SELECTIVE ENRICHMENT OF THE RAW MILK MICROBIOTA IN CHEESE PRODUCTION: CONCEPT OF A NATURAL ADJUNCT MILK CULTURE

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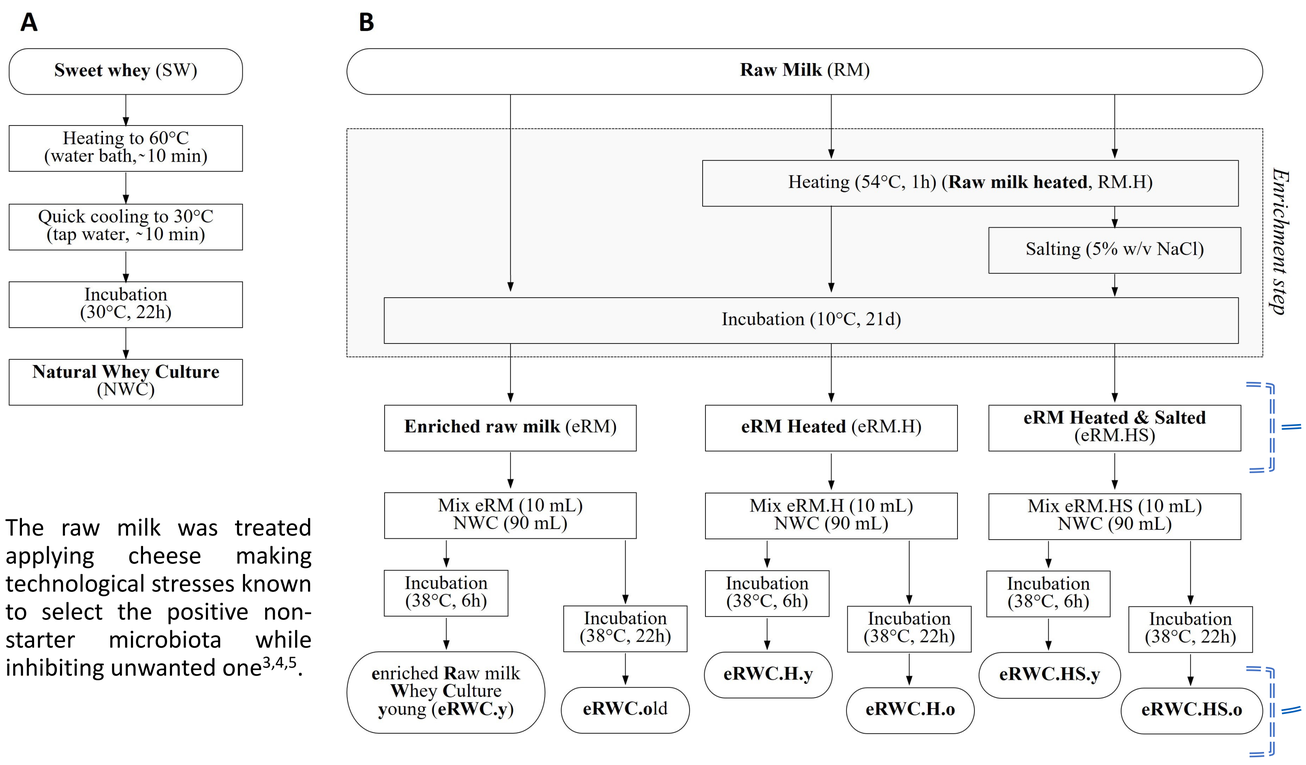
INTRODUCTION

Cheeses with Geographical Indication owe their popularity to the complex organoleptic profile developed by the traditional cheesemaking technology applied, in addition to the activity of autochthonous microorganisms important for the ripening process[1]. This microbial component arise from the farm and dairy environment and reach the cheese through the raw milk[2], and it is called *non-starter* to be differentiated from the *starter* one which is instead intentionally added in the form of a culture. In the last decades, the improvement of the hygienic milking and cheesemaking conditions has resulted in the depletion of useful non-starter lactic acid bacteria (LAB) microbiota. This diminishing biodiversity resulted in the production of cheeses with less flavour in the industrial setting.

To develop a practical tool for introducing a culture rich in autochthonous microorganisms to traditional cheese, we investigated the production of an enriched raw milk whey culture (eRWC), an artisanal adjunct produced from mixing an enriched raw milk with a natural whey culture (NWC). The optimization of such a tool could be an alternative to the practice of isolating, geno-pheno-typing, and formulating mixed-defined-strain adjunct cultures that require knowledge and facilities not always available for artisanal cheese makers.

CULTURE DESIGN

Fig. 1. Natural adjunct culture production flow chart: (A) natural whey culture; (B) raw milk enrichment step and final enriched raw milk whey culture production. **Reference for the samples' abbreviation**.



The raw milk was treated applying cheese making technological stresses known to select the positive non-starter microbiota while inhibiting unwanted one[3,4,5].

