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Generation of aroma compounds in sourdough: Effects of stress exposure and lactobacilli–yeasts interactions

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Abstract

The effects of the interaction between *Saccharomyces cerevisiae* LBS and *Lactobacillus sanfranciscensis* LSCE1 and of their responses to acid, oxidative or osmotic stress on alcohol and aroma production were assessed. The exposure of *S. cerevisiae* LBS and *L. sanfranciscensis* LSCE1 cells to oxidative, acid or osmotic sub-lethal stress gave rise to a common or specific responses. γ-decalactone, 2(5H)-furanones and aldehydes were overproduced by LAB following oxidative stress. The acid stress induced both in yeasts and LAB, as well as in their co-cultures, a relevant accumulation of isovaleric and acetic acids and higher alcohols. A cross-exposure of yeasts and LAB to their preconditioned media, generated in *S. cerevisiae* a release of esters including esters of long-chain unsaturated fatty acids coming from membrane phospholipids. These esters were excreted also by yeasts following a pressure stress.

Keywords: Sourdough lactic acid bacteria; Sourdough yeasts; Microbiota interactions; Environmental stresses; Volatile compounds

1. Introduction

The selective pressure exerted by the environmental conditions encountered by microbial cells during sourdough fermentation, accounts for the consolidated dominance of selected species of lactobacilli and yeasts. The impact of the process variables and their changes during continuous propagation enable the association of *Lactobacillus*/yeasts to endure also for years (De Vuyst and Neysen, 2005). The nutrient availability and limitation are likely the factors that modulate the microbial ecology of sourdough. However, within the sourdough ecosystem there are numerous mechanisms whereby one species may influence the growth of another species. In fact, lactobacilli and yeast growth generates an array of metabolites some of which are toxic or favourable to other species. The concept of quorum sensing, as a mechanism by which microbial cells communicate with each other and regulate population growth has yet to be considered in the context of systems as wine (Bisson, 1999) and sourdough both characterized by the co-fermentation of yeasts and lactobacilli. Quorum sensing represents a new concept in microbial ecology whose significance in sourdough fermentation and generation of aroma compounds has to be deeply investigated. It has been reported that yeasts like *Saccharomyces cerevisiae* or *Candida albicans* co-opt their own metabolites like isoamyl and isobutyl alcohols or tyrosols and terpenes, regarded as important key odorants for fermented foods, to promote and coordinate, when nutrient become limiting, cell differentiation (Lorenz et al., 2000; Hornby et al., 2001).

The incomplete knowledge of physiological responses when yeasts and lactobacilli undergo a variety of stresses, including their metabolite-mediated interactions, makes their performance somewhat difficult to predict. It is known that the exposure of microbial cells to stressful conditions during fermentation process involves a broad transcriptional response with up to hundreds of induced or repressed genes (Erasmus et al., 2003). The complex network of such responses, involving several metabolic activities will reflect upon the composition and organoleptic properties of the dough and final products. This paper was focused on the study of the effects of some stresses on: (1) the release of flavour components by individual cultures and co-cultures of *Lactobacillus sanfranciscensis* and

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S. cerevisiae and (2) the metabolite-mediated interactions lactobacilli and yeasts in model and real systems.

2. Methods

2.1. Strains and growth media

L. sanfranciscensis strain LSCE1 and *S. cerevisiae* strain LBS belong to Dipartimento di Scienze degli Alimenti (DISA) of Bologna University (Italy).

Cell viability of LSCE1 was verified by plate counting on sourdough bacteria medium (SDB) (Kline and Sugihara, 1971) containing 0.1 g/L of cycloheximide after incubation at 30 °C for 72 h in anaerobiosis (Anaerocult A system, VWR International, Milan, Italy). Yeasts were counted on Sabouraud Dextrose Agar (SDA, Oxoid, Basingstoke, UK) containing 0.2 g/L chloramphenicol after incubation at 28 °C for 72 h.

2.2. Stress exposure

The medium used for the stress exposure was wheat flour hydrolysed (WFH) broth (Gobbetti et al., 1994). Overnight cells of *L. sanfranciscensis* and *S. cerevisiae*, respectively, grown in SDB at 30 °C and Sabouraud Dextrose Broth (Oxoid, Basingstoke, UK) at 28 °C, were collected and inoculated, in order to obtain a cell density, respectively, of 8 ± 0.3 and 7 ± 0.2 log cfu/ml, in WFH (control) and WFH acidified with lactic acid (pH 3.6) (acid stress), WFH added with H_2O_2 5 mM (oxidative stress) or sucrose 40% (w/v) (osmotic stress). After 2 and 12 h of incubation at 30 °C the cell suspensions were centrifuged (2500*g* for 10 min, 4 °C) and the supernatants (CMs) were used for cross-exposure and GC–MS/SPME analysis.

2.3. Cross-exposure of the two strains to conditioned media (CMs)

Overnight cells of *L. sanfranciscensis* and *S. cerevisiae*, respectively, grown in SDB at 30 °C and Sabouraud Dextrose Broth at 28 °C were centrifuged as above described. The cells of LSCE1 and LBS were resuspended for 120 min at 30 °C, at a concentration, respectively, of 8 ± 0.4 and 7 ± 0.3 log cfu/ml, in the various CMs obtained as above described, after the growth of LBS and LSCE1, respectively. After 2 h of exposure at 30 °C the suspensions were centrifuged as above described and analysed with gaschromatography–mass spectrometry-solid phase microextraction (GC–MS/SPME).

