Effect of Temperature on Threshold Values for Citric Acid, Malic Acid and Quinine Sulphate—Energy of Activation and Extreme-Value Determination*

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(*Manuscript received 30 March, 1971*)

The thresholds for citric acid, malic acid and quinine sulphate were determined at three temperatures to ascertain the effect of temperature on taste sensitivity. Temperature had an effect, but judge–substance interactions were just as important. The energy of activation for taste response usually was in the range 5–20 kcal. The data suggest that extreme-value methods should be useful in predicting the percentage of individuals whose thresholds will be below some certain level.

Introduction

THE present study had three objectives: (1) to ascertain the effects of temperature on threshold levels for citric acid, malic acid and quinine sulphate; (2) to estimate the energy of activation involved in detecting these substances, and (3) to examine the applicability of extreme-value methods to the estimation of threshold levels.

The literature relative to the effect of temperature on taste thresholds is not extensive and much of it is in conflict. Bitterness, for example, has been reported to be more detectable at low temperatures, at high temperatures, at room temperature, or that temperature makes no difference. The same is true for sourness although there is greater concurrence that temperature has little effect.

Komuro[1] reported a doubling of sensitivity to acid, salt, sugar and quinine when the temperature was raised from 10° to 20°C, that sensitivity remained on a plateau from 20° to 30°C, but that it then fell off from 30° to 40°C. Sensitivity to quinine sulphate has, on the other hand, been shown[2] to decrease progressively with an increase in temperature in the range 17° to 42°C. At 17–22°, 32° and 42°C, the respective thresholds were $2{\cdot}0 \times 10^{-6}$, $2{\cdot}7 \times 10^{-6}$ and $6{\cdot}7 \times 10^{-6}$ M (0·000149%, 0·000202% and 0·000500%), respectively. Howell[3] stated that a temperature between 10°C and 30°C gives the optimum reaction for taste. Maurizi & Cimino[4] found that the threshold values for bitterness, acidity and saltiness were lower at 35–40°C than at 15–20°C.

Several investigators[5–9] concluded that an increase in temperature increases the response to sweetness, decreases it to saltiness and bitterness, and has little effect on sourness.

Salmon & Blakeslee[10] considered that changes in temperature had no influence on threshold levels. Their conclusions were not restricted to sourness. Beidler,[11] using an electrophysiological method, observed no change in response magnitude for sodium chloride at 20°C, 24°C and 30°C. Cameron[6] stated that the optimum temperature of response to sucrose and hydrochloric acid was 35–50°C, for saltiness 18–35°C, and for quinine 10°C.

Part of the reason for the discrepancies above is the difficulty of relating temperature to threshold values. Unless the judgment about the taste attribute is made within a few milli- or centi-seconds after taking a small amount of the solution into the mouth, the temperature of the solution will be at body temperature. In fact, some of the determinations reported to be at 20° or 30°C, for example, may truly be values at 37°C simply because the taster took too long to make a decision and the solution had come to body temperature.

There are ways around this problem such as dipping the tongue into a solution maintained at the desired temperature or constantly flowing a warm (or cold) solution over the tongue, but these procedures require that the response to taste be sufficient to override the sensation of pain or numbness caused by prolonged contact with the solution.

Apart from the problem of being able to know the temperature at the moment the presence of the compound is detected, there is also the problem of variability among individuals. Salmon & Blakeslee[10] showed that the taste sensitivity of an individual may vary within the course of a few hours. Histograms, illustrating within- and between-judge differences in sensitivity, have been recently published.[12]

For the three compounds used in the present study, threshold values have been reported over fairly wide ranges. Fabian & Blum,[13] for example, found the threshold level for malic acid to be 0·00075 M (0·010%) and for citric acid 0·00070 M (0·013%). These threshold values were higher than those reported by Berg *et al.*[14] The threshold for malic acid was 0·0030 g/100 ml (0·0030%) and for citric acid 0·0025 g/100 ml (0·0025%). Pfaffman[7] reported the threshold for malic acid as 0·0016 N (0·0107%) and for citric acid 0·0023 N (0·0152%). A lower threshold value for citric acid, 0·000208 M (0·004%), was reported by Schutz & Pilgrim[15] but the lowest value of 0·0025 g/100 ml (0·0025%) seems to be that of Berg *et al.*[14]