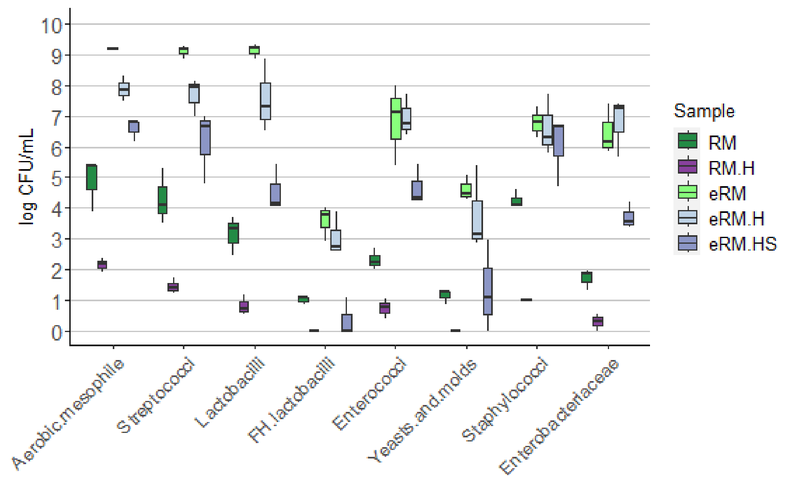
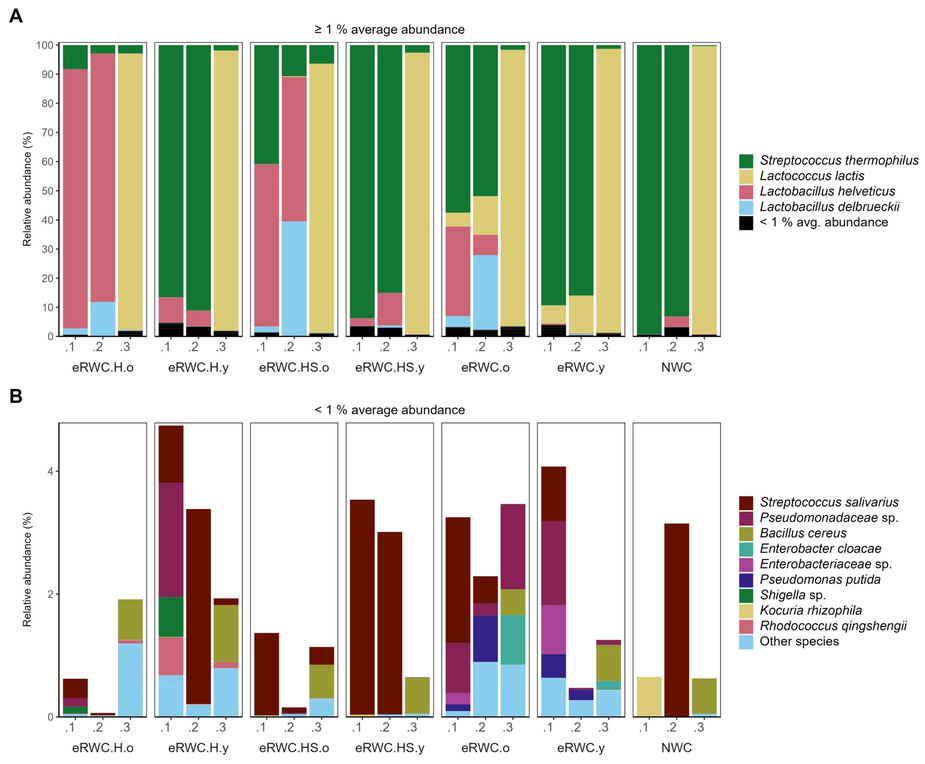


Fig. 2. Boxplot of counts of viable microorganisms (Colony Forming Units/mL.) in the **enriched milks after the incubation period**.

Fig. 3. NGS results of NWC and eRWCs: stacked bar plot of species' relative abundance (%). (A) Species with and average abundance ≥1%. (B) Species with and average abundance <1%. Refer to Figure 1 for samples' abbreviation; the three samples' replicates are reported



CHEESEMAKING

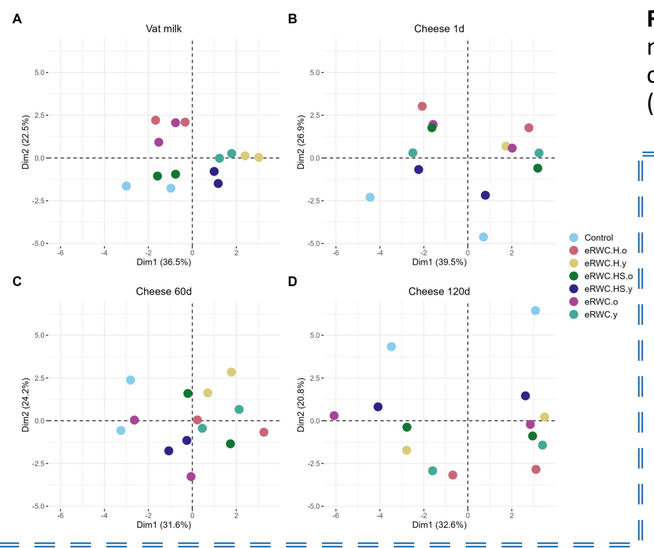


Fig. 4. Ordination plots of principal component analysis: (A) vat milk, microbial features (n=14); (B) 1d cheese, microbial (n=14) and chemical features (n=7); (C) 60d cheese microbial features (n=14); (D) 120d cheese, microbial (n=14) and chemical features (n=29).

