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# Effect of Sodium Metabisulphite on Blood Metabolic Status of Wistar Rats

**Fatima Zohra El Kadi[1], Amina Imène Bénali[1], Mohammed Bénali[1], Slimane Belbraouet[2*]**

[1]Laboratoire de Biotoxicologie, Département de Biologie, Faculté des Sciences de la Nature et de la vie, Université D Liabés, Sidi Bel Abbés, Algérie
[2]École de Nutrition, Université de Moncton, Moncton, Canada
Email: elkadiftimazohra@yahoo.fr, benalimene@yahoo.fr, benalimo@yahoo.fr,
[*]slimane.belbraouet@umoncton.ca

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## Abstract

**The objective of this work was to determine the sodium metabisulphite (NaMBS) subchronic toxicity used as a food additive in Algeria. Three groups of female Wistar rats were treated with 0.25%, 1% and 4% of NaMBS in their drinking water for 90 days. An immunization protocol was conducted during the experiment. Mortality, comportmental and weight modifications, and food and water consumption were recorded. At the end of the experiment, the control and experiment rats were killed, and their blood and organs were removed. Immunoglobulin levels were evaluated; biochemical and hematological parameters were investigated. Our results showed that the administration of NaMBS at 1% and 4% had significant effects on body weight, food and water consumed. There was an increase in biochemical parameters (calcium, urea, creatinine, uric acid, transaminases) and decrease immunoglobulin levels. The hematology revealed a decrease in red blood cells and hemoglobin, as well as leucocytosis. Physiological study showed enlarged spleen, kidney, liver and stomach. In light of our results, we can conclude that subchronic intake of NaMBS 1% and 4% seems to alter immune function, biochemical, hematological and physiological parameters in Wistar rats.**

## Keywords

**Sodium Metabisulphite, Subchronic, Haematology, Biochemistry, Physiology, Wistar Rat**

## 1. Introduction

Sulphites are substances naturally present in foods and in the body. They are also regulated food additives that

[*]Corresponding author.

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are used as preservatives to maintain the color and prolong the shelf life of food. They protect the preparations against enzymatic or non-enzymatic browning [1]. The European Union has classified them among the food preservatives [2]. Endogenous sulphites are normally generated in the human body by degradation of amino acids such as methionine and cysteine-sulphur. They can also be generated by polymorphonuclear neutrophils [3]. Several mammalian cells produce sulphites from $H_2S$ [4]. Exogenous sources of sulphites include food, drinks, ambient air and pharmaceutical products [5].

There are several amino acid preparations used for parenteral nutrition that contain high levels of sulphites [6]. Although sulphites are very efficient, they are subject to restrictions of use due to their toxic effects. They have the ability to react with several molecules of biological importance such as DNA and may also be neurotoxic molecules by generating free radicals such as superoxide [7]. These radicals can also react with proteins and lipids [8]. Similarly, sulphites are additives and allergens to which the majority of asthmatics are sensitive [9].

Consumption of 0.25% $Na_2S_2O_5$ supplemented food is equivalent 72 mg/kg of $SO_3^{2-}$ intake daily. With this finding, Joint Expert Committee in the World Health Organization (WHO) has added 100 fold safety factor (by multiplying this number with 0.01) and has established acceptable daily intake (ADI) level of sulfites as 0.7 mg/kg/body weight [10].

Thus, the aim of this study was to evaluate, in a sub-chronic study, the effects of ingestion of sodium metabisulphite (NaMBS) at levels of 0.25%, 1% and 4% on the biochemical, hematological and physiological parameters of Wistar rats.

## 2. Material and Methods

### 2.1. Animals and Experimental Design

Twenty four females Wistar rats (weighing 130.41 ± 22.68 g, 8 weeks old), were used in this study. During the entire experimental period, the animals were housed under standard conditions of temperature (21˚C ± 2˚C) and humidity (55% ± 10%) with a 12-hour light/dark cycle and received a specific diet and water *ad libitum* according to the method of Lane-Peter and Pearson [11]. food and water were measured every day and body weight weekly. The animals were separated into four groups (n = 6) and received daily three different doses of NaMBS (0.25%, 1%, and 4%) in water for 12 weeks, the doses chosen are based on the value of acceptable daily intake ADI [12]. A 42-day immunization protocol was used [13].

