PE4.78 Generation of selected volatile compounds in yeast-fortified dry-cured hams 274.00

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***Abstract*— A strain of the yeast *Candida famata* isolated from dry-cured ham was inoculated on dry-cured ham surface during maturation, to increase the surface population contributing to the generation of organic volatile compounds useful for dry-cured ham aroma. Some aldehydes, alcohols and ketones, selected for their known influence on dry-cured ham aroma, were monitored in yeast-fortified and control dry-cured hams by HD-SPME/GC-MS analysis. Dry-cured hams inoculated with *C. famata* showed a remarkable generation of 2-methyl-propanal, 3-methyl-butanal, benzaldheyde, 2-pentanone, 2-esanone, 2-eptanone, while 3-methyl-butanol and 2-methyl-butanol gave lower signals than the control dry-cured hams. The overmentioned organic volatile compounds showed a pattern in real yeast-fortified dry-cured hams differing from dry-cured ham like model systems inoculated with the same yeast; results suggest that the model system, though relevant to display yeast metabolism, is not a reliable marker of *C. famata* contribution to dry-cured ham aroma. The sensory analysis of yeast-fortified and control dry-cured hams was made, giving a different sensory profile for the inoculated samples.**

***Index Terms*—volatile organic compounds, yeasts, *C. famata*, dry-cured ham.**

I. INTRODUCTION

During the manufacturing process of dry-cured ham, many biochemical changes, originating from available substrates like free amino acids and fatty acids, give rise to volatile organic compounds (VOCs) which contribute to the characteristic flavor of dry-cured ham.

The role of microorganisms in the generation of VOCs is well documented for dry-cured meat products [1-3], and a microbial population is abundant on the surface of dry-cured ham [4], made up of moulds, yeasts and bacteria.

On ham surface, a high yeast population is witnessed by the so called “patina farinosa”, i.e. a thin white powder layer covering ham mask; when the range of water activity (0.85-0.92), temperature (10-28°C) and moisture favorable to growth occurs, yeasts outnumber bacteria and form a film on the whole ham. *Candida famata* strains were recently isolated from the muscle surface of Parma dry-cured ham [5] and showed the capability of growing up to high populations on a dry-cured pork surface [6]. Species of yeasts and moulds naturally occurring on the surface of dry-cured ham can contribute to the development of typical flavor throughout the ripening process of dry-cured meat products [7-8]. Several studies has been focused on the study of the volatile compounds of dry-cured ham; the use of solid-phase micro extraction (SPME) coupled with GC-MS has permitted the isolation and identification of a high number of volatile compounds that contributes to the overall aroma of dry-cured ham [9-11].

The aim of this study was to monitor the effect of the inoculation of a well-growing strain of *Candida famata* on the surface of Parma dry-cured ham and in a dry-cured-ham- like model system, to evaluate both the generation of volatile compounds and the sensory profile of the yeast-fortified dry-cured hams.

II. MATERIALS AND METHODS

A. Yeast strain

The strain of *C. famata* was isolated from typical Parma dry-cured ham, cultured, purified and stored in the yeast collection of SSICA [5]; the strain was maintained frozen (-80°C) in Malt Extract Broth (MEB) containing 15% glycerol (w/v).

B. Dry-cured ham inoculation

Six dry-cured hams were labeled after the washing step; than, three of them were inoculated with a suspension of *C. famata* GS78 strain [5], suitable to achieve a surface population of about 10^5-10^6 cfu/g, while the remaining dry-cured hams were not inoculated (control group), allowing the natural uncontrolled surface population to proliferate. Yeast counts of ham muscle surface portions were obtained at several times during ham maturation and ageing, on dichloran-glycerol agar plates (DG-18, Oxoid), after incubation at 25°C for 4-5 days. At the end of the established ageing time of dry-cured hams (18-20 months), a surface portion of 2.5 mm-thickness was

taken from each ham and stored at -20°C until the analysis.

