Sulphur dioxide in commercially produced microalgae

Gideon Ashworth investigates the source of Sulphur Dioxide identified in microalgae and the need for further research in this field.

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The EU wide recall by Nutrisure Ltd of Organic Chlorella Power (Chlorella vulgaris) Batch O.CH-DF-160330 (BBE Mar 2019) in April 2018 due to the presence of sulphites posed questions on the origins of the allergen Sulphur Dioxide (SO₂) within the microalgae. When the recall notice was first posted, one logical explanation pointed towards whether this could be a false positive reported by the laboratory that carried out the analysis. Extensive further testing (circa 70 samples) of multiple batches of Chlorella and also Spirulina from suppliers in different continents (Europe, Asia and North America) was therefore carried out. Samples were sent to two separate UKAS accredited laboratories for SO₂ analysis, both used the optimised Monier-Williams method. The results returned positive readings at levels above the permitted limit of <10ppm for 100% of tested Spirulina and Chlorella products, across both organic and conventional lines (Table 1).

These results were alarming; not only is the presence of SO₂ and/or sulphites a breach of Organic regulations but sulphites are also a listed allergen and therefore must be declared on pack/ at point of bulk sale. Any allergen must be segregated during storage, transportation and further Table 1: Examples of SO₂ levels measured in product samples from different regions

| Product              | Country of origin | Sulphur dioxide |
|----------------------|-------------------|-----------------|
| Chlorella powder     | China             | 52              |
| Organic Spirulina    | China             | 31              |
| Powder               |                   |                 |
| Organic Chlorella    | China             | 55              |
| Powder               |                   |                 |
| Organic Chlorella    | India             | 13              |
| Powder               |                   |                 |
| Spirulina powder     | Hawaii            | 17              |
| Chlorella powder     | Portugal          | 40.3            |
| Spirulina powder     | USA               | 25.8            |
| Chlorella powder     | Korea             | 15.2            |
| Spirulina powder     | France            | 18.4            |
Another explanation for the positive result could have been that the producer had deliberately added the SO$_2$ either during the production process to increase yields in the growing pools or during postharvest processing for increased durability and shelf life. However, the product already has a 36-month shelf life so there seems to be no benefit in using additives to extend this. Sulphur dioxide addition had not been declared by the supplier during the rigorous approval process, nor by any other supplier or competitor in the marketplace. An alternative explanation is that the SO$_2$ could have been present as a contaminant in the water used for growing the algae, however investigations did not show evidence of this.

Since Chlorella powder has been traded for decades now, it is unclear why no previous complaints relating to SO$_2$ content of algal food products and allergies have been logged or reported.

**Origins of sulphur dioxide in microalgae**

Sulphur dioxide is ubiquitous and can be detected in the atmosphere from both naturally occurring and man-made sources. It is emitted during volcanic eruptions and is a by-product of burning fossil fuels. It is a precursor to sulphuric acid. Sulphur dioxide is present as a gas at varying concentrations throughout the atmosphere and is water soluble. The method of measuring SO$_2$ density in the atmosphere is recorded in Dobson units. There is an ongoing monitoring schedule, driven partly by the role of SO$_2$ in climate change.

Sulphur compounds are required for cell division, protein development and fatty acid synthesis by microalgae. In commercial production, sulphur is intentionally added to the fertiliser used in the growing pools, along with carbon, nitrogen and phosphorous. However, SO$_2$ production from sulphur requires heat at temperatures above the boiling point of water, while the optimum growing temperature for microalgae is between 20 and 35°C.

The SO$_2$ combines with oxygen in the air, or oxygen generated during microalgae photosynthesis to create SO$_3^{2-}$ (sulphites) and SO$_4^{2-}$ (sulphates) which potentially aids absorption by the microalgae.

Information in the scientific literature about SO$_2$ content of microalgae food products is limited. However, a 2013 Novel Food Application in Spain for the microalgae ‘Tetraselmis chuii’ reports that the sulphites analysis returned a positive result of 15ppm. The authors state that this organism is ‘similar to the species Chlorella’. Allergy assessments were then carried out on both healthy individuals and those with known allergies to sulphites; there were no reports of reactions in any individual involved in the trial.