2.4. Solid system (dough)

The characteristics of the wheat flour and the dough making process were the same adopted by Di Cagno et al. (2003). Overnight cells of *L. sanfranciscensis* LSCE1 and *S. cerevisiae* LBS, respectively, grown in SDB at 30 °C and Sabouraud Dextrose Broth (Oxoid, Basingstoke, UK) at 28 °C were collected and inoculated (individually or co-inoculated), in order to obtain a cell density, respectively of 7.8 ± 0.5 and 7.1 ± 0.3 log cfu/g, in dough (control) and dough acidified with lactic acid (pH 3.6) (acid stress), dough added with H_2O_2 5 mM (oxidative stress) or sucrose 40% (w/v) (osmotic stress). The fermentation of the different doughs, having dough yield 146, was carried out at 30 °C. During the fermentation the different samples were periodically analysed in order to obtain the GC–MS/SPME profiles.

2.5. GC–SPME analysis and mass spectrometry

A divinylbenzene/carboxen/polydimethylsiloxane coated fibre (65 μm) and a manual SPME holder (Supelco Inc., Bellefonte, Pennsylvania, USA) were used in this study after preconditioning according to the manufacturer's instruction manual. Before each head space sampling, the fibre was exposed to the GC inlet for 5 min for thermal desorption at 250 °C in a blank sample. In total, 3 ml of the liquid medium (WFH) or 3 g of solid system (dough) were placed in 10 ml vials and the vials sealed. The samples were then equilibrated for 10 min at 60 °C. The SPME fibre was exposed to each sample for 20 min and finally the fibre was inserted into the injection port of the GC for a 5 min sample desorption. These conditions were chosen on the basis of previous experiments with pure compounds added to dough and analysed after different times and temperatures (45, 50, 60, 70, 75 °C).

GC–MS analyses were carried out on an Agilent 6890 gaschromatograph (Agilent Technologies, Palo Alto, California, USA) coupled to an Agilent 5970 mass selective detector operating in electron impact mode (ionization voltage 70 eV). A Chrompack CP-Wax 52 CB capillary column (50 m length, 0.32 mm ID) was used (Chrompack, Middelburg, The Netherlands). The temperature programme was: 50 °C for 2 min, then programmed at 1 °C/min to 65 °C and finally at 5 °C/min to 220 °C which was maintained for 22 min. Injector, interface and ion source temperatures were 250, 250 and 230 °C, respectively. Injections were performed in splitless mode and helium (1 ml/min) was used as carrier gas. Compounds were identified by use of available mass spectra databases (NIST/EPA/NIH Vers. 1998 and Wiley Vers. 1996). The quantification of ethanol was performed by means of calibration curve prepared using known concentrations of pure standard (Sigma–Aldrich, Milan, Italy).

2.6. Statistical analysis

The data reported are the means of three repetitions. Standard deviation (SD) is represented on graphs as error bars. The statistical treatment of data was performed using Microsoft Office Excel 2002 version.

3. Results

3.1. Ethanol production

All the stress conditions considered did not affect *L. sanfranciscensis* and *S. cerevisiae* viability (data not shown).

In Table 1, the extent of ethanol produced in 2 h by cells of *S. cerevisiae* and *L. sanfranciscensis*, when incubated under the various stress conditions in WFH, and after 4 and 12 h in dough are reported. In addition ethanol produced in 12 h in WFH co-inoculated with *L. sanfranciscensis* and *S. cerevisiae* is reported.

Both *L. sanfranciscensis* and *S. cerevisiae* produced small amount of alcohol when individually inoculated in WFH. However, when *S. cerevisiae* was inoculated in cell free conditioned media (CMs), in which *L. sanfranciscensis* had grown, a significant amount of ethanol was produced particularly in the control. The enhancing effect was observed also when the two strains were co-inoculated in WFH after 12 h. In the control and under oxidative stress *S. cerevisiae* produced more ethanol in dough than in WFH. However, its co-inoculum in dough with *L. sanfranciscensis* enhances its performance particularly under acid stress. This effect was more evident after 4 h than after 12 h. It is interesting to observe that under osmotic conditions the co-inoculum favoured ethanol production in WFH but not in dough.

3.2. Effects of stress factors on the release of flavour compounds

Fig. 1a reports the metabolic profiles of *L. sanfranciscensis* when fresh cells were inoculated for 2 h in the various modified WFH. Due to scale problem the ethanol was not included in all GC-profiles. The metabolites release was remarkably affected by the conditions. In particular, the acid stress gave rise to a relevant increase of isovaleric and medium chain fatty acids (FAs). Moreover two molecules, whose release has been recently reported in *L. helveticus*, were identified (Ndagijimana et al., 2006). They are, according to the following mass spectrometry data, 4-methyl-3-hydroxy-2(5H)-furanones with an alkyl substituent in position 5, named from now on as furanone A and furanone B. Their characteristic ion fragments were, respectively, 41(39), 57 (80), 67 (28), 69 (25), 79 (18), 85 (8), 97 (100), 99 (73), 109 (40), 123 (7), 143 (9) and 43 (28), 57 (47), 69 (11), 83 (17), 97 (100), 111 (10), 123 (16), 137 (10), 151 (4), 165 (4), 180 (10).

