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PSYCHOPHYSICAL ASSESSMENTS OF SOURNESS IN CITRIC ACID-ETHANOL MIXTURES[1, 2]

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Summary.—The effect of ethanol in modulating the intensity and duration of the perceived sourness induced by citric acid was studied. Magnitude Estimation-Converging Limits method was applied to rate the sourness of seven solutions (3–70 mM) of citric acid in aqueous solution presented alone and mixed with 8% V/V or 15% V/V ethanol. Dynamic sourness ratings of 5, 15, and 45 mM citric acid alone and mixed with the same two ethanol levels were assessed by the Time Intensity Method (TI). Results were consistent with both methods. Sourness changed with citric acid concentration and ethanol levels. From TI measurements, a similar interactive pattern was obtained for parameters as duration, area under the curve, peak and average intensity.

Human psychophysical studies for sourness of citric acid in ethanolic vehicles show complex results. For example, weak aqueous solutions of ethanol are perceived as bitter (Martin & Pangborn, 1970; Mattes & DiMeglio, 2001) and bitter and sweet (Scinska, Koros, Habrat, Kukwa, Kostowski, & Bienkowski, 2000). As concentration increases, chemesthetic responses from the trigeminal and vagus nerves such as numbing, burning, tingling, and irritation are elicited. These properties can change with other variables like the lingual locus of the stimulation, salivary status, duration of stimuli in the mouth, the 6-*n*-propylthiouracil status, gender, age of judges, and others (Martin & Pangborn, 1978; Green, 1988; Mattes & DiMeglio, 2001;

Duffy, Peterson, & Bartoshuk, 2004). For a review on the major factors involved in taste-taste interaction see Keast and Breslin (2002).

Citric acid elicits trigeminal sensations in addition to perception of a sour taste. At high concentrations, it induces oral and, presumably, intranasal irritation as well as a bitter taste (Settle, Meehan, Williams, Doty, & Sisley, 1986). When applied to the mucosa of the oral cavity, it can elicit pain (Gilmore & Green, 1993). Similar to other organic acids, it has an astringent effect (Rubico & McDaniel, 1992). It has also been reported that tactile sensations like dryness, puckering, and roughing of oral tissues are observed with exposure to acids (Lawless, Horne, & Giasi, 1996). Martin and Pangborn (1970) reported a reduction of sourness with the addition of 4% V/V ethanol to citric acid. More complex model solutions showed that mixtures suppressed the perception of sourness, i.e., Fischer and Noble (1994) found that the increase of ethanol in wine from 8% to 14% diminished sourness significantly. The two chemicals elicit various sensory perceptions at different concentrations in complex solutions. For example, in salty and sour samples mixed with capsaicin in broth solutions, Cowart (1987) concluded that taste and oral irritation are largely independent and that the observed effects depend mainly on procedural variations.

Psychophysical studies on taste intensity have been preferentially devoted to quantifying single static judgments by Magnitude Estimation (Stevens, 1975). A modified version of this conventional method is Magnitude Estimation-Converging Limits (MECL) (Guirao, 1991). Under MECL, participants are allowed to use a flexible scale that represents a compromise between category and ratio scales. Another difference is that participants know in advance the size of the intensity range to be estimated. This procedure allows more consistent judgments and produces less individual scatter in the data than the conventional Magnitude Estimation (Eisler & Guirao, 1997). Halo-dumping effect refers to response bias induced by the instructions given to the participants. According to Clark and Lawless (1994), when they are not given appropriate rating scales to score a sensation, they dump this sensation onto the only available scale. By applying the MECL method, possible halo-dumping effects can be attenuated because before starting the experiments the participants are asked to set their own subjective range and are also asked to identify the taste to be rated. As noted by Clark and Lawless (1994), prior knowledge of which attributes are to be rated should abolish halo-dumping effects. The MECL method has also been effective for quantifying different sensory and perceptual modalities (Guirao, 1987, 1991; Zamora, 1995).

