PE4.59 Volatile compounds in dry-fermented sausage "Espetec" subjected to high pressure processing. A comparison of dynamic headspace and solid-phase microextraction. 208.00

Ana Rivas-Cañedo (1) ana.rivas@inia.es, C Juez-Ojeda(1), M Nuñez 1, E Fernández-García 1
(1)National Institute for Research on Agricultural and Food Science (INIA), Spain

***Abstract*—The effect of high pressure processing (400 MPa, 10 min, 12ºC) on the volatile profile of a low-acid fermented sausage Espetec was investigated by comparing two extraction techniques: dynamic headspace and solid-phase microextraction.**

Both techniques were suitable for monitoring the changes originated in the volatile fraction. However, SPME provided a richer volatile profile.

Minor changes were observed in the volatile fraction of Espetec after both refrigerated storage and pressure-treatment.

Lower levels of some compounds coming from citrate metabolism, namely acetoin and 2,3-butanediol, as well as those associated with lipid oxidation, such as octane, pentanal, 1-pentanol and 1-octanol, were found after storage and pressure-treatment. Diacetyl and hexanal showed significant lower levels after refrigerated storage.

The pressure treatment applied in this study might be especially indicated for extending shelf life of Espetec without remarkable changes in its aroma.

All authors are with the National Institute for Research on Agricultural and Food Science (INIA), Carretera de La Coruña, Km. 7, Madrid 28040, Spain.

Rivas-Cañedo, A. (telephone 34-91-3473955; fax 34-91-3572293; e-mail ana.rivas@inia.es).
Juez-Ojeda, C. (e-mail crisjuez@inia.es).
Nuñez, M. (e-mail nunez@inia.es).
Fernández-García, E. (e-mail fgarcia@inia.es).

***Index Terms*—dynamic headspace extraction, low-acid fermented sausages, high pressure processing, SPME, volatile compounds.**

I. INTRODUCTION

"Espetec" is one of the most consumed dry-fermented sausages in Spain. This low-acid fermented product of small diameter is manufactured from a mixture of chopped meat, lard, salt, additives, starter cultures (optional) and spices [1]. The moderate fermentation process of this meat product leads to the development of characteristic sensory properties which, together with its image of a traditional product, are very appreciated by the consumers. Although contamination is not to be expected in dry-fermented products, the slightly acidic conditions of Espetec may favor microbial growth, which could shorten its shelf life. To improve safety as well as to lengthen the shelf life the application of a non-thermal treatment such as high pressure processing (HPP) might be especially suitable for meat based products.

HPP is a relatively new processing method with increasing importance in the production of "minimally processed" foods, improving food safety due to its ability to kill microorganims and to inactivate enzymes [2,3] while retaining the characteristics of fresh products and their nutritional value [4,5]. Another advantage of HPP is its acting uniformly and nearly instantaneously throughout the food product with independence of its geometry, being especially indicated for products presented in bulks or pieces. However, HPP might cause protein denaturation, changes in enzyme-substrate interactions and changes in the polymer carbohydrates and fats [6] that could affect the final quality of dry-fermented sausages [7].

The volatile fraction of a food product is closely related to its actual aroma. Thus, the study of the volatile profile can be a useful tool to identify flavor alterations induced by any treatment. Volatile compounds can be extracted by different techniques, there not being an ideal method. Since different extraction techniques often lead to different volatile profiles [8], the combination of more than one extraction technique may be advisable.

Reports on the effect of HPP on the volatile fraction of meat and meat products are scarce, most of the identified changes being related to microbiota alterations [9,10].

The aim of the present work was to study the effect of HPP on the volatile profile of Espetec by means of two different headspace extraction techniques, viz. dynamic headspace extraction and solid-phase microextraction.

II. MATERIALS AND METHODS

2.1. Sausages and high pressure treatment. Sausages from the same manufacture batch were purchased at a local supermarket. Casings were taken off and sausages were cut into pieces of approximately 12 cm length. Eighteen equivalent pieces were wrapped in aluminum foil and successively vacuum packaged in two multilayer plastic bags (HT 3050, Cryovac Sealed Air Corporation, Milan, Italy). Six portions were immediately frozen at – 35 ± 1 ºC ("*controls*"), 6 portions were pressure-treated the following day (400

MPa, 10 min, 12 ºC, "*HPP-samples*") in a 100 L capacity discontinous isostatic press at NC Hyperbaric (Burgos, Spain) whereas the rest of the samples were kept untreated ("*refrigerated*" samples). After HPP, both refrigerated and HPP samples were storaged at 4 ºC for three days and then frozen at – 35 ± 1 ºC for 30 days.

2.2. Dynamic headspace extraction (DHE). Ten grams of sausage were homogenized in a mechanical grinder (IKA Labortechnik, Staufen, Germany) with 20 g of anhydrous sodium sulfate (Na_2SO_4) and 20 µL of an aqueous solution of 670 mg/L cyclohexanone as internal standard. An aliquot of the mixture (3.5 g) was subjected to volatile extraction in an automatic dynamic headspace apparatus (Purge and Trap, HP 7695, Agilent), coupled to GC-MS, for 20 min at 45 ºC using helium (45 mL/min) with 10 min of previous equilibration. Volatile compounds were concentrated in a Vocarb 4000 trap (Tekmar, Manson, OH) maintained at 35 ºC, with 4 min dry-purge, and desorbed during 2 min at 260 ºC through a transfer line heated at 200 ºC, directly into the injection port at 220 ºC with a split ratio of 20:1 and 1.4 mL/min helium flow.

