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ScienceDirect
Food Chemistry 108 (2008) 1217–1225
Food Chemistry
www.elsevier.com/locate/foodchem

Influence of starch addition and dough microstructure on fermentation aroma production by yeasts and lactobacilli

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Received 17 February 2007; received in revised form 15 June 2007; accepted 27 June 2007

Abstract

The work hypothesis of this paper was that during fermentation processes the chemico-physical interactions of the microbial metabolites with food matrix affects not only their retention, but the metabolic activity of the microorganisms. The influence of starch addition to liquid fermentation systems simulating sourdough and inoculated with pure and mixed population of *Saccharomyces cerevisiae*, *Candida milleri* and *Lactobacillus sanfranciscensis* significantly enhanced the production of selected metabolites. Moreover the starch addition interfered with the response of *S. cerevisiae*, *C. milleri* and *L. sanfranciscencis* when exposed to conditioned media of *L. sanfranciscensis* resulting in an increasing production of alcohols, isovaleric acid and of a key odorant like γ-decalactone. Under real conditions, the microstructure of the dough matrix significantly affected the metabolites production and cell activity.

Keywords: Sourdough; Flavour compounds; *Lactobacillus*; Yeasts

1. Introduction

Extensive research has been carried out to study the essential role of food ingredients and their properties in the modification of the microstructure and textural properties as well as their physicochemical interactions with food aroma compounds (Terta, Blekas, & Paraskevopoulou, 2006). In particular it has been shown that, in several food systems, polysaccharides such as starch are involved in the retention of a wide spectrum of ligand molecules, for instance flavour compounds (Guichard, 2002; Heinemann, Conde-Petit, Nuessli, & Escher, 2001; Heinemann, Escher, & Conde-Petit, 2003). Linear ligands are thought to be located in the hydrophobic cavities of the amylose helix. In contrast bulky ligands, such as *n*-butanol and *n*-pentanol, may be located between the amylose helices (Helbert & Chanzy, 1994).

The partitioning of the molecules between different phases of a food system is based principally on two mechanisms (Terta et al., 2006): (1) diffusion decrease, as predicted by Stokes–Einstein equation where diffusion is inversely proportional to viscosity and (2) specific molecular interactions ligands/matrix with complexation, encapsulation and hydrogen bonding. The importance of these phenomena on the flavour retention, release and perception by consumers has been extensively investigated (Goubet, Le Quere, & Volley, 1998; Heinemann et al., 2003). An additional, but not less important, effect of the interactions matrix/ligands should be involved in the regulation of the fermentation processes in solid or viscous systems. In fact in liquid systems the metabolites production by starter and non starter microorganisms, follows dynamics which are described as exponential in presence of limiting factor: the accumulation of volatile metabolites like alcohols, esters, aldehydes, etc. is a limiting factor for the fermentation performances. On the other hand, in viscous systems such as dough or sour-

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doi:10.1016/j.foodchem.2007.06.050

dough for bakery products the bacterial cells or yeasts become entrapped within the matrix. It can be hypothesized that slow nutrient and metabolite diffusion to and from cells affects their fermentation performance making the effects of the food microstructure possibly as relevant to microbial growth as environmental factors such as temperature, Aw or pH.

The work hypothesis of this preliminary paper is that in liquid systems added with hydrocolloids or viscous systems, characterized during fermentation by the continuous uptake of nutrients and release of microbial metabolites or signalling molecules (Guerzoni, Vernocchi, Ndagijimana, Gianotti, & Lanciotti, 2007), the chemicophysical interactions of the volatile molecules produced by microrganisms with the various constituents of the matrix will affect not only the retention of the aroma compounds and their sensorial perception, but also the ability of the various microbial species to survive, predominate and produce metabolites. The inclusion or complexation of metabolites in the gel matrix can dramatically reduce their vapour pressure and their inhibiting effect on microbial cells (Caccioni, Gardini, Lanciotti, & Guerzoni, 1997; Gardini, Lanciotti, Caccioni, & Guerzoni, 1997; Guerzoni, Nicoli, Massini, & Lerici, 1997). In fact the ability of some food components such as proteins, fats and starch to neutralize the antimicrobial activity of natural compounds, including metabolites, has been described in other systems (Devlieghere, Vermeiren, & Debevere, 2004). A model system simulating sourdough characterized by different sugar concentrations was added with an hydrocolloid and particularly starch in order to study its interaction with microbial flavour production and metabolite-mediated relationship between lactic acid bacteria (LAB) and yeasts. Moreover the technological properties of the dough have been modulated in order to simulate different industrial conditions and particularly dough yield (DY) values. In fact, according to the demand of production facilities and flavour preferences of different countries and regions a broad variety of sourdough processes, including liquid fermentation, have been developed (Carnevali, Ciati, Leporati, & Paese, 2007). However the importance of the dough microstructure or polysaccharides on fermentation performances have not yet been extensively studied.