At the end of the experiment (day 90), animals were fasted for approximately 16 h and were sacrificed by exsanguinations sunder urethane anesthesia. The sacrifice was done according to the guidelines of the regional Committee of Ethics of Animals, consistent with those agreed upon by the European Community on 24 November 1986 (86/609/EEC). Samples blood for hematological and clinical chemistry examinations were withdrawn from the abdominal aorta of rats, under conditions of food and water deprivation. Blood aliquots were put in different anticoagulants, according to the type of investigations. For the evaluation of hematological parameters, an aliquot of blood per animal was placed in ethylen-diamino-tetracetic-acid (K3-EDTA). For the evaluation of biochemical parameters, one aliquot of blood per animal was placed in a tube containing lithium-heparin and centrifuged at 4000 × g for 10 minutes at room temperature to obtain serum. The obtained serum is aliquoted in volumes of 50 µL in Eppendorf tubes and frozen at −20˚C. For the determination of immunochemical parameters blood samples were then collected to the nonanticoagulated tubes centrifuged and aliquoted for until analysis.

The organs (liver, spleen, thymus, kidney, and stomach) were carefully collected, rinsed with physiological saline (0.9% NaCl), weighed, snap-frozen in liquid nitrogen, and stored at −80˚C until further analysis.

### 2.2. Methods

The chosen test substance was NaMBS- $Na_2S_2O_5^-$ (Biochem International Chemopharma: 7681-57-4; Canada). The provider indicates a 95% degree of purity of the product. The substance presents itself as a very fine powder of white colour, pungent and very soluble in water.

The hematologic analyses were determined on the same day after the blood samples were taken from the rats,using automated Advia 60 CT (Bayer DC): white blood cells, red blood cells, hematocrit; haemoglobin was determined by the colorimetric method of Drabkin (cyanomethemoglobin) [14].

Biochemical assays were measured by spectrophotometer using commercial assay kits. Kidney functions, creatinine was measured by Jaffé. Colometric-kinetic method and urea was assayed by Berthelot. Enzymatic

colorimetric method [15] using Spinreact kits (Spain) and recorded at spectrophotometer with wavelength 492 nm and 580 nm, respectively. The level of uric acid was measured using enzymatic colorimetric test (PAP-aminophenarose method) with lipid clearing factor (LCF) and recorded at spectrophotometer with wavelength 520 nm, using Human kits (Germany) [16] [17].

Total Cholesterol (TC) was estimated by enzymatic colorimetric assay (CHOD-PAP method) [18], triglycerides (TG) was determined by Glycerol Phosphate Oxidase/Peroxidase(GPO-PAP) method [19] [20] and recorded at spectrophotometer with wavelength 500 nm, using Human kits (Germany).

Total protein (TP) was assayed by using the Biuret, colorimetric method and recorded at spectrophotometer with wavelength 540 nm [21], using Spinreact kits (Spain).

Liver functions, the activities of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were adapted to determine in blood serum according to the kinetic method without activation by the pyridoxal phosphate [22], using Human kits (Germany).

Calcium value was determined by colorimetric method according to Stern & Lewis [23], using Biomaghreb kits (France) and recorded at spectrophotometer with wavelength 570 nm.

The rate of immunoglobulins is assayed by the reverse radial immunodiffusion technique of Mancini. The principle consists in depositing the antibody solution to be assayed in dug wells in a gel containing antigen. Antibodies diffuse into the gel and form rings of precipitation when the relative concentrations of the two elements are close to the equivalence point. The disc surface is proportional to the concentration of antibody [24]. For the optimization of this technique, we based our procedures on the protocols described by Mancini [25] and Vearman [26].

### 2.3. Statistics

The data is expressed as mean ± standard deviation (X ± SD). Statistical test one way ANOVA was applied to find significant difference between values of various parameters recorded for control and treated animals. $p < 0.05$ was considered statistically significant.

## 3. Results

Regular monitoring of animals receiving water with 4% sulphites resulted in the following observations: diarrhea from the 2nd week; blood around the nose from the 4th week; and loss of hair from the 6th week. Anorexia and inhibition of growth were also observed.

