International Journal of Agriculture Innovations and Research
Volume 4, Issue 1, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 27/06/2015 | Accepted on : 05/07/2015 | Published : 14/07/2015

Microbiological Quality Analyses of Camembert Cheese Packed in Modified Atmosphere using Perforated Film

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Abstract – **Camembert is a soft cheese that is highly perishable because of its high nutritional value and moisture content. This study examined the effect of using two different films of different permeability on the extension of the shelf-life of the cheese samples. The samples were prepared at Cheese cellar, London, and analysed at the food science laboratory of London South Bank University, London. The two films used were of the same food grade material i.e. polyethylene terephthalate (PET). One film had perforations (*Mp*), while the other is a non-perforated film (*M*). Without perforations, the permeability of the film for water, O_2, CO_2 and N_2 is 3.9g/m^2 /24hrs at 38^{O}C , 3cm^3/m^2 /24hrs at 23^{O}C, 30cm^3/m^2 /24hrs at 23^{O}C and 3cm^3/m^2 /24hrs at 23^{O}C respectively.**

The two set of samples were stored at 4^{o}C ± 0.5 and studied for a period of three weeks. The samples were randomly selected in duplicates on day 0 (of week 1), day 7 (of week 2), and day 14 (of week 3). Changes in the microbiological quality of the food samples were studied. The effect of the two films on the Total Viable Count (TVC) and Yeast /Mould Count (YMC) of the samples was examined. Malt Extract Agar (M.E.A) and Plate Count Agar (P.C.A) were used for YMC and TVC respectively. Initially, the TVC and YMC were similar for both sample groups. Later, there was difference in the TVC of the samples in the different packages. Although, not statistically significant (P>0.05), the sample in the package sealed with non-perforated film exhibited higher TVC.

The study did not reveal any statistically significant difference in YMC for the two set of samples. i.e. P>0.05). However, the package sealed with non-perforated film supported the growth of some greenish-black yeast colonies which were not present in the package sealed with perforated film.

Keywords – **Film perforation, Modified Atmosphere, Total Viable Count, Yeast Mold Count.**

I. Introduction

Camembert is a cylindrical, cream coloured, mould-ripened soft cheese which is commonly consumed in various countries for its unique taste and nutritional value. Bacteria, yeasts and moulds are important microbial contaminants in the dairy industry (Beresford, Fitzsimmons, Brennan, & Cogan, 2001).

It has been observed that cheeses having high pH and moisture content, such as Camembert are very susceptible to microbial spoilage, by moulds, yeasts, and enteric bacteria. (Papaioannou et al, 2007).

The quality and shelf-life of this cheese is usually few days after ripening, depending on factors such as packaging technology used, oxidation of fats, microbiological contamination, loss of moisture (Air products, 1995)

Consumer demands for natural, healthy, preservatives-free products with extended shelf-life, have led food technologists and researchers to develop new packaging concepts. Among these new packaging concepts is the modified atmosphere packaging (MAP).MAP alters the natural gas composition surrounding the product in the package in order to delay deteriorative changes (Air products, 1995; Maria R. B., 2009). MAP technologies increase commercial life of cheeses because they combine the protection against oxidation and dehydration with the inhibition of undesirable microorganisms (Olivares *et al*, 2012)

MAP of different cheese varieties has been studied in the last decades (Rodriguez-Aguilera *et al*, 2011), but scarce information related to MAP of Camembert cheese is available.

The permeability and the transmission rate of the packaging film to O_2, CO_2, and water vapour are among the most essential factors which determine the gas composition in the package, which may influence the product's deterioration rate (Mullan and McDowell 2003; Church 1994).Therefore, the MAP design for a product requires careful handpicking of the packaging film type and size of packaging for the product (Farber et al. 2003).The changes in water activity (a_w) and moisture content (Mc) that accompanies the maturation and storage of cheeses influences their microbiological and physicochemical properties (Saurel, Pajonk, & Andrieu, 2004). Therefore, the control of a_w and moisture content is very important for the preservation of quality and safety of cheeses. For packaged cheeses, the thermodynamic driving force for water transfer out of the cheese and out of the package depends also on the barrier to moisture that the package offers (Holm, Mortensen, & Risbo, 2006).

Traditionally, Camembert tends to be sold whole in thin, round, wooden containers, but it is also sold in tins, with a ring-pull tab for opening. The product is also commonly wrapped dry in a paper/foil wrapper. These systems of packaging may lead to excessive water loss from the product. On the other hand, using a barrier film that has very low permeability to water will not produce a desirable quality for these cheeses. The Camembert cheese under study does not last more than seven days after it has been unwrapped, cut and repacked by the retailer in a MAP, using non-perforated film.