Since taste intensity can change over the duration of tasting, a dynamic procedure like the time-intensity (TI) method is an effective tool to analyze the temporal changes of a given tastant in a mixture. In fact,

chemosensory research of sourness complex matrices like beverages and food have shown that the gustatory strength or chemesthetic effect for a specific acid is a function of both time and stimulus concentration, as well as contextual effects (Kallithraka, Bakker, & Clifford, 1997; Lugaz, Pillias, Boireau-Ducept, & Faurion, 2005). The experimental procedure was similar to the one described in previous studies (Guinard, Pangborn, & Shoemaker, 1985; Guirao, 1991; Calviño, Garrido, & García, 2000; Guirao, Greco Driano, Zunino, Evin, & Sleiman, 2008).

Testing of various organic acids has revealed different potencies. Lugaz, *et al.* (2005) evaluated the time-intensity of the sour taste of various organic acids, reporting that citric acid has the greatest potency. Previously, Ough (1963) analysed several acids and reported that citric acid was judged as the most sour when it was added to wine. From these findings, it was hypothesized that a citric acid and ethanol is a good combination to explore perceptual interactions and further understand the effects of typical ethanol concentrations in alcoholic beverages over an extended range of sourness produced by this potent organic acid. Thus, the present experiments were designed to provide static and dynamic psychophysical data to clarify the role played by ethanol in modulating the intensity and duration of the sour taste of citric acid.

Method

Material and General Procedure

Aqueous solutions of pure citric acid (CA Parafarm) in deionized water, 8% V/V and 15% V/V ethanol, were prepared. Samples were stored in glass bottles at 4–8 °C and served in plastic medicine cups (30 ml). The solutions and a container of deionized water for rinsing between stimuli were heated in circulated water baths to 37 °C to render the stimuli and rinses thermally neutral to the mouth. At this temperature, measurement of pH of the solutions was determined by triplicate. Mixed and unmixed solutions were in the low pH range (pH<3.0). All experiments took place in specialized sensory testing room.

The participants were instructed to place the solution in their mouths, to hold for about 5 sec., to expectorate it and to rate the sourness intensity. A period of about 3 min. between samples was allowed to cleanse their palate with CMC 0.5%W/V and distilled water to reduce carryover effects from prior samples. Samples with and without ethanol were presented in the same experimental session and the presentation order was randomized.

Participants

Ten participants (aged 20–45 yr.), recruited from the University of Buenos Aires campus, gave consent and were paid to participate in the two series of experiments. They knew that their responses were anony-

mous and that they could withdraw at any time. They were nonsmokers, non-frequent alcohol drinkers, and were asked to refrain from smoking, eating, or drinking foods or beverages for at least one hour prior to their scheduled session.

Prior to the first data collection participants were asked to identify, under free recall, the taste qualities by presenting them with weak and strong pure and mixed samples. All samples tasted sour for all participants and mixed samples were described as presenting also an "alcohol-like" component. They were also asked to focus on the sourness intensity and disregard any other perceived attribute.

Magnitude Estimation-converging Limits (MECL)

Seven women and three men took part in two experiments. In Experiment 1, seven citric acid concentrations (3, 5, 10, 15, 30, 45, and 70 mM) dissolved in water and 8% V/V ethanol aqueous solution were presented. In Experiment 2, the same citric acid concentrations were presented alone and dissolved in 15% V/V ethanol aqueous solution. In both experiments the fourteen stimuli (unmixed and mixed) were replicated in two sessions scheduled at least one day apart; the order of the stimuli within each session was randomized between participants.

Before data collection, participants were presented with one weak (3 or 5 mM) and one strong (45 or 70 mM) solution of citric acid. After each presentation, they were instructed to assign a number to sourness intensity. In the subsequent trials they were told to assign numbers appropriate to the sourness intensity. They were told that they could use smaller or larger numbers than the first two and during the experiment, and they could ask for any of their previous ratings.

Time Intensity

Three sets of experiments were performed under the time intensity method. Each set consisted of three sessions in which each of three concentrations (5, 15, and 45 mM) of citric acid were presented alone and mixed with the same two ethanol solutions used in the magnitude estimation series. In one condition, participants rated sourness alone, and in another, they rated citric acid mixed with 8% ethanol, and in a third, they rated citric acid mixed with 15% ethanol.

