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**COMPARATIVE STUDY ON
FREEZE-DRIED LACTIC CHEESE
STARTERS AND RIPENING
CULTURES FOR THE PRODUCTION
OF CAMEMBERT CHEESE**

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Food Technology

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ABSTRACT

Background and Methodology

The key to success in producing cheeses is the performance of the starter cultures (Parente and Cogan, 2004). Storage of freeze-dried cheese cultures at refrigeration and ambient temperature or higher provides convenience to culture handling and transportation, as well as reduce cost. This study investigated the effects of 4 storage temperatures: -18°C, 4°C, 20°C and 37°C on the stability of mesophilic lactic cheese starters and ripening cultures intended for Camembert production. In phase one, a 2² randomized complete block design (RCBD) was used to determine the potential of 14 commercial freeze-dried direct-vat-set (DVS) mixed cultures to produce Camembert after 5 months storage at the 4 temperatures. The cultures used were: O-type: *Lactococcus* (*L.*) *lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*; LD-type: *L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetylactis* and *Leuconostoc* species (*Leuconostoc* (*Leuc.*) *lactis* and *Leuc. mesenteroides* subsp. *cremoris*) and a mould, *Penicillium* (*P.*) *camemberti*. During storage, the cultures were analysed for cell viability, acid production, colour and species composition. The characterised cultures were screened to select the most stable cultures with good potential for Camembert production. In phase two, a 2³ RCBD design was used to study the potential of the cultures to produce prototype Camembert cheese using I-Make® Limited domestic cheese kits. The prepared cheeses were characterised for acidity, viable cell counts content, texture, volatile aromatic compounds and proteolysis using standard procedures.

Results and Discussion

Viable cell counts and acidification potential of cultures decreased ($P < 0.05$) during storage at selected temperatures for 5 months. Cultures stored at 37°C were the most affected. Proportion of citrate-fermenting lactic acid bacteria (LAB) in LD-type starters also decreased in a similar pattern. Cell inactivation at high temperature was probably attributed to high oxidation, browning reactions, lactose crystallization, changes in glass transition temperature (T_g) of culture-lactose matrix and loss of β -galactosidase enzyme activity, which were possibly also affected by water activity (a_w) of the culture during storage (Higl et al., 2007; Kurtmann et al., 2009c). Viability and activities of cultures stored at 4 and 20°C after 5 months were comparable to those of -18°C cultures and levels normally used in industry. Thus, the cultures demonstrated good potential for Camembert cheese production.

Similar patterns of microbial growth (LAB and *P. camemberti*) and acidification were observed in both cheeses (O- and LD-types) during cheese fermentation. However, cheeses fermented with O-type starters had better growth and acidification activity ($P < 0.05$), which may be attributed to compositional differences of culture, leading to variable metabolic patterns (Mcsweeney and Fox, 2004). Cheeses produced with cultures stored at 4 and 20°C had lower levels of cell growth and acidity ($P < 0.05$), suggesting that the microorganisms could have been affected by prolonged storage at relatively high temperatures.

During cheese ripening, changes in microbial content, acidity, proteolysis, texture and aroma compounds, were similar, and significantly changed ($P < 0.05$) with ripening time. Viable cell counts of LAB reduced, while pH and *P. camemberti* counts increased. Increase of pH may result from lactate metabolism by *P. camemberti* creating an alkaline environment due to the deamination activity of the mould (Spinnler and Gripon, 2004). Proteolysis of cheeses was correlated ($P < 0.05$) with LAB and *P. camemberti* activity as well as the pH of

samples. Softening of cheese was associated with increased proteolysis and pH due to the growth of *P. camemberti* (Spinnler and Gripon, 2004). A range of volatile organic compounds, dominated by fatty acids, alcohols and aldehydes were identified in cheese samples as reported in other studies (Sable and Cotteceau, 1999). Changes in 3-methylbutanal and 3-methylbutanol profiles of samples reflected the degradation of leucine,, synthesis of the aldehyde and its degradation to branched alcohols as a consequence of peptidolytic activity of LAB (Yvon and Rijene, 2001) and enzymatic activity of *P. camemberti* (Molimard and Spinnler, 1996). Increased concentrations of 2-heptanone, 2-nonanone and butyric acid in cheese samples suggested lipolytic activity in all samples (Molimard and Spinnler, 1996). The activity of *P. camemberti* involved in β -oxidation pathway for producing methyl ketones was also demonstrated confirmed by identified metabolites.

