

Effects of sulfate and nitrate on the taste of water: a study with a trained panel

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ABSTRACT

The role of sulfate and nitrate in the taste of drinking water is not entirely clear. Taste threshold concentrations (TTCs) were estimated for these two species by the 3-AFC method by a trained panel, as sodium and calcium salts. In both cases TTCs for nitrate were about 100 mg NO₃/L, twice the usual reference value of international recommendations. TTCs for sulfate were about 160 mg SO₄/L (sodium salt) and 80 mg SO₄/L (calcium); 75% detection values were also estimated for both anions. An experiment with a series of duo-trio tests plus preference showed that sulfate at low concentrations tended to improve the taste of water. However, at high concentrations it was perceived negatively: salty and bitter were the main descriptors used. The reversion concentration took place between 176 and 259 mg SO₄/L. These results indicate that nitrate has to be considered an important issue for health, but with no relevant role in the taste of water. Sulfate shows a positive influence at moderate concentrations but becomes negative at high levels. The usual aesthetic-based level of 250 mg/L established by international regulations appears to be reasonable. The findings of this study with trained panellists are preliminary. Future research with consumer panels is recommended.

Key words | drinking water, flavour, nitrate, sulfate, taste

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INTRODUCTION

'The need to understand the role of minerals in the taste and nutritional value of drinking water is timely for the drinking water industry'. This statement was made ten years ago (Burlingame *et al.* 2007) and it is obvious that the scientific community has gained knowledge on this subject and that water supply companies are taking advantage of it to improve the taste of tap water.

On the other hand, it is crucial to think about the exponential growth of membrane treatments and the remineralization processes that they require. Membrane technologies improve the taste of water because they reduce (sometimes almost remove) the organic matter present in raw water. Permeates, on the other hand, are very poor in salts and need remineralization. These changes in the total dissolved solids (TDS) and the contribution of the different ions are

perceived by consumers. Therefore, the design of the remineralization processes has to consider taste considerations in addition to health and aggressivity reasons.

Various relevant approaches about taste and minerals in waters can be found in the literature over the last decade, for example:

- Influence of TDS as an aggregate parameter of the mineralization level on the taste of water (Teillet *et al.* 2010; Dietrich & Gallagher 2013)
- Role of specific ions (Burlingame *et al.* 2007; Marcussen *et al.* 2013a, 2013b; Platikanov *et al.* 2017)
- Perception of metallic sensation – today we know its important retronasal contribution (Ömur-Özbek & Dietrich 2011; Sain & Dietrich 2015)

- Improvement in the taste of tap water thanks to membrane use (Devesa *et al.* 2010; Raich-Montiu *et al.* 2014; Vingerhoeds *et al.* 2016)
- Critical evaluation of organoleptic-based standards (Dietrich & Burlingame 2015).

In previous works on the mineral composition and liking of water, sulfate and nitrate showed a significant influence on liking scores by trained and volunteer panels (Platikanov *et al.* 2013; Platikanov *et al.* 2017). Sulfate presented a positive effect, even though the concentration of this cation has been traditionally limited in international regulations for aesthetic and health reasons. And the effect of nitrate was negative, although the possibility of a spurious correlation with sodium was considered (Platikanov *et al.* 2013). The purpose of the work was to gain knowledge on the role of these two species' flavour thresholds and effects (positive/neutral/negative) on the taste depending on their concentration.

NITRATE AND SULFATE: LEVELS AND REGULATIONS

Nitrate and sulfate are major ions in natural water composition. Both are included in international regulations and recommendations due to their effects on health and the taste of drinking water.

Nitrate

The main sources of nitrate in water are anthropogenic: fertilizers and livestock manure, human sewage and landfill leachates. Nitrate does not generally have a geological origin. Nitrate may also come from acid rain (oxidation of nitrogen oxides) or from soil as a result of the oxidation of nitrogenated natural organic matter. Water bodies most vulnerable to nitrate contamination are in agricultural and cattle farming areas. Nitrate presents high mobility because practically all of its salts are highly soluble in water. Small concentrations of nitrate of just 1–3 mg N/L are indicative of human influence (Madison & Brunett 1985; Dubrovsky *et al.* 2010).