The weight change of the control and experimental rats is illustrated in **Figure 1**. The values of weight gain in the control group and those receiving 0.25% and 1% of NaMBS were respectively 100.83 ± 6.49 g, 115.33 ± 7.55 g and 72.66 ± 13.75 gr Variations between these groups remain insignificant. In contrast, the rats treated with 4% of NaMBS showed very low weight gain and the inhibition of growth, and the difference was highly significant compared to the controls ($p < 0.0001$). At the highest doses of NaMBS food and water intakes were strongly diminished in the treated rats with 4% of NaMBS compared with controls during the 12 weeks of observation (**Figure 2**).

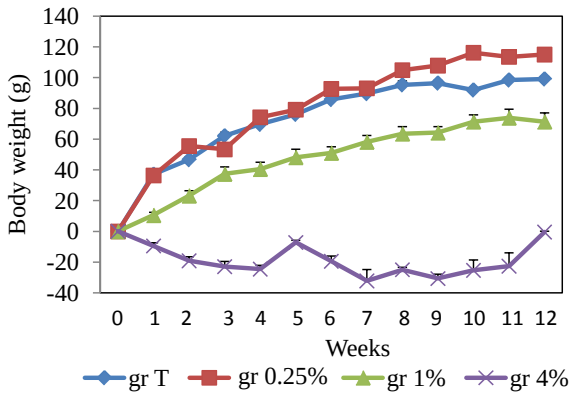


**Figure 1.** Body weight changes of control rats and rats treated with 0.25%, 1% and 4% of sodium metabisulfite daily for 12 weeks. Each value represents the mean ± SD.

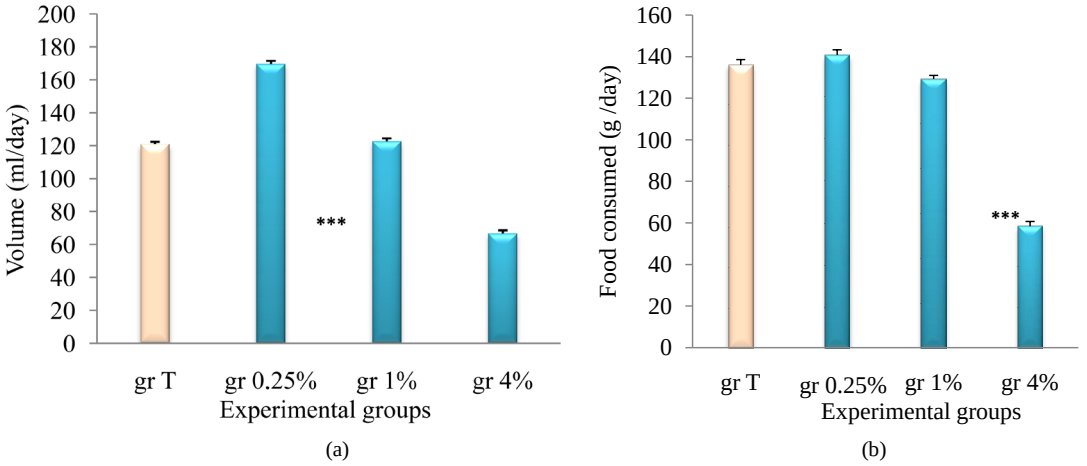


**Figure 2.** (a) Water intake (ml/day) of control rats and rats treated with 0.25%, 1% and 4% of sodium metabisulfite daily for 12 weeks. Each value represents the mean ± SD; (b) Food consumption (g/day) of control rats andrats treated with 0.25%, 1% and 4% of sodium metabisulfite daily for 12 weeks. Each value represents the mean ± SD. ***Statistically significant compared to control group; $p < 0.001$.

According to the immunochemical test results, there was a decrease in the diameter of precipitates (immune complex antibody-antigen Ab-Ag) of the experimental groups receiving 1% and 4% of NaMBS compared with the control (**Figure 3**).

At the end of the experiment, various hematological parameters of treatment rats were measured and were compared with those of controls (**Table 1**). The results of the hematological study revealed a decrease in the number of red blood cells and the content of haemoglobin associated with leucocytosis in the group treated with 4% of NaMBS compared with the control group. The results of the different biochemical assays of the serum in the treatment groups compared to the control group are presented in **Table 2**. Rats treated with high doses of NaMBS (4%) expressed a hypercalcemia and high rates of urea combined with hypercreatinemia and hyperuricemia. Increased serum transaminases were also found.