Higher proteolysis and softness in LD-cheeses than O-type, suggested a higher degree of cheese ripening (Ardö, 1999), which may be attributed to proteolytic and peptidolytic activity of LD-starters (Tzanetaki et al., 1993). Higher proteolysis may be also associated with higher pH of cheese curd at draining, which facilitated higher syneresis. Increased whey content of curd may retain higher concentration of coagulant enzyme in the curd (Guinee and Wilkinson, 1992) and effectively stimulate the growth of *P. camemberti*, thus probably allowing proteolysis to occur more readily (Grappin et al., 1985). A relatively higher concentration of 3-methylbutanal was found in O-type cheeses than in LD-type. This suggests that LAB in O-type starters may exhibit higher activity in degrading leucine to 3-methylbutanal than LD-type starters (Yvon and Rijene, 2001). 2,3-butandione was suspected in LD-type cheeses but not in O-type samples, demonstrating the active role of citrate-fermenting bacteria of LD-starters (Mcsweeney and Fox, 2004).

Results indicate that storage temperature of cultures had a significant ($P<0.05$) impact on viable cell counts and acidity of samples. In spite of reduced cell counts, proteolysis, texture and aroma of the prototype cheese samples were not affected ($P<0.05$). Although there were no differences between the Camembert cheeses, 4 and 20°C cultures used in cheese-making may enhance the ripening process (Ardö, 1999) than -18°C cultures, as indicated by relatively higher proteolysis and degree of softening. Lower levels of 3-methylbutanal in samples containing 4 and 20°C cultures was probably due to the reduced aminotransferases activity of LAB (Yvon and Rijene, 2001) after prolonged storage at the two temperatures. The slightly higher levels of 2-heptanone, 2-nonanone and butyric acids in samples with 4 and 20°C cultures were probably due to increased lipolytic activity of enhanced growth of *P. camemberti* (Molimard and Spinnler, 1996) during cheese ripening.

Conclusion

LAB starter cultures and *P. camemberti* can be stored for 5 months at 4 and 20°C without affecting their activities and the quality of prototype Camembert produced. Camembert cheese samples produced in this study had typical characteristics of this type of cheese. Cheese fermented with LD-type starters showed extra flavour enhancement potential and the products had higher degree of softening due pronounced proteolysis. Cultures stored at 37°C for 5 months were characterised by poor viable cells and capability to the produce acid, therefore, they were not suitable for Camembert cheese production.

