Uracil as an Index of Lactic Acid Bacteria Contamination of Tomato Products

ALYSSA HIDALGO,* CARLO POMPEI, ANTONIETTA GALLI, AND SARA CAZZOLA

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche (DiSTAM), Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy

The aim of this research was to evaluate the suitability of uracil as an hygienic quality index of tomato products. Whereas uridine was naturally present throughout tomato fruits' ripening, uracil appeared only after microbial contamination. In tomato pulp inoculated with nine different microbial strains, all five lactic acid bacteria (LAB) studied released relevant quantities of uracil (150−1040 mg/kg of dm), with a correlated partial or total decrease of uridine. Uracil production by yeasts and molds was very low or nonexistent; the starting uridine concentration (∼960 mg/kg of dm) remained constant or increased. Uracil thermostability was also verified. Twenty-six samples of tomato paste (30 °Brix) were collected from bag-in-drums produced in an industrial processing plant, some with evident swelling symptoms. All of the samples with high microbial count presented uracil. Uracil was also present in samples with microbial contamination under the detection limit and Howard mold count below legislation limits, implying the reprocessing, at least partial, of altered tomato product. The results indicate that uracil presence in tomato products is an index of LAB contamination that has occurred before heat treatment.

KEYWORDS: Lactic acid bacteria; microbial contamination; molds; tomato; tomato products; uracil; uridine; yeasts

INTRODUCTION

The Howard mold count (HMC), introduced by Howard (*1*) and adopted by several legislatures (*2*, *3*), is the most common method to evaluate the hygienic quality of tomato products. Different parameters, relying mainly on the assessment of compounds or degradation products of molds cell walls, were later proposed (*4*−*8*). Another parameter considered by European legislation (*3*) is volatile acidity, linked to the presence of microbial metabolites (lactic and acetic acid) in the product.

Even if tomatoes undergo microbial alteration by lactic acid bacteria (LAB), molds, and yeasts (*9*−*11*), the contamination of packed tomato products as a consequence of insufficient heat treatment or recontamination is mainly due to LAB (*12*, *13*). However, if the altered tomato product is reprocessed, no traces of LAB may remain. Illegal remanufacturing of altered tomato products is a practice widely performed by producers of all over the world, because it is, at present, undetectable: therefore, the quest for a chemical index able to detect LAB contamination and not influenced by the technological phases of the production process is extremely relevant.

Uracil formation as a consequence of the enzymatic hydrolysis of uridine by microorganisms (*14*−*17*) was successfully utilized to evaluate the hygienic quality of egg products. Uracil presence has been correlated to the odor of egg products with various degrees of alteration (*18*). More recently, it has been proposed as a chemical index in egg products (*19*) and fresh egg pasta (*20*) and for the evaluation of the hygienic conditions of raw eggs used for egg product manufacture.

The purpose of this research was to assess the potential of uracil as an index of hygienic quality in tomato products. To this end, research was carried out to determine if uridine and uracil are natural components of tomato fruits, either in good hygienic conditions or spoiled by microorganisms. Subsequently, the influence of the growth of selected microbial strains on uridine and uracil content was studied. Finally, uracil thermostability and its presence in tomato products sampled from an industrial processing plant and from commercial channel were evaluated.

MATERIALS AND METHODS

Uracil and Uridine in Tomato Fruits. Seven tomato samples of different cultivars or commercial types (Ciliegina, 1; Ramato, 2; da insalata, 3; San Marzano, 4; Cencara, 5; Santa, 6; Cuore di bue, 7) from the market were analyzed at three tomato ripening stages: pink to light red (samples 1−7), red (samples 1, 2, and 6), and/or spoiled fruits, obtained by leaving red tomatoes at 5 ± 1 °C in a cold chamber until the appearance of spoilage symptoms (samples 1, 3, and 4).

The tomatoes were peeled, cut in four pieces, and deseeded by screening through a 20-mesh sieve. The juice and pulp were then blended with a Waring blender, model 32B/70 (New Hartford, CT). Dry matter (dm), uracil, and uridine contents were determined.

Uracil and Uridine Development in Tomato Pulp Inoculated with Microbial Strains. Nine 700-g bottles of tomato pulp of the same brand

* Author to whom correspondence should be addressed (telephone +39 02 50316639; fax +39 02 50316632; e-mail alyssa.hidalgovidal@unimi.it).

and batch were bought in a supermarket. From each bottle, 50-mL aliquots of tomato pulp were aseptically transferred into 14 sterile containers. Each of the nine series of samples was then inoculated with a strain of different microorganisms [five LAB (*Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Pediococcus* sp., and *Leuconostoc mesenteroides*; two yeasts (*Saccharomyces cerevisiae* and *Debaryomyces hansenii*), and two molds (*Alternaria solani* and *Cladosporium sp.*)] and incubated at 30 °C, with the exception of *Le. mesenteroides*, kept at room temperature. Microbial cell count and dry matter, uracil, and uridine determinations were performed at 48−72-h intervals, up to 28−30 days of incubation. At time 0, chemical analyses were performed on the tomato pulp, whereas microbial count was carried out on the freshly inoculated samples.

Uracil Thermostability Evaluation. The contents of nine 700-g bottles of tomato pulp of the same brand were carefully mixed with 300 mL of uracil solution, to obtain a tomato pulp with ∼30 mg/kg uracil. Samples (330 g) of uracil-laced tomato pulp were then transferred to 17 tin cans that, after cover sealing, underwent different heat treatments in an autoclave (Fedegari, Italy). To detect the time−temperature profile of each heat treatment, the temperature at the center of the container was recorded every 8 s with a thermocouple connected to a PC recording system (SmartReader Plus 6, ACR System Inc., British Columbia, Canada), using the software TrendReader version 1 (ACR System Inc.).