2. Materials and methods

2.1. Strains and growth media

Lactobacillus sanfranciscensis LSCE1 strain, *Saccharomyces cerevisiae* LBS strain, *Candida milleri* LBC strain used in this work belong to Dipartimento di Scienze degli Alimenti (DISA) of Bologna University (Italy).

Sourdough bacteria medium (SDB) (Kline & Sugihara, 1971) and Sabouraud Dextrose (Oxoid, Basingstoke, UK) were used, respectively, for the growth of *L. sanfranciscensis* (30 °C) and the two yeasts (28 °C).

Cell viability of LSCE1 was evaluated by plate counting on SDB 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. Model system simulating sourdough

The medium used for the growth and the stress exposure of *L. sanfranciscensis* was wheat flour hydrolyzed (WFH) broth (Gobbetti, Corsetti, & Rossi, 1994). Overnight cells of *L. sanfranciscensis* LSCE1 strain, grown in SDB, were inoculated (5 log CFU/ml) in WFH control, WFH added with sucrose 40% (w/v) (osmotic stress), WFH control with 10 g/l of starch (potatoes starch) and WFH added with sucrose 40% (w/v) (osmotic stress) and with 10 g/l of starch. After 10 h of incubation at 30 °C the cell suspensions were centrifuged (2500*g* for 10 min, 4 °C) and filter-sterilized. The cell free supernatants (CMs) were then used for GC–MS/SPME analysis and subsequent cross exposure of yeasts and *L. sanfranciscensis*.

For the latter experiment, overnight cells of *S. cerevisiae* and *C. milleri* or *S. cerevisiae*, *C. milleri* and *L. sanfranciscensis* were resuspended, at a concentration respectively of 8 ± 0.4 log CFU/ml for lactobacilli and 7 ± 0.3 log CFU/ml for yeasts in the various CMs obtained as above described. After 4 h of exposure at 30 °C the suspension, after cell removal by centrifugation (2500*g* for 10 min at 4 °C), were analyzed with gas chromatography–mass spectrometry–solid phase microextraction (GC–MS/SPME).

2.3. 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 strain and *S. cerevisiae* LBS strain respectively grown in SDB at 30 °C and Sabouraud Dextrose Broth (Oxoid, Basingstoke, UK) at 28 °C were collected and individually inoculated, in order to obtain a cell density, respectively of 7.8 ± 0.5 log CFU/g and 7.1 ± 0.3 log CFU/g, in dough with different dough yield (DY) values (146, 165, 190 and 220). The DY is expressed as dough amount, as percentage, per 100 g flour. The fermentation of the different doughs, was carried out at 30 °C for 12 h. During the fermentation the different samples were periodically analyzed in order to obtain the GC–MS/SPME profiles.

2.4. Scanning electron microscopy

The firmness of the dough was investigated by scanning electron microscope (SEM) according to Ndagijimana et al. (2006).

2.5. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) analysis

Genomic DNA was extracted from pure cultures by the procedures described by Querol, Barrio, Huerta, and Ramon (1992), for yeast and for bacteria was used an Insta Gene Matrix Kit (Bio-rad).

For the extraction of total DNA from sourdough, each sample was initially diluted 1:10 with a lysis solution (0,1 NaOH, 1% SDS) and homogenised for 3 min. The preparation was allowed to settle for 30 min at room temperature and a 1 ml sample was centrifuged at 5500*g* for 2 min the aqueous phase was removed, added with 1 M $NaClO_4$ and nucleic acids were first purified by chloroform extraction and hence precipitated with ethanol.