Henning[16] summarised the threshold values for quinine sulphate as ranging from $6{\cdot}69 \times 10^{-7}$ to $6{\cdot}69 \times 10^{-6}$ M (0·00005–0·0005%). Using 38 subjects, Harris & Kalmus[17] reported threshold values for quinine sulphate to range from $2{\cdot}4 \times 10^{-4}$ to $5{\cdot}7 \times 10^{-6}$ M (0·779%–0·000185%), while Pfaffman[7] established a threshold of 0·000008 M ($5{\cdot}98 \times 10^{-4}$%). The lowest threshold level reported appears to be $2{\cdot}9 \times 10^{-6}$ M (0·000217%).[18]

Threshold values have also been established for quinine. Scholl & Munch[19] reported the threshold as 2·5 mg/l (0·00025%). Moncrief[20] stated that thresholds for quinine range from 4×10^{-5} to $1{\cdot}5 \times 10^{-6}$ M ($1{\cdot}30 \times 10^{-3}$–$4{\cdot}87 \times 10^{-5}$%). Lugg,[21] using Malayan negritoes as subjects, found a value of $1{\cdot}3 \times 10^{-6}$ M ($4{\cdot}22 \times 10^{-5}$%), which appears to be the lowest recorded threshold for quinine at that time.

Experimental

Determination of thresholds

In order to establish a general threshold range, each individual was provided with a solution containing 0·00025% of either malic acid (mol. wt. 134·09) or citric acid (mol. wt. 192·12), or a solution containing 0·00000075% quinine sulphate (mol. wt. 746·93), all made from reagent-grade chemicals.

Using the triangular procedure, each solution was taste tested against distilled water. Each panellist tasted a minimum of ten sets of three. Some sets contained two samples

* Journal Paper No. 1046, University of Georgia, College of Agriculture Experiment Stations, College Station, Athens, Georgia 30601, U.S.A.

of distilled water and one of the test substance whereas others contained the opposite. The ten sets were randomised so that the location of the odd sample within each set varied as did the order of receiving sets containing two samples of water and one of test substance, or the opposite.

Initially, the descending system was used to locate approximately the threshold level of the panellist; then ascending–descending trials were employed to narrow down the range. The panellist had the option of moving on to the next level based on ten sets or of choosing to sample 20 sets (sometimes 30 sets) in an effort to establish significance at the level being tested. From one to three sets were taste-tested each day with a rest period between sets.

If the number of correct choices was statistically significant, the panellist moved down to the next lowest level, the level usually being 50% less concentrated. For the ascending series, the strength of each solution was double that of the one below it.

Five panellists were used for the citric and malic acid trials, seven for the first phase of the quinine sulphate study and 63 for a second phase.

Temperature

Taste thresholds were established for each of the three chemicals at three temperatures. For citric and malic acid, the temperatures were 2°C, room temperature (20·5°C) and 41°C. For quinine sulphate, they were 3°C, room temperature (22°C) and 38°C. The trials at 2° and 3°C were carried on in a walk-in meat ageing room, and the 38° and 41°C trials in a walk-in incubator. All equipment (glassware, pipettes, taste solutions, distilled water, stock solutions and disposable, plastic 'shot' glasses used as the tasting vessels) were placed in the appropriate room one day prior to tasting.

The panellists were instructed to make a decision relative to the identity of the sample almost immediately upon taking it into the mouth so as to minimise any changes in temperature.

Statistical analysis of the data

Absolute and difference thresholds were calculated according to the A.S.T.M. method.[22] The statistical threshold was obtained by comparing the number of correct choices of the odd sample with that required for statistical significance at the 5% level.[23]

To compare the effects of temperature, a pooled chi-square analysis was performed.

