Effect of respirative and catalase-positive cultures of *Lactobacillus casei* on the production of Cheddar cheese

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Introduction

The effect of respiratory metabolism on the growth and fitness of some lactic acid bacteria (*Lactococcus lactis*, *Lactobacillus casei*, *L. plantarum*) has been widely investigated in synthetic media, demonstrating that respirative phenotypes have improved technological and stress response properties (Pedersen et al. 2012; Zotta et al. 2014; Ianniello et al. 2015) compared to the strains grown in anaerobic conditions.

To our knowledge, however, no respiration-competent LAB strains have been yet successfully tested and used for the production of fermented foods.

In this study, the effect of respirative and catalase-positive phenotypes of *L. casei* N87 and *L. casei* N2014 (Zotta et al. 2014; Ianniello et al. 2015) was evaluated, for the first time, on the production and quality of Cheddar cheese.

Materials and Methods

L. casei N87 and *L. casei* N2014 (Zotta et al. 2014) cultivated in anaerobic (AN) and respiratory (RS; aerated growth with hemin and menaquinone in the substrate) conditions (Ianniello et al. 2015) were used as adjuncts for the production of Cheddar. Cheeses inoculated only with a defined mixture of *Lc. lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* (Ciocia et al. 2013) were used as controls. After 14, 30, 60, 120 and 180 days of ripening, cheeses were subjected to microbiological, chemical and biochemical analyses. Proteolysis (total free amino acids; casein degradation by urea-PAGE; peptide profile by RP-HPLC), production of volatile organic compounds (VOCs by SPME/GC-MS) and the capability of starter and adjuncts to scavenge toxic free (DPPH- and hydroxyl) radicals and prevent lipid (content of malondialdheyde) and protein (content of carbonyl compounds) peroxidation were also evaluated.

Effect of AN or RS adjuncts of *L. casei* on microbial counts, gross composition and proteolysis

Lactococcal starters decreased during the ripening (up to 2 log cfu/g reduction after 180 days), while the number of non-starter LAB increased up to 8 log cfu/g. The addition of AN or RS cultures of *L. casei* N87 or N2014 did not cause significant changes in gross composition and casein degradation of Cheddar cheese.

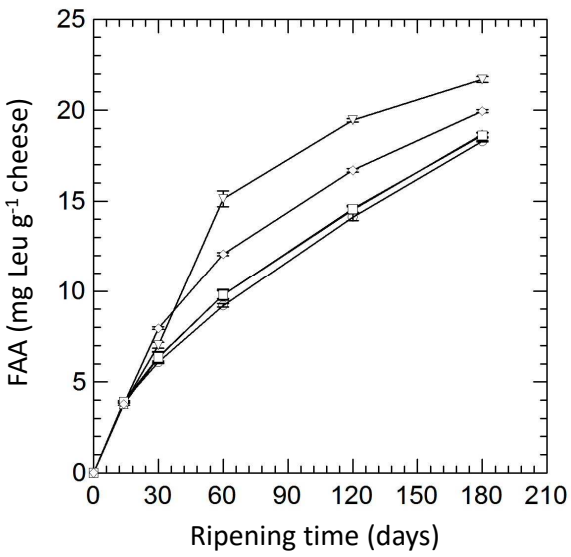


Fig. 1: **Concentration of total free amino acids** (FAA) during the ripening of control cheeses (circle), cheeses with AN (up triangle) or RS (down triangle) *L. casei* N87, and cheeses with AN (square) or RS (diamond) *L. casei* N2014.

On the contrary, RS cells of *L. casei* N87 and N2014 caused a noticeable increase in **free amino acid** (FAA; Fig. 1) and **peptide production** (Figs. 2A, 2B) compared with AN adjuncts and the starter culture alone.

The PC1-axis, in PCA graph, separated the samples on the ripening time, while the PC2-axis discriminated starter and/or adjunct cultures. Several peaks with high loadings were affected by ripening time alone or by both ripening time and starter/adjunct system.