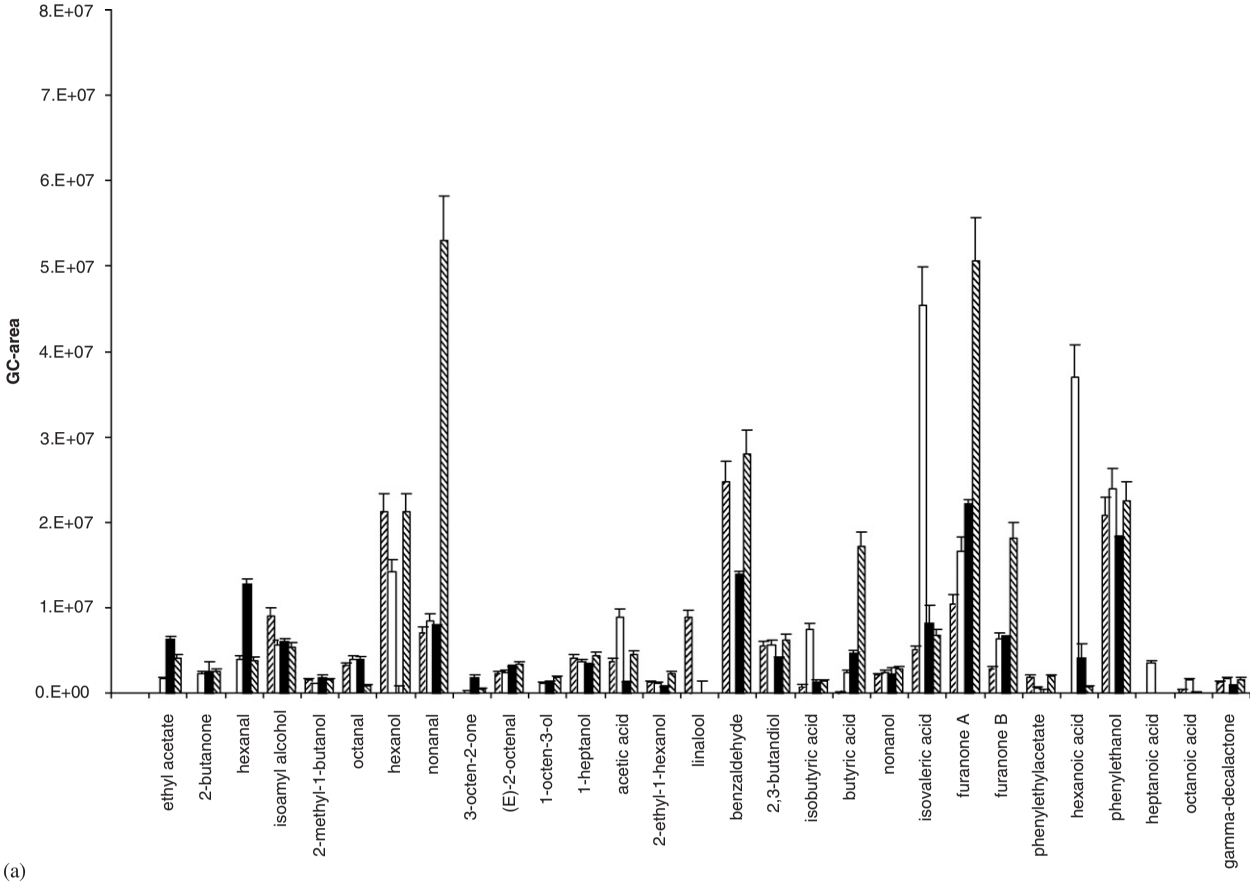
The release of these two molecules was particularly elevated when the cells were exposed to oxidative stress. This latter condition, caused also the accumulation of aldehydes such as nonanal and benzaldehyde, and of some metabolites such as ethyl acetate, 2-butanone, hexanal, 1-octen-3-ol, heptanoic acid and γ-decalactone which were absent in the control. When the cells were exposed to the above reported conditions in dough systems, acetic acid, acetoin, furanone B and ethanol were the most important compounds (data not shown). However, their production was quantitatively reduced (by 10–20-fold) with respect to WFH and not affected by the stress condition.

The metabolites produced by *S. cerevisiae* LBS, exposed for 2 h to the various stresses in WFH, are shown in Fig. 1b. Isovaleric acid was the principal metabolite released when the yeast cells were exposed to acid and osmotic stresses. This molecule was associated to the presence of medium chain FAs. In contrast with what has been observed with *L. sanfranciscensis*, *S. cerevisiae* produced more metabolites such as ethanol, isobutanol, phenylethanol when inoculated in dough than in WFH (Fig. 2). The release of these molecules by yeasts decreased with the stress exposure and especially they tend to disappear under osmotic stress. In fact, the ethanol accounted for 0.138–2.8 g/L under different experimental conditions in WFH and for 0.051–12.38 g/kg in different experimental conditions in dough.