Energy of activation

Energies of activation (E_a) for detection of each chemical by taste were calculated by the formula method. First, the percentage of correct choices was plotted on the logarithmic scale of semi-logarithmic graph paper *vs.* the solution concentration on the arithmetic scale. The slope, or the rate of change in taste response with concentration, was designated k. To calculate the energy of activation, the k_2 and the k_1 values for the highest and lowest temperatures under consideration were inserted into the Arrhenius equation. This mathematical method is similar to the graphical one described by Charm.[24] Some E_a values were calculated by both methods, and agreed within ~3% of each other.

Extreme-value analysis

After the three series of trials had been completed and the data were being analysed, the use of extreme-value analysis was considered. Powers *et al.*[25] had used extreme-value analysis to predict the number of cans which, upon being pressure-sterilised for various lengths of time, would have received less heat than that required for sterilisation. Estimation of the percentage of the population, the thresholds of which for a compound might be less than that observed in small-panel trials, is a similar problem. In order to compare actual thresholds with the percentage of the population predicted to have thresholds below some certain level, a further set of taste-testing trials became necessary. A panel of 63 was assembled and tested for sensitivity to quinine sulphate in the manner described above except that the trials were confined to room temperature.

The statistical theory of extreme-value analysis has been described by Gumbel,[26] and applications have been described.[27,28] Lieblein's method of calculation [28] was followed.

Results and Discussion

Citric and malic acid

Tables I and II show the threshold values obtained at the three temperatures. The values are listed on three different bases, i.e. the 50%, the 75% and the statistically significant level.

The 50% level is defined as the absolute threshold or the stimulus which is noticed 50% of the time.[22] For the triangular test, this level is statistically significant only when 30 or more sets have been examined. For fewer than 30 replicate determinations, more than 50% of the odd samples must be noticed.

The columns headed 75% are for the difference threshold as defined by the A.S.T.M.[22] Again, this level does not coincide with statistical significance. Above six sets, the 75% threshold will always be higher than that required for statistical significance. For six or fewer replications, the opposite is true. The third set of values gives the threshold values based on the 5% probability level.

For citric acid (Table I), the threshold was higher for each of the five panellists at 2°C than at the other two temperatures. As between 20·5°C and 41°C, the lower thresholds tended to be at 20·5°C. Chi-square analysis of the data showed that there were distinct judge–temperature interactions. The optimum for all judges was not at the same temperature, but temperature did significantly affect the response of all judges.

TABLE I

Taste threshold for citric acid according to different methods

Taste-tester	Concentration, moles[a]								
	50%[b]			75%[b]			Statistical[c]		
	2°C	20·5°C	41°C	2°C	20·5°C	41°C	2°C	20·5°C	41°C
LW ♂	74	7	7	102	25	22	97	23	14
KR ♂♀	30	9	—	50	26	11	48	23	9
HH ♂	—	—	4	122	27	39	98	6	36
DL ♂♀	39	11	12	45	30	23	44	16	22
JP ♂	74	—	2	122	32	23	134	10	20

[a] All values should be multiplied by 10^{-7}
[b] A.S.T.M. method
[c] 0·05 probability level

Table II shows that the thresholds for malic acid also tended to be higher at 2°C than at the other two temperatures, but for one judge the values were almost identical irrespective of the temperature. Unlike citric acid, the threshold values tended to be lower at 41°C than at 20·5°C.

The data from Tables I and II support the comments of Blakeslee & Salmon[29] that 'each of us lives in a different (taste) world'. Some tasters whose thresholds were high for citric acid at a given temperature did not have high thresholds for malic acid at the same temperature. This observation gives added evidence to earlier findings[30] that hydrogen-ion concentration alone is not responsible for sourness, though there are many who explain sourness on this basis. The pHs of citric acid and malic acid at the same concentrations are close to each other.[31] The difference in response suggests that the structure of the undissociated molecule is also a determinant of sourness.