To evaluate the intensity of each treatment, the F_0 value was computed with the equation

$$F_0 = \int_0^t \frac{dt}{10^{(T_r - T/z)}} \quad (1)$$

where F_0 is the time in minutes at the reference temperature (T_r = 121 °C), t is the time of the treatment in minutes, T is the actual temperature of the treatment (°C), and z (10 °C) represents the increase in temperature that causes a 10-fold spore decrease (*21*).

Before and after heat treatment, dry matter, uracil, and uridine contents were determined. The results reported are the means of two evaluations carried out on each container. One-way analysis of variance was performed to determine significant differences among untreated and heat-treated samples. When significant differences were detected, the least significant difference (LSD) test was performed.

Tomato Paste from an Industrial Processing Plant. From a continuous industrial production plant of medium tomato paste (28−30 °Brix), 26 samples were collected. The production was carried out in September, and the sampling was done at the beginning of November when some 200-kg bag-in-drums started showing clear spoilage symptoms, such as swelling. Samples of the product were thus aseptically transferred to sterile 200-mL plastic containers from a series of bag-in-drums containing tomato paste produced every 4 h.

Dry matter, uracil, uridine, and lactic and acetic acid contents and volatile acidity were determined on all samples; total LAB, yeasts, molds, mesophilic aerobic/facultatively anaerobic (*Bacillus* sp.), and strictly anaerobic spore formers (*Clostridium* sp.) were evaluated. HMC was carried out on selected samples.

Tomato Products from the Commercial Channel. Twenty-three samples of different kinds of tomato products [juice (J, 5 °Brix), canned tomatoes (CT, 5.6 °Brix), tomato pulp (PU, 8 °Brix), and light (L-PA, 19.6 °Brix), medium (M-PA, 30 °Brix), and extra heavy (XH-PA, 38 °Brix) tomato paste] were acquired in the market. One sample was from a non-EU country. Total microbial count, dry matter, uracil, uridine, and lactic and acetic acid contents were determined on all of the samples; volatile acidity and HMC were measured on uracil-containing samples.

Chemical Analyses. Dry matter content (grams per 100 g of product) was determined following AOAC official gravimetric method 964.22 (*22*); Brix degrees were measured using a digital refractometer (model DBX-55, Atago, Tokyo, Japan); volatile acidity, expressed as grams of acetic acid per 100 g of dm, was determined following the European official method (*3*). Uracil, uridine, and lactic and acetic acid analyses were performed using the analytical HPLC system reported by Hicks et al. (*23*) and following the sample preparation procedure reported by Morris (*24*), with slight modifications as suggested by Rossi and Pompei (*25*). Sample preparation slightly differed only for tomato paste samples, which were diluted with water in a proportion of 0.53:1.0 (w/v) before weighing. Uracil and uridine were detected at 260 nm, using a Millipore Waters 996 series photodiode array detector (Milford, MA) controlled by the software Millenium^{32} Chromatography Manager (Waters Chromatography Division, Millipore, Milford, MA). The wavelength range used was 200−290 nm. Lactic and acetic acids were detected using a refractive index detector (model 1037A, Hewlett-Packard, Geneva, Switzerland) connected to a D-2500 chromatointegrator (Merck-Hitachi, Tokyo, Japan).

For peak quantification, calibration curves were built using 16 different concentrations (between 0.2 and 50 mg/L) of the uracil standard (Merck, Darmstadt, Germany), 9 different concentrations (between 5 and 85 mg/L) of the uridine standard (Sigma Chemical Co., St. Louis, MO), 15 different concentrations (between 4 and 4000 mg/L) of the lactic acid standard (Supelco, Bellefonte, PA), and 8 different concentrations (between 28 and 497 mg/L) of the acetic acid standard (BDH Laboratory Supplies, Poole, U.K.) in water. On the basis of the calibration curves, the detection limits were calculated as the intercept value of the regression line plus 3 times the standard error of the estimate (*26*). The results are expressed as milligrams per kilogram of dm. Uracil, uridine, and lactic and acetic acid calibration curves were linear ($r^2 = 1$; $p \leq 0.001$) in the concentration ranges considered and showed detection limits in the standard solution of 0.7, 0.7, 20.7, and 6.6 mg/L respectively, corresponding to 36.3, 32.5, 987, and 314 mg/kg of dm in tomato pulp and to 19.4, 17.3, 526, and 168 mg/kg dm in tomato paste. The values relating to tomato pulp and tomato paste refer to mean dry matter contents of 8 and 28 g/100 g, respectively. The results were computed considering the different samples quantity utilized for the analysis of the two product types.

To detect possible chromatographic interference of uracil- and uridine-like substances, standards of uridine diphosphate-glucose (UDP-glucose), of nucleotides uridine monophosphate (UMP), uridine diphosphate (UDP), and uridine triphosphate (UTP) (Fluka BioChemika, Buchs, Svizzera), of nucleosides adenosine, guanosine, cytidine (Merck, Darmstadt, Germany), and thymidine (Sigma Chemical Co., St. Louis, MO), and of bases adenine, guanine, cytosine, and thymine (Sigma Chemical Co.) were also injected. UDP-glucose and the injected nucleotides (UMP, UDP, and UTP) were not retained by the column and showed a retention time (t_r) similar to that of the solvent front peak, excluding any nucleotide interference in the uracil and uridine analysis. With regard to the nucleosides adenosine, guanosine, cytidine, and thymidine, only the cytidine peak overlapped the uridine peak (t_r = 12.5 min). Nevertheless, the sensitivity of the method for cytidine was very low: at equal concentration, the cytidine solution peak area was 8800 times smaller than the uridine solution peak area. Furthermore, thymidine had a t_r = 16.7 min, excluding the overlapping between its peak and uridine's peak, even with a comparable method sensitivity. The purinic nucleosides adenosine and guanosine were not detectable with the adopted analysis method. The peaks of the bases adenine, guanine, cytosine, and thymine did not interfere with the uracil peak (t_r = 20 min) because they had longer retention times, that is, 24 min for adenine and 28 min for guanine, cytosine, and thymine.