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LIST OF ABBREVIATIONS

| | | |
|--|---|--|
| ALA | = | α -acetolactate (α -acetolactic acid) |
| ArAAs | = | Aromatic amino acids |
| ANOVA | = | Analysis of variance |
| ASN | = | Acid soluble nitrogen |
| a_w | = | Water activity |
| BcAAs | = | Branched chain amino acids |
| C | = | Carbon |
| CCP | = | Colloidal calcium phosphate |
| CAR-PDMS | = | Carboxen/Polydimethylsiloxane |
| Cit ⁺ | = | Citrate-fermenting |
| Cit ⁻ | = | Non-citrate-fermenting |
| cfu/g | = | Colony forming unit per gram |
| cfu/ml | = | Colony forming unit per milliliter |
| CN | = | Casein nitrogen |
| CO ₂ | = | Carbon dioxide |
| (Ca ₃ (PO ₄) ₂) | = | Calcium phosphate |
| Cd | = | Cadmium chloride (CdCl ₂) |
| C/F | = | Casein-to-fat ratio |
| CV% | = | Coefficient of variation |
| d | = | Day |
| Da | = | Dalton |
| DVS | = | Direct vat set |
| DVI | = | Direct vat inoculation |
| DMC | = | Dry matter content |
| DNA | = | Deoxyribonucleic acid |
| DSS | = | Defined-strain starters |
| DMS | = | Dimethyl sulfide |
| DMDS | = | Dimethyl disulfide |
| DMTS | = | Dimethyl trisulfide |
| FA | = | Fatty acid |
| FFA | = | Free fatty acid |
| FAO/WHO | = | Food and Agriculture Organization/World Health Organization |
| FSANZ | = | Food Standard Australia New Zealand |
| FDM | = | Fat-in-day matter |
| g | = | Gram |
| GLM | = | General linear model |
| GLY pathway | = | Glycolytic pathway |
| h | = | Hour |
| H ₂ O | = | Water |
| HCl | = | Hydrochloric acid |
| HS-SPME/GC-MS | = | Headspace solid phase microextraction/gas chromatography-mass spectrometer |
| i.d. | = | Internal diameter |
| IDF | = | International dairy federation |
| IMCU | = | International milk clotting units |

| | | |
|-----------------------|---|--|
| Ile | = | Isoleucine |
| <i>k</i> | = | Rate constant |
| k-casein | = | Kappa-casein |
| KHP | = | Potassium hydrogen phthalate Solution |
| kg | = | Kilogram |
| LAB | = | Lactic acid bacteria |
| Leu | = | Leucine |
| Log cfu/g | = | Logarithm colony forming unit per gram |
| LPL | = | Lipoprotein lipase |
| mmol/L | = | millimoles per litre |
| MRS | = | Molten de Man Rogosa Sharpe |
| MSS | = | Mixed-strain starters |
| MNFS | = | Moisture in non-fat substance |
| Met | = | Methionine |
| <i>m/z</i> | = | Mass-to-charge ratio |
| mV | = | millivolts |
| N | = | Nitrogen |
| n | = | mole |
| nm | = | nanometer |
| NaOH | = | Sodium hydroxide |
| NaCl ₂ | = | Sodium chloride |
| NSLAB | = | Non-starter lactic acid bacteria |
| NPN | = | Non-protein nitrogen |
| NH ₃ | = | Ammonia |
| NQ | = | Not quantified |
| PA | = | Polyacrylate |
| pH 4.6-SN | = | Soluble nitrogen at pH 4.6 |
| PTA-SN | = | Phosphotungstic acid soluble nitrogen |
| PCA | = | Principle component analysis |
| PDA | = | Potato dextrose agar |
| PK pathway | = | Phosphoketolase |
| Phe | = | Phenylalanine |
| pI | = | Isoelectric point |
| ppm | = | parts per million |
| Pa.s | = | Pascal-second |
| <i>r</i> ² | = | Correlation coefficient |
| RTE | = | Ready-to-eat |
| RH | = | Relative humidity |
| RSM | = | Reconstituted skim milk |
| RT | = | Retention time |
| RCBD | = | Randomized complete block design |
| RNA | = | Ribonucleic acid |
| SD | = | Standard deviation |
| SN | = | Soluble nitrogen |
| T _g | = | Glass transition temperature |
| T.A. | = | Titrateable acidity |
| TCA | = | Trichloroacetic acid |
| TCA-SN | = | Trichloroacetic acid soluble nitrogen |
| TN | = | Total nitrogen |

| | | |
|-------|---|--|
| Tyr | = | Tyrosine |
| Trp | = | Tryptophan |
| TAG | = | Triacylglyceride |
| Trace | = | Trace amount |
| μ | = | Micrometer |
| μg | = | Microgram |
| U | = | Unit |
| v/v | = | Volume/volume |
| Val | = | Valine |
| VC | = | Volatile compounds |
| w/v | = | Weight/volume |
| WSN | = | Water soluble nitrogen |
| X-gal | = | 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside |
| Ø | = | Diameter |