The threshold levels for the two acids were, in general, lower than those reported by others. The levels listed, however, are for difference levels, not recognition levels. The judges were able to select the odd sample because it was different, but they did not necessarily attribute sourness to the solution.

Quinine sulphate solutions

Table III shows the results for the quinine sulphate determinations at the three temperatures. The sensitivity of five of the seven judges was significantly influenced by temperature. Most published reports state that sensitivity to bitterness is greater the lower is the temperature. There was a significant difference in response between 3°C and 22°C, but greater sensitivity occurred at 22°C than at 3°C. Generalisations about response to bitterness are difficult to make. As in the citric and malic acid trials, there was strong judge–temperature interaction (Fig. 1). The present findings do not

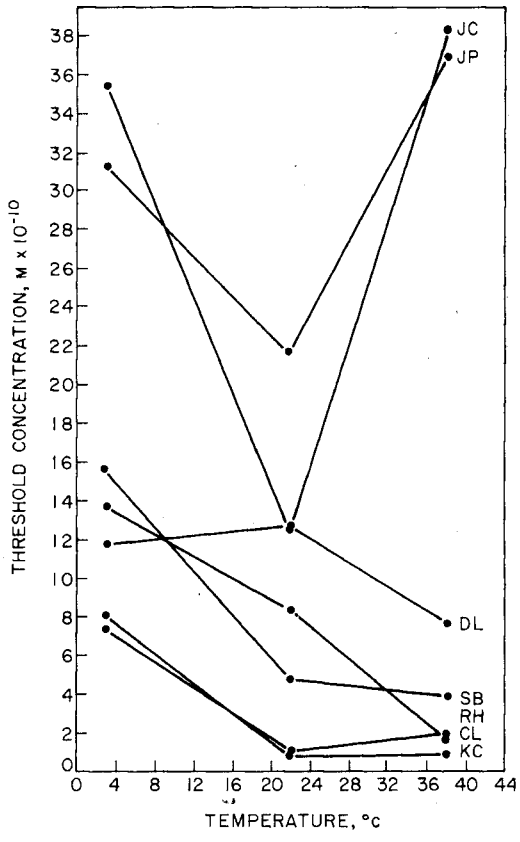

FIG. 1. *Influence of temperature on the thresholds of judges for quinine sulphate*

Initials refer to individual judges

TABLE II

Taste threshold for malic acid according to different methods

Taste-tester	Concentration, moles[a]								
	50%[b]			75%[b]			Statistical[c]		
	2°C	20·5°C	41°C	2°C	20·5°C	41°C	2°C	20·5°C	41°C
LW ♀	94	—	22	248	41	28	224	30	27
KR ♀	12	14	5	26	25	7	24	23	7
HH ♀	36	6	—	74	28	19	68	24	16
DL ♀	74	8	6	138	30	7	126	26	7
JP ♂	30	38	32	81	61	47	41	43	44

[a] All values should be multiplied by 10^{-7}
[b] A.S.T.M. method
[c] 0·05 probability level

TABLE III

Taste threshold for quinine sulphate according to different methods

Taste-tester	Concentration, moles[a]								
	50%[b]			75%[b]			Statistical[c]		
	3°C	22°C	38°C	3°C	22°C	38°C	3°C	22°C	38°C
DL ♀	105	121	63	156	148	124	11·7	12·7	7·6
JP ♀	289	180	324	393	328	380	31·3	21·6	27·0
SB ♀	98	38	29	168	49	68	15·4	4·7	3·8
JC ♀	217	83	334	84	258	—	35·4	12·4	38·4
KC ♀	39	14	10	216	17	18	8·0	0·8	0·8
RH ♀	56	—	17	92	30	19	7·4	1·0	1·8
CL ♂	91	56	16	146	88	20	13·6	8·3	1·6

[a] All values should be multiplied by 10^{-10}
[b] A.S.T.M. method
[c] 0·05 probability level

bear out the idea that sensitivity to bitterness, at least in the case of quinine sulphate, is greater the lower is the temperature.

As for the acids, the threshold levels themselves were well below those reported by others.

