EFFECT OF SODIUM REDUCTION ON VOLATILE ORGANIC COMPOUNDS OF TRADITIONAL ITALIAN SAUSAGE

A. Pinna, N. Simoncini, C. Bacchieri and R. Virgili

Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), viale Tanara 31/A, 43121, Parma, Italy.

Abstract – The effect of low levels of NaCl (2%, 1.6%, 1.2%) and partial replacement with KCl (0.4% - 0.6%) in Italian traditional sausages was evaluated. As a rule, sausage standard recipes are formulated with 2.4 - 2.7% NaCl in the fresh mince. The changes in sausage water activity (a_w) were monitored during the processing time, and the volatile compound profile was analysed by HS-SPME-GC-MS method. The signals due to terpenes coming from added spices were affected by salt content ("salting out effect") and their release in the headspace was found to be decreased by salt reduction. As a consequence, to avoid an impairment of typical sausage aroma, the decrease in salt should be counteracted by an increase in spice addition.

Key Words – low sodium, HS-SPME-GC-MS, volatile compounds, terpenes, salting out effect.

I. INTRODUCTION

Health organizations worldwide have called for a reduction in the average sodium intake of the population [1]. A high sodium consumption is related to the increase in blood pressure, stomach cancer and osteoporosis. Processed foods are recognized as a main source of sodium chloride, and producers are working to achieve new products with lower salt content. NaCl is a key additive of meat products because of its role on texture, flavour and shelf-life. Salt reduction in meat products can cause adverse effects on water binding, sensory quality and microbiological safety. As a consequence, the replacement of sodium chloride by other salts has been suggested as an alternative for manufacturing meat products having at the same time a healthier appeal and an adequate sensory quality. The reduction of NaCl and its partial replacement by other salts has been assayed at different percentages by many authors, assessing the effect of replacement on sensory quality and microbiological safety of dry-cured meat products [2, 3]. KCl has been recognized as safe, showing an antimicrobial effect similar to NaCl [4].

In this study, the changes in sausage aroma affected by the decrease in NaCl have been studied by using Headspace Solid Phase Microextraction coupled with gas-chromatography and mass spectrometry (HS-SPME-GC-MS). The aim was to identify the volatile compounds of sausages affected by the different amounts of sodium chloride and sodium / potassium ratios used in the trial.

II. MATERIALS AND METHODS

Three batches of sausages (named B1, B2 and B3) were manufactured at SSICA pilot plant, and made with lean pork and back fat grounded through a plate with 7mm diameter holes. The following additives were added for each treatment to the meat mixture: white wine (5 g/Kg), black pepper (0.6 g/Kg), white pepper (0.3 g/Kg), sodium nitrite (0.15 g/Kg), potassium nitrate (0.08 g/Kg) and microbial starter culture (SM 170, Chr. Hansen) (0.125 g/Kg). The batches were mixed with different sodium and potassium chloride amounts (Table 1) and underwent the traditional processing stages until nearly 30% weight loss, at 30 days processing time.

Table 1. Na^+ and K^+ in fresh sausage mince

Batch	NaCl [a]	KCl [a]	Na^+ [b]	K^+ [c]	Na^+:K^+ [d]
B1	2.0	0.5	0.87	0.58	2.5:1
B2	1.6	0.6	0.74	0.65	2.0:1
B3	1.2	0.4	0.54	0.71	1.3:1

a: added salt (g / 100 g fresh sausage mince)
b: Na^+ total content of fresh sausage mince (g / 100 g fresh sausage mince)
c: K^+ total content of fresh sausage mince (g / 100 g fresh sausage mince)
d: Na^+:K^+ molar ratio in fresh sausage mince

The Na^+ and K^+ content was determined in duplicate in each fresh sausage mince according to AOAC (2002) [5]. The molar ratio reported in Table 1 takes into account Na^+ and K^+ given by natural and added content in the mince. Batches B1, B2 and B3 were made with a Na^+ reduction corresponding to nearly 20, 36 and 52% calculated by comparison with the NaCl amount currently added to standard Italian typical dry-cured sausage (≈ 2.5%).