Levels

The US Geological Survey (Madison & Brunett 1985) found that 20% of 124,000 wells presented concentrations above 3 mg N/L, and 6% were above the primary water level of 10 mg N/L. A more recent study (Dubrovsky *et al.* 2010) predicts that significant areas in several states are affected by nitrate contamination. In Europe, a study covering almost 900 samples of bottled and tap water obtained a median of 1.3 mg/L and a 95% percentile of 15.4 mg/L (Reimann & Birke 2010). Tap waters presented significantly higher values (mean = 7.9 mg/L), with several samples between 20 and 45 mg/L.

Regulations

The main hazard of nitrate occurs when it reduces to nitrite in the digestive system. The nitrite reacts with haemoglobin in the red blood cells to form methaemoglobin. In this situation, blood lacks the capacity to transport oxygen, causing the veins and skin to appear blue. This effect is only serious for children under six months of age, and is known as 'blue baby syndrome'. The risk of methaemoglobinaemia increases in case of gastrointestinal infections, which increase endogenous nitrite formation. For this reason, most of the reported cases of this illness are associated with consumption of water from private wells not properly disinfected.

The WHO has established a guideline for nitrate of 50 mg/L (as NO₃), based on epidemiological data. In the European legislation (EU 2015), it is also regulated at this value, and in Canada at 45 mg/L (Health Canada 2014). In the USA, the maximum concentration level (MCL) is established at 10 mg/L as N (USEPA 2012), equivalent to 44.3 mg/L as NO₃.

Sulfate

Sulfates occur naturally in different minerals, soil and rocks. Typical sulfate minerals include salts of calcium (gypsum and anhydrite), magnesium (epsomite), sodium (mirabilite and thenardite), barium (barite), and others. Sulfate is also formed when sulfide minerals are oxidized. Sulfide, the

reduced state of valence of the element, is the most abundant form of the element in the earth's crust.

Several anthropogenic activities are also sources of sulfate: mining, the pulp and paper industry, tanneries, the chemical industry, etc. Sulfate also comes from rainfall, both from marine aerosol and from sulfur dioxide emissions (acid rain). Once in solution, sodium, potassium and magnesium sulfates are very soluble in water, but calcium sulfate is not. Barium sulfate is highly insoluble. Sulfate concentrations in natural waters seem to be generally limited by gypsum solubility.

Levels

Sulfate concentration in natural waters presents a wide range, from a few mg/L to more than one thousand. A survey performed in the USA indicated a median value of 24 mg/L and a 99th percentile of 1,200 mg/L (USEPA 2003). Similar surveys in Canada showed mean concentrations between 12.5 and 22.5 mg/L in Ontario and Nova Scotia, but higher results of medians up to 368 mg/L in Saskatchewan, with individual values above 2,000 mg/L. A study performed in the European Union for tap and bottled water (Reimann & Birke 2010) showed a median of 20 mg/L and a 95th percentile of 809 mg/L.

Regulations

Sulfate is known to produce laxative effects at high concentrations above 1,000–1,200 mg/L (WHO 2004) because of its osmotic gastrointestinal activity. Taking into account the usual levels of the anion in drinking water, no health-based maximum concentration is proposed by WHO guidelines (WHO 2011). However, some regulations have established a maximum recommendable value of 500 mg/L (USEPA 2012; Health Canada 2014).

In addition, sulfate is generally regulated from an aesthetic point of view because of its salty taste. Generally, 250 mg/L maximum is recommended (EU 2015; USEPA 2012). Taste threshold concentrations (TTCs) have been reported by several studies but the results show large differences (Burlingame *et al.* 2007). The counter-ion seems to be very significant.

EXPERIMENTAL

Panel and tasting sessions

All the experiments were performed by the Aigües de Barcelona expert panel (Devesa *et al.* 2004) consisting of 15 panellists, 5–7 per session, trained according to the Flavour Profile Analysis (FPA) method (APHA, AWWA, WEF 2012). The sensory tests used in this study are not of a descriptive nature (the taste-and-odour wheel is not used) and therefore do not require in-depth training. In any case, this expert panel was used for availability reasons.

Samples were always presented coded and without giving the panellists any information which could influence their appreciation. The tasting took place in a room specifically intended for this purpose: comfortable and free from interfering odours. The flavour test was carried out using glasses on samples maintained at 25 °C.

Sensory methods

Taste threshold determination by 3-AFC

TTCs were determined by the three-alternative forced-choice (3-AFC) method, following the general instructions of ASTM E-679 (ASTM 2011). In this method, a series of triangle tests are presented, always with two identical samples (water reference or blank) and one water containing the stimulus, the compound, or species to be tested. The panellists were required to choose one sample as different in each series of three (forced version of the test).