Microbial Analyses. Tomato pulp was inoculated with microbial strains from the DISTAM collection (*La. brevis*, *La. plantarum*, *Pediococcus* sp., *S. cerevisiae*, and *A. solani*) or isolated from tomatoes naturally altered during cold chamber storage (*Le. mesenteroides*, *D. hansenii*, and *Cladosporium* sp.) and from spoiled tomato paste of industrial origin (*La. fermentum*). The cells for the mother suspensions used for inoculation were obtained by centrifugation from overnight broth culture of each microorganism and then put into a pH 7 Tripton salt solution (NaCl, 8.5 g; Tripton, 1 g; distilled water, 1000 mL). To have a proper inoculum concentration, cell counts in the mother suspensions for LAB and yeasts was done with a Burker chamber at the optical microscope, whereas for molds cell count was performed by plate counting.

Each microorganism was inoculated in a different series of containers with 50 mL of sterile tomato pulp, to have starting concentrations of at least 10^4 cfu/g for bacteria and yeasts and 10^2 cfu/g for molds.

LAB, yeast, and mold development was monitored by plate counting, using the serial decimal dilution technique. LAB counting was

Table 1. Uracil and Uridine Contents in Pink to Light Red Tomatoes

sample	cultivar or commercial type	uracil (mg/kg of dm)	uridine (mg/kg of dm)
1	Ciliegina	nd[a]	569
2	Ramato	nd	777
3	da insalata	nd	548
4	San Marzano	nd	408
5	Cencara	nd	536
6	Santa	nd	393
7	Cuore di bue	nd	528
mean ± SD[b]			537 ± 127

[a] Not detectable. [b] Standard deviation.

performed by pour plates of Man Rogosa Sharpe Agar (Merck), with incubation at 30 °C for 48−72 h under anaerobic conditions (*27*); *Le. mesenteroides* incubation was carried out at room temperature. Yeasts and molds were counted by spread plates in yeast glucose chloramphenicol (Merck) and incubated at 30 °C for 3−5 days (*28*).

Only plates with 30−300 colonies were considered for computing the colony-forming units (cfu) using the equation of Peeler and Maturin (*29*). The results are expressed as total number of microbial cells per gram of analyzed product (cfu/g).

Microbial development in tomato paste samples from an industrial processing plant was monitored as reported above. The isolated LAB were identified at genus level according to Smeath et al. (*30*); the species were determined only for lactic rods by molecular methods, sequencing the 16 SrDNA with an ABI PRISM 310 Genetic Analyzer (Applied Biosystem, CA). The sequence was elaborated by Chromas 2.13 (Technelsym Pty., Ltd., Helenswale, Queensland, Australia) and compared with sequences available online in the NCBI database (*31*).

Mesophilic aerobic/facultatively anaerobic (*Bacillus* sp.) and strict anaerobic (*Clostridium* sp.) spore formers were determined by enrichment of 10 and 1 g of tomato paste in an appropriate liquid medium: tryptic soy broth (Difco, Sparks, MD) (*32*) for *Bacillus* sp., and reinforced clostridial medium (Oxoid Ltd., Basingstoke, Hampshire, U.K.) (*33*) for *Clostridium* sp. The enrichments were previously pasteurized in a water bath at 80 °C for 10 min and incubated at 37 °C for 48 h; gas-pack anaerobic conditions were adopted for *Clostridium* sp. To evaluate the presence of spore formers, confirmatory tests were then performed in tryptic soy agar (*32*) and in reinforced clostridial agar (*33*) at the same incubation conditions outlined above. The colonies were observed at a phase-contrast-viewing microscope and tested for catalase. The results were referred to 10 g of tomato pulp.

HMC determination on tomato paste samples from the industrial processing plant and from the market (XP-PA2) and on commercial canned tomato (CT5) samples was performed following the official AOAC 965.41 and AOAC 945.90 (*34*) methods, respectively.

Total microbial count in the tomato products from the commercial channel was performed by pour plates of tryptic soy agar (*32*) with incubation at 32 °C for 72 h.

All chemical and microbiological determinations were performed in duplicate.

RESULTS AND DISCUSSION

Uracil and Uridine in Tomato Fruits. Uracil and uridine contents in pink to light red tomatoes of seven cultivars/commercial types are presented in **Table 1**. Uridine was naturally present in tomato fruits, with an average value of 537 mg/kg of dm in the tested samples, whereas uracil was always absent.

Table 2 reports uracil and uridine contents in pink to light red, red, and spoiled tomatoes. During tomato ripening uracil was always absent, whereas uridine content increased, probably as a consequence of catabolic reactions of the RNA nucleotides by increased ribonuclease activity during senescence (*35*). In spoiled tomatoes, uracil appeared in the three samples, although

Table 2. Uracil and Uridine Contents in Pink to Light Red, Red, and Spoiled Tomatoes

	uracil (mg/kg of dm)		uridine (mg/kg of dm)	
sample	pink to light red	red	pink to light red	red
1	nd[a]	nd	569	1125
2	nd	nd	777	881
6	nd	nd	393	688

	uracil (mg/kg of dm)		uridine (mg/kg of dm)	
sample	pink to light red	spoiled	pink to light red	spoiled
1	nd	139	569	1142
3	nd	232	548	nd
4	nd	40	408	972

[a] Not detectable.

in different quantities, whereas uridine increased in samples 1 and 4 but disappeared in sample 3.

Therefore, uracil in tomato remains absent throughout the ripening and appears only after microbial contamination.

Uracil and Uridine Development in Tomato Pulp Inoculated with Microbial Strains. Figures 1−3 show uracil and uridine evolution during microbial growth in tomato pulp samples inoculated with different microbial strains. Before inoculation, uracil was absent in all samples, whereas uridine was present with an average value of 959 mg/kg of dm and a variation coefficient of ∼12%.

With respect to uracil evolution in samples inoculated with LAB, **Figure 1** shows that all five strains were able to release such compound, already detectable 1−2 days after inoculation and growing until a maximum value, reached at different times depending on the different strains; afterward, the concentration was constant even after a decrease in LAB content.

