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

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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 2^2 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, *Penicillum* (*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 2^3 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 Cottenceau, 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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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_2O	=	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
k	=	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
m/*z*	=	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
r^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.	=	Titratable 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