The concentration of the species increases in steps from one triangle to the next by a constant factor. Individual thresholds are calculated as the geometric mean between the first concentration from which all the responses are correct and the concentration immediately lower. The group threshold is calculated as the geometric mean of the individual values. No stopping rule was applied (Lawless 2010); that is, panellists completed all the steps independently of their answers. Eight steps were used in all the experiments. A 5-minute pause was maintained between steps, except between the fourth and fifth when a 20-minute break was established.

Duo-trio test

This is a difference test in which three samples are presented. The first one is a reference, followed by two test samples, one of which matches the reference and the one which is different. Subjects are told to identify the unknown samples. Two versions of the tests are possible, with the reference sample being balanced or with the reference kept constant.

Reference waters

Four different reference waters (blanks) were used in the study. TTCs depend on the water used as a reference (blank) in terms of type of water (main ions) and level of mineralisation. Different waters have been reported in the literature, depending on the author's criteria and availability. In this work, sodium and calcium salts at two common TDS levels have been used as representative of different composition. Both ions are major species and confer a different taste to water (Burlingame *et al.* 2007).

The two sodium bicarbonate waters were prepared at the laboratory, dissolving the required amount of this salt in purified water: 158 mg/L for 100 mg/L TDS and 412 mg/L for 260 mg/L TDS. All TDS values are referred at 180 °C. At this drying temperature, bicarbonate decomposes into carbonate with the release of water and carbon dioxide (Hounslow 1995; APHA-method 2540, APHA, AWWA, WEF 2012).

Calcium bicarbonate water of TDS = 100 mg/L was obtained by a proper dilution with Milli-Q water of a mineral bottled water of TDS = 116 mg/L with negligible concentration of sodium. A bottled calcium bicarbonate water of TDS = 260 mg/L was used directly. Table 1 shows the composition of the reference waters used. Sodium, potassium, calcium, and magnesium were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Perkin Elmer Optima 4300 DV). Bicarbonate and pH were determined by a robotic titrosampler (MetröhM modules 855 and 856). Chloride and sulfate concentrations were analysed by ion chromatography (Dionex ICS-2000). TDS (total dry residue) at 180 °C was determined gravimetrically.

Table 1 | Chemical composition of the reference waters

Parameter	Calcium bicarbonate water TDS = 116	Calcium bicarbonate water TDS = 260
Sodium (mg Na/L)	< 0.5	5.1
Potassium (mg K /L)	< 0.5	1.1
Calcium (mg Ca/L)	26.9	59.4
Magnesium (mg Mg/L)	7.7	25.6
Bicarbonate (mg HCO ₃ /L)	121.6	284
Sulfate (mg SO ₄ /L)	3.0	21.3
Chloride (mg Cl/L)	0.52	7.4
TDS 180 °C (mg/L)	100	260
pH (units pH)	7.8	7.9

RESULTS AND DISCUSSION

Nitrate

TTCs for nitrate were measured according to the Three-Alternative Forced-Choice procedure (3-AFC), with ascending concentration series (ASTM 2011). Two nitrate salts were studied: sodium and calcium. The experiment with sodium nitrate was performed using the calcium bicarbonate water of 100 mg/L TDS as reference water (blank). For the calcium nitrate determination, the 100 mg/L sodium bicarbonate was used.

The experiments were designed to minimize the uncertainty of the results. Preliminary experiments were performed for each compound in order to find the proper working range. In both cases eight steps were considered with a concentration factor of 1.75. The working concentrations were the same for both species, from 16.3 to 820 mg NO₃/L (corresponding to the established value of 50 mg/L at the third step).

The results following ASTM E679-04, as geometric mean, are presented in Table 2. An alternative calculation method for TTC is from the psychometric function (Piriou *et al.* 2009; Lawless 2010), the well-known sigmoidal curve of percentage of detection (correct answers) vs. logarithm of the concentration. Abbott's formula (Lawless 2010) for guessing correction was not used because only answers with all subsequent higher concentration answers correct were considered to calculate the percentage. The linear portion of the function permits the

Table 2 | TTCs for nitrate, as sodium and calcium salts

Ion	Co-ion	Reference water		Number panellists	Geometric mean	25% Perception	50% Perception	75% Perception
Nitrate	Sodium	Calcium bicarbonate 100 mg/L TDS		13	111 mg/L	56 mg/L	110 mg/L	215 mg/L
Nitrate	Calcium	Sodium bicarbonate 100 mg/L TDS		15	100 mg/L	36 mg/L	100 mg/L	281 mg/L

All the results are expressed as NO₃ in mg/L.

calculation by regression of the 50% perception concentration (usually a value close to the geometric mean), but also makes it possible to calculate the concentrations at other perception percentages. For example, in this work the 25% and 75% perceptions have been calculated. Regression lines are presented in the Supporting Information (Figures S1 and S2), available with the online version of this paper.