The assessment of the relative weights of taken organs of control and experimental rats treated with 1% showed an enlargement in spleen and liver. The group receiving 4% expressed a hypertrophy in spleen, liver, stomach, kidneys. However, no significant change was found in the weights of the thymus in the different experimental groups compared with the control group (**Table 3**).

## 4. Discussion

In this work, the effects *in vivo* of subchronic NaMBS consumption at doses of 0.25%, 1% and 4% were studied. The doses of sulfite administered in this project was determined in reference to previous studies designed to investigate the effects of high level of sulfite exposure via consuming certain foods and beverages containing sulfite [12] [27] [28]. In particular, there are several amino acid preparations utilized in total parenteral nutrition (TPN) solutions that contain large amounts of sulfites [6]. Sulfite toxicity is also considered possible with peritoneal dialysis fluids, some of which contain $Na_2S_2O_5$ in concentrations of 0.005% - 0.012% [29]. Considering that many foods such as sausage, dried fruit, beer and wine contain $SO_2^{3-}$, the daily level of normal $SO_2^{3-}$ intake can easily be exceeded [30].

The results show that the effect of subchronic high-dose (4%) of NaMBS administration in rats translates to clinical and behavioural changes (diarrhea, anorexia, hair loss). These neurobehavioural changes can be explained by the neurotoxic effect of sulphites, the toxicity of which is attributed to the release of sulphur and oxygen free radicals that can damage the central nervous system [8] [31] [32]. Reist *et al.* [33] showed that sulphite exerts toxic effects on neuronal cells grown directly or in combination with peroxynitrite. In addition, individuals suffering from a congenital disease caused by a deficiency of sulphite oxidase, a key enzyme in the metabolism of sulphites in the body that causes their oxidation to sulphate, develop severe neurological abnormalities with mental retardation, attenuated brain growth and early death [34].

The results obtained showed a significant decrease in food and water consumption associated with body

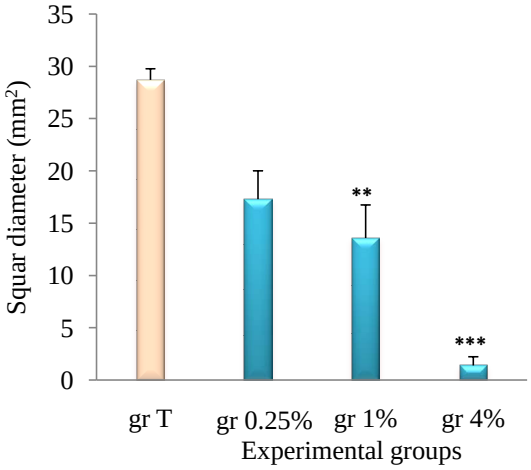


**Figure 3.** Square diameter ($D^2$) of the precipitate Ab-Ag of controls rats andrats treated with 0.25%, 1% and 4% of sodium metabisulfite daily for 12-weeks. Each value represents the mean ± SD. **Statistically significant compared to control group; $p < 0.01$; ***Statistically significant compared to control group; $p < 0.001$.

**Table 1.** Blood levels of some hematological parameters in controls rats and daily treated with 0.25%, 1%, and 4% of sodium metabisulphite for 12 weeks.

| Parameters | Controls | Experimental | | |
|---|---|---|---|---|
| | | 0.25% | 1% | 4% |
| Red blood cell ($10^6$/mm$^3$) | 7.17 ± 0.14 | 7.72 ± 0.24 | 6.56 ± 0.44 | 6.13 ± 0.34* |
| White blood cell ($10^3$/mm$^3$) | 3.13 ± 0.42 | 3.54 ± 0.22 | 2.5 ± 0.11 | 5.8 ± 0.17** |
| Haemoglobin) (g/dl) | 13.98 ± 0.25 | 14.9 ± 0.4 | 12.7 ± 0.43 | 11.55 ± 0.43* |
| Hematocrit (%) | 40.11 ± 1.17 | 40.14 ± 1.67 | 40.85 ± 1.83 | 36.97 ± 1.76 |

*Statistically significant compared to control group; $p < 0.05$; **Statistically significant compared to control group; $p < 0.01$; Results are expressed as Mean + SD standard deviation.