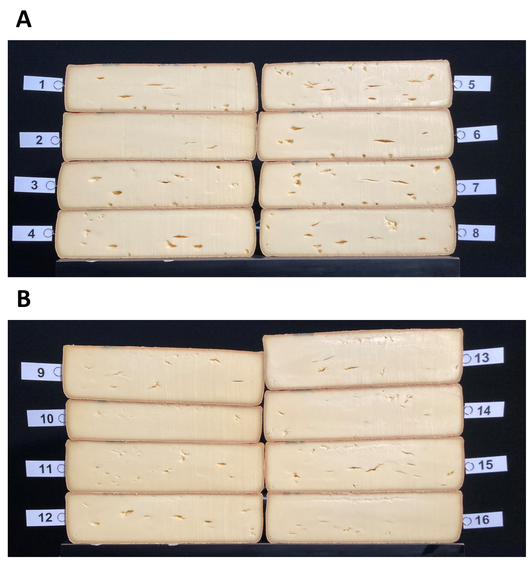


Fig. 6. Cheese cross sections after 120 days of ripening. A) First replicate; B) Second replicate. 1 and 9 = control, raw milk; 2 and 10 = control, thermized milk; 3 and 11 = eRWC.y; 4 and 12 = eRWC.H.y; 5 and 13 = eRWC.HS.y; 6 and 14 = eRWC.o; 7 and 15 = eRWC.H.o; 8 and 16 = eRWC.HS.o

A

Adjunct culture	Prod. day	Observed	Chao1	ACE	Shannon	Simpson	Fisher
Control	1	25.0	25 ± 0	25 ± 2	1.5	0.6	2.5
	2	18.0	18 ± 0	18 ± 1.6	1.1	0.5	1.7
eRWC.y	1	23.0	23 ± 0.5	-	1.3	0.6	2.1
	2	20.0	20 ± 0	20 ± 1.8	1.2	0.5	1.9
eRWC.o	1	24.0	24 ± 0	24 ± 1.6	1.4	0.7	2.2
	2	27.0	27 ± 0	27 ± 1.6	1.3	0.6	2.6
eRWC.H.y	1	27.0	27 ± 0.5	27.5 ± 1.4	1.5	0.7	2.6
	2	29.0	29 ± 0	29 ± 1.9	1.7	0.7	2.7
eRWC.H.o	1	28.0	28 ± 0.5	28.3 ± 1.9	1.5	0.7	2.7
	2	27.0	27 ± 0	27 ± 1.4	1.7	0.7	2.6
eRWC.HS.y	1	25.0	25 ± 0.5	25.6 ± 1.4	1.5	0.7	2.3
	2	24.0	24 ± 0	24 ± 1.6	1.5	0.7	2.2
eRWC.HS.o	1	27.0	27 ± 0.2	28.1 ± 2	1.4	0.7	2.6
	2	24.0	24 ± 0	24 ± 1.8	1.4	0.6	2.3

B

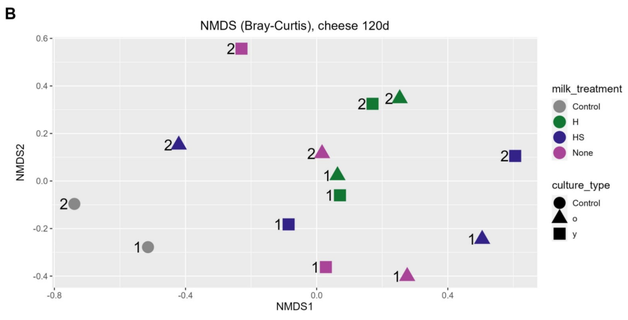


Fig. 5. NGS results of 120d ripened cheese produced with different adjunct cultures. (A) Alpha diversity parameters; (B) beta diversity, ordination plot of non-metric multidimension scaling (NMDS) on Bray–Curtis dissimilarities ("1"=production day 1; "2"=production day 2.)

CONCLUSIONS

This study provides new insights into the possibility to enrich the raw milk microbiota for the production of cheese. Our results showed that the applied raw milk enrichment protocols were able to increase the concentration of autochthonous LAB, and that the combination of heating and osmotic stresses at this step are effective in control the presence of some undesired microbial groups. This allowed us to produce natural adjunct cultures harboring diverse microbiota.

Chemical and microbiological results suggested that this microbial diversity influenced the early stages of cheese making, but its effect decreased over time during ripening, showing an inferior effect than that of raw milk microbiota. More research is needed to optimize the culture production, including testing different treatments that could more specifically select desired NSLAB present in raw milk, or try different ratios of eRM-NWC mixing since our results showed a strong influence of the NWC on the eRWC microbiota.

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