2.3 Solid-phase microextraction (SPME). Fifteen grams of sausage, together with 15 g of Na_2SO_4 and 20 L of an aqueous solution of 670 mg/L cyclohexanone were homogenized in a mechanical grinder. Twelve grams of the final mixture were weighed in a 40 mL headspace glass vial sealed with a PTFE faced silicone septum (Supelco, Bellefonte, PA, USA). After equilibration at 40 ºC for 1 h in a thermostatic bath (D3 model, HAAKE, Berlin, Germany), the SPME fiber (2 cm x 50/30 µm Divinylbenzene/Carboxen/ Polydimethylsiloxane DVB/CAR/PDMS, Supelco, Bellefonte, PA) was exposed for 1 h to the headspace. Volatile compounds were desorbed for 10 min at 260 ºC in the GC injection port (splitless mode).

2.4. Gas chromatography - mass spectrometry (GC-MS). GC-MS analyses were carried out in triplicate in an HP-MSD HP 5973 apparatus (Agilent Technologies, Palo Alto, CA). All samples were thawed overnight at 5 ºC. A Zebron 100 % polyethylene glycol capillary column was used (60 m long; 0.25 mm i.d., 0.50 µm film thickness; ZB-WAXplus, Phenomenex, CA) with 1 mL/min helium flow. Chromatographic conditions were: GC injection port: 220 ºC for DHE and 260 ºC for SPME; temperature program: 16 min at 45 ºC, first ramp 4 ºC/min to 110 ºC, 9 min at 110 ºC, second ramp at 15 ºC/min to 230 ºC and 3 min at 230 ºC. A third ramp was added to SPME runs (10 ºC/min to 250 ºC and 2 min at 250 ºC) in order for higher boiling point compounds to elute.

Detection was performed with electron impact ionization, with 70 eV ionization energy operating in the full-scan mode (33-280 a.m.u.; 2.97 scans/s). Source, quadrupole and interface were at 230, 150 ºC and 280 ºC, respectively. Compound identification was carried out by injection of commercial standards, by spectra comparison using the Wiley7Nist05 Library (Wiley & Sons Inc., Germany), and/or by calculation of linear retention indices (LRI) relative to a serie of alkanes (C_5-C_{20}). The sum of up to four characteristic ions per compound was used for semi-quantitative determination. Areas have been referred to the IS (compound peak area multiplied by 10^3 and divided by the IS peak area).

2.5. Statistics. SPSS Win 12.0 software (SPSS Inc., Chicago, IL) was used for the analysis of variance (ANOVA), with treatment and headspace extraction method as main effects. One-way ANOVA was further performed on the treatment effect for each extraction method. Data were grouped by chemical family in order to make them more manageable.

III. RESULTS AND DISCUSSION

3.1. Volatile fraction of Espetec. Comparison of techniques. A total of 113 compounds were detected in the volatile fraction of Espetec. However, the results obtained by means of each method differed. DHE allowed the extraction of 85 compounds, whereas 94 compounds were identified by SPME (data not shown). Alkanes, diacetyl, secondary alcohols and linear aldehydes were more efficiently extracted by DHE. However, SPME extracted benzene compounds, acetoin and fatty acids more efficiently than DHE.

Fig. 1 shows the volatile profiles (relative percentages) of the main chemical families of control samples extracted by both DHE and SPME. DHE provided a volatile fraction mainly consisting of 51% of terpenoids, 15% of secondary alcohols, 7% of methyl ketones and 5 % of linear alkanes. The relative percentage of terpenoids was similar (40%) when using SPME, however, this technique provided a better extraction of acetoin and a higher percentage of methyl ketones (23%) as well as of benzene compounds (7%) and fatty acids (6%).

3.2. Treatment. Twenty-one compounds were significantly affected by the treatment. 2,3-Butanedione (diacetyl) showed significantly lower levels after 4 days of refrigerated storage ($P< 0.05$) independently of HPP (Fig. 2). 3-Hydroxy-2-butanone (acetoin) and 2,3-butanediol also decreased during storage, but not significantly ($P> 0.05$). HPP seemed to further decrease the level of these compounds during storage, although differences between refrigerated HP-treated and untreated samples were not significant. Diacetyl and its reduction products, acetoin and 2,3-butanediol, originate from the metabolism of carbohydrate and citrate by different bacteria,

especially lactic acid bacteria and staphylococci [11, 12]. Since the equilibrium between these compounds is very sensitive to the redox conditions, the changes in the redox potential induced by storage, together with the HPP killing of microorganisms may be responsible for these results.