The shelf-life of this type of cheese could be extended by allowing water loss, but at a lower rate. Selecting a packaging film with the right permeability to water could be challenging, but identifying the correct packaging film for cheeses has been noted to be the key to extending the shelf-life of this type of cheeses (Simal, Sa´nchez, Bon, Femenia, & Rossello´ , 2001). The use of a packaging system with a tailor-made moisture barrier, which allows for water loss, but at a lower rate, is a way of extending the cheese's shelf-life **(**Pantalea˜o I. et al., 2006**).** MAP applications for a product are developed from different trials and test of different methods (Air product, 1995). The final choice on the packaging film to be used should be made based on vast evaluations and in collaboration with the packaging suppliers (Sandhya, 2010). Although, MAP with 30% CO_2 and 70% N_2) is recommended for soft cheeses, it is not used for mould-ripened soft cheeses (Air product, 1995). Also, perforated films are often used for fresh produce such as fruits and vegetables i.e. this is the first study evaluating the effect of MA and a perforated film on Camembert cheese.

The study examined the effect of using two films of same food grade material (PET), but of different permeability, and a modified atmosphere of 60% CO_2 and 40% N_2 on the microbiological quality of Camembert cheese.

II. Materials and Method

The methodology used for the study is described below.

2.1 Food sample preparation

The samples were collected from the delivery unit of *Cheese cellar*, London, and taken to the production unit (of the same food company), were each was unwrapped and cut into 8 wedges, using a clean wedge line cutter. The wedges were placed in trays (each wedge weighing approximately 30g).

The packaging trays were then separated into 2 groups.

The Multivac's MAP machine which uses preformed tray and lidding film (PTLF) was used for the sample preparation. The Multivac's MAP machine was used to evacuate air from the package and to seal the trays with the 2 different films, after the addition of protective gases (CO_2, N_2)at pre-set levels. The gas mixture used for the study was 60% $CO_2$and 40% N_2.

The samples were then stored at 4°C ± 0.5 in the storage room of the factory. Duplicate samples were randomly collected and analysed. The samples were held at ambient temperature during the evaluation of the microbiological quality of the samples. A two weeks trial study was conducted, before the final three weeks study. The different samples were labelled using different code. Letter *M* was used for the camembert cheese packaged using non-perforated film, while letter *Mp* was used for the camembert cheese packaged using perforated film. Numeric subscripts such as 1, 2, and 3 were used to differentiate samples collected in different weeks (i.e.*1* for day 0 of week 1, *2* for day 7 of week 2, and *3* for day 14 of week 3)

2.2 Microbiological analyses

(a) Serial Dilution

In sterile conditions, 10 g of each sample was homogenized for 2 min with 90ml of 0.1% sterile peptone water, using a Stomacher Lab Blender 400 (Seward medical, London, England).

1ml portion of the homogenates of each sample was added to different McCartney bottles containing 9ml of sterile 0.1% peptone water, with the aid of sterile pipettes. Further serial dilutions were carried out for the estimation of viable bacteria, yeasts and moulds according to the serial dilution technique described by Carl, V., Don F. S. (1992)

(b) Total Viable Count (TVC) and Yeast/Mould Count (YMC)

The total viable count (TVC) of bacteria and yeast/mould count (YMC) were determined by pour plating method using plate count agar and malt extract agar, respectively. 1ml of an appropriate dilution of each sample was transferred to sterile plates. The plates were then covered with the appropriate agar medium, that have been cooled to about 45°C, for the enumeration of the total viable bacteria, yeasts/moulds. For the total viable bacterial counts, inoculated plates were incubated at 37± 0.5°C for 24- 48hrs. For the yeast and mould counts, the inoculated plates were incubated at 25±0.5 °C for 5 days.

After the incubation period, the agar plates were observed and the total numbers of colonies counted and recorded as average of duplicate count. Microbial counts were expressed as colony forming units per gram (cfu/g wet weight) of the samples.

(c) Identification of the organisms

Representative bacterial colonies were isolated and sub-cultured until pure colonies were obtained. The isolates were then stored in an agar slant, in the refrigerator at 4°C for further identification. The pure cultures obtained were examined for cultural and microscopic characteristics.

Gram's staining was used for presumptive bacterial identification, while simple staining using methylene blue dye was used for presumptive yeast/mould identification

The presumptive identification of the bacterial isolates was done by using the cultural characteristics of the bacterial colonies, and the characteristic image of the organisms captured by Zeiss Primo StariLED Halogen/LED Microscope.