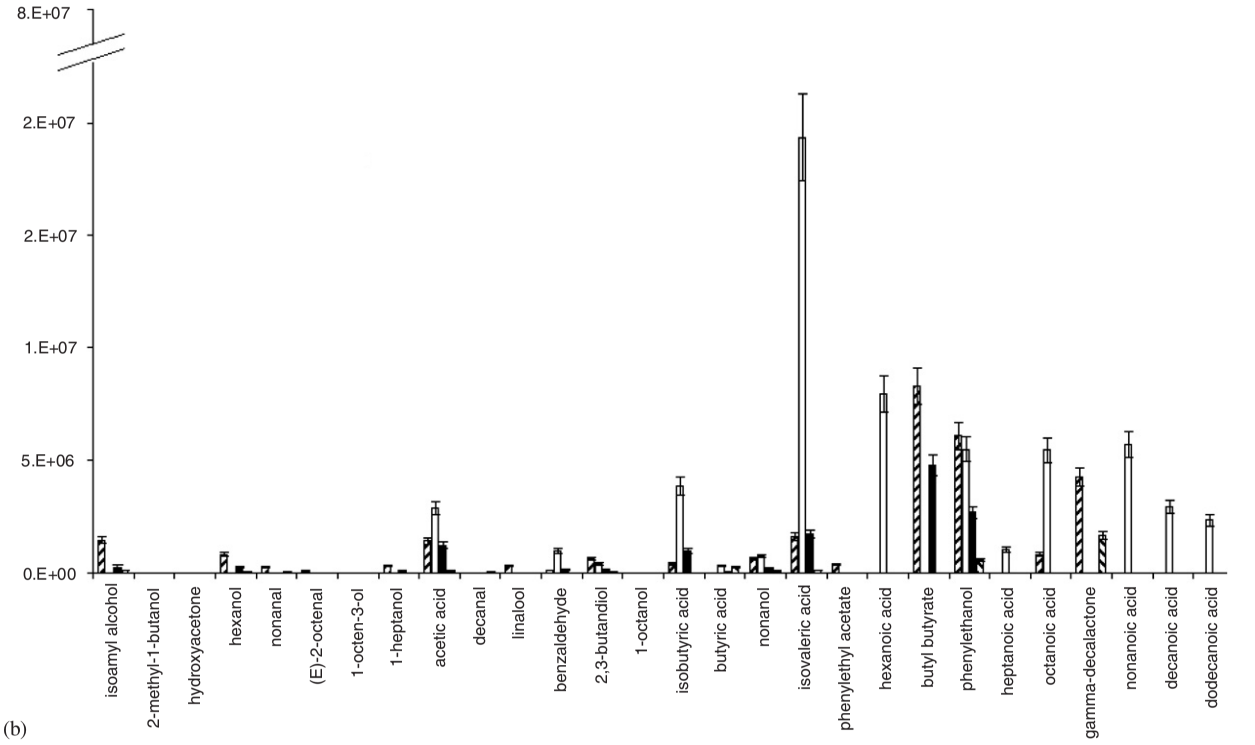
Table 1
Ethanol detected (as g/L or g/kg) in WFH and dough inoculated with *L. sanfranciscensis* LSCE1 and/or *S. cerevisiae* LBS under different stress conditions

WFH	Incubation time (h)	Control	pH 3.6	40% sucrose	$H_2O_{25\,mM}$
LSCE1 in WFH	2	0.06±0.01	0.09±0.01	0.13±0.01	0.06±0.01
LSCE1 in CMs[a] of LBS	2	0.09±0.01	0.01±0.006	0.06±0.01	0.05±0.01
LBS in WFH	2	0.11±0.01	0.06±0.01	0.15±0.02	0.01±0.006
LBS in CMs of LSCE1	2	2.85±0.28	1.66±0.17	1.67±0.17	1.64±0.16
LBS + LSCE1 co-inoculated	12	3.12±0.31	3.04±0.31	9.94±0.99	2.52±0.25
DOUGH					
LSCE1	4	0.17±0.02	0.02±0.007	n.d	0.04±0.003
LBS	4	6.28±0.63	n.d[b]	0.36±0.04	5.60±0.59
LBS + LSCE1 co-inoculated	4	9.64±0.96	9.80±1.28	n.d	9.94±1.49
LSCE1	12	0.05±0.01	0.03±0.008	n.d	n.d
LBS	12	9.40±0.94	8.56±0.86	0.12±0,01	8.50±0.85
LBS + LSCE1 co-inoculated	12	9.50±0.95	7.41±0.76	0.11±0.01	7.24±0.72

[a]CMs = cell free supernatants of *L. sanfranciscensis* or *S. cerevisiae* cultures previously exposed to acidic, osmotic or oxidative stress conditions.
[b]Under the detection limit.



(a)



(b)

Fig. 1. (a) SPME–GC profiles of *L. sanfranciscensis* (LSCE1) after 2 h of stress exposure condition: (▨) WFH control, (□) WFH acidified to pH 3.6, (■) 40% sucrose, (▩) H_2O_2 5 mM. (b) SPME–GC profiles of *S. cerevisiae* LBS after 2 h of stress exposure condition: (▨) WFH control, (□) WFH acidified to pH 3.6, (■) 40% sucrose, (▩) H_2O_2 5 mM.