C. Ham-like model system inoculation

The preparation of the yeast suspension was carried out according to the procedures of Pinna et al. [12]. Briefly, suspensions of *C. famata* GS78 were used to inoculate (3% v/v) 100 ml flasks containing 40 ml of ham-like model system (54 g/l of freeze-dried dry-cured ham). At different times (2, 5, 8 and 13 days), cells were removed by centrifugation (15,300×g, 15 min., 4 °C). Aliquots of 7 ml of the supernatant were placed in a 15 ml vial and sealed with a PTFE faced silicone septum (Supelco) for volatile compounds determination. At each time, triplicate samples were collected and maintained at - 20°C until analysis. Yeast counts in the ham-like medium were determined on Malt Extract Agar (MEA) after incubation at 25°C for 4-5 days. Fifty µl of a 0.1 mg/L solution of chlorobenzene were added as internal standard in the vials containing the ham-like system. Control flasks were also made (no inoculation).

D. Analysis HD-SPME/GC-MS

VOCs were collected by using a solid-phase microextraction (SPME) device (Supelco, Bellafonte, PA), with a 75 µm CAR-PDMS coating. Prior to analysis, the fiber was preconditioned at 300°C for 30 minutes. The separation and identification of VOCs was performed by a Trace Gas-Chromatograph Ultra equipped with a DSQ II mass selective detector (electronic impact source, Thermo Electron). The compounds adsorbed by the fiber were desorbed in the injection port at 250° for 1 min. in splitless mode. The compounds were separated in a SLB 5-ms column (60 m × 0.25 mm × 0.25 µm, Supelco). The carrier gas was elium with a 1.3 ml/min flow. The oven temperature was held at 36°C for 15 min. and programmed to rise at 4°C/min. to 120°C, then at 20°C/min. to a final temperature of 250°C, held for 10 min. The transfer line was kept at 280°C, the detector temperature was set at 250°C. The mass range was 35-350 amu in full scan mode, the scan time was 0.71s.

1.5 grams of minced dry-cured ham were placed in 20 ml glass vial capped with a PTFE septum. The vials were left in the TriPlus autosampler at 40°C. The conditioning phase was 15 min. and then the fiber SPME was exposed to the headspace for 30 min. to allow equilibration of the volatile in the headspace. Three analyses were replicated for each dry-cured ham. In the case of dry-cured ham-like model system, the vials were placed in a water bath at 35°C for 15 min. (conditioning phase), and then the fiber SPME was exposed to the headspace for 30 min. For dry-cured ham analysis, 100 µl of a 2.5 µg/L solution of ethyl propionate were used as external standard and the TIC area was used to correct samples for equipment variability.

The amount of volatile compounds was calculated by SIM (Single Ion Monitoring) integration. The selection of the characteristic ion for the SIM quantification, is performed by studying the molecular fragmentation and choosing the ion that differentiates from background and coeluting compounds. Peaks were integrated by ICIS method of the software Excalibur V 1.4 (Thermo Electron).

E. Sensory analysis

The sensory analysis of dry-cured hams was carried out to assess the odor of the aged dry-cured hams. The panel consisted of six trained assessors; 4 samples were tested at each session, replicating two samples. The judges used a 0-5 scale where 0 is the absence of the perception of the descriptor, 5 is used for the maximum intensity perceived.

The flavor descriptor used were: aged, rancid, cheese- and mould-like.

F. Data analysis.

SPSS 11.5.1 for Windows was used for statistical analyses (DESCRIPTIVES procedure). Percent piled bar and radar graphs were obtained by using Excel 2000.

III. RESULTS AND DISCUSSION

A. Yeast growth in control and C. famata-fortified dry-cured hams

The counts of yeast population are reported in table 1 and table 2 for dry-cured hams (surface layer) and dry-cured ham like model system.

The counts of yeasts population made at established times of processing, show that the surface of dry-cured hams inoculated with *C. famata* reached a yeast population exceeding 10^8 cfu/g between 5^{th} and 7^{th} months and remained 10^7 cfu/g until the last count at 12 months (requested processing time for branding), while control dry-cured hams achieved yeast counts of about 10^6 cfu/g at each monitored maturing time.

Yeast counts ranged from 10^4 to 10^7 cfu/g during the incubation period, reaching a maximum at day 2; then they decreased until 10^6 and remained nearly steady up the end of the incubation, showing that model system composition and nutrients are adequate for the growth of *C. famata* GS78.