The water activity (a_w) was measured over processing time (0, 3, 6, 10, 13, 17, 27 days) in a 0.5 cm-thick slice taken from the center of the sausage, using a LabMaster Aw (Novasina, Switzerland) according to ISO [6].

Three sausages were randomly taken from each batch at 3, 13 and 30 days, and a 1cm-thick inner slice was removed for the analysis of volatile compounds and stored under vacuum at -20°C until HS-SPME-GC-MS analysis.

The frozen slice was divided in four sections for replicate analysis; each section was fragmented with a knife and 1 g was weighted into 20 ml glass vial. The vial was tightly capped with a PTFE septum and left for 10 min at 40°C to allow temperature equilibration. Volatile components were extracted by Head Space-Solid Phase Microextraction technique (HS-SPME), piercing the septum covering each vial with a needle equipped with a Carboxen/PDMS/DVB (Supelco, Bellefonte, PA) coated fibre. Prior to collection of volatiles, the fibre was preconditioned at 300°C for 40 minutes in the GC injection port and exposed to the headspace for 120 min at 40°C. Then the fiber was inserted into the injector port of the GC for 1 min at 250°C using the split mode (split ratio 1:15). Temperature, time of incubation and injection were controlled by means of the TriPlus autosampler (Thermo Electron Corporation) using the Excalibur 1.4 software (Thermo Electron Corporation).

The volatile compounds were separated using a SLB-5ms column (Supelco; 60 m × 0.25 mm id × 0.25 μm film thickness) installed on a Trace GC Ultra (Thermo Electron Corporation). The carrier gas was helium. The oven temperature was held at 36°C for 15 min, programmed to rise 10°C/min to 250°C and held for 5 min. The GC-MS transfer line temperature was set at 280°C. The mass spectrometer operated in the electron impact mode, with an electron energy of 70 eV, a scan rate of 1.4 s^{-1} over a range of *m/z* from 35 to 350 in full scan mode for data collection.

The identification of volatile compounds by GC–MS was carried out with a DSQ II mass-selective detector (Thermo Electron Corporation). Volatile compounds were tentatively identified by comparison with reference spectra from the NIST 2005 version 2.0 spectral library database and with Kovats retention indices in agreement with literature (NIST, Gaithersburg, MD, USA).

Peak integration and relative quantification was based on the signal area, computed on an arbitrary scale, according to the Single Ion Monitoring (SIM) mode and based on a single ion selected for each compound according to its fragmentation pattern, to improve selectivity and remove noise due to background and co-eluting peaks. Two replicates were run and averaged for each sample.

Sixty-two volatile organic compounds (VOCs) were identified by HS-SPME-GC-MS, mostly belonging to chemical categories of terpenes, ethyl esters, alcohols, aldehydes, ketones, aromatic and aliphatic hydrocarbons.

VOCs data were analyzed by Kolmogorov-Smirnov test for normality. Then, sampling time and batch were the main effects included in the model and the least square means (LSM) were computed for the effect "batch" with General linear Model (GLM) (SPSS package ver.14 for Windows). Bonferroni t-test was used to statistically separate LSM when $P < 0.05$.

III. RESULTS AND DISCUSSION

The sausages underwent the ripening process of traditional Italian sausages [7], achieving similar appearance, colour, weight loss, and pH in the three batches (data not shown).

Differences among the batches were found in a_w values during processing time, as displayed in Fig. 1.

B1 underwent a sharp drop at the end of monitored processing, achieving on average 0.930 a_w, while B2 and B3 had a slow decrease and were featured by higher a_w values (0.943 and 0.949 respectively). Measured a_w values are in agreement with salt additions made according to Table 1.

