IMPLEMENTATION OF RAPID AND NON-INVASIVE QUALITY CONTROL THROUGH PTR-TOF-MS FOR ANHYDROUS MILK FAT QUALITY CONTROL

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Abstract– In this pilot study, Proton Transfer Reaction Mass Spectrometry coupled with a time-of-flight mass analyzer and an automatic sampler was evaluated as rapid a rapid tool for the quality control of anhydrous milk fat. Two different types of packaging for storage – cardboard and bag-in-box - were evaluated during accelerated shelf life at 50°C for 11 days.
Preliminary results show the possibility to identify markers related to both products packaging and shelf life, thus indicating the possible application of Proton Transfer Reaction Mass Spectrometry in agroindustry quality control programs.

Keywords: *PTR-MS, rapid quality control, anhydrous milk fat, VOCs*

1. INTRODUCTION

Quality control (QC) in agroindustry faces new challenges due to the growing awareness of consumers towards food quality. Sensory evaluation and, at the same time, instrumental methods to warrant product quality and consumer satisfaction are necessary [1]. Food volatile organic compounds (VOCs) are the ideal target to be addressed by instrumental analysis since they are produced and affected in most stages of the food-production chain and they are key drivers of food perceived quality both before, during and after food consumption [2]. Gas chromatography-mass spectrometry (GC-MS) is the actual reference method for the instrumental analysis of VOCs [3].

In the last years, the need to develop faster and simpler methods [4] to overcome GC poor time resolution, led to the development of new rapid, non-invasive and high sensitive methods. Among these, Proton Transfer Reaction-Mass Spectrometry (PTR-MS) is an accurate, highly sensitive, non-destructive, direct-injection technique, suitable for a rapid characterization of food products and for the monitoring of processes in agroindustry without any pre-treatment [2]. It is based on an efficient implementation of chemical ionization by proton transfer [5]. The gas mixture under investigation is continuously injected in the reaction region – the drift tube – where the VOCs are ionized by proton transfer from H_3O^+ ions produced by the hollow cathode ion source. The generated ions are then analyzed according to their mass/charge ratio (m/z) using a time-of-flight (ToF) mass analyzer which provides high mass resolution (6000 m/Δm in the V-mode) and a broad mass range [6]. The outcome is a rapid mass-resolved fingerprint of the total volatilome of the samples with ultra-high sensitivity (sub ppt).

Anhydrous milk fat (AMF) is defined by the Codex Alimentarius as a fatty product made exclusively from milk and containing at least 99.8% dairy fat obtained by eliminating quasi-entirely water and non-fat dry matter. Due to its characteristics - higher stability and longer shelf life when compared to normal butter - industry interests for this matrix are globally increasing.
More than 230 VOCs have been identified in different types of butter as well as in butter oil [7]. PTR-MS technique was used by Van Ruth *et al.*[8, 9] to classify butter oils and to verify the geographical origin of European butters in terms of headspace volatile composition. The method was deemed as a promising approach for control of regulations and quality.

In industry, AMF is usually wrapped in a plastic film and stored in cardboard packages (CT) of about 25 kg or, directly after production, inserted in the so called bag-in-box (BIB) containers which are more expensive. Bag-in-box are sealed and do not allow exchange with the atmosphere, preventing oxidation but, at the same time,

accumulating VOCs and off-flavors possibly generated during production or storage.

In this work, PTR-ToF-MS rapid and automated headspace analysis coupled to a multipurpose GC automatic sampler is applied in order to (1) verify the possibility to identify the effects of different type of packaging and (2) to characterize changes in VOCs profile during accelerated shelf life at 50°C (ASL).

2. EXPERIMENTAL

Three production lots from the same producer were sampled in 3 different days. Each lot was then stored for 180 days at 4°C in the different packaging conditions under control humidity (65%). On the first day of measurement (day 0) all samples were melted in a thermal bath (50°C). For each sample, five 2.5 mL aliquots of anhydrous milk fat were transferred into sampling vials. Vials were then closed, labelled and stored at 50°C till measurements that were done at day 0, 2, 4, 7, 9 and 11. Empty vials were used as blanks and for all time points the procedure was the same.

A commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) was used for the headspace measurements. The instrumental conditions in the drift tube were as follows: drift voltage 557 V, drift temperature 110 °C, drift pressure 2.30 mbar affording an E/N value of 141 Td (1 Td= 10^{-17} V cm^2). Sampling was performed with a flow rate of 35 sccm. The mass resolution (m/Δm) was at least 3800. Measurements were performed in an automated way by using a multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) as previously described [10, 11]. The measurement order was randomized to avoid possible systematic memory effects. All vials were incubated for equilibration at 50 °C for 25 min before PTR-MS analysis. Each sample was measured for 60 s, at an acquisition rate of one spectrum per second. Data where then processed according to the procedure described by Cappellin *et al.*[12, 13] and concentrations (in ppbV) were obtained according to the formula described by Lindinger *et al.*[5] by using a constant reaction rate coefficient (k_R = 2 x 10^{-9} cm^3/s). The ensuing matrix with the estimated concentrations has 222 rows corresponding to the measured samples and 396 columns for the m/z values of the extracted peaks. Analysis of variance (ANOVA) with Bonferroni correction was performed on the whole data set for selection of mass peaks significantly higher in the samples than in the blanks. Principal Component Analysis (PCA) was then conducted on the first and the last day of measurement reduced and scaled dataset. The median ± s.d. of the 5 replicates for each extracted peak was then plotted to have an idea of the ASL trends.