III. Results and Discussion

3.1 Microbiological analyses

The total viable bacterial counts estimated for the different camembert cheese samples are shown in *table 1*and *2*.For the preliminary study, the mean total bacterial count for samples packaged using non-perforated film ranged from 3.65×10^7 to 1.04×10^8 cfu/g, while the mean total viable count for samples packaged using perforated film ranged from 2.55×10^7 to 4.00×10^7 cfu/g.

For the final study, the mean total bacterial count for samples packaged using non-perforated film ranged from 2.25×10^6 to 1.63×10^7cfu/g, while the mean total viable count for samples packaged using perforated film ranged from 2.04×10^6 to 2.46×10^6 cfu/g. This is consistent with high TVC reported by Kalogridou-Vassiliadou (1994) for Anthotyros cheese.

Table 1. Mean TVC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu ml^{-1})

	Preliminary Study	
Week	MAP with non-perforated film (M)	MAP with perforated film (Mp)
1	*3.65 x 10⁷*	*4.00 x 10⁷*
2	*1.04 x 10⁸*	*2.55 x 10⁷*

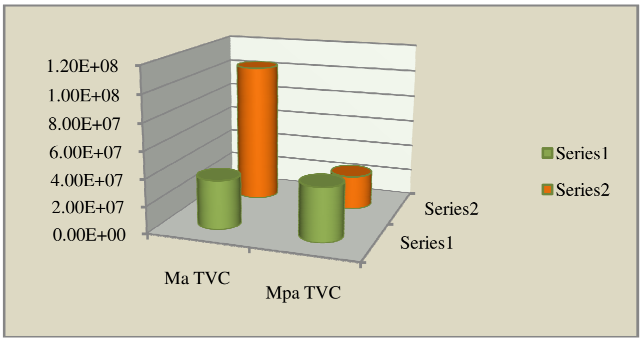

Fig.1. Mean TVC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu ml^{-1})

Table 2: Mean TVC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu ml^{-1})

	Final Study	
Week	MAP with non-perforated film (M)	MAP with perforated film (Mp)
1	*2.25 x 10⁶*	*2.46 x 10⁶*
2	*1.63 x 10⁷*	*2.43 x 10⁶*
3	*4.23 x 10⁶*	*2.04 x 10⁶*

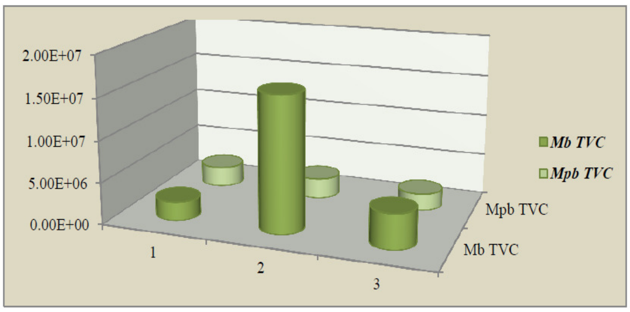

Fig.2. Mean TVC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu ml^{-1})

For the preliminary study, the mean yeast/mould count (YMC) for samples packaged using non-perforated film ranged from $7.85 \times 10^5$to 1.68×10^7cfu/g, while the mean yeast/mould count for samples packaged using perforated film ranged from $7.35 \times 10^5$to 2.80×10^7 cfu/g.

For the final study, the mean yeast/mould count (YMC) for samples packaged using non-perforated film ranged from 3.28×10^6 to 1.94×10^7cfu/g, while the yeast/mould count for samples packaged using perforated film ranged from $2.85 \times 10^6$to 2.61×10^7 cfu/g.

Table 3: Mean YMC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu g^{-1})

	Preliminary Study	
Week	MAP with non-perforated film (M)	MAP with perforated film (Mp)
1	*7.85 x 10⁵*	*7.35 x 10⁵*
2	*1.68 x 10⁷*	*2.80 x 10⁷*

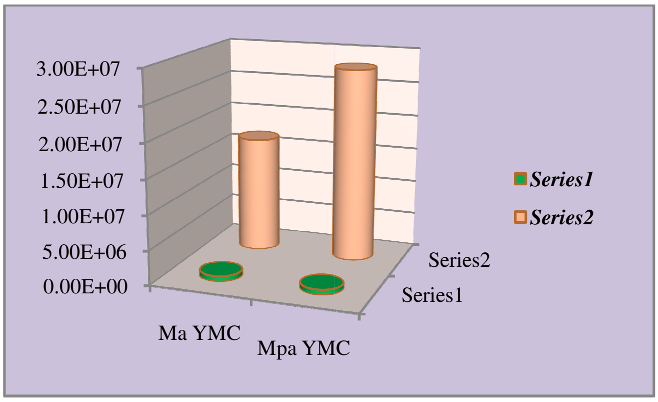

Fig.3. Mean YMC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu g^{-1})