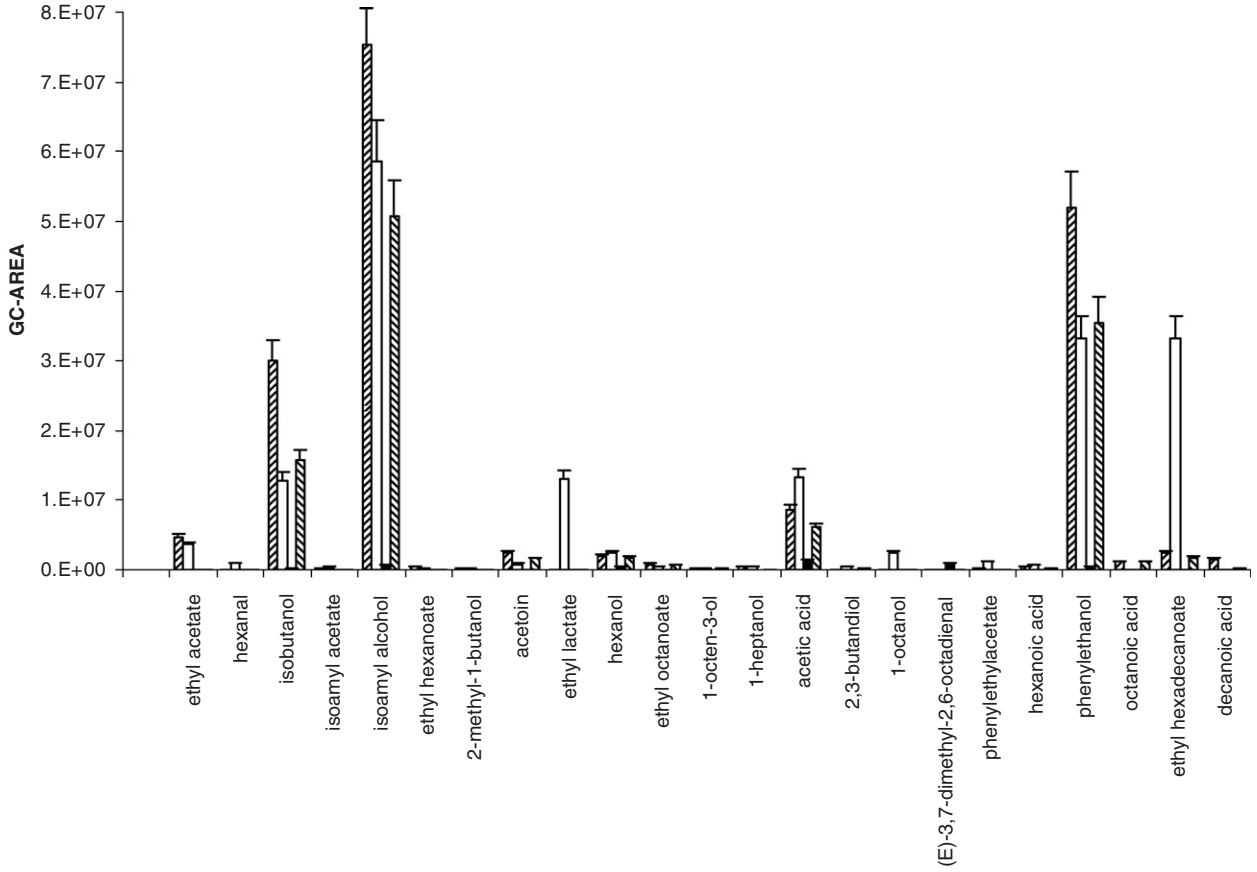


Fig. 2. SPME–GC profiles of *S. cerevisiae* LBS after 2 h of inoculum in: (▨) dough control, (□) acidified to pH 3.6, (■) 40% sucrose, (▨) H_2O_2 5 mM.

Fig. 3a reports the comparison of the metabolic profiles of fresh cells of *S. cerevisiae* (7 log cfu/ml) exposed for 2 h to the various CMs in which *L. sanfranciscensis* had been previously incubated for 2 h. The most important effect of the exposure to the various CMs was the release of decanoic and dodecanoic acid and of ethyl esters of the mono unsaturated FAs *cis*-9-hexadecenoic acid (C16:1Δ9) and *cis*-11-hexadecenoic acid (C16:1Δ11).

The accumulation of these esters was enhanced when *S. cerevisiae* was exposed to CMs of cultures in which *L. sanfranciscensis* had been exposed to oxidative or acid stresses.

When *L. sanfranciscensis* was incubated for 2 h in CMs, in which *S. cerevisiae* (Fig. 3b) had been previously inoculated an increase of isovaleric acid and the furanone A and furanone B, with respect to the CM profiles (Fig. 1b) in the control and under the various stress conditions, was observed. Moreover, octanoic and decanoic acid were the most important metabolites in the control.

3.3. Co-culture conditions in WFH and dough

Figs. 4a and b report the comparison of the SPME–GC profiles of the metabolites released after 12 h, in WFH and dough, when *S. cerevisiae* and *L. sanfranciscensis* were co-inoculated. The prevalent metabolites released by co-cultures of *S. cerevisiae* and *L. sanfranciscensis* in WFH were different from those occurring in dough under the same conditions. Higher alcohols and acetic acid were dominant in dough while medium, long-chain FAs and their esters, as well as medium chain FAs and isovaleric acid were the most important volatiles in WFH. In the latter system acetic acid was the dominant molecule under sugar stress.

The presence of the esters of the long-chain FAs C16:1Δ9 and C16:1Δ11 and medium chain FAs, generally regarded as associated with biosynthesis or breakdown of FAs, confirmed that their excretion is associated with a response of *S. cerevisiae* to *L. sanfranciscensis* or its metabolites. The co-culture of *S. cerevisiae* and *L. sanfranciscensis* in WFH remarkably enhanced the effect of some stress factors with respect to the solid medium. In particular, the exposure to osmotic stress induced about 20-fold release of acetic acid, isoamyl alcohol, ethyl octanoate, ethyl decanoate, ethyl dodecanoate and phenylethanol and of ethyl esters of C16:1Δ9 and C16:1Δ11.

4. Discussion

Sourdough microflora is usually composed of stable associations of lactobacilli and yeasts based on their requirement and metabolic interactions. The study of the