**Table 2.** Serum levels of biochemical parameters in controls rats and treated daily with 0.25%, 1%, and 4% of sodium metabisulphite for 12 weeks.

| Parameters | Controls | Experimental | | |
|---|---|---|---|---|
| | | 0.25% | 1% | 4% |
| T. protein (gr/L) | 67.7 ± 2.13 | 65.6 ± 2.16 | 69.75 ± 1.46 | 67 ± 1.73 |
| Calcium(mmol/L) | 2.26 ± 0.12 | 2.37 ± 0.21 | 2.21 ± 0.08 | 2.78 ± 0.02* |
| Urea (mmol/L) | 11.77 ± 0.02 | 11.72 ± 0.02 | 10.92 ± 0.01 | 11.99 ± 0.01* |
| Creatinine(µmol/L) | 42.47 ± 2.76 | 42.92 ± 6.9 | 52.92 ± 3.37 | 57.7 ± 2.93* |
| Uric acid (µmol/L) | 160.05 ± 28.07 | 159.46 ± 20.95 | 61.89 ± 23.8 | 232.05 ± 13.75* |
| T. cholesterol (mmol/L) | 2.21 ± 0.15 | 2.16 ± 0.17 | 1.91 ± 0.17 | 1.83 ± 0.04 |
| Triglycerides (mmol/L) | 0.98 ± 0.29 | 0.54 ± 0.05 | 0.76 ± 0.08 | 1.08 ± 0.03 |
| ASAT (IU/L) | 68.66 ± 4.27 | 69.5 ± 4.46 | 131 ± 22.69* | 151 ± 44.3* |
| ALAT (IU/L) | 35 ± 2.78 | 41.6 ± 10.63 | 39.8 ± 6.64 | 30.8 ± 0.38 |

T: total; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; Results are expressed as mean + SD standard deviation; *Statistically significant compared to control group; $p < 0.05$.

**Table 3.** Relative organ weights (g) in controls rats and treated daily with 0.25%, 1%, and 4% of sodium metabisulphite for 12 weeks.

| Organ | Controls | Experimental | | |
|---|---|---|---|---|
| | | 0.25% | 1% | 4% |
| Spleen | 0.37 ± 0.01 | 0.38 ± 0.01 | 0.45 ± 0.02* | 0.50 ± 0.03** |
| Thymus | 0.12 ± 0.01 | 0.17 ± 0.007 | 0.13 ± 0.01 | 0.2 ± 0.02 |
| Stomach | 0.69 ± 0.01 | 0.76 ± 0.09 | 0.76 ± 0.05 | 1.48 ± 0.09** |
| Liver | 3.13 ± 0.15 | 2.93 ± 0.11 | 3.85 ± 0.12* | 4.65 ± 0.40** |
| Kidneys | 0.66 ± 0.02 | 0.7 ± 0.02 | 0.68 ± 0.03 | 0.98 ± 0.0** |

Results are expressed as mean + SD standard deviation. *Statistically significant compared to control group; $p < 0.05$. **Statistically significant compared to control group; $p < 0.01$.

weight loss in the group receiving the highest dose (4% of NaMBS). Sulphites have an unpleasant taste and smell so that their administration in drinking water might cause a decrease in the water consumption. In addition, the destruction of thiamine by sulphite is an important factor in the toxicological evaluation of this food additive. Thiamine destruction not only occurs when sulphite is added to the food, but also when it is present in the organism It thus appears as an antivitamin factor B1 [35]. This may explain the decrease in weight gain of animals treated with the highest dose.

These results are consistent with those of other studies, including those Till *et al*. [36], which showed that male and female rats fed high doses of NaMBS Joglur (0% - 8%) have reduced food consumption and weight growth. In a follow-up study [37], it was shown that a dose of 1.72% of NaMBS Joglur added to the diet causes a decrease in growth and feeding in male and female pigs during an experimental study of 12 months. Similarly, a study by inhalation was conducted by Miyata *et al*. [38]. Six rabbits exposed to dioxide of sulphur (186.2 $mg/m^3$) for 6 weeks. The main symptom was a decrease in growth.

The significant decrease in immunoglobulin in a manner inversely proportional to levels of administered of NaMBS Joglur explains the immunomodulatory effects of NaMBS. Several studies have been conducted to measure the impact of exposure to known concentrations of sulphites on the normal immunological mechanisms. The results show that sulphites in particular can influence the production of antibodies and agglutinins [39].

In order to assess the actual toxicity of NaMBS Joglur, different hematological and biochemical parameters were measured at the end of the experiment in treated rats and were compared to those of control rats. Our results show that