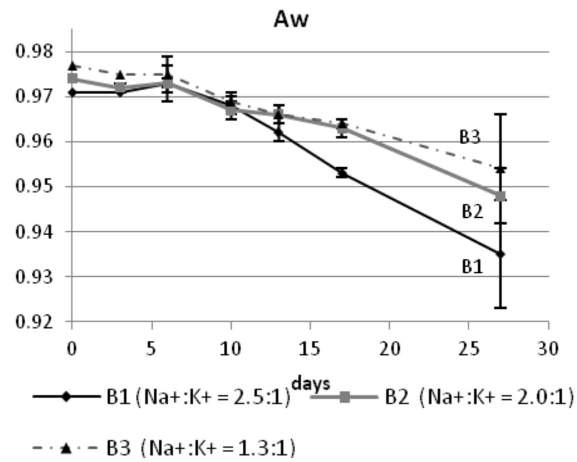

Figure 1. Water activity changes during the monitored processing time

Volatiles ascribed to bacterial activity in carbohydrate fermentation, amino acid catabolism, and lipid β-oxidation [8], are mainly branched aldehydes, alcohols, ketones, organic acids, and esters; the lipid autoxidation generated linear aldehydes, alcohols and hydrocarbons.

VOCs from added spices (black and white pepper, garlic) are mainly terpenes and sulfur compounds.

Table 2. Effect of salt decrease on VOCs (LSMs of areas expressed in arbitrary units)

	Batch			Sign
	B1	B2	B3	*P*
Ethanol	2128^{a}	1754^{b}	1791^{b}	0.031
2-butanone	1043^{c}	1540^{b}	2158^{a}	0.000
Octane	20.5^{b}	27.5^{a}	25.5^{a}	0.034
β –pinene	1975^{a}	1654^{a,b}	1026^{n}	0.046
1,8-cineol	18.3^{a}	16.6^{a}	9.53^{b}	0.017
α - copaene	7.31^{a}	4.75^{b}	3.31^{c}	0.000
α - caryophillene	0.31^{a}	0.32^{a}	0.20^{b}	0.013
β - caryophillene	131.2^{a}	88.3^{b}	69.4^{b}	0.007
α - selinene	1.63^{a}	1.27^{a,b}	0.83^{b}	0.036

LSMs within a row with different lower case letters are different (Bonferroni multiple test, P < 0.05) between batches B1, B2, B3 corresponding to Na^+:K^+ molar ratios 2.5:1, 2.0:1 and 1.3:1 respectively.

Differences in salt between batches did not generate differences in most signals due to microbial activity or lipid autoxidation, while several terpene signals were found to be affected by salt differences between sausage batches.

The LSMs of VOC areas with significant differences due to sodium decrease in mince formulations are reported in Table 2 (P < 0.05).

Terpenes intensely contribute to the overall flavour of the product. They represent on average the 47% of the integrated areas. Salt decrease affects (P < 0.05) 6 terpenes in the headspace (among 21 identified terpenes), that showed a remarkable drop in the B3; the mechanism involved can be ascribed to the “salting out effect”, causing a major release of less polar substances in the headspace. The sharp salt reduction made for the B3 can increase the interaction between terpenes and mince matrix, lowering the release in the headspace.

The replacement of NaCl with KCl did not affect VOCs profile coming from microbial metabolism, with the exception of ethanol and 2-butanone (carbohydrate fermentation), showing an opposite trend. Ethanol production rised in sausages with the highest salt content and Na^+/K^+ ratio, while 2-butanone increased in the case of lowest salt and Na^+/K^+ ratio. Other studies showed that ethanol is involved in the microbial biosynthesis of ethyl esters [9], and this mechanism may be favoured by salt decrease. In the case of 2-butanone, the increased production in less salty formulations may be related to a carbohydrate fermentation pathway more present in the case of lower control by salt.

Even if salt has been reported as a pro-oxidant [10], the oxidation markers were not affected by salt reduction, with the exception of octane. Alkanes are regarded as oxidation products, but in the present study octane increased in less salty sausages.

CONCLUSION

This study showed that the reduction of salt and the replacement of NaCl with KCl, though causing a less pronounced decrease in a_w values over processing, slightly affected the volatile profile, with the exception of terpenes coming from spices. The decrease in chlorides (NaCl and KCl) in sausages, caused a less intense “salting out effect” lowering the release of some terpenes in the headspace. This disadvantage may be counteracted by increasing the amount of spieces in sausage mince formulations. As to a_w values in formulations with strong sodium reduction, an extension of processing time is suggested to achieve a more pronounced a_w decrease in final product.

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