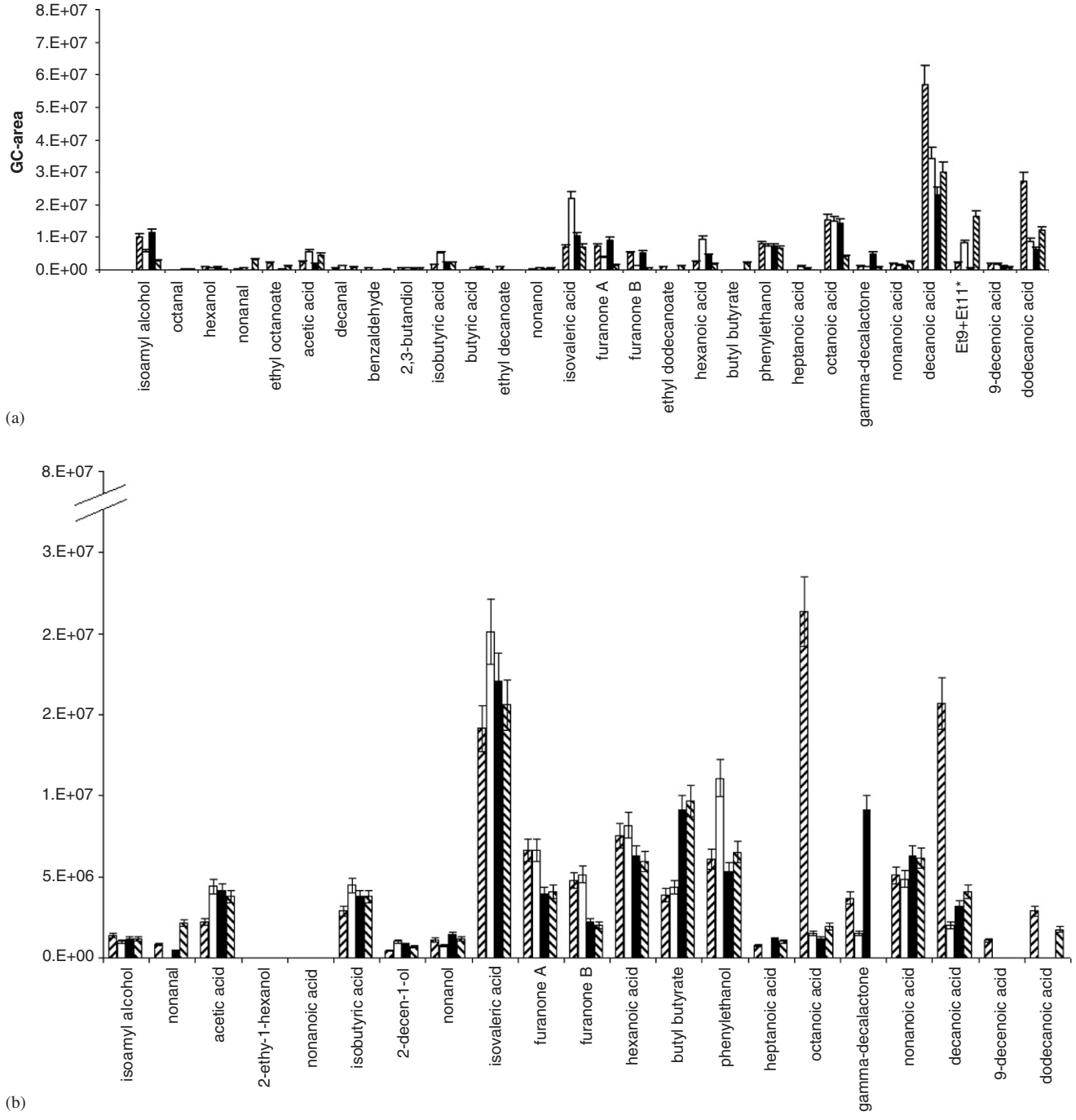


Fig. 3. (a) SPME–GC profiles of *S. cerevisiae* LBS inoculated, for 2 h, in various CMs in which *L. sanfranciscensis* LSCE1 was previously exposed to different stresses in WFH: (▨) CM from WFH control, (□) CM from WFH acidified to pH 3.6, (■) CM from WFH added with 40% sucrose, (▧) CM from WFH added with H_2O_2 5 mM. *Et9 + Et11 = ethyl esters of C16:1Δ9 and C16:1Δ11. (b) SPME–GC profiles of *L. sanfranciscensis* LSCE1 inoculated, for 2 h, in various CMs in which *S. cerevisiae* LBS was previously exposed to different stresses in WFH: (▨) CM from WFH control, (□) CM from WFH acidified to pH 3.6, (■) CM from WFH added with 40% sucrose, (▧)CM from WFH added with H_2O_2 5 mM.

antagonistic and synergistic interactions between the two microbial groups has been prevalently focused on the metabolism of carbohydrates and aminoacids (De Vuyst and Neysen, 2005). However, bacteria and yeasts can produce an extensive repertoire of secondary metabolites and can respond to a wide variety of chemicals in their environment. In recent years, particular families primary and secondary metabolites have been characterized for their role in the regulation of gene expression in a cell/density dependant manner (Keller and Surette, 2006).

The principal aim of this work was to study the physiological alterations of *L. sanfranciscensis* and *S. cerevisiae* when exposed in model and real systems to