Table 4: Mean YMC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu g^{-1})

	Final Study	
Week	MAP with non-perforated film (M)	MAP with perforated film (Mp)
1	*1.94 x 10⁷*	*2.61 x 10⁷*
2	*3.28 x 10⁶*	*2.85 x 10⁶*
3	*7.56 x 10⁶*	*3.97 x 10⁶*

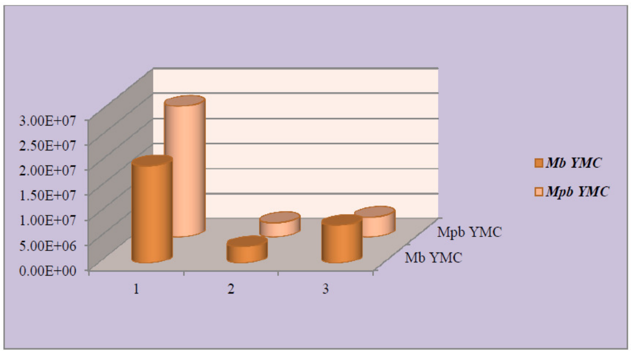

Fig.4. Mean YMC of Camembert cheese packed in MAP with non-perforated film (M) and perforated film (Mp) (values in cfu g^{-1})

For the final study, the initial microbial counts of the Camembert cheese packaged using both films were similar. This could be a result of the films and the MA not having significant effect on the microbial load of the product on day 0 of sample analyses.

The TVC recorded during the study was in the range 10^6–10^8 g^{-1}. This high bacterial count is consistent with the report of Kalogridou- Vassiliadou et al. (1994)for a cheese with high moisture content.

There was numeric difference in the microbial count for both samples. This was especially true for the TVC. The mean TVCs of the cheese samples packaged using perforated film (*Mp*) were numerically lower than the mean TVCs of the cheese samples packaged using non-perforated film, though the difference was not statistically significant. ($P>0.05$). However, it must be noted that statistical analyses may not reflect the difference that exist in reality. *Table 5*shows the result of the statistical analysis, using *SPSS* software.

The differences in TVCs for the two packages possibly reflect differences in the effect of the % CO_2 on the survival of sensitive microorganisms during the storage of the samples.

It is known that the gas mixture used may influence the growth of specific groups of micro-organisms. The non-perforated film allowed low O_2% (0.63 - 3.91% - which is desirable for food products) and a high % CO_2(57 - 66.1%), while the perforated film allowed high % O_2(between 20.9 to 22.4%) and very low % CO_2(between 0.15 - 3.1%).

The difference in the concentration of CO_2 may have affected the solubility and antimicrobial effects of CO_2 in both samples.

The fungi static and bacterio static effect of $CO_2$was expected to be more pronounced for sample *M*, packaged using non-perforated film. Indeed, at some point during the study, the high % CO_2 in sample *M* adversely affected the growth of the mould. On the other hand the mould growth (typical of *Penicillium*), was not adversely affected for sample *Mp*, and packaged using the perforated film.

The high CO_2 level and the low O_2 level did not affect the growth of some yeasts and bacteria present in sample *M*. Yeasts are facultative anaerobes that can survive under aerobic and anaerobic conditions, while most moulds are aerobes which need high amount of O_2 to survive. Also some psychrotrophic bacteria are known to survive under high amount of CO_2 (Maniar et al, 1994; Alves et al, 1996). According to Helen B., James W. (2003), the effect of carbondioxide is not universal, as it has little effect on yeast cells. Helen B., James W. (2003) also noted that the growth of lactic acid bacteria is improved in the presence of carbon dioxide and lower oxygen levels. Lactic acid bacteria such as *Lactococcus* have also been noted to be very important in the proteolysis of cheeses such as Cheddar (Fox et al, 1992).

Some of the organisms observed during this study were yeasts and bacteria that appeared to be Lactic acid bacteria. These were more in the package sealed with non-perforated film (*M*), accounting for its early deterioration. The inhibition of the mould growth by the low O_2 and high CO_2may have encouraged the proliferation of some spoilage yeasts and bacteria in sample *M*.

Of the two packaging films, the perforated film was the most effective for the reduction of TVCs perhaps due to the less inhibitory effect of the low concentration of CO_2 and high O_2 on the mould growth.

The final study revealed that the high % CO_2may not be appropriate for mould-ripened cheese, such as Camembert. The high % CO_2under the non-perforated film affected the mould used for ripening. In addition, high yeast and bacterial counts were also noticed for sample *M*. The suppression of the mould growth may have given rise to successful competition by anaerobic or micro aerophilic yeasts and bacteria.