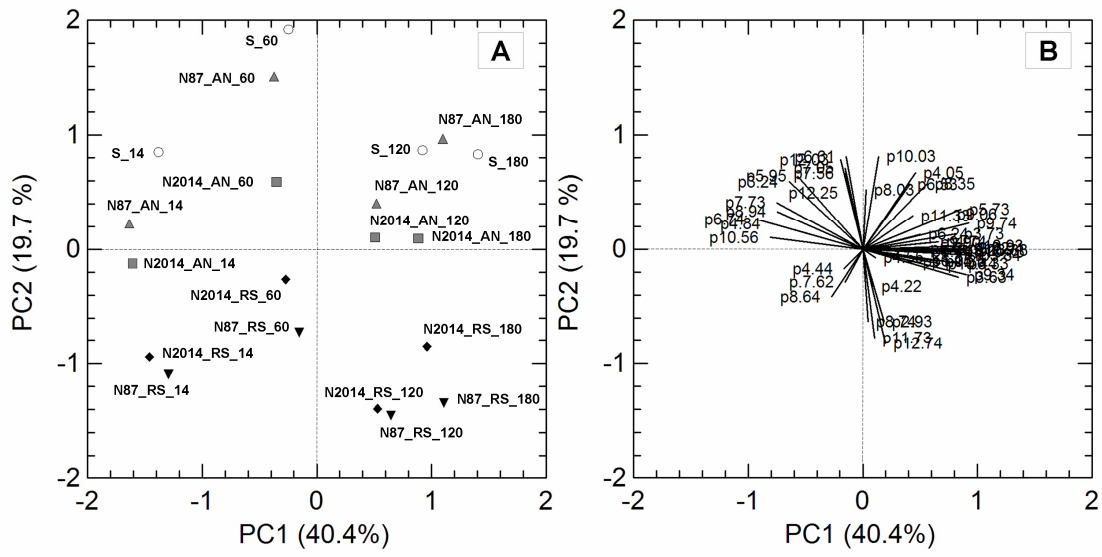
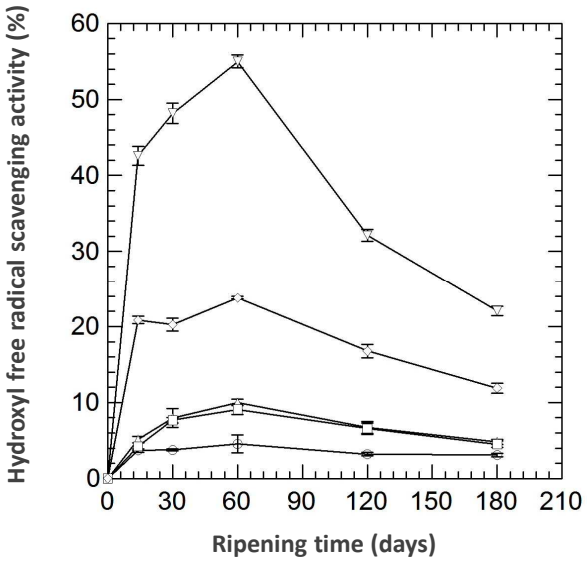


Fig. 2: Score (panel **A**) and loading (panel **B**) plots of the **Principal Component Analysis** (PCA) carried out on the Pearson's *r* correlation matrix of **RP-HPLC data set**. Symbols in the panel A: empty circles, control cheeses; grey up triangles, cheeses with AN *L. casei* N87; black down triangles, cheese with RS *L. casei* N87; grey squares, cheese with AN *L. casei* N2014; black diamonds, cheese with RS *L. casei* N2014. Numbers in panel B indicated the retention time of chromatographic peaks.

Effect of AN or RS adjuncts of *L. casei* on free-radical degradation and lipid and protein oxidation



The **content of hydroxyl free radicals** (Fig. 3) and the degree of **lipid and protein oxidation** (Figs. 4a, 4b) were significantly lower in Cheddar produced with RS adjuncts.

The RS cells of *L. casei* N87 and *L. casei* N2014, moreover, reduced the redox potential more quickly than lactococcal starter or *L. casei* N87 and *L. casei* N2014 cultivated under AN conditions, preventing the oxidative processes during the cheese ripening.