3. RESULTS AND DISCUSSION

The final dataset on which analysis were performed was composed of 129 peaks significantly higher than the blanks ($p < 0.05$ with Bonferroni correction). In figure 1 it is possible to see the example of one mass peak plotted.

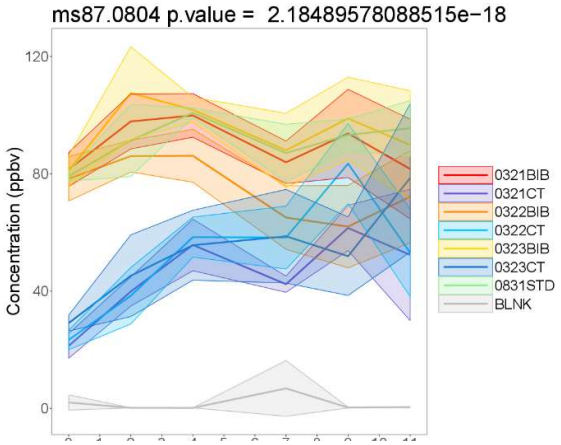

Figure 4: Time evolution (day 0-day11) of the concentration of the peak at m/z= 87.080 ($C_5H_{10}OH^+$) for all investigated samples.

Based on available literature [14-16], the peak at m/z 87.080 can be tentatively identified as 2-pentanone. 2-pentanone with a caramel/cream-like aroma has been found as one of the VOC constituents of butter, cream and ghee aromas [7, 17, 18]. In the figure, it is possible to clearly see that the two types of packaging have a different effect on the concentration of this molecule.

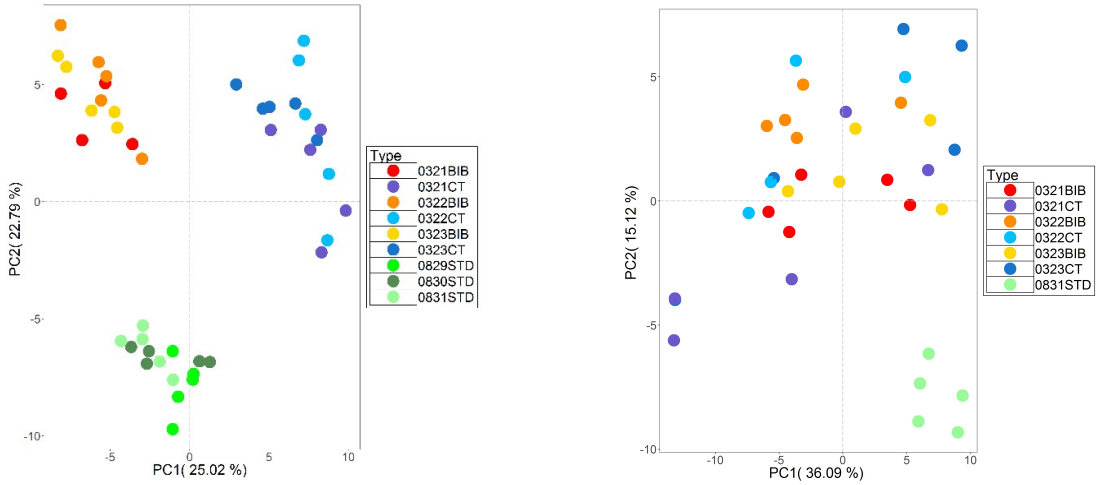

Figure 5: Score and loading plots of principal component analysis of BIB, CT and reference samples (STDs) at day 0 (A) and at day 11 (b). Different colors indicate the different sample categories.

In particular, BIB has similar levels and trends with the fresh product (0831STD) and a higher concentration than CT, indicating a better preservation of this VOC. Moreover, it is possible to observe that, while at day 0 the concentration difference between BIB and CT is clear, during ASL at 50°C these differences are reduced. This tendency is observable also in the PCA presented in fig. 2: at day 0, samples are clearly discriminated. In particular, figure 2(a) shows that BIB and CT samples are separated on the first PC (explaining 25.02% of total variance) while the second PC (explaining 22.79% of total variance) separates the samples from the reference samples (STDs). At day 11 (fig 2(b)) the separation between BIB and CT is not observable anymore while the STDs are separated on the second PC (explaining 15.42% of the total variance). This effect may be explained by the fact that during the ASL at 50°C samples are kept in the vials. Oxidation processes may take over the initial VOC profile differences produced by the different packaging type.

4. CONCLUSIONS

This study demonstrates the feasibility of the rapid non-invasive and automated quality control of AMF by PTR-ToF-MS. In particular, PTR-ToF-MS was shown as a valid method to identify both initial differences between different types of packaging or fresh standards and to monitor the effect of storage at 50°C.

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