Table 1: Yeast counts during processing (3, 5, 7, 9, 12 months) in dry-cured hams not inoculated (control) and inoculated with *Candida famata* GS78

processing months	control (log cfu/g)	inoculated (log cfu/g)
3	6.16	5.98
5	6.35	8.84
7	6.5	8.05
9	6.35	7.60
12	6.21	7.10

Table 2: Yeast counts (0, 2, 5, 8, 13 incubation days) in dry-cured ham like model systems inoculated with *Candida famata* GS78.

days	*dry-cured ham-like model system (cfu/ml)*
0	4.40
2	7.20
5	6.41
8	6.30
13	6.85

B. VOCs identifiction and integration

Seventy six VOCs were identified and integrated by SIM mode in dry-cured hams samples. The means of the signals obtained from three replicates/sample were calculated. Seventy eight VOCs were identified and integrated by SIM mode in the dry-cured ham like-model system. The means of the signals obtained from three replicates/sample at 4 incubation times were calculated.

Since the number of VOCs is very high, only chemical categories and compounds regarded as mainly representative for their contribution to aroma [5-11] and yeast presence [3-13] have been taken into account. These categories are aldehydes (contribution to aroma), alcohols (yeast metabolism) and ketones (contribution to aroma and microbial metabolism).

Aldehydes play an important role in the ham aroma because of their low odor thresholds. They are often suggested among the most important VOCs to the characterization of dry-cured ham flavor. Aldehydes reported in this work (Fig.1) are generated by degradation of amino acids released by means of proteolysis during dry-cured ham ageing; 2-methyl-propanal contributes to aroma with toasted fruity pungent notes, 3-methyl-butanal fruity, almond-like toasted, but also nutty and salty notes [14]. Benzaldehyde provides bitter almond notes. The pattern of aldehydes in assayed headspaces differs noticeably: in dry-cured-ham-like model system the antioxidant effect of the yeast is remarkable, leading to the disappearance of aldehydes, while in dry-cured hams fortified with *C. famata* signals of aldehydes are higher than in the control. Alcohols display a complementary trend to aldehydes (Fig.2), being their reduction products; the odor threshold of alcohols is higher than aldehydes. In the case of ketones (Fig.3), both yeast-inoculated systems (real and model systems) are higher than the control ones (with the exception of 2-eptanone in the model system). High levels of these compounds in dry-cured ham has been reported [15]; 2-pentanone has been characterized by the sensory attributes green, fruity and tropical fruit-like, 2-hexanone contributes to the sensory perception "ethereal", 2-heptanone contributes to "blue cheese" flavor. Data reported show the ability of *C. famata* in increasing these ketones.

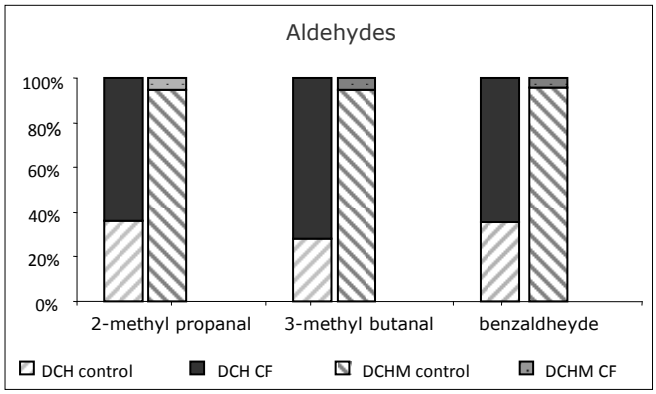

Fig. 1. Percent piled bars displaying the changes of selected aldehydes in real dry-cured hams and dry-cured ham-like model systems inoculated with the yeast C. famata GS78 (CF). DCH: dry-cured ham, DCHM: dry-cured ham model system.