La. brevis produced the highest uracil quantity, ∼1040 mg/kg of dm, followed by *Pediococcus* sp. (530 mg/kg of dm), *La. fermentum* (320 mg/kg of dm), *La. plantarum* (250 mg/kg of dm), and finally *Le. mesenteroides* (150 mg/kg of dm). Uracil increase was combined with a decrease in uridine, confirming the hydrolytic activity of bacteria on this substance. Several authors have studied the properties of nucleoside phosphorylases obtained from different microorganisms: these enzymes catalyze the splitting of *N*-riboside linkage and produce ribose-1-phosphate and the corresponding base (*14*−*17*). However, in the case of *La. brevis* and *La. fermentum* uracil increase went on for a few days even after the complete disappearance of uridine. A possible explanation is the ability of some microorganisms to deaminate cytidine to uridine, by the cytidine deaminase enzyme (*14*), allowing further uracil formation. Evidently the uridine-to-uracil transformation rate is greater than cytidine deamination, making uridine levels analytically undetectable. Only *La. brevis*, *La. fermentum*, and *La. plantarum* completely metabolized the nucleoside, whereas *Pediococcus* sp. caused a rapid uridine concentration decrease during the first 3 days and leveled off at ∼100 mg/kg of dm. *Le. mesenteroides* caused a gradual decrease of uridine during the 30 days of incubation, from 1143 to 741 mg/kg of dm.

Figure 2 depicts the behavior of the yeasts: *S. cerevisiae* did not release uracil, consumed all of the available uridine in 2 days, and then began to produce uridine, reaching levels higher than the starting concentration. *D. hansenii* produced uracil, but fluctuations in the content of the two products were evident during incubation. This behavior was confirmed by a second trial, as shown in **Figure 2**, carried out to verify if the results

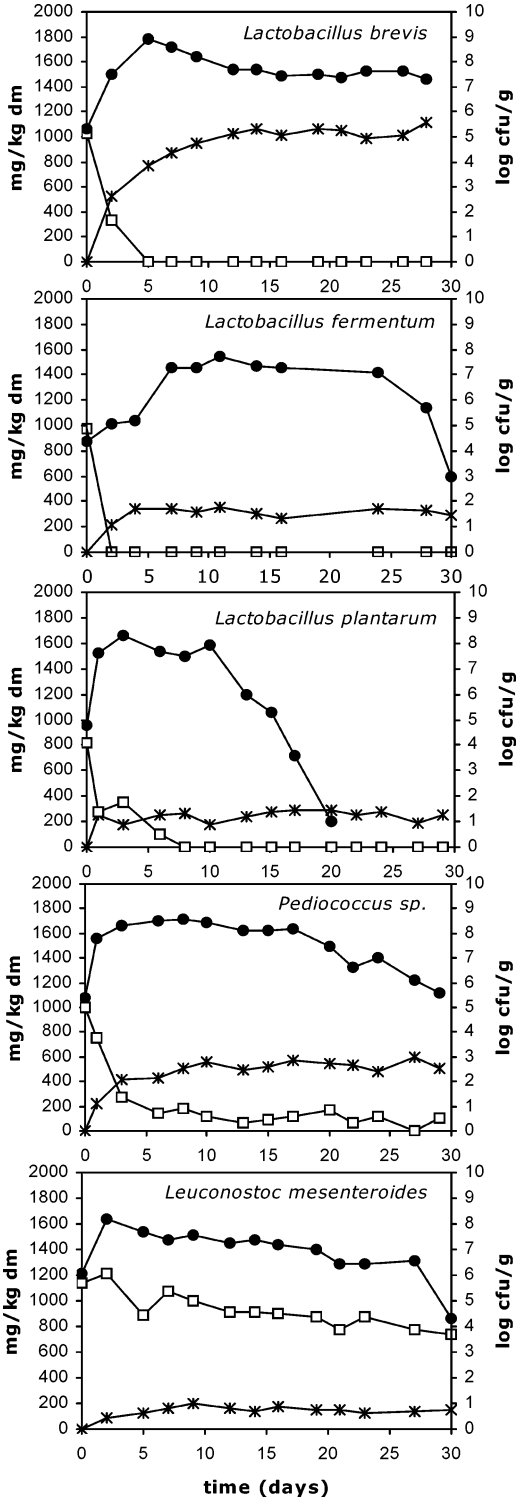

Figure 1. Uracil (∗), uridine (□), and microbial count (●) evolution during incubation of tomato pulp inoculated with five lactic acid bacteria strains.

were correct or were a consequence of experimental errors. This behavior remains unexplained.

Figure 3 reports the results of the molds: *A. solani* started producing uracil only after 22 days, reaching 75 mg/kg of dm after 28 days. This mold produced instead much uridine, increasing from 756 to 1958 mg/kg of dm in 28 days. The other mold, *Cladosporium* sp., started producing little uracil after 5 days of incubation and caused a significant increase of uridine, with concentration ∼50 mg/kg of dm).