Similar to diacetyl, hexanal decreased significantly ($P< 0.05$) after 4 days of refrigerated storage with independence of HPP (Fig. 3). Other compounds (pentanal, 1-pentanol and 1-octanol) also decreased during storage, although not significantly ($P> 0.05$). Octane and 1-octen-3-ol decreased significantly during storage only in HP treated samples. These compounds are generally associated with lipid oxidation but some of them (specifically 1-octen-3-ol) might also originate from linoleic and linolenic catabolism [13] by the action of lipolytic microorganisms, such as molds. HPP is supposed to enhance lipid oxidation; however, it seems to exist a critical pressure under which no lipo-oxidation products are formed. Some authors have suggested 500 MPa in chicken breast [14]. In the present study, mold growth might have been inhibited by the lack of oxygen during vacuum storage, which may have caused a decrease in mold related compounds. HPP could have enhanced this decrease by the killing of these microorganisms and also by the inhibition of molds´ enzymes.

IV. Conclusions

Both volatile extraction techniques are suitable for monitoring the changes occurring in the volatile fraction of dry fermented sausage caused by HPP and subsequent refrigeration. However, different information was provided by each extraction method.

HPP had a minor effect on the volatile profile of Espetec. Most of the observed changes might be related to an alteration of the microorganisms by the action of pressurization.

According to the results of this preliminary study, a pressure treatment of 400 MPa/10 min might be valuable to delay spoilage microorganisms' growth in Espetec with minimum sensory effects. However, a deeper study dealing with the effect of a long-term storage would be needed.

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Fig. 1 Relative percentages of the main chemical families of the volatile compounds of Espetec extracted by both DHE and SPME

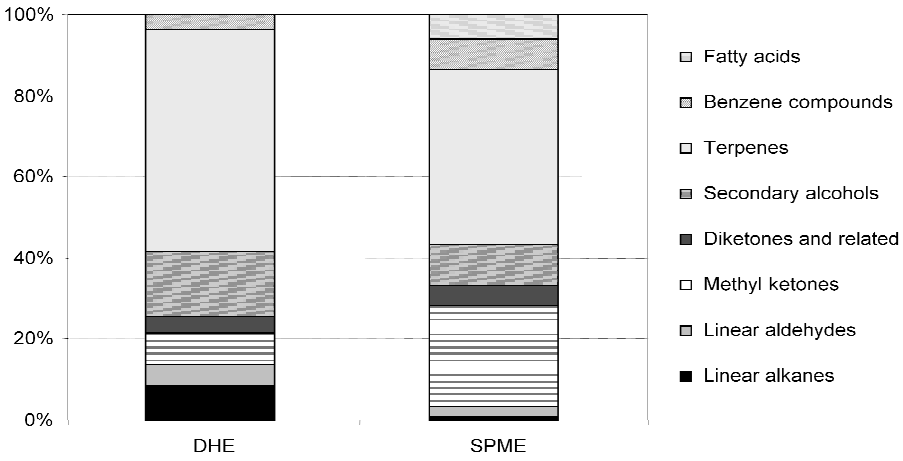
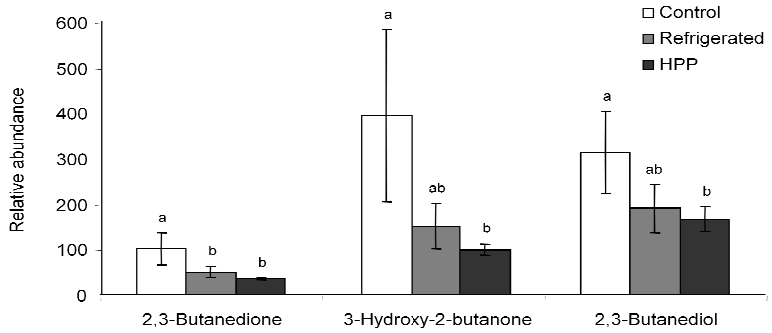
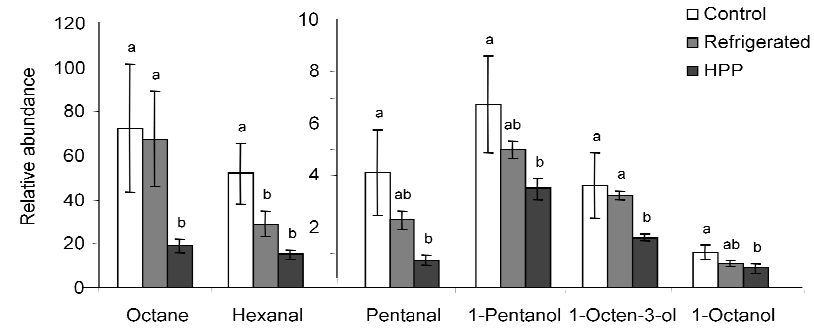


Fig. 2 Relative abundance of the volatile compounds of Espetec extracted by SPME significantly affected by the treatment



[ab] Means with different letters for each volatile compound differ significantly (P < 0.05).

Fig. 3 Relative abundance of the volatile compounds of Espetec extracted by DHE significantly affected by the treatment



[ab] Means with different letters for each volatile compound differ significantly (P < 0.05).