For both salts, detectable concentrations were clearly above the generally recommended value of 50 mg/L NO₃. Therefore, it is confirmed that nitrate is an important health-related issue but without organoleptic relevance in normal conditions.

Sulfate

Sulfate TTCs

Thresholds for sulfate were determined using the 3-AFC method with identical design to that indicated above for nitrate. Again, sodium and calcium salts were studied, with the same reference waters (blanks). Preliminary experiments determined the working range for each species. In both cases, eight steps with a factor = 1.5 were used. The concentrations ranged from 49 to 843 mg SO₄/L for sodium sulfate, and from 33 to 562 mg SO₄/L for the calcium salt.

The results (Table 3) show that the detectable concentrations are below the usual reference value of 250 mg SO₄/L. They are clearly lower than previous values from the literature indicated above. For this reason, some additional experiments were performed in order to gain knowledge on the influence of sulfate on the taste of

water. Regression lines are presented in the Supporting Information (Figures S3 and S4), available with the online version of this paper.

Role of sulfate: duo-trio tests with preference

The experiment was designed as a series of duo-trio tests in constant reference mode and with ascending concentration of sulfate in the odd sample. The first duo-trio test consisted of presenting three samples: one reference water not containing sulfate and two others, one of them identical to the reference and the other the same water spiked with a certain concentration of sulfate. The next duo-trio test or step had the same design but with a higher concentration of sulfate in the odd sample. Eight duo-trio tests were presented in this way. The reference water and the concentration factor between steps were always the same.

The instructions were the same for all the duo-trio presentations. Tasters were told to: (a) identify the sample that was different from the reference; (b) indicate their preference; that is, whether the water that they chose as different was better, worse, or equal/no preference with respect to the constant reference; and (c) explain why, the reason for the preference.

The reference sample (constant) was always tasted first. Indeed, the same glass was used for the reference water in all the steps. The other two samples were presented in a balanced order. Once the subjects had tasted the samples in the specified order, they were allowed to re-taste them as they preferred.

Four experiments were performed, with two sulfate salts (sodium and calcium) at two levels of TDS of the reference

Table 3 | TTCs for sulfate, as sodium and calcium salts

Ion	Co-ion	Reference water		Number panellists	Geometric mean	25% Perception	50% Perception	75% Perception
Sulfate	Sodium	Calcium bicarbonate 100 mg/L TDS		13	159 mg/L	98 mg/L	167 mg/L	282 mg/L
Sulfate	Calcium	Sodium bicarbonate 100 mg/L TDS		14	83 mg/L	28 mg/L	71 mg/L	178 mg/L

All the results are expressed as SO₄ in mg/L.

(a)	Step	1	2	3	4	5	6
TDS =100 mg/L	Conc. SO4 (mg/L)	100	150	225	337	506	759
Sodium sulfate							
	Panelist 1						
	Panelist 2		X				
	Panelist 3						
	Panelist 4		X	X			
	Panelist 5	X	X	X			
	Panelist 6						
	Panelist 7						
	Panelist 8						
	Panelist 9						
	Panelist 10		X				
	Panelist 11	X	X				

(b)	Step	1	2	3	4	5	6
TDS =260 mg/L	Conc. SO4 (mg/L)	100	150	225	337	506	759
Sodium sulfate							
	Panelist 1	X					
	Panelist 2						
	Panelist 3			X	X		
	Panelist 4						
	Panelist 5	X					
	Panelist 6						
	Panelist 7	X					
	Panelist 8	X	X				
	Panelist 9			X			

(c)	Step	1	2	3	4	5	6
TDS =100 mg/L	Conc. SO4 (mg/L)	80	120	180	270	405	607.5
Calcium sulfate							
	Panelist 1						
	Panelist 2	X					
	Panelist 3						
	Panelist 4						
	Panelist 5						
	Panelist 6						
	Panelist 7						
	Panelist 8	X					
	Panelist 9						

