Cheese. (eds.), 2017,379-387. Ed. Elsevier.
17. McSweeney PLH. Biochemistry of cheese ripening. International Journal Dairy Technology,2004:57:127-144.
18. Messaoudi K, Nadjet A. Contribution A L'étude Des Effets De L'addition Des Huiles Essentielles De Genévrier Oxycèdre Et Phénicie Et De L'activation De Système Lactoperoxydase Sur Les Caractéristiques Physicochimique Du Lait Cru De Vache, 2019.
19. Messaour H. L'étude de l'effet de l'incorporation de l'huile essentielle d'orange dans le fromage fondu de la laiterie et fromagerie Boudouaou, 2018.
20. Morgan F. Lipolyse du lait de chèvre et qualité organoleptique des fromages. Le lait,2001:609:36-37.
21. Nadjib M, Ferhat A, Kameli A. Méthodes d'extraction et distillation des huiles essentielles. Revue Agrobiologia,2019:9:1653-1659.
22. Peleg Micha. The Hurdle Technology Metaphor Revisited. Food Engineering Reviews,2020:12:309–320.
23. Peng J, Tang J, Diane M, Shyam S, Anderson N, et Joseph R. Powers. Thermal pasteurization of ready-to-eat foods and vegetables: Critical factors for process design and effects on quality. Food Science and Nutrition,2015:57:2970-2995.
24. Predrag Putnik, Branimir Pavlic, Branislav Šojic, Sandra Zavadlav, Irena Žuntar, Leona Kao, *et al.* Innovative Hurdle Technologies for the Preservation of Functional Fruit Juices. Foods,2020:9:699.
25. Sahraoui N, Abchich H, Mellal M. Optimisation par plan d'expériences de l'extraction de l'huile essentielle du (thymus pallescens). International Journal ofEssential Oil Therapeutics,2016:1:83-90.
26. Seifu E, Elna M. Quality aspects of Gouda cheese made from goat milk preserved by the lactoperoxidase system. International Dairy Journal,2004:14:581–589.
27. Vignola CL, Michel JC, Laquin P, Moineau M, Ponliont M et Simpson R. Science et technologie du lait : transformation du lait. technique et documentation Lavoisier, 2002, 600.
28. Zarei M, Shahriari A, Tarazoudar F, Paknejad M. Comparing the activity and thermal inactivation behavior of Lactoperoxidase in Iranian cow and buffalo milk and whey. Journal of Food Quality and Hazards Control,2016:3:141-145.
29. Zantar S, HM Zerrouk, M Zahar, B Saidi, Z Notfia, A Laglaoui. Effet de l'utilisation des huiles essentielles (du thym, du romarin, de l'origan et du myrte) sur les propriétés physicochimiques, microbiologiques et sensorielles du fromage de chèvre frais et semi-affiné. Options Méditerranéennes,2013:108:183-190.
30. Zhang M, Dong X, Huang Z, Li X, Zhao Y, Wang Y, *et al.* Cheese consumption and multiple health outcomes: an umbrella review and updated meta-analysis of prospective studies. Advances in Nutrition,2023:14:1170-1186.
31. Zhu D, Kebede B, Chen G, Comb K, Frew F. Effects of the vat pasteurization process and refrigerated storage on the bovine milk metabolome. Journal of Dairy Science,2020:103:2077-2088.