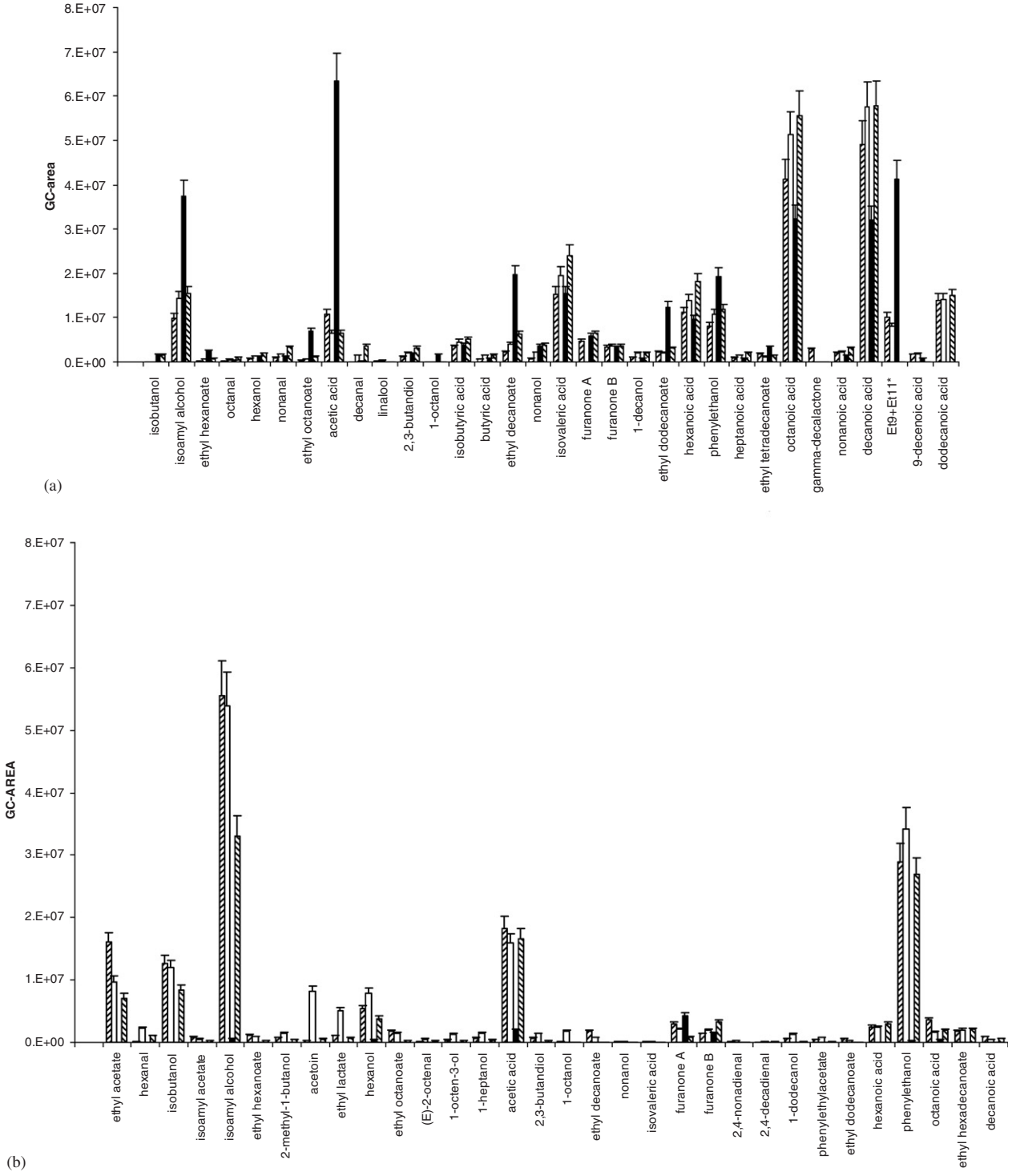


Fig. 4. (a) SPME–GC profiles of *S. cerevisiae* LBS and *L. sanfranciscensis* LSCE1 co-inoculated for 12 h in WFH: (▨) WFH: control, (□) WFH acidified to pH 3.6, (■) 40% sucrose, (▧) H_2O_2 5 mM. Et9 + Et11 = ethyl esters of C16:1Δ9 and C16:1Δ11. (b) SPME–GC profiles of *S. cerevisiae* LBS and *L. sanfranciscensis* LSCE1 co-inoculated for 12 h in dough: (▨) dough control, (□) acidified to pH 3.6, (■) 40% sucrose, (▧) H_2O_2 5 mM.

chemico-physical perturbations or to their reciprocal cell/cell, metabolites/cells interactions.

The SPME GC–MS approach, which is, able to analyse, identify and quantify a wide range of chemicals having molecular weight up to 200–290, as depending on their chemical composition, was adopted. It provided different metabolic fingerprints accounting for the responses of *L. sanfranciscensis* and *S. cerevisiae* to sub-lethal stress exposure. In addition to primary metabolites like ethanol, 2,3 butandiol, acetic acid, etc., some molecules whose physiological origin and potential role have to be better elucidated, were overproduced under specific conditions.

In particular an overproduction of isovaleric acid, as a specific response to acid stress, was observed both in *L. sanfranciscensis* and *S. cerevisiae* but only in the liquid medium WFH and not in dough. Isovaleric acid is associated, like isobutyrate, 3-methyl butanal and 2-methyl butanal, with branched-chain aminoacid metabolism and is regarded as a key odorant in various fermented foods and particularly cheeses. Its sensorial contribution to bakery products has not been yet evaluated. It derives from the oxidation, by aldehyde dehydrogenase, of 3-methyl butanal which is a precursor of 3-methyl butanol. In its turn, isovaleryl-CoA it is a priming substrate for the branched chain FAs associated with the membrane phospholipids (PL) of several bacteria (Ratledge and Wilkinson, 1989; Zhu et al., 2005). A signalling role of isovaleric acid in the dormancy release of *Agaricus bisporus* has been also reported (Lösel, 1989).

Also medium chain FAs having a carbon chain number from six to ten in *L. sanfranciscensis* and from 6 to 12 in *S. cerevisiae* were accumulated under acid conditions in WFH. Only traces of these FAs were released in dough. Medium-chain FAs were also released by *L. sanfranciscensis* and *S. cerevisiae* during cross-exposure to their cell free CM and when the two species were co-inoculated in WFH. The FA released by cells exposed to chemico-physical stresses has been associated with an interrupted biosynthesis of FAs in yeasts cultures or to a peroxidation of PL integrated unsaturated FA following an oxidative stress and subsequent degradation (Guerzoni et al., 1999). In fact hydroperoxidation of monounsaturated FAs such as C18:1 results in medium chain FAs and aldehydes formation (Priault et al., 2002). Medium-chain FAs could be involved in communication phenomenon. In fact it has been reported that in *Myxococcus xanthus* a mixture of straight and branched FAs has an autocide activity (Rosenbluh and Rosenberg, 1993).