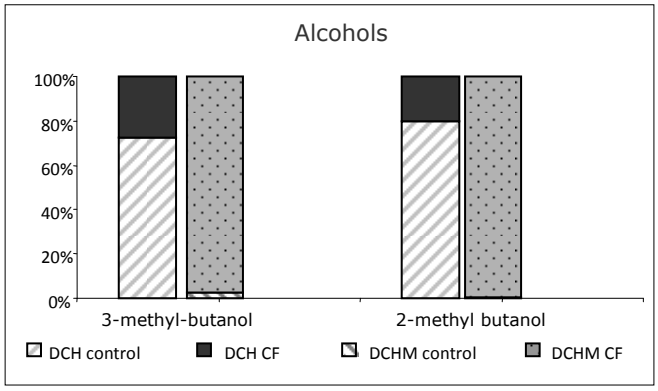

Fig. 2. Percent piled bars displaying the changes of selected alcohols in real dry-cured hams and dry-cured ham-like model systems inoculated with the yeast C. famata GS78

(CF). DCH: dry-cured ham, DCHM: dry-cured ham model system.

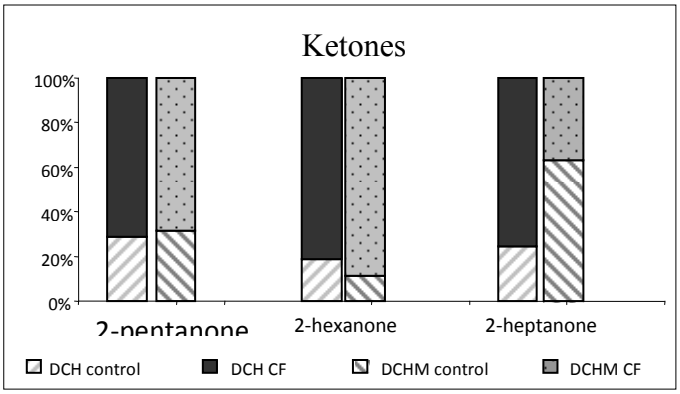

Fig. 3. Percent piled bars displaying the changes of selected ketones in real dry-cured hams and dry-cured ham-like model systems inoculated with the yeast C. famata GS78 (CF). DCH: dry-cured ham, DCHM: dry-cured ham model system.

C. Dry-cured ham sensory analysis

Results of sensory evaluation of dry-cured hams show the superimposed profiles of control and *C. famata*-fortified dry-cured hams. A higher perception of cheese-like flavour is the distinctive trait between yeast inoculated and control samples.

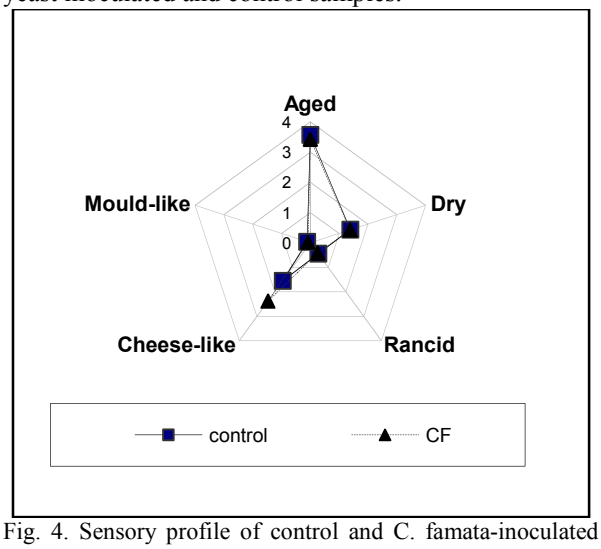

Fig. 4. Sensory profile of control and C. famata-inoculated dry-cured hams.

IV. CONCLUSION

Results suggest that the inoculation with the yeast *C. famata* of both real dry-cured hams and dry-cured ham-like model systems, changes the volatile profile of the fortified samples if compared to the control ones. Real dry-cured hams and model systems showed different patterns in the signals from representative aldehydes, alcohols and ketones, as a possible consequence of differences in yeast growing substrate properties (nutrient amount and ratio, water activity, time of incubation, microbial populations). Inoculation of dry-cured ham with *C. famata* may be a tool for influencing the flavor of final outcome by mean of a naturally occurring yeast of this product. The work is in progress with other autochthonous yeasts, an higher number of dry-cured hams, different processing managements.

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