Ratings were assigned by 10 women who received training in three sessions, during which they learned how to operate a computerized technique designed to record time-intensity responses. They practiced assessing sourness intensity in the three concentrations with and without ethanol. As prompted by the computer, they operated a mouse to track changes from onset to extinction of the taste sensations. Each sample was tasted only once per session and was evaluated in triplicate in three different sessions. Again, the instruction was to rate sourness intensity.

Data Analysis

As intensity ratings from the MECL tend to be distributed log-normally across participants, numerical judgments were subjected to log transformation prior to the parametric analysis. To compare the ratings of sourness, two-way repeated measures analyses of variance (ANOVA) were run, with citric acid (3, 5, 10, 15, 30, 45, and 70 mM) and ethanol (8, 15% V/V) concentrations as factors. Least Significance Difference tests were performed on the main effects to assess the nature of the differences. When interactions were significant, differences in simple effects were examined using *post hoc* analysis with Student *t* test. Measures of effect size were calculated.

Time intensity curves were averaged according to the methods developed by Liu and MacFie (1990). Four summary measures were derived from each time-intensity curve: peak intensity (IMAX), total duration from onset to offset (Ttot), area under the curve (AUC), and average intensity (Iav). Starting points of time-intensity curves were the averaged time required to reach the first intensity larger than zero. Two-way repeated-measures ANOVA was performed. The ratings were analyzed by SPSS Version 11.5. For both experiments, $p \leq .05$ was considered statistically significant.

<div align="center">RESULTS AND DISCUSSION</div>

Magnitude Estimation—Converging Limits

As shown in Fig. 1, the unmixed and mixed sour psychophysical functions were clearly separated. There was a significant interaction between citric acid concentration and ethanol 8% ($F_{6,114}=2.56$, $p=.02$, $\eta^2=0.12$) as well as ethanol 15% ($F_{6,114}=4.50$, $p=.0003$, $\eta^2=0.19$). Thus, the two ethanol concentrations increased sourness but it was confirmed that this effect was dependent on citric acid concentration. Analysis of simple effects showed that at 8%, ethanol increased only the sourness of 3 mM citric acid ($t_{19}=-2.54$, $p=.02$, $d=-1.17$), but adding 15% ethanol increased sourness of 3 mM ($t_{19}=-2.34$, $p=.03$, d$=-1.07$) and 5 mM citric acid ($t_{19}=-2.29$, $p=.03$, $d=-1.05$), and decreased sourness of 45 and 70 mM citric acid ($t_{19}=2.50$, $p=.02$, $d=1.15$). All these results confirmed the robust interactive pattern between citric acid and ethanol components in mixture.

Also the presence of ethanol shortened the range of the perceived sourness. Plotted in logarithmic coordinates (Fig. 2) the two functions follow Stevens's power law with the same exponential value (0.63) for the citric acid samples. Citric acid slopes also highlight the test-retest reliability of data ($t_{19}=-0.06$), revealing non-significant differences across experiments. These values are the same as those obtained before by Garcia Medina (1981) and close to those reported by Meiselman (1971), Moskowitz (1974), and Gilmore and Green (1993). Figures 2a and 2b showed that the
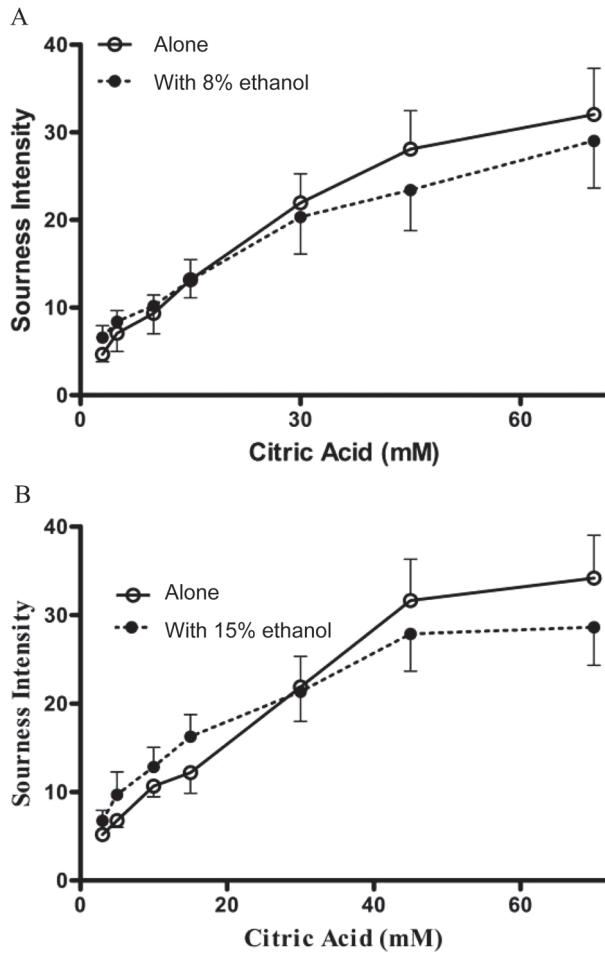
FIG. 1. A. Geometric means of perceived sourness intensity of citric acid solutions presented alone (—) and mixed with 8% ethanol (----). Error bars indicate the SEM ($N=20$ replications). B. The same as in A, but citric acid solutions were presented alone (—) and mixed (----) with 15% ethanol. Error bars indicate the SEM ($N=20$ replications).