Both the cross-exposure of *S. cerevisiae* to cell free CMs, in which cells of *L. sanfranciscensis* had been incubated under the various stress conditions, and the co-inoculum of the two strains in WFH gave rise to release of ethyl esters of the unsaturated long-chain FAs C16:1Δ9 and C16:1Δ11. In WFH co-inoculated with the two strains these esters were accompanied by minor extent of ethyl tetradecanoate, ethyl dodecanoate, ethyl decanoate (particularly under osmotic stress) while the co-inoculum of *S. cerevisiae* and *L. sanfranciscensis* in dough gave rise to ethyl decanoate, ethyl dodecanoate, ethyl hexadecanoate, but not to C16:1Δ9, C16:1Δ11 esters. It has been reported that the presence of reactive oxygen species, generated when cell are subjected to sub-lethal injury (Dodd et al., 1997), induces the release of long-chain unsaturated FAs and their peroxidation products (Kocsis and Weselake, 1996). In the fission yeasts long-chain free FAs trigger apoptosis mediated by diacylglycerol (Zhang et al., 2003). Therefore, the physiological rationale of the esterification of C16:1Δ9, C16:1Δ11 and medium chain FAs and of their release can be identified in an homeostasis mechanism to reduce the deleterious effects of accumulated FAs. In *S. cerevisiae,* the synthesis of ethyl esters of unsaturated FAs implies an "a priori" deacylation of the FAs from membrane PL. In fact, double bonds are introduced in aliphatic acyl-chains of saturated FAs when the latter are already incorporated in the membrane PL (Keweloh and Heipieper, 1996). A release of ethyl esters of long-chain FAs by *S. cerevisiae* when cells were subjected to high pressure homogenization treatment has been reported (Guerzoni et al., 1999). This provide further evidence that these esters and their acyl moieties come from damaged cells. New light on the possible physiological role of ester synthesis, including signalling pathways, has been provided by recent research on *S. cerevisiae* (Black and Di Russo, 2006; Verstrepen et al., 2003). Esters of long-chain FAs have been identified as signalling molecules also in higher organisms (Thoma et al., 2003).

If the C16:1Δ9 and C16:1Δ11 esters release can be regarded as a stress marker, the interaction between *L. sanfranciscensis* and *S. cerevisiae*, also mediated by their metabolites, can results in the improvement of fermentative performances as indicated by the ethanol production increase.

The incubation of *S. cerevisiae* in CMs in which *L. sanfranciscensis* had been grown, enable the former to produce significant ethanol amount. When it was individually inoculated in WFH having a high sugar concentration the ethanol production was inhibited.

Also the release of two 2(5H)-furanones can be regarded as a specific response of *L. sanfranciscensis* particularly under an oxidative stress. They had been previously detected in whey in which *L. helveticus* had been exposed to oxidative stress and met a number of criteria to be included into cell/cell signalling molecules (Ndagijimana et al., 2006). In particular, these metabolites have a biological effect on cells of the same species and the exposure of fresh cells of *L. helveticus* to CMs containing these molecules induced the production of known and new autolysins and cell morphological changes (Ndagijimana et al., 2006).

The overproduction of the furanones A and B under oxidative stress and the contemporaneans release of medium chain FAs, as well as, the already reported formation of epoxides by *L. helveticus* following oxidative stress (Guerzoni et al., 2001), suggest that epoxidated chain membranes FAs may be precursors of the two 2(5H)-furanones. It is known that in many organisms, reactive oxygen species, such as H_2O_2 and superoxide ion, are produced under many physiological conditions. They can oxidize unsaturated acyl chain integrated in membrane phospolipids. 2(5H) furanones can be subsequently produced throughout a sequence of shortening by β oxidation and lactonization reactions. In fact also γ-decalactone was produced in WFH under oxidative, osmotic and acid stress by *L. sanfranciscensis*. The precursors of lactones were proved to be hydrolysed FAs of C18 chain length through the above-reported mechanisms (Waché et al., 2003). The 2(5H)-furanones, likewise the 2(3H)-furanones are

important key odorants for a wide range of fermented foods including bakery products (Hodge et al., 1963). Also, γ-decalactone has a dinstinct fruity flavour and is a key aroma compound.

In general, a possible signalling role can be attributed to the major part of the molecules released in WFH after stress exposure. On the other hand, their accumulation in dough was often negligible and less dependant on the stress conditions. It is well known that the micro-structure of food systems affects the microbial growth performances (Guerzoni et al., 1997).

Up to now, insufficient attention has been paid to the ecological explanation of why bacteria produce signalling molecules and respond to both intraspecific and interspecific signals. However, it has been suggested that signalling molecules function as probes to provide individual cells with information about the diffusive properties of the immediate environment. Consequently, the reduction of the extent and number of the metabolites released by *L. sanfranciscensis* in dough, respect to WFH, could be due to the interference of the solid phase of dough with the cellular mechanisms of release, diffusion and communication.

The major part of the molecules produced, in WFH, by *L. sanfranciscensis* and *S. cerevisiae* under stress conditions or when cross-exposed to their CM, can have an organoleptic impact whose extent has to be better investigated in the baked products. In fact, on the basis of the retention times and molecular characteristics of phenyl ethyl alcohol, furanones, lactones or long-chain FAs and their esters, it can be assumed that they can be retained or complexated by the solid matrix during baking (Heinemann et al., 2001).

The study of the metabolites released, as a consequence of the stress exposure, can contribute to the understanding of the mechanisms which regulate the microbial interactions and the metabolic alterations induced by stress. Moreover, this approach can be useful to identify technological conditions inducing micro-organisms to produced desirable metabolites. Appropriated taylor made sequences of individual or multiple inocula of lactobacilli and yeasts can result in fact in the generation of flavouring molecules or aroma precursors and can contribute to rationalize the process and optimize the products.

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