Identification and characterisation of malty/chocolate flavour compounds generated in a semi-hard cheese by HS-SPME-GC-MS, HS-ITEX-VD-GC-MS-O and GCxGC heart-cut

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Introduction

Hints of malt or chocolate can sometimes be perceived in Swiss Raclette cheese in various intensities. According to literature, these aromas are due to the presence of 2- and 3-methylbutanal[1][2][3]. These two compounds are dependent on the thermal process during cheese making and the medium in which strains develop. Some Agroscope strains are known to inhibit this aroma often negatively perceived. This odour can change depending on the balance between the responsible compounds.

A selection of various Swiss Raclette references having high (''malt positive'') or low (''malt negative'') intensity of malt/chocolate aroma have been studied. The compounds responsible for this aroma have been identified and characterised by gas-chromatography mass-spectrometry (GC-MS) and GC-MS-O (olfactometry). These studies have been performed on strain cultures and mini-cheeses to validate the results. Furthermore, kinetic measurements were performed in order to understand their formation.

Methods

The following methods and conditions were used to analyse the aromatic compounds from the strains cultivated during 18 hours in milk and filtered De Man, Rogosa and Sharpe (MRS) broth at 30°C, and the mini cheeses designed with a fabrication process of Agroscope:

Sample analyses by GC-Olfactometry:

- The perceived odours were described and evaluated by a minimum of 8 trained panelists per sample on a scale from 1 to 5 (1: barely perceivable, 5: highly perceivable).
- AcquiSniff® software was used to process the obtained aromagrams taking into account the frequency of detection, indicated intensities and the class of vocabulary used by the panelists.

Sample analyses by headspace in-tube extraction (HS-ITEX) [4] **:**

- GC-MS: T= 55°C, $t_{incubation}$ = 10 min, $t_{extraction}$ = 5 min, trap: Tenax TA/Carbosieve S III
- GC-MS-O : *T*= 60°C; $t_{extraction}$= 45 min, same trap as above

Sample analyses by headspace solid phase microextraction (HS-SPME) [5][6] **:**

- GC-MS : *T*= 60 °C; $t_{extraction}$= 60 min, fiber: DVB/CAR/PDMS 50/30 µm 2 cm

Results

GC-Olfactometry

The aromagrams obtained by statistical processing with AcquiSniff® software are the mean of evaluations given by the panelists who classified the perceived odours in 13 different classes. Figures 1 to 3 show the aromagrams giving an overview on the malt aroma smelled by the panelists on a strain culture and two mini cheeses[7][8]. Mixtures of strain cultures were used as references for the ''malt positive'' (Raclette 27) and ''malt negative'' (Raclette 18).

Culture 27

«**Malt positive**» reference, *culture in milk of Swiss Raclette 27*, consisting of 6 Agroscope strains

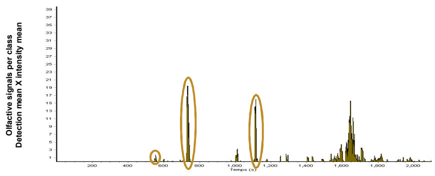
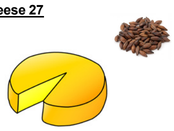


Fig. 1 Aromagrams of malt odours perceived in the sample of Swiss Raclette culture 27

Cheese 27



«**Malt positive**» reference, *Swiss Raclette cheese 27*, consisting of 6 Agroscope strains

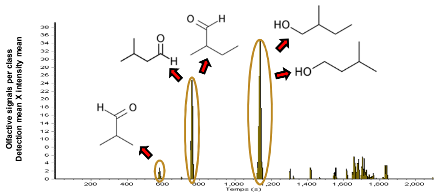
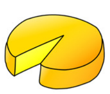


Fig. 2 Aromagrams of malt odours perceived in a Swiss Raclette mini cheese 27

Cheese 18



«**Malt negative**» reference, *Swiss Raclette cheese 18*, consisting of 6 Agroscope strains

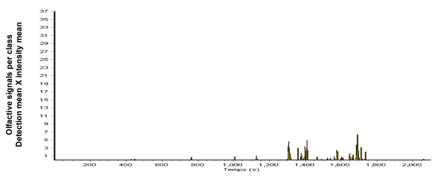


Fig. 3 Aromagrams of malt odours perceived in a Swiss Raclette mini cheese 18

Kinetic analyses

The kinetics of the three compounds identified as co-responsibles for the malt/chocolate aroma were tracked in order to know when they were formed and if their concentration remained constant from the strain culture to the cheese ripening. The ''malt positive'' reference (Raclette 27) was used for the kinetic analyses in the mini-cheese (Fig. 4). A pure culture mainly responsible for the strong malt aroma (FAM-17841) was used for the ones in fermented milk (FM) and in our model filtered MRS medium (Fig. 5-6).