functions with citric acid + ethanol had lower exponents for both ethanol concentrations, at 8% V/V ($t_{19}=4.82$, $p<.0001$, $d=2.21$) and citric acid + ethanol 15% V/V ($t_{19}=4.37$, $p<.0001$, $d=2.00$). Both functions were flattened by an effect of compression: equal increments in molarities produced progressively smaller increments in sourness intensity for the acid mixed with ethanol than for acid alone.
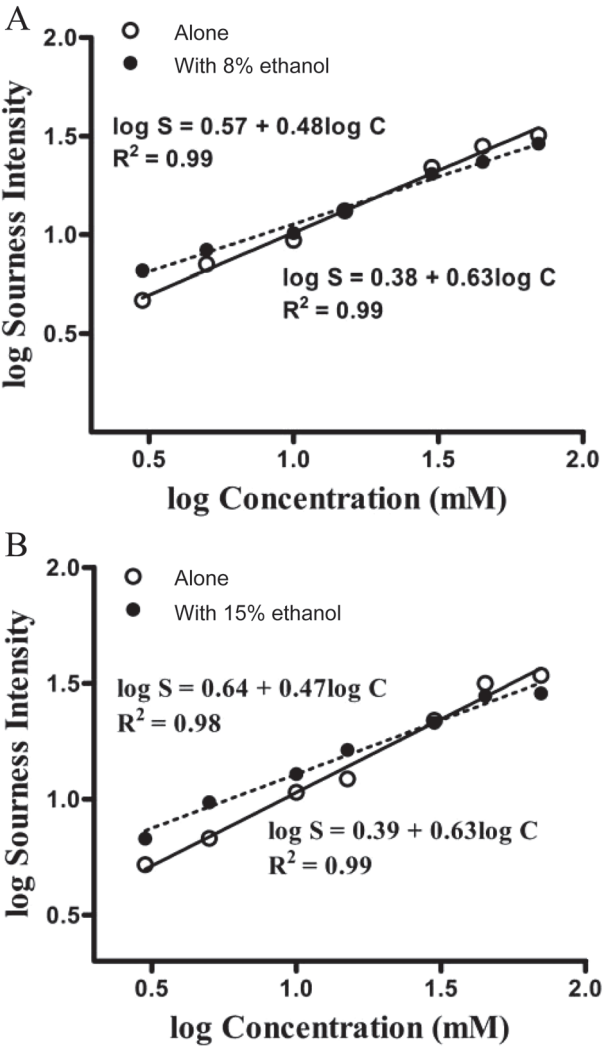
FIG. 2. A. Data plotted in log-log coordinates show two power functions for citric acid solutions presented alone and mixed with 8% ethanol. Both power functions intersected near the middle of the range of concentration. B. Two power functions for citric acid solutions presented alone and mixed with 15% ethanol show the same shape as those plotted in A.

These results only partially replicated the data presented by Martin and Pangborn (1970), who were working with four concentrations of ethanol (4, 8, 12, and 24%) and applying other psychophysical methods. They reported that sourness intensity was depressed at all ethanol concentra-

tions. Functions presented here are similar to those obtained by Yau and McDaniel (1992); mixing citric acid (0, 02–29% W/V) with CO_2, they found that carbonation compressed the sourness ranges and lowered the exponents of the power functions.