The spoilage of dairy products (such as Camembert cheese) can be attributed to yeast and bacterial growth (Air products, 1995)

The study therefore revealed that the perforated film may have the potential of extending the shelf-life of the camembert cheese more than the non-perforated film.

3.2 Statistical analysis

The statistical analysis of the sets of data obtained was done using *SPSS* software (version 15). An Independent t-test (2- tail) was conducted to determine if a difference existed between the mean TVC of the two sets of camembert cheese samples. The Independent t-test was also used for data from YMC, and headspace gas analysis.

Table 5: Statistical analysis (Independent Samples Test) for TVC

	Barrier_Film	N	Mean	Std. Deviation	Std. Error Mean
Total_Viable_Count	*Non_Perforated_Film*	*3*	*7593333.3333*	*7604908.50263*	*4390695.97115*
	Perforated_Film	*3*	*2310000.0000*	*234307.49028*	*135277.49258*

		Levene's Test for Equality of Variances		*t-test for Equality of Means*						
									95% Confidence Interval of the Difference	
		F	*Sig.*	*t*	*df*	*Sig. (2-tailed)*	*Mean Difference*	*Std. Error Difference*	*Lower*	*Upper*
Total_ Viable_ Count	*Equal variances assumed*	*12.994*	*.023*	*1.203*	*4*	*.295*	*5283333.33333*	*4392779.42892*	*-6912977.61030*	*17479644.27696*
	Equal variances not assumed			*1.203*	*2.004*	*.352*	*5283333.33333*	*4392779.42892*	*-13582991.23181*	*24149657.89848*

Table 6: Statistical analysis (Independent Samples Test) for YMC

	Barrier_Film	N	Mean	Std. Deviation	Std. Error Mean
Yeast_Mould_Count	*Non_Perforated_Film*	*3*	*10080000.0000*	*8350233.52967*	*4821009.57615*
	Perforated_Film	*3*	*10973333.3333*	*13112041.53949*	*7570240.71245*

		Levene's Test for Equality of Variances		*t-test for Equality of Means*						
		F	*Sig.*	*t*	*df*	*Sig. (2-tailed)*	*Mean Difference*	*Std. Error Difference*	*95% Confidence Interval of the Difference* Lower	Upper
Yeast_ Mould_ Count	*Equal variances assumed*	*1.441*	*.296*	*-.100*	*4*	*.926*	*-893333.33333*	*8975002.94027*	*-25811936.31597*	*24025269.64930*
	Equal variances not assumed			*-.100*	*3.393*	*.926*	*-893333.33333*	*8975002.94027*	*-27672040.90682*	*25885374.24015*

Although there was numeric difference in the TVC of the two groups, the difference was not statistically significant i.e. P>0.05. Also, there was no statistically significant difference between the mean YMC of the two sets of samples at alpha level of 0.05 and confidence level of 0.95. The results of the statistical analyses as produced by the *SPSS* version 15 software are shown below.

IV. Conclusion

The study revealed that the high % CO_2may not be appropriate for mould-ripened cheese, such as Camembert. The high % CO_2under the non-perforated film affected the mould used for ripening. At some point during the study few colonies typical of the *Penicillium camemberti* were observed, whereas high yeast and bacterial counts were noticed for the samples with non-perforated film (M).The growth of spoilage organisms, such *as Lactococcus, Streptococcus, Lactobacilli*, *Actinobacteria* and yeasts was observed more in the package sealed with non-perforated film (*M*).The suppression of the mould growth in sample *M* may have given rise to successful competition by anaerobic or microaerophilic yeasts and bacteria.

There was numeric difference in the TVCs for both samples, although the difference was not statistically significant (It must be noted, however that sometimes, statistical analyses do not reflect the difference that exist in reality)

Therefore, it could be concluded from this study that the use of the perforated film has a good potential for extending the shelf-life of the Camembert cheese.

The result of the study is consistent with the reasoning of some specialists on MAP that mould-ripened cheeses should not be preserved using modified atmosphere (Air products, 1995), because the CO_2 may affect the mould growth and hence the quality of the cheese.

The research revealed that low level CO_2 in the range of 0.2 -4% may be desirable for mould-ripened soft cheeses, such as Camembert. To the best of our knowledge, this is the first study reporting on the use of MAP and perforated film for extension of the shelf-life of Camembert cheese. It must, however, be noted that these recommendations on MAP packaging conditions for Camembert cheese correspond to samples from one cheese factory and thus their general application is yet to be verified.

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