Two set of primers were used for PCR amplification for bacteria: HDA6-f-GC-5′-AAA CCG GAG GAA GGT GGG GA-3′, L1395-r-5′-CCC GGG AAC GTA TTC ACC G-3′; for yeasts: LIVE-f-GC-5′-TTG TTG AAA GGG AAG GG-3′, LIVE-r-5′-CAT TAC GCC AGC ATC CT-3′. PCRs were performed with T 3000 Thermocycler (Biometra®). For the amplification of bacterial DNA the reaction mixture (50 μl) contained 1× PCR buffer, 2.5 mM $MgCl_2$, 200 μM dNTP, 0.2 μM each primer (HDA6-f-GC-/L1395-r), 1.25 U of *Taq* polymerase and template DNA (2 μl). After an initial incubation at 94 °C for 5 min, 35 cycles of the following cycle were used: 94 °C for 20 s, 58 °C for 20 s and 72 °C for 40 s. A final extension at 72 °C for 7 min was performed. The PCR mixture used for the amplification of yeast DNA was the same as for bacterial DNA except for the concentrations of $MgCl_2$ and primers (LIVE-f-GC/LIVE-r), 1.5 mM and 0.5 μM, respectively. The amplification program was 94 °C for 5 min, 35 cycles of 94 °C for 20 s, 51 °C for and 72 °C for 40 s and elongation step at 72 °C for 7 min (Gatto & Torriani, 2004).

PCR products were analyzed by using a Dcode apparatus (Bio-rad). Samples (30 μl) were applied to 8% (w/v) polyacrylamide gels (acrylamide/bisacrylamide, 37.5:1) in 1× TAE buffer (2 M Tris base, 1 M glacial acetic acid, 50 mM EDTA, pH 8). Optimal separation of PCR fragments was achieved with a 30–60% urea-formamide denaturant gradient (100% denaturing solution contained 40% (w/v) formamide and 7.0 M urea) increasing in the direction of electrophoresis. Gels were electrophoresed for 6 h at 60 °C with constant voltage of 130 V, stained with ethidium bromide, rinsed in distilled water and photographed under UV illumination (Gatto & Torriani, 2004). All separate DGGE gel images were analyzed by Quantity – One 1 D Analysis Software (Bio-rad).

2.6. GC–SPME analysis and mass spectrometry

A divinylbenzene/carboxen/polydimethylsiloxane coated fiber (65 μm) and a manual SPME holder (Supelco Inc., Bellefonte, PA, USA) were used in this study after preconditioning according to the manufacturer's instruction manual. Before each head space sampling, the fiber was exposed to the GC inlet for 5 min for thermal desorption at 250 °C in a blank sample. Three milliliter 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 fiber was exposed to each sample for 20 min and finally the fiber 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 the dough and WFH, and analyzed after different times (10, 20, 30, 50 min) and temperatures (45, 50, 60, 70, 75 °C) GC–MS analyses were carried out on an Agilent 6890 gaschromatograph (Agilent Technologies, Palo Alto, CA, 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 program 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. The identification of the molecules has been based on comparison of their retention times with those of pure compounds (Sigma–Aldrich, Milan, Italy) added to uninoculated model system (WFH). The identification of the metabolites was confirmed comparing mass spectra of compounds both with those contained in available databases (NIST/EPA/NIH Vers. 1998 and Wiley Vers. 1996) and those of pure standards.

Calibration curves (Ndagijimana et al., 2006) were prepared for each metabolite identified using known concentrations of pure standards in WFH at different sugar concentrations. The data are reported as mg/l of WFH. Non calibration curves were prepared for the various doughs: results are reported as chromatographic peak area.

2.7. Statistical analysis

The data reported are the means of three repetitions. Standard deviations were reported in the results. The statistical treatment of data was performed using Microsoft Office Excel 2002 version.

3. Results

3.1. Effect of starch addition on the metabolic profiles

The effect of osmotic stress and starch addition on the concentration of metabolites produced by *L. sanfranciscensis* LSCE1 strain after 10 h of incubation in WFH are shown in Table 1. The levels of the various volatile metabolites produced by this strain did not exceed 180 mg/l with the exception of isovaleric acid which attained a level of 300 mg/l under osmotic stress and starch addition. The

Table 1
Effect of starch addition on selected metabolites released (after 10 h of incubation) by pure cultures of *L. sanfranciscensis* in WFH and by mixed cultures of *S. cerevisiae*, *C. milleri*, and *L. sanfranciscensis* or *S. cerevisiae* and *C. milleri* after 4 h of incubation in the CMs of *L. sanfranciscensis* (data are expressed as mg/l)