Time Intensity

Table 1 summarizes the effect of ethanol on each time-intensity parameter and Fig. 3 depicts the average curves for 5, 15, and 45 mM citric acid, respectively. The effect of ethanol on dynamic sourness depends again on the concentration of the two chemicals in the mixture. The effect was different for each one of the acid concentrations and for the two (ethanol) concentrations. Enhancement of four parameters—peak intensity, duration, area, and average intensity—at the two ethanol concentrations occurred in the weakest acid solution, as shown in Fig. 3a. At moderate concentration (15 mM), two different effects were observed. Fig. 3b shows that peak intensity and total duration also showed a reduction when the acid was mixed with 8% ethanol but peak intensity, duration, area, and average intensity were again enhanced when ethanol was raised to 15% V/V. At the highest concentration (45 mM), the effect of ethanol was not observed.

TABLE 1
MEAN VALUES OF THE TIME-INTENSITY PARAMETERS AND THEIR STANDARD ERROR ($N=30$)

Citric Acid/ Ethanol (mM/%V/V)	IMAX		Ttot		AUC		Iav	
	M	SEM	M	SEM	M	SEM	M	SEM
5/0	25.36[a]	3.62	19.65[a]	1.27	314.05[a]	49.08	15.98[a]	2.12
5/8	31.24[b]	3.68	24.41[b]	1.97	478.70[b]	66.33	19.61[b]	2.06
5/15	40.17[c,d]	3.78	28.99[d]	2.99	716.16[c]	171.55	24.70[c,d]	2.38
15/0	44.46[d]	3.37	29.23[c,d]	2.78	802.28[c]	88.74	27.45[d]	1.77
15/8	36.21[b,c]	3.23	26.08[b,c]	2.67	576.89[b]	88.31	22.12[b,c]	1.82
15/15	49.13[e]	3.87	34.08[e]	2.32	1029.57[d]	117.23	30.21[e]	2.19
45/0	54.48[e]	3.65	32.99[d,e]	2.23	1104.53[d]	137.07	33.48[f]	2.16
45/8	48.58[e]	3.65	32.40[d,e]	2.00	972.90[d]	129.68	30.03[e,f]	2.11
45/15	46.72[e]	4.32	33.54[e]	2.45	991.94[d]	147.32	29.57[e,f]	2.49

Note.—Means followed by different superscripts differ significantly ($p<.05$) according to LSD test. Indicators are: IMAX = Peak, Ttot = Duration, AUC = Area under the curve, Iav = Average intensity

Similar results have been obtained when citric acid was mixed with CO_2. Working with two acidulated systems, Yau and McDaniel (1992) measured the effects of CO_2 concentration on sourness of citric acid and found that carbonation enhanced sourness ratings at the lower acid levels and had no effect at higher acid levels. It is possible that a burning component
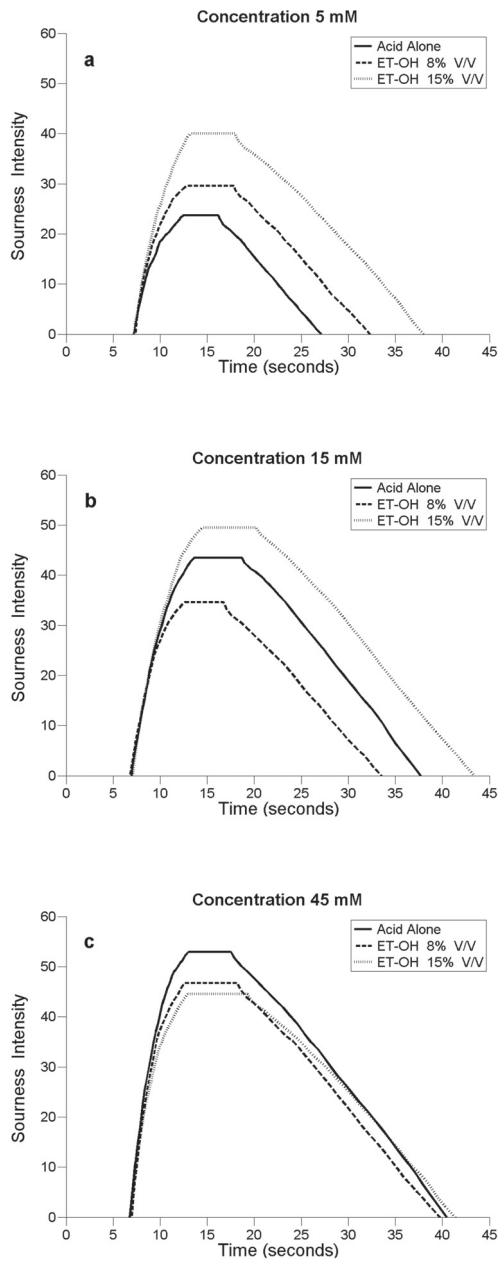
FIG. 3. Time-intensity average curves for citric acid alone and mixed with 8 and 15% v/v ethanol (ET-OH). Data were processed as stated by Liu and MacFie, 1990.