(d)	Step	1	2	3	4	5	6
TDS = 260 mg/L	Conc. SO4 (mg/L)	80	120	180	270	405	607.5
Calcium sulfate							
	Panelist 1		X	X			
	Panelist 2						
	Panelist 3						
	Panelist 4						
	Panelist 5						
	Panelist 6	X					
	Panelist 7						
	Panelist 8						
	Panelist 9	X					
	Panelist 10			X			

X	Incorrect answer.
	Correct answer. Odd sample (spiked with sulfate) likes more
	Correct answer. No preference among samples
	Correct answer. Odd sample (spiked with sulfate) dislikes more

Figure 1 | Duo-trio test results with sulfate spiked in the odd sample.

water, 100 and 260 mg/L. The experiments with sodium sulfate were performed taking the calcium bicarbonate waters as a reference, and those with calcium sulfate taking the sodium bicarbonate waters.

Results of duo-trio plus preference tests are presented in Figure 1. Although different behaviours can be observed among individual panellists as usual in this type of experiments in sensory analysis, some general trends can be drawn. Spiked water with sulfate (odd sample) is generally preferred at the beginning of the experiment, when the sulfate concentrations are low. However, in the last steps when the concentrations are high, the odd sample rich in sulfates is disliked. In some cases, tasters define a transition zone where no preference is given. This general behaviour, and in particular the improvement at low concentration of sulfates, seems to be more clear with calcium sulfate than with the sodium salt.

The experiment also permits an estimation of the mean concentration above which sulfates present a negative influence on taste (Table 4). This is calculated in a similar way to the 3-AFC method: the geometric mean between the first value where the spiked water is evaluated as worse and the value immediately below it. In Figure 1, it corresponds to the geometric mean between the first value of the continuous black strip to the right of the figure and the preceding value. For panellist 4 in Figure 1(d) the value was obtained as the geometric mean between the last concentration and the one theoretically immediately above.

With regard to the tasters' reasons for their preference, two different situations were observed. In those cases, during the first steps, in which the spiked sample was preferred, the most common descriptors were 'less bitter' and 'less sweet'. After the reversion, when the sample spiked with sulfate was less appreciated, the common descriptors were 'salty'/'very salty' and 'bitter', and less frequently 'metallic' and 'sweet'.

These findings confirm that the usual aesthetic-based level of 250 mg/L by USEPA and other international

regulations is consistent, as pointed out by Dietrich & Burlingame (2015).

CONCLUSIONS

TTCs were estimated by the 3-AFC method by a trained panel for nitrate and sulfate, as sodium and calcium salts. TTCs for nitrate were 111 mg NO₃/L (sodium salt) and 100 mg NO₃/L (calcium), about twice the usual reference value of international recommendations of 50 mg NO₃/L; 75% detection was estimated as 282 and 178 mg NO₃/L, respectively.

TTCs for sulfate were 159 mg SO₄/L (sodium salt) and 83 mg SO₄/L (calcium); 75% detection was estimated as 282 and 178 mg SO₄/L, respectively. These values are below the international maximum recommended concentration of 250 mg SO₄/L.

An experiment with a series of duo-trio tests plus preference with constant reference showed that sodium sulfate at low concentrations tended to improve the taste of water: panellists qualified the water as 'less bitter' or 'less sweet'. This improvement at low concentrations was significantly clearer with calcium sulfate, the most common situation in natural and tap waters. In contrast, sulfate at high concentrations was perceived negatively with both salts: salty and bitter were the main descriptors. The experiment also permitted an estimation of the concentrations for the appearance of the negative influence of sulfate on taste, between 176 and 259 mg/L depending on the blank water used. In short, nitrate is an important health issue as is well known, but plays no relevant role in the taste of water. The usual maximum recommended level of 250 mg/L for sulfate in international regulations is consistent from the aesthetic point of view in agreement with Dietrich & Burlingame (2015).

Given the innate variability of humans in taste sensitivity and the small number of participants in trained panels, the findings of this paper should be considered a preliminary study to assess the effects of nitrate (irrelevant) and sulfate (negative/neutral/positive depending on the concentration) on taste of water with regard to drinking water regulations. Future research with consumer panels is recommended.

Table 4 | Geometric mean concentrations above which sulfate presents a negative influence on taste

	Sodium sulfate	Calcium sulfate
TDS = 100 mg/L	198 mg/L	176 mg/L
TDS = 260 mg/L	210 mg/L	259 mg/L

All the results are expressed as SO₄ in mg/L.

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