	L. sanfranciscensis inoculated in WFH				*L. sanfranciscensis*, *S. cerevisiae*, and *C. milleri* inoculated in the CMs of *L. sanfranciscensis*				*S. cerevisiae* and C. *milleri* inoculated in the CMs of *L. sanfranciscensis*			
	WFH[a]	WFH S[b]	WFHO[c]	WFHOS[d]	CM[e]	CMS[f]	CMO[g]	CMOS[h]	CM	CMS	CMO	CMOS
Ethanol	n.d[i]	n.d	n.d	n.d	3952 ± 345	4680 ± 421	6147 ± 593	6728 ± 622	814 ± 74	5225 ± 503	1947 ± 185	7745 ± 764
Isoamyl alcohol	n.d	n.d	n.d	n.d	453 ± 41	344 ± 31	226 ± 20	310 ± 27	179 ± 18	843 ± 85	364 ± 37	952 ± 96
Acetic acid	89 ± 8	56 ± 4	104 ± 10	65 ± 6	152 ± 13	312 ± 27	414 ± 38	462 ± 40	22 ± 2	149 ± 12	66 ± 5	147 ± 12
Isobutyric acid	40 ± 4	29 ± 3	151 ± 15	147 ± 15	362 ± 35	248 ± 22	271 ± 24	273 ± 24	47 ± 5	111 ± 10	165 ± 16	187 ± 17
Isovaleric acid	46 ± 5	32 ± 3	290 ± 26	300 ± 30	780 ± 72	824 ± 81	539 ± 51	743 ± 72	80 ± 6	200 ± 23	358 ± 36	385 ± 32
Hexanoic acid	n.d	n.d	36 ± 4	35 ± 4	120 ± 11	70 ± 7	61 ± 6	60 ± 6	n.d	27 ± 3	47 ± 5	171 ± 16
Phenylethanol	116 ± 30	88 ± 9	81 ± 7	85 ± 9	n.d	763 ± 75	662 ± 57	715 ± 70	147 ± 15	324 ± 33	129 ± 19	516 ± 50
Octanoic acid	33 ± 3	n.d	141 ± 14	145 ± 14	1024 ± 99	765 ± 75	539 ± 49	663 ± 64	97 ± 10	453 ± 41	453 ± 40	694 ± 64
γ-Octalactone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	19 ± 2	n.d	n.d	n.d	n.d
γ-Decalactone	n.d	n.d	n.d	n.d	n.d	n.d	n.d	14422 ± 1435	n.d	n.d	n.d	n.d
Ethyl-9-hexadecenoate	n.d	n.d	n.d	n.d	18 ± 2	39 ± 3	27 ± 3	13 ± 1	n.d	152 ± 15	n.d	486 ± 41
Decanoic acid	59 ± 6	62 ± 6	178 ± 18	99 ± 8	540 ± 48	843 ± 82	826 ± 81	785 ± 76	119 ± 12	210 ± 22	258 ± 21	1720 ± 155

[a] WFH: control.
[b] WFHS: control added with starch.
[c] WFHO: WFH added with sucrose 40%.
[d] WFHOS: WFH added with sucrose 40% and starch.
[e] CM: conditioned medium of *L. sanfranciscensis* grown in WFH.
[f] CMS: conditioned medium of *L. sanfranciscensis* grown in WFH added with starch.
[g] CMO: conditioned medium of *L. sanfranciscensis* grown in WFH added with sucrose 40%.
[h] CMOS: conditioned medium of *L. sanfranciscensis* grown in WFH added with sucrose 40% and starch.
[i] Under the detection limit.

growth under osmotic stress resulted also in an increase of isobutyric acid and medium chain fatty acids. The other metabolites identified were not affected by the conditions taken into consideration.

In order to establish if starch addition interferes with the response of *S. cerevisiae* and *C. milleri* to the metabolites released by *L. sanfranciscensis*, fresh cells (7 ± 0.3 log CFU/ml) of the two yeast species were co-incubated with fresh cells of *L. sanfranciscensis*. (8 ± 0.4 log CFU/ml) for 4 h in the CMs of *L. sanfranciscensis* grown under the conditions indicated in Table 1. The comparison of the results indicates that when the three species were inoculated together under osmotic stress and starch addition, the ethanol and acetic acid production increased. The most interesting effect of osmotic stress and starch addition was the production of relevant extent of γ-decalactone accompanied by minor level of γ-octalactone when the three species were inoculated together.

To better evidence, these differences Fig. 1a and b report, respectively, the SPME profiles obtained when the three microorganisms were incubated together in the CMs of *L. sanfranciscensis* grown under osmotic stress without and with starch addition. The starch supplementation was associated, under osmotic stress, to a significant increase of the majority of metabolites including ethanol, isoamyl alcohol, acetic acid, isovaleric acid, medium chain fatty acids, ethyl esters of medium and long chain fatty acids and to an unusual production of γ-octalactone and γ-decalactone. On the other hand when only the two yeasts were co-inoculated in the CMs of *L. sanfranciscensis* under the same conditions (Fig. 2a and b) γ-decalactone and γ-octalactone were absent, suggesting that their biosynthesis requires the simultaneous contribution of yeasts and *L. sanfranciscensis*. Moreover in absence of this latter species a greater amount of isoamyl alcohol and phenyl ethanol were produced as effect of starch addition under osmotic stress. Short and medium chain fatty acids production was also favoured by starch addition (Fig. 1).