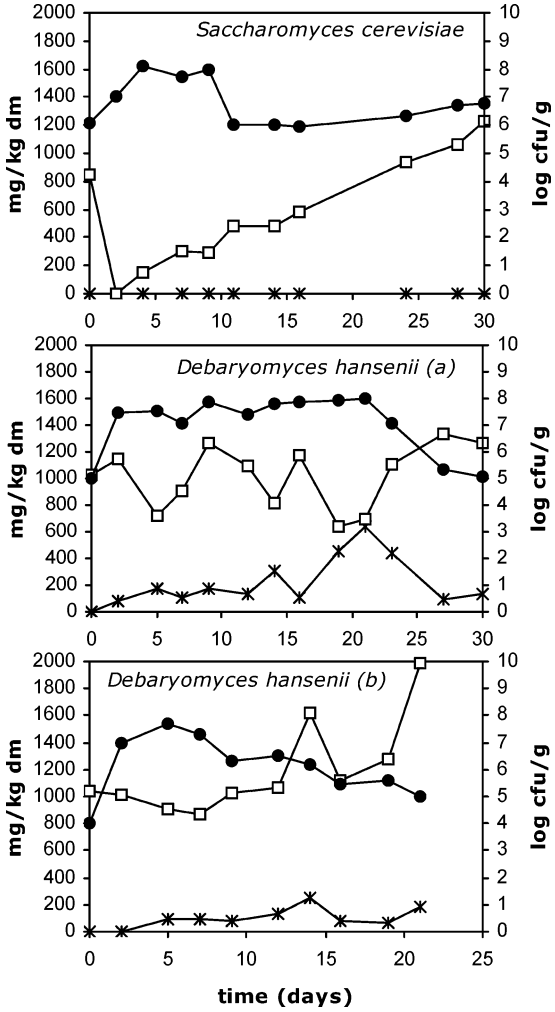

Figure 2. Uracil (∗), uridine (□), and microbial count (●) evolution during incubation of tomato pulp inoculated with two yeast strains; a and b are replicated assays of the same strain.

The results reported in **Figures 1**−**3** allow a better interpretation of uridine and uracil contents in the spoiled tomato fruits reported in **Table 2** (samples 1, 3, and 4). Sample 3 in **Table 2** was probably contaminated by LAB, such as *La. fermentum* and/or *La. brevis* and/or *La. plantarum*, as suggested by the complete disappearance of uridine and the production of high quantities of uracil. Uridine at levels similar to those of red tomatoes and the high uracil content in sample 1 suggest a mixed contamination by yeasts, mold, and/or LAB such as *Le. mesenteroides.* Two hypotheses are instead appropriate for sample 4: the sample was analyzed in the initial stages of a LAB contamination, when uridine contents are still high and uracil is low (40 mg/kg of dm), or the sample was mostly contaminated by yeasts and molds, which release only small quantities of uracil.

These results suggest general conclusions on the effect of the growth of different microorganisms (LAB, yeasts, and molds) on uracil and uridine contents in tomato pulp. All of the LAB strains studied released relevant quantities of uracil, with a correlated partial or total decrease of uridine. Particularly, uracil contents >150 mg/kg of dm indicate a LAB contamination that has reached levels of at least 10^6 cfu/g. Uracil production by yeasts and molds, on the other hand, was variable: some species produced significant quantities, whereas others yielded small or zero quantities. Yeasts and molds, in contrast to LAB, maintained the starting uridine concentration

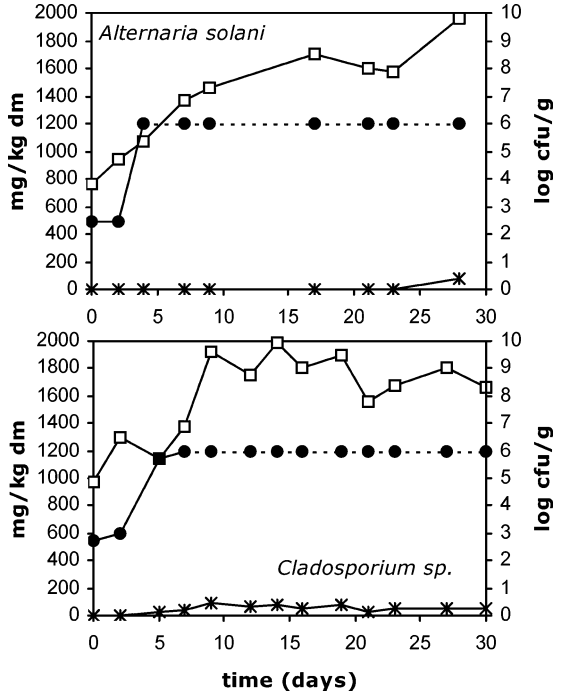

Figure 3. Uracil (∗), uridine (□), and microbial count (●) evolution during incubation of tomato pulp inoculated with two mold strains. Dotted lines indicate microbial counts above 10^6 cfu/g.

Table 3. Uracil and Uridine Contents in Tomato Pulp Samples before and after Heat Treatments[a]

	before heat treatment	after heat treatment			
		1	2	3	4
F_0 (min)	0	5.3	62	62	5264
uracil	418	411	408	412	407
(mg/kg of dm)	416	393	413	415	419
	426	407	413	422	–[b]
	–	379	418	380	–
	–	390	398	393	–
mean ± SD[c]	420a ± 5	396a ± 13	410a ± 7	404a ± 17	413a ± 9
uridine	1098	1421	1271	1319	1304
(mg/kg of dm)	1122	1472	1282	1314	1334
	1159	1462	1314	1360	–
	–	1402	1304	1249	–
	–	1397	1237	1277	–
mean ± SD	1126a ± 30	1431c ± 34	1282b ± 30	1304b ± 42	1319b ± 22

[a] Values in the same row, per each variable, bearing different letters are significantly different at $p \leq 0.05$. [b] Not analyzed. [c] Standard deviation.

or increased it many fold. This research does not allow a precise assessment, for each strain, of the metabolic paths followed by uridine phosphorolysis products. Even though an exact mass molecular balance between the degraded uridine and the formed uracil is lacking, it has to be stressed that uracil does not disappear and its content does not decrease after formation. On the other hand, uridine levels increase or decrease depending on the microbial strain and growth stage.