Fig. 3: **Radical degradation** in control cheeses (circle), cheeses with AN (up triangle) or RS (down triangle) *L. casei* N87, and cheeses with AN (square) or RS (diamond) *L. casei* N2014.

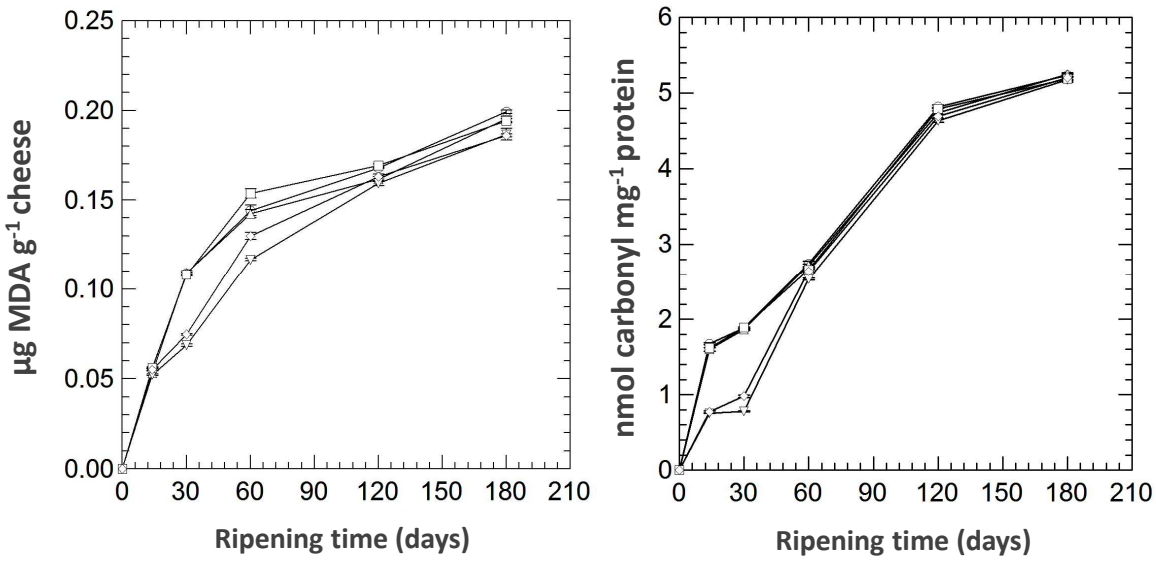


Fig. 4: **Lipid** (µg MDA g^{-1} cheese; panel A) and **protein oxidation** (nmol carbonyls mg^{-1} protein; panel B) measured during the ripening of control cheeses (circle), cheeses with AN (up triangle) or RS (down triangle) *L. casei* N87, and cheeses with AN (square) or RS (diamond) *L. casei* N2014.

Effect of AN or RS *L. casei* on aroma compounds

Diacetyl and **acetoin** were the prevalent VOCs in cheeses produced with RS cells. The use of RS adjuncts reduced the content of aroma compounds resulting from lipid and protein oxidation (i.e., nonanal, 2-pentanone, 2-heptanone, 2-nonanone) or involved in development of moulded ripened/blue cheese flavour (methyl ketones and secondary alcohols) or Cheddar off-flavour (esters and alcohols with sweet and/or fruity notes).

CONCLUSIONS

- This is the first study that confirms the benefits of respirative LAB strains in food matrices.
- The conditions of growth of adjunct cultures, prior to the inoculum in milk, may significantly affect the biochemistry (i.e. proteolysis, aroma compounds, oxidation of lipids and proteins) of cheese ripening.
- The increased production of aroma compounds by respirative *L. casei* strains may be commercially relevant for dairy industry.

References

Ianniello RG, Ricciardi A, Parente E, Tramutola A, Reale A, Zotta T. 2015. *LWT-Food Sci Technol*, 60: 817-824.
Pedersen MB, Gaudu D, Lechardeur M, Petit MA, Gruss A. 2012. *Annu Rev Food Sci Technol*, 3: 37-58.
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