In order to establish whether during solid fermentation in dough the microstructure characteristics affect the metabolite accumulation, *L. sanfranciscensis* and *S. cerevisiae* were individually inoculated in doughs having the following DY values: 146, 165, 190 and 220. Fig. 3a and b reports the SEM microphotographs of the doughs having 220 and 146 DY values. When the DY values was 146 the microstructure was characterized by a compact gluten network including starch granules. On the other hand, when the DY values was 220 the gluten matrix was not structured and organized.

The comparison of the concentrations of the various metabolites identified with GC–MS/SPME (Table 2) suggests that the complex network, formed when the dough yield was 165 (data not shown) or 146 with respect a dough of 220 DY, favours, when the dough was inoculated with *L. sanfranciscensis*, the production of isobutanol, acetoin, 1-hexanol, ethyl octanoate and butyric acid. When the

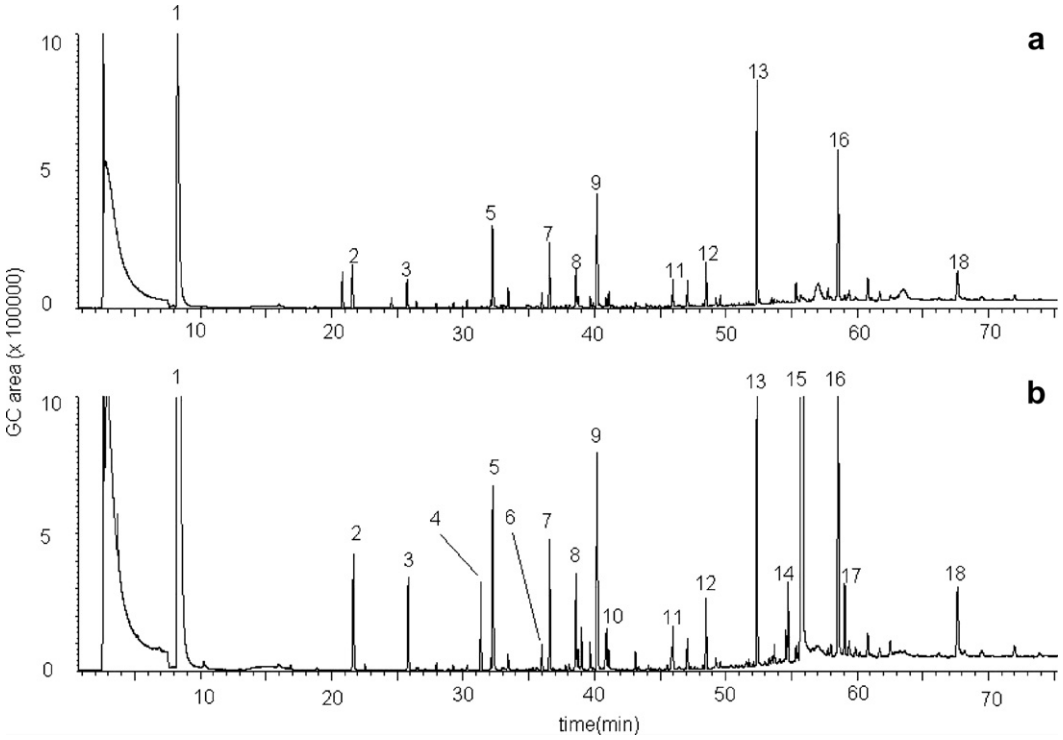

Fig. 1. SPME profiles of the coculture of *S. cerevisiae*, *C. milleri*, *L. sanfranciscensis* in the CMs of *L. sanfranciscensis* without (a) and with (b) starch. (1) ethanol; (2) isoamyl alcohol; (3) acetoin; (4) ethyl octanoate; (5) acetic acid; (6) 1-octanol; (7) isobutyric acid; (8) butyric acid; (9) isovaleric acid; (10) ethyl-9-decenoate; (11) hexanoic acid; (12) phenylethanol; (13) octanoic acid; (14) γ-octalactone; (15) γ-decalactone; (16) decanoic acid; (17) ethyl-9-hexadecenoate; and (18) dodecanoic acid.