Uracil Thermostability Evaluation. Uracil and uridine contents of uracil-laced pulp samples before and after heat treatments are reported in **Table 3**. All treatments were markedly more intense (F_0 from 5.3 to 5264 min; $T_r = 121$ °C) than those performed in industrial manufacturing of tomato products (*36*). The analysis of variance did not show significant differences between uracil contents of untreated and thermically treated samples, emphasizing the high thermostability of uracil. Uridine

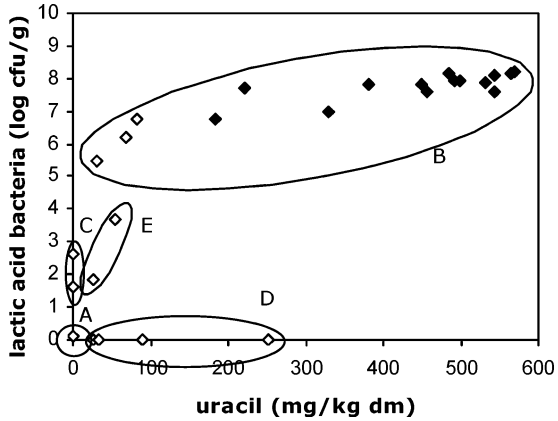

Figure 4. Lactic acid bacteria count as a function of uracil content in tomato paste sampled from bag-in-drums of industrial product manufactured every 4 h. Solid symbols indicate samples collected from swollen bags, whereas the elliptical coded areas represent hypothetical samples clusters.

contents, instead, were significantly different ($p \leq 0.001$). The LSD test ($p \leq 0.05$) showed that the samples from treatments 2−4 had a significantly greater uridine content than the untreated sample, whereas the sample from the least severe treatment (treatment 1; $F_0 = 5.3$ min) showed the highest uridine content. This increase is at present still unexplained; however, uridine, the precursor of uracil, never decreased after heat treatment.

Evaluation of Tomato Paste from an Industrial Processing Plant. The microbial analysis showed LAB contamination in many samples. In samples from nonswollen bag-in-drums the contamination was due to *Pediococcus* sp., whereas the samples from swollen bags were contaminated by *La. fermentum*. Only one sample had significant yeast contamination, whereas another sample showed a minimal presence of *Bacillus* sp. spores. *Clostridium* sp. molds or spores were never detected.

Figure 4 reports the relationship between uracil content and bacterial contamination of the 26 industrial tomato paste samples. The samples may be divided in five groups (A−E). Group A includes only one sample, with no LAB and uracil content: in fact, uracil is naturally absent in unspoiled tomato fruits and tomato products. Group B samples have LAB counts $>10^5$ cfu/g and significant uracil contents (31−569 mg/kg of dm). The presence of uracil is related to microbial development, particularly to LAB; interestingly, all of the swollen bag-in-drums (solid symbols in **Figure 4**) belong to this group. Group C samples present a LAB count from 10 to 10^3 cfu/g, but uracil is absent: possibly the contamination is too low to induce the formation of analytically detectable uracil. Group D samples present microbial counts under the detection limit, but uracil is present in variable quantities (from 27 to 252 mg/kg of dm). Probably, raw materials of poor hygienic quality was employed for the production of tomato pastes; alternatively, they were obtained by remanufacturing a certain percentage of altered product along with a good hygienic quality product, and the heat treatment destroyed all living bacteria. However, the heat treatment did not reduce the content of the thermostable uracil. Group E samples display a microbial count similar to those of group C samples, but whereas in group C samples LAB contamination was not enough to induce uracil formation, in group E samples uracil is instead detectable. In this case, the same hypotheses already mentioned for group D samples can be proposed; additionally, the end-product was recontaminated, reaching microbial contents of 10^2−10^4 cfu/g.

The relevant spread of uracil contents in the samples of groups B and D may be associated with the natural development

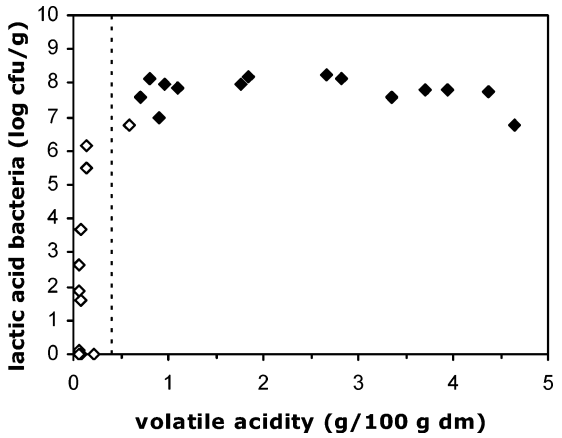

Figure 5. Lactic acid bacteria count as a function of volatile acidity in tomato paste sampled from bag-in-drums of industrial product manufactured every 4 h. Solid symbols indicate samples collected from swollen bags. Dotted line indicates European legal limit (*3*).

sequence of the different LAB in the fermentation processes (*37*), because uracil formation depends on both microbial type and growth stage. A further source of variation could be related to the fact that contamination may have occurred before and/or after heat treatment.

Figure 5 shows the relationship between LAB count and volatile acidity. Only samples with a microbial count of 10^7 cfu/g or higher presented volatile acidity above European legislation limits (0.4 g/100 g of dm) (*3*). The samples with high uracil content and absence of LAB (group D in **Figure 4**) presented moderate values of volatile acidity (<0.4 g/100 g of dm). Obviously, the low volatile acidity values are at least a partial consequence of their removal during the concentration step, achieved by evaporation. This suggests that volatile acidity in tomato paste in not a reliable index to evaluate the hygienic quality of the raw material (i.e., the product before tomato paste manufacture). The same holds true also for lactic and acetic acids, two variables that resulted correlated to volatile acidity ($r^2 = 1$; $p \leq 0.001$) (data not presented).

HMC was performed on selected samples: positive fields for the group A samples (**Figure 4**) were 24%, for group B samples, between 20 and 22%, and for group D samples, between 20 and 32%. All HMCs were therefore lower than the U.S. (*2*) and European (*3*) legislations limits. Hence, group D samples, for the legislation, are products of acceptable hygienic quality, whereas uracil presence denounces the reprocessing, partial or total, of spoiled tomato product.