All three series of trials indicated that there were differences in threshold level as a result of temperature, but judge-specific compound effects often overrode temperature effects. Fig. 2 shows the responses of two judges who were panellists in each series. Some of the discrepancies in relating temperature to optimal sensitivity undoubtedly arose because different judges have different optima, and thus conclusions depend more on the composition of the panel than on the effects of temperature. This is true for all sensory evaluation and present results indicate the need for large panels or appropriate statistical analysis, such as the extreme-value method to be described below, and preferably both.

Energy of activation

Table IV lists E_a values in the different temperature ranges. If the plot of $\ln k$ *vs.* $1/T$ sloped upward to the left, E_a was designated as positive, and if it sloped downward to the left, E_a was designated as negative. In this context, the energy of activation may be interpreted much as it is for bacterial growth. Below the point of optimal growth, the energy of activation is looked upon as the energy needed to stimulate growth.

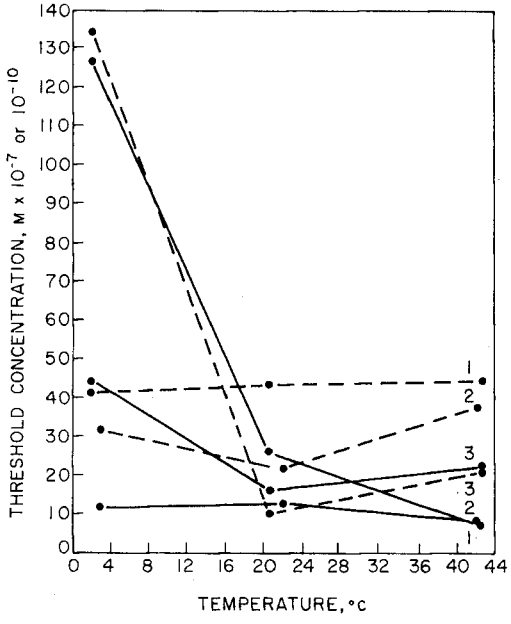

FIG. 2. *Illustration of judge–compound–temperature interactions*

(1) malic acid; (2) quinine sulphate; (3) citric acid, ●——● judge DL; ●– – –● judge JP. Threshold concentrations for citric acid and malic acid should be multiplied by 10^{-7} and for quinine sulphate by 10^{-10}

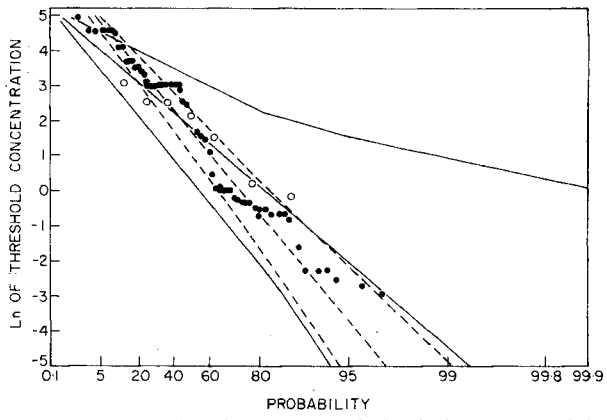

FIG. 3. *Extreme-value plot for threshold levels for quinine sulphate*

The regression lines were calculated as natural logarithms. −23·0259 should be added to the values on the ordinate to yield the natural logarithm of the threshold value. The original seven determinations, the regression line and the 5% confidence limits are shown as solid lines and open symbols. The subsequent plot based on 70 values is shown as dashed lines and closed symbols.