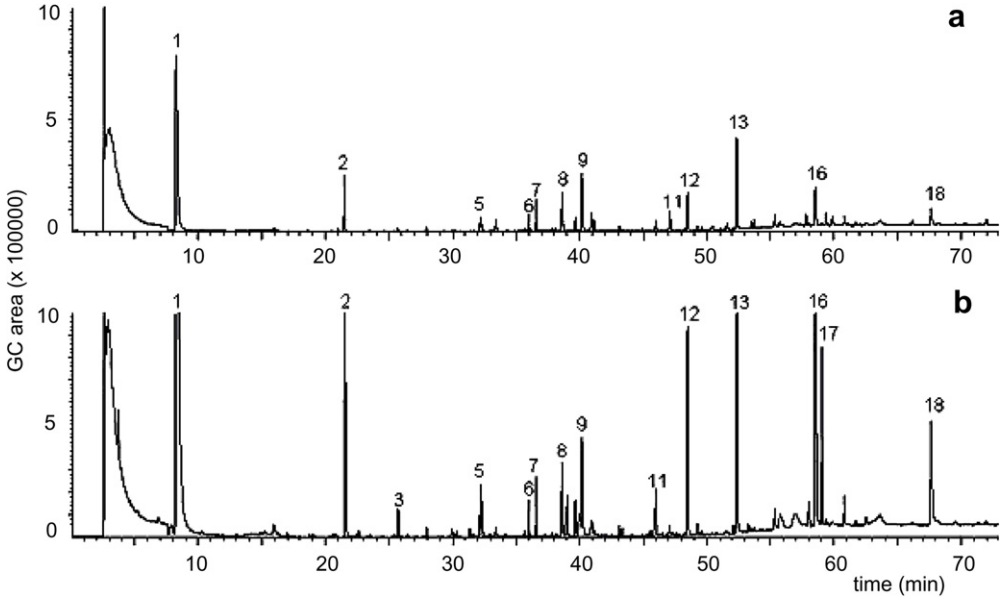

Fig. 2. SPME profiles of the coculture of *S. cerevisiae* and *C. milleri* in the CM of *L. sanfranciscensis* without (a) and with (b) starch. (1) ethanol; (2) isoamyl alcohol; (3) acetoin; (4) ethyl octanoate; (5) acetic acid; (6) 1-octanol; (7) isobutyric acid; (8) butyric acid; (9) isovaleric acid; (10) ethyl-9-decenoate; (11) hexanoic acid; (12) phenylethanol; (13) octanoic acid; (14) γ-octalactone; (15) γ-decalactone; (16) decanoic acid; (17) ethyl-9-hexadecenoate; and (18) dodecanoic acid.

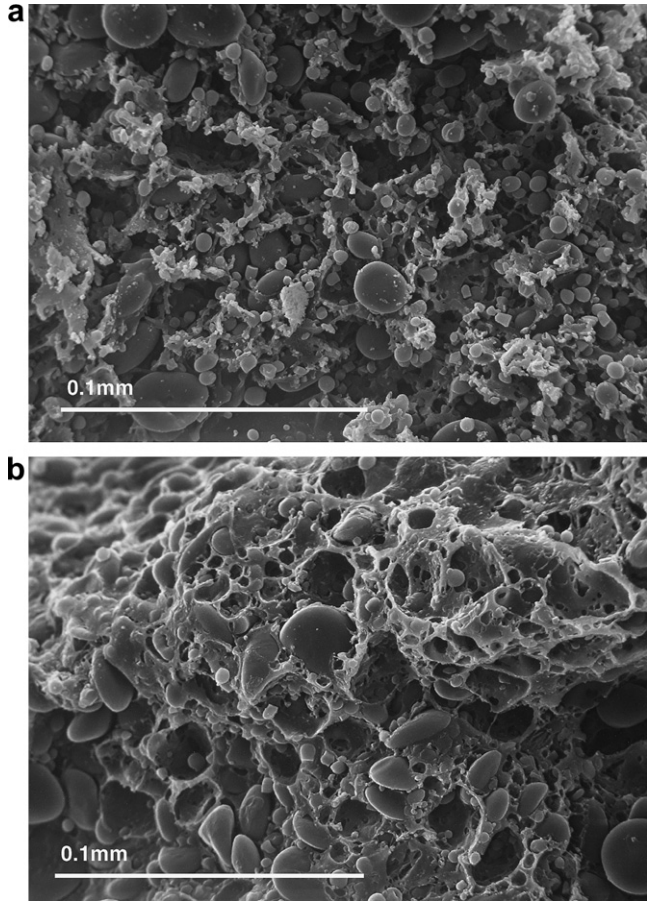

Fig. 3. Scanning electron microscopy (SEM) micrographs of dough having a DY of 220 (a) and 146 (b).