Tomato Products from the Commercial Channel. Uracil, uridine, and lactic and acetic acid contents of 23 samples of six different tomato product types collected from the market are reported in **Table 4**. Even if all of the samples presented a total microbial count under the detection limit, uracil was found in one sample of canned tomatoes (CT5) (92 mg/kg of dm) and in one sample of extra-heavy tomato paste (XH-PA2) (133 mg/kg of dm). In both cases, uridine presence suggests either an initial step of contamination, mainly due to LAB, or a mixed contamination by LAB, yeasts, and molds. High quantities of lactic (20.32 g/kg of dm) and acetic (4.99 g/kg of dm) acids were detected in the canned tomatoes sample, whereas the concentrations of the two acids were lower in the extra-heavy tomato paste, even in the presence of a higher uracil content. The volatile acidity values of the two samples were 0.43 and 0.15 g/100 g of dm, respectively, higher than the European legislation threshold (*3*) in the canned tomatoes sample. The different volatile acidity values of the two samples are justified by the fact that canned tomatoes are sterilized inside a closed container and lactic and acetic acid present in the raw material remain in the end-product. In tomato paste, instead, volatile acids are, at least partially, lost by evaporation.

Table 4. Uracil, Uridine, Lactic Acid, and Acetic Acid Contents in Tomato Juice (J), Canned Tomato (CT), Tomato Pulp (PU), and Light (L-PA), Medium (M-PA), and Extra-Heavy (XH-PA) Tomato Paste Samples Collected from the Market

sample	uracil (mg/kg of dm)	uridine (mg/kg of dm)	lactic acid (mg/kg of dm)	acetic acid (mg/kg of dm)
J1	nd[a]	623	nd	1028
J2	nd	951	1282	2386
CT1	nd	573	3001	2783
CT2	nd	406	1221	1862
CT3	nd	810	3790	2539
CT4	nd	636	nd	1489
CT5	92	1064	20316	4986
PU1	nd	901	3316	2061
PU2	nd	656	nd	1322
PU3	nd	1035	2954	2939
PU4	nd	656	nd	1362
PU5	nd	860	nd	1357
PU6	nd	616	1432	2254
PU7	nd	989	nd	1467
PU8	nd	789	3564	2545
L-PA	nd	797	2338	2064
M-PA1	nd	838	nd	922
M-PA2	nd	752	6813	1773
M-PA3	nd	713	nd	831
M-PA4	nd	794	2065	1565
M-PA5	nd	867	2641	1913
XH–PA1	nd	739	1333	1053
XH–PA2	133	569	4919	2187

[a] Not detectable.

With regard to the HMC, CT5 presented 26% of positive fields, lower than the European legislation threshold (*3*) but higher than the U.S. regulation (*2*). A similar remark is valid also for the XH-PA2 sample, with 64% of positive fields. The two products are therefore marketable under the European legislation, but uracil presence implies the utilization of LAB contaminated products in their manufacture.

Conclusions. When detected in sterile end-products with low HMC, uracil, because of its thermostability, indicates the remanufacturing of tomato products contaminated by LAB. In tomato, uracil is derived from LAB metabolism and is therefore a clear index of poor microbial quality, even when found in barely detectable quantities. Uracil analysis provides additional information about microbial contamination history, in particular from LAB, and, therefore, should be coupled to HMC.

ABBREVIATIONS USED

cfu, colony-forming units; F_0, defined for a given heat treatment as the time required to get, at the reference temperature of 121 °C, the same spore destruction of the heat treatment; dm, dry matter; HMC, Howard mold count; HPLC, high-performance liquid chromatography; LAB, lactic acid bacteria; LSD, least significant difference; nd, not detectable; r^2, determination coefficient; SD, standard deviation; p, probability; T_r, reference temperature; T, temperature; t, time; UDP-glucose, uridine diphosphate-glucose; UDP, uridine diphosphate; UMP, uridine monophosphate; UTP, uridine triphosphate; t_r, retention time; RNA, ribonucleic acid; z, increase in temperature that causes a 10-fold spore decrease.

LITERATURE CITED

(1) Howard, B. J. Tomato ketchup under the microscope with practical suggestions to insure a clean product. U.S. Department of Agriculture, Bureau of Chemistry, Circular 68; U.S. GPO: Washington, DC, 1911.

(2) U.S. Food and Drug Administration (FDA). http://www.cfsan.fda.gov/~dms/qa-ind5p.html.

(3) EEC. Regulation (EEC) 1764/86, May 27, 1986, Commission. *Off. J. Eur. Communities* **1986**, *L 153*, 1-17.

(4) Jarvis, B. A chemical method for the estimation of mould in tomato products. *J. Food Technol.* **1977**, *12*, 581−591.

(5) Hubbard, J. D.; Seitz, L. M.; Mohr, H. E. Determination of hexosamines in chitin by ion-exchange chromatography. *J. Food Sci.* **1979**, *44*, 1552−1553.

(6) Pettipher, G. L.; Williams, R. A.; Gutteridge, C. S. An evaluation of possible alternative methods to the Howard Mould Count. *Lett. Appl. Microbiol.* **1985**, *1*, 49−51.

(7) Ghiretti, G. P.; Spotti, E.; Strina, F.; Sandei, L.; Mori, G.; Attolini, G.; Leoni, C. Ergosterol production by different types of moulds able to colonize tomatoes. *Ind. Conserve* **1995**, *70*, 3−12.

(8) Potts, S. J.; Slaughter, D. C.; Thompson, J. F. Measuring mold infestation in raw tomato juice. *J. Food Sci.* **2001**, *67*, 321−325.

(9) Casolari, A.; Ercolani, G. L. Variazioni delle caratteristiche microbiologiche del pomodoro dalla raccolta alla lavorazione. *Ind. Conserve* **1965**, *40*, 306−311.

(10) Zacconi, C.; Causarano, A.; Dallavalle, P.; Casana A. Monitoring of contaminating microflora in the production of tomato products. *Ind. Conserve* **1999**, *74*, 133−144.

(11) Villari, P.; Costabile, P.; Fasanaro, G.; De Sio, F.; Laratta, B.; Pirone, G.; Castaldo, D. Quality loss of double concentrated tomato paste: evolution of the microbial flora and main analytical parameters during storage at different temperatures. *J. Food Process. Preserv.* **1998**, *18*, 369−387.