is added to the sour quality when the concentration of the acid increases. Animal studies indicated that high concentrations of citric acid presented to the tongue elicit neuronal activity in the mandibular nerve (Carstens, Kuenzler, & Handwerker, 1998) as well as sensation of irritation (Gilmore & Green, 1993; Dessirier, O'Mahony, Iodi-Carstens, & Carstens, 2000).

Concerning duration, the fact that strong acid samples were unaffected by ethanol was somewhat expected as it was noted previously, relative to other oral irritants, that the pungency of ethanol had the shortest perceived onset and overall duration (Cliff & Heymann, 1992). Citric acid also induces short-lived irritation (Dessirier, *et al.*, 2000).

With regard to the taste quality, as reported by the participants, even at the higher ethanol level, the sourness quality in the mixtures remained constant. This sourness constancy has been noticed in other contexts and solutions. Simons, O'Mahony, and Carstens (2002) reported no changes in the sour quality after pretreatment with an irritant compound like capsaicin. Furthermore, while looking at the interactions between CO_2 oral pungency and taste, Cometto-Muñiz, García-Medina, Calviño, and Noriega (1987) suggested that the perceptual attributes of sourness and saltiness are qualitatively closer to oral pungency than are the attributes of sweetness and bitterness. Also when looking at the correspondence among oral wine descriptors, it was found that sourness is clearly an independent attribute with no significant relationship except pungency and lemon (Guirao & Zamora, 2000). It was also noted that most of the panelists tended to use the terms "sourness" and "prickling" as synonymous (Zamora & Guirao, 2002).

Since citric acid as other organic acids at high concentration can be detected by nasal inhalation (Settle, *et al.*, 1986), probably an association between the properties of ethanol and the acid taste via gustatory, trigeminal, and retronasal olfaction are inducing a sour flavor that is perceptually localized in the mouth.

In psychophysical experiments in which capsaicina was used to stimulate receptors on the tongue, oral chemical irritation was mediated by more than one sensory pathway (Green, 1991). Furthermore, when participants rated the perceived intensity of individual sensation qualities in a mixture with capsaicin, it was found that desensitization dramatically reduced the burning and stinging/pricking components of irritation, but left the sensations of numbness and chemogenic warmth unchanged (Green, 1991).

Regarding ethanol, it has been postulated that chemesthetic sensations of numbness may be mediated by different neural pathways than the sensation of burning and stinging. On the basis of these observations, although trigeminal sensations were not tested in the present study, it

could be interpreted that from the perceptual integration of the taste and chemesthetic sensory properties, new composite flavors emerged: sourness/numbing at the weak and sourness/irritation at the strong combinations.

In summary, the present results suggest that with application of static and dynamic psychophysical methods, consensus was obtained through the range of concentrations used here: the effect depends on the ethanol and citric acid concentrations. At low concentrations, intensity and duration of sourness are enhanced. These properties are decreased when moderate acid solutions are mixed with a low ethanol level but enhanced at the higher level. Stronger acid concentrations result as sour alone, as in the mixed solutions. These interactions make it difficult to summarize a unique effect of ethanol on the perceived of sourness of citric acid. To test the generality of these results, it will be necessary to evaluate the weight of sourness and of the ethanol trigeminal sensations in the flavor of the mixture with a larger number of panelists.

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