Table 2
Selected metabolites produced by *L. sanfranciscensis* and *S. cerevisiae* inoculated in dough having various dough yield (data are expressed as peak chromatographic area)

	L. sanfranciscensis		*S. cerevisiae*	
	DY 220	DY 146	DY 220	DY 146
Ethanol	63293434	66212708	129293261	221365648
Isobutanol	1613000	28117594	14451271	20639882
Isoamyl alcohol	39896697	26683712	59811236	93480545
Acetoin	2836897	4723769	502035	1470985
1-Hexanol	53980421	90941565	8640500	12030904
Ethyl octanoate	5317238	8978118	1229401	362860
Acetic acid	7103807	218806	2682232	7851410
Ethyl decanoate	n.d[a]	n.d	1336378	322831
Butyric acid	1152420	7362258	715068	1130854
Hexanoic acid	43161776	21242489	5918416	9672060
Phenylethanol	58942717	1932554	75407744	86460933
Octanoic acid	4880221	4698348	3271418	2471577
γ-Octalactone	n.d	n.d	7178303	6003708
Ethyl hexadecanoate	n.d	n.d	1541342	2354196

Standard deviation values of the three repetitions in this experiment were under 10%.
[a] Under the detection limit.

doughs were inoculated with *S. cerevisiae* a value of 146 DY resulted in a greater production of the major part of metabolites including alcohols and short and medium chain fatty acids.

3.2. Cell viability and DGGE analyses

In order to assess whether the metabolic activity changes, in the experiments with WFH and dough, were

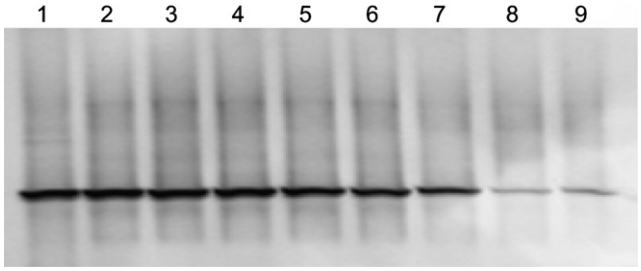

Fig. 4a. DGGE profiles for *L. sanfranciscensis* in WFH at different starch addition and dough with different DY: (1) *L. sanfranciscensis*; (2) DY 220; (3) DY 190; (4) DY 165; (5) DY 146; (6) WFH with starch 40 g/l; (7) WFH with starch 20 g/l; (8) WFH with starch 10 g/l; and (9) WFH with starch 5 g/l.

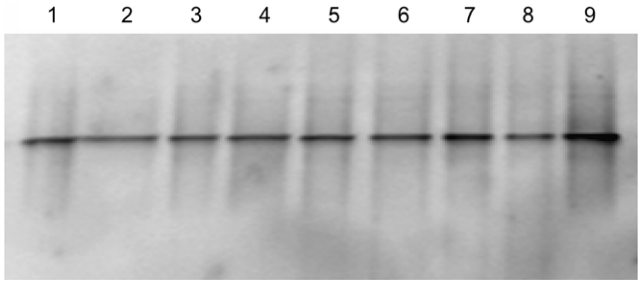

Fig. 4b. DGGE profiles for *S. cerevisiae* in WFH at different starch concentration: (1–3) WFH with starch 5 g/l; (4–6) WFH with starch 10 g/l; (7–9) WFH with starch 40 g/l; and (10) *S. cerevisiae* LBS.

related to a different yeast or LAB viability, the viable cells of *L. sanfranciscensis* and yeasts were evaluated on the basis of plate counting with selected media. The starch addition to WFH gave rise for *L. sanfranciscensis* in a viable cell increase of 1.1 ± 0.3 and 1.2 ± 0.4 log CFU/ml when the starch addition was respectively of 20 and 40 g/l. When *S. cerevisiae* was incubated in WFH, with different starch amounts an increase of 0.9 ± 0.3 log CFU/ml was observed when starch was 40 g/l.

Fig. 4 reports the results of PCR–DGGE of *L. sanfranciscensis* and yeasts. In Figs. 4a and 4b the band intensity respectively of *L. sanfranciscensis* and *S. cerevisiae* in WFH added with different amounts of starch is shown. Moreover in the Fig. 4a also *L. sanfranciscensis* band intensity in dough at different DY values is reported. Although PCR–DGGE is not specifically regarded as quantitative method, the band intensity both of *L. sanfranciscensis* and *S. cerevisiae* in WFH increased with starch concentration. The difference in band intensity were less significant in dough.