(12) Gould, W. Spoilage of canned tomatoes and tomato products. In *Tomato Production, Processing and Technology*, 3rd ed.; CTI Publications: Timonium, MD, 1992; pp 419−431.

(13) International Commission on Microbiological Specifications for Foods (ICMSF). Fruits and fruit products. In *Micro-organisms in Foods 6. Microbial Ecology of Food Commodities*; Blackie Academic and Professional: London, U.K., 1998; pp 252−273.

(14) Sakai, T.; Tochikura, T.; Ogata, K. Metabolisms of nucleosides in bacteria. Part II. Studies on nucleoside-N-ribosyl group transfer reaction in bacteria. *Agric. Biol. Chem.* **1965**, *29*, 742−750.

(15) Leer, J. C.; Hammer-Jespersen, K.; Schwartz, M. Uridine phosphorylase from *Escherichia coli*. Physical and chemical characterization. *Eur. J. Biochem.* **1977**, *75*, 217−224.

(16) Utagawa, T.; Morisawa, H.; Yamanaka, S.; Yamazaki, A.; Yoshinaga, F.; Hirose, Y. Properties of nucleoside phosphorylase from *Enterobacter aerogenes*. *Agric. Biol. Chem.* **1985**, *49*, 3239−3246.

(17) Avraham, Y.; Grossowicz, N.; Yashphe, J. Purification and characterization of uridine and thymidine phosphorylase from *Lactobacillus casei*. *Biochim. Biophys. Acta* **1990**, *1040*, 287−293.

(18) Morris, C. E.; Hoerning, E. F.; St. Angelo, A. J. Uracil as potentially useful indicator of spoilage in egg products. *J. Food Sci.* **1989**, *54*, 581−583.

(19) Hidalgo, A.; Rossi, M.; Pompei, C.; Casiraghi, E. Uracil as an index of egg product hygienic quality. *Ital. J. Food Sci.* **2004**, *4*, 429−436.

(20) Alamprese, C.; Rossi, M.; Casiraghi, E.; Hidalgo, A.; Rauzzino, F. Hygienic quality evaluation of the egg product used as ingredient in fresh egg pasta. *Food Chem.* **2004**, *87*, 313−319.

(21) Kessler, H. G. Pasteurization-Sterilization-Heating Methods. In *Food Engineering and Dairy Technology*; Verlag A. Kessler: Freising, Germany, 1981; Chapter 6, pp 139−207.

(22) AOAC International. Vegetable products, processed. In *Official Methods of Analysis of AOAC International*, 16th ed.; Cunnif, P., Ed.; AOAC International: Gaithersburg, MD, 1995; Vol. 2, Chapter 42, p 4.

(23) Hicks, K. B.; Sondey, S. M.; Lim, P. C.; Foglia, T. A.; Raupp, D. L.; Holsinger, V. H. Analysis of uridine and uracil in caprine milk by high performance liquid chromatography. *J. Dairy Sci.* **1985**, *68*, 300−306.

(24) Morris, C. E. Determination of uracil, uridine and formic acid in egg products by high-performance liquid chromatography. *J. Chromatogr.* **1987**, *394*, 408−413.

(25) Rossi, M.; Pompei, C. Changes in some components and analytical values due to hen age. *Poult. Sci.* **1995**, *74*, 152−160.

(26) Miller, J. C.; Miller, J. N. Errors in instrumental analysis, regression and correlation. In *Statistics for Analytical Chemistry*, 2nd ed.; Ellis Horwood Publishers: Chichester, U.K., 1988; pp 101−136.

(27) De Man, J. C.; Rogosa, M.; Sharpe, M. E. A medium for the cultivation of *Lactobacilli*. *J. Appl. Bacteriol.* **1960**, *23*, 130−136.

(28) IDF Standards. Milk & milk products—Yeasts and moulds; Brussels, Belgium, 1990; 94B.

(29) Peeler, J. T.; Maturin, L. J. Aerobic plate count. In *Bacteriological Analytical Manual*, 7th ed.; AOAC International: Arlington, VA, 1992; Chapter 3, pp 17−26.

(30) Smeath, P. H. A.; Mair, N. S.; Sharpe, M. E.; Holt, J. G. *Bergey's Manual of Sistematic Bacteriology*; Williams & Wilkins: Baltimore, MD, 1986; Vol. 2, pp 1075−1076, 1209−1234.

(31) National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/.

(32) Stevenson, K. E.; Segner, W. P. Mesophilic aerobic sporeformer. In *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed.; Vanderzant, C., Splittstoesser, D. F., Eds.; American Public Health Association, technical committee on microbiological methods for foods: Washington, DC, 1992; pp 265−274.

(33) Oxoid. *Manuale Oxoid*; Unipath: Garbagnate Milanese (MI), Italy, 1993; pp 197−199.

(34) AOAC International. Extraneous materials: isolation. In *Official Methods of Analysis of AOAC International*, 16th ed.; Cunnif, P., Ed.; AOAC International: Gaithersburg, MD, 1995; Vol. 1, Chapter 16, pp 63−64.

(35) Dangl, J. L.; Dietrich, R. A.; Thomas, H. Senescence and programmed cell death. In *Biochemistry and Molecular Biology*; Buchanan, B. B., Gruissem, W., Jones, R. L., Eds.; American Society of Plant Physiologists: Rockville, MD, 2000; pp 1044−1100.

(36) Hidalgo, A.; Pompei, C.; Zambuto, R. Heat damage evaluation during tomato products processing. *J. Agric. Food Chem.* **1998**, *46*, 4387−4390.

(37) Pederson, C. S. Pickles and sauerkraut. In *Commercial Vegetable Processing*; Luh, B. S., Woodroof, J. G., Eds.; Avi Publishing: Westport, CT, 1982; Chapter 11, pp 458−463.

Received for review August 11, 2004. Revised manuscript received September 14, 2004. Accepted November 3, 2004.

JF0486489