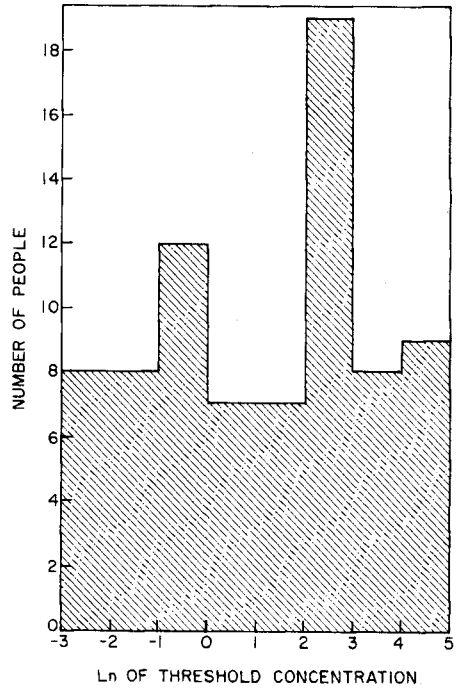

FIG. 4. *Frequency distribution of threshold values among 70 individuals*

−23·0259 should be added to the values on the abscissa to yield the natural logarithm of the threshold values

TABLE IV

Energies of activation (kcal) for sensing of malic acid, citric acid and quinine sulphate

Taste-tester	Malic acid			Citric acid			Quinine sulphate		
	2°–20·5°C	20·5°–41°C	2°–41°C	2°–20·5°C	20·5–41°C	2°–41°C	3°–22°C	22°–38°C	3°–38°C
LW			12·54			5·48			
KR	3·02	14·86				3·16			
HH	6·48	−·27		13·39	4·27				
DL	10·02	20·65		−7·77	3·12		4·84	−5·79	
JP			5·22	2·51	9·26		−1·02	10·67	
SB							57·53	−11·13	
JC							3·90	−6·93	
KC							31·31	−6·13	
RH							·59	17·26	
CL									14·71
Average	6·51	11·75	8·88	2·71	5·55	4·32	16·19	0·34	14·71

Above the optimum, the value may be looked upon as the energy leading to deactivation of the organisms. In the same fashion, the inflection points in Figs 1 and 2 suggest that each judge had an optimum for each compound and the energy changes above or below the optimum represent activation or deactivation of response.

With some exceptions, the E_a values were in the range 5–20 kcal, suggesting that a process such as adsorption may be involved.

Extreme-value analysis

Fig. 3 shows an extreme-value plot for the thresholds of the seven judges at room temperature and within the confidence limits of this plot an extreme-value plot for 63 additional judges. The extreme-value plot was highly effective in predicting the range within which the thresholds of a greater number of people might occur. The solid lines and limits and the seven open symbols are for the original seven determinations. The dashed lines and closed symbols are for the additional 63 values. It should be noted that both the regression line and the limits of the latter fall within the confidence limits of the plot based upon only the seven values.

The ordinate values in Fig. 3 are natural logarithms but not the logarithms of the threshold values themselves. For simplicity in calculation or graphing, the factor 10^{-10} was disregarded, e.g. if the threshold value were $3{\cdot}78 \times 10^{-10}$, only the 3·78 part was dealt with. The values in Fig. 3 can be converted to the natural logarithm of the threshold value by adding −23·0259 to the value shown.

For the extreme-value analysis, it was assumed initially that the threshold values would follow a logarithmic function. This was born out by ranking the 70 values available. They formed a distinct logarithmic curve.

Fig. 4 shows a histogram of the number of panellists whose thresholds were within the seven classes.

The use of extreme-value methods should have particular merit in threshold predictions. For good or natural flavour itself, one is generally more interested in supra-threshold ranges, but for off-flavour or for added substances such as anti-oxidants or preservatives, one is interested at the level first detectable and one would like to make predictions about the percentage of individuals who will be able to detect the off-flavour at different concentration levels. The same is true for perfumes or for pharmaceuticals, except that for perfumes one is interested in 'wrong' notes and for pharmaceuticals one is interested in the masking of distasteful ingredients. For air and water pollution standards, extreme-value methods should have particular applicability. One can generally sample only a few hundred people to ascertain concentration levels which are objectionable. The question then arises: the threshold having been determined for the most sensitive among 25, or 100, or 200 people, what proportion of the population is even more sensitive than the most sensitive individual actually observed? Extreme-value calculations should be of material help in making this decision.

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