4. Discussion

It has been reported that the stress caused by the exposure of microbial cells to their own cell free conditioned media, containing metabolites and bioactive compounds including "quorum sensing" molecules, promotes cell differentiation, autolysis and overproduction of specific metabolites (Guerzoni et al., 2007; Lorenz, Shane Cutler, & Heitman, 2000; Ndagijimana et al., 2006; Vannini et al., 2007). The rationale of this work is that the interaction or binding of the metabolites with the system microstructure or added hydrocolloids affect not only their volatility but also their limiting effect on producing cells.

According to the results obtained in this preliminary work both the properties of the dough and the addition of starch to the liquid media under osmotic stress resulted in a significant enhancement of the production of primary and secondary metabolites including γ-decalactone and γ-octalactone. Lactones, and in particular γ-decalactone, are volatile compounds which contribute to the natural flavour of many fruits and are generated through the action of yeast cells in several fermented foods. γ-Decalactone results from lactonisation of 4-hydroxydecanoic acid. Many works have dealt with the biotranformation pathway which involve β-oxidation steps (Aguedo, Waché, Coste, Husson, & Belin, 2004). The principal problem for the industrial production of these odorants is that their accumulation is strongly limited by their toxicity against producing cells (Aguedo, Waché, Mazoyer, Sequeira-Le Grand, & Belin, 2003). The trapping of γ-decalactone "in situ" by hydrophobic sorbents is regarded as a good system to maintain yeast cells viability during its microbial production with *Yarrowia lipolytica* (Dufossè, Souchon, Feron, Latrasse, & Spinnler, 1999). Specific research demonstrated that starch and particularly linear amylose is able to form inclusions or complexes with lactones (Heinemann et al., 2003). The overproduction of γ-decalactone in presence of starch can be attributed to the ability of this hydrocolloid to reduce the lactone toxicity as free molecule. The contribution of lactobacilli to the synthesis of lactones should be associated with the production of their precursors i.e. hydroxyacids (Kishimoto et al., 2003). According to Aguedo et al. (2005), the yeast γ-decalactone pathway is controlled by the NAD^+ dependent 3-OH acyl-CoA dehydrogenase. Under high osmotic conditions the NAD^+ availability is associated with the osmoregulatory response in *S. cerevisiae*. In fact yeasts adapt to increased osmotic stress by enhanced production of intracellular glycerol as the main compatible solute (Erasmus, van der Merwe, & van Vuuren, 2003). The key step of glycerol synthesis is catalyzed by a NADH dependent cytosol glycerol-3-phospatidyl dehydrogenase with the production of NAD^+. An increase of glycerol requires therefore an equimolar increase of cytoplasmic NADH formation in order to maintain redox balance. Under high osmotic pressure this requirement is in general partially met by the decreased reduction of acetaldehyde to alcohol on one hand and on the other by an increased oxidation to acetate (Blomberg & Adler, 1989). Here one might imagine that, under the conditions taken into consideration, the redox balance can be maintained also by using NAD^+ also in the lactone production.

The growth of *L. sanfranciscensis* under osmotic stress resulted in a relevant accumulation of isovaleric acid. Its synthesis is associated with the branched chain amino acids metabolism, is NAD^+ dependent and produces NADH during the reaction (Ward et al., 2000). The isovaleric acid accumulation, as predominant metabolite had been already observed in model systems simulating sourdough also as a consequence of osmotic acid or oxidative stress (Guerzoni et al., 2007).

The co-fermentation of yeast and LAB in model system simulating sourdough renders an unambiguous interpretation of the results only in terms of redox balance difficult. Moreover, as reported by Erasmus et al. (2003), in *S. cerevisiae*, exposed to osmotic stress, many genes, including those codifying the sugar and branched chain amino acids transporter-like proteins, are highly up-regulated with respect to the control. The osmotic response of *L. sanfranciscensis* has not been deeply investigated. However, the role of the starch addition to pure and mixed cultures consistently resulted in an enhancement of the majority of metabolites having an antimicrobial effect. The addition of this hydrocolloid should work as a continum extraction system of the molecules such as alcohols, lactones, etc. released by the microbial cells.

Although the interactions of the metabolites or flavour compounds produced by microorganisms with the microstructure or macromolecules of the food matrix should be more systematically investigated, the findings of this preliminary work could have useful implications not only for fermented food but also for microbial production of flavour compounds.

Acknowledgement

Mrs. Luciana Perillo is acknowledged for the precious technical assistance.

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