Recent research suggests that microalgae, including Chlorella, are good SO$_2$ scavengers. There have been a number of studies into the potential for using microalgae to reduce pollution from coal generated flue gases by absorbing SO$_2$. There is also evidence that SO$_2$ application can decrease the pH of water, which would be undesirable in the commercial production of microalgae, since pH control is a fundamental parameter. Japanese researchers reported in 1997 that a controlled increase of SO$_2$ concentrations in growing pools decreased yields of the microalgae Nannochloropsis salina and Phaeodactylun tricornutum. The research suggested that an application of SO$_2$ at 50ppm decreased the pH of the water from 7 to 6, whilst SO$_2$ concentrations of 400ppm significantly decreased pH to less than 4, resulting in unfavourable
conditions for the microalgae to develop. These findings therefore suggest that there is no overall yield advantage to be gained by the deliberate addition of SO$_2$ as a growth enhancer.

The presence of naturally occurring SO$_2$ in foods has been common knowledge for a number of years. Alliums (onion, garlic, chives etc.) as well as some Brassica (including cabbage) contain levels of naturally occurring sulphites. Indeed, researchers have developed methods to determine if the presence of sulphites in these foods is naturally occurring or if it has been intentionally added.

The presence of SO$_2$ in herbal medicines has also been reviewed. Although the research did not include microalgae, it highlights that levels of SO$_2$ were detected above the permitted levels in food (Reg (EU) 1169/2011) of 10ppm, using the Monier-Williams method.

Microalgae production methods

Overall production methods for Spirulina and Chlorella are very similar, using fresh water growing pools. Optimum growth for microalgae, specifically commercial production of Spirulina and Chlorella, requires a pH of 8 to 9. The pH levels are continually monitored and managed with CO$_2$ addition to help reduce the risk of microbial contamination and to optimise productivity. The optimum air and water temperature for production is between 20 and 35°C. The density of microalgae, which affects the growth rates and nutrient uptake, is measured daily. The water flows at a rate of 10m min$^{-1}$ within the raceway pools and is replaced quarterly after multiple harvests, there is usually an onsite facility to monitor microbial activity. The depth of the water is circa 20cm$^3$; each standard pool can contain circa 400m$^3$.

The microalgae are cultured in an onsite lab, prior to being transferred to the pools. Growth rates vary with the seasons, pH, temperature, density and fertiliser use. Sulphur is added to the pools in the organic fertiliser, which often contains rapeseed meal and castor seed meal, or other organic fertilisers.

Conclusions

Recent evidence has emerged of sulphite levels in microalgae (Spirulina and Chlorella) food products from a wide range of different sources exceeding the permitted limit of <10ppm. There is no evidence of the SO$_2$ having been deliberately added by any producer during or post production and research into microalgae usage to scavenge SO$_2$ suggests that it could be absorbed during photosynthesis and then stored. It appears that the microalgae products may contain naturally occurring SO$_2$. Therefore, the recommendation by the competent authorities to include the allergen declaration ‘Contains naturally occurring sulphites’ on pack remains.

Although trials are underway as commercial production of the microalgae continues, currently there is insufficient evidence that control measures to reduce SO$_2$ concentrations during production (increased cleaning of the pools, increased harvest frequency to reduce density in the pools, filtering air in the drying room and adding more fresh water during postharvest cleaning) will reduce SO$_2$ levels to a consistently compliant level.

This area of investigative research requires urgent escalation and attention by the relevant authorities, including FSA, Defra and government bodies globally (including the UN’s FAO), to identify the reasons for (and the consequences of) the SO$_2$ presence in commercially grown microalgae. Furthermore, there is potential for additional testing methods to be developed to corroborate measurements by the standard Monier-Williams method. This will require the cooperation of research institutions and funding bodies.

References are provided on the next page.

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