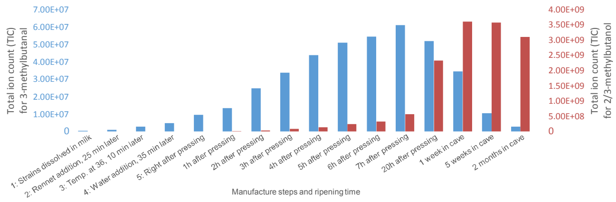


Fig. 4 Kinetics of 3-methybutanal and 2/3-methylbutanol in a mini-cheese during making and ripening given by GC-MS TIC (total ion count)

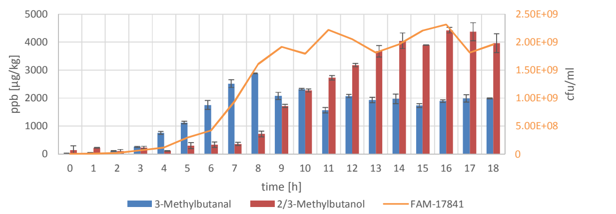


Fig. 5 Kinetics of 3-methylbutanal, 2/3-methylbutanol and colony formation of the strain FAM-17841 cultivated in FM during 18 hours (in µg/kg)

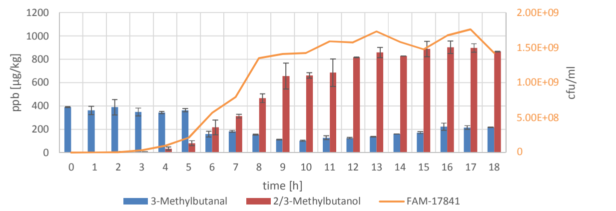


Fig. 6 Kinetics of 3-methylbutanal, 2/3-methylbutanol and colony formation of the strain FAM-17841 cultivated in filtered MRS during 18 hours (in µg/kg)

Conclusion

More than 50 strain mixtures of Swiss Raclette have been cultivated in milk to find the references for detection by smell as ''malt positive'' or ''malt negative''. Two mixtures of strains and one single strain have been chosen as references for the GC-MS-O analyses and kinetic measurements.

Apart from 2-methylbutanal and 3-methylbutanal – originally identified as main responsibles for the malt/chocolate aroma in dairy products – this study proposes three new compounds (2-methylpropanal, 2- and 3-methylbutanol) as co-responsibles of this odour. Two other molecules (phenylacetaldehyde and 2-phenyl-2-butenal) were further identified with heart-cut GC-GC-MS-O[9] as potential markers.

Kinetic measurements showed that 2- and 3-methylbutanal were formed at the beginning of the cheese making and their concentration decreased during the ripening time. Instead, 2- and 3-methylbutanol were formed after the pressing step and their absolute concentration increased during the first hours in the cheese cellar before maintaining their concentration. This could not only be observed during cheese making, but also during the 18 hours of strain culturing resulting in this strong malt aroma. Leading us to the hypothesis that 2- and 3-methylbutanal were reduced to 2- and 3-methylbutanol respectively. This hypothesis can be supported by the fact that 3-methylbutanal is not further produced when already present in its growing media. Instead, it rather seems to be converted to 3-methylbutanol as the strains are exponentially growing. However, when 3-methylbutanal is not present, it must be first generated before being converted to 3-methylbutanol which would explain the slower increase of the latter in fermented milk in comparison to filtered MRS broth.

These results will allow us to better understand the formation pathway of compounds responsible for the malt aroma negatively perceived in order to control them during the cheese making.

The authors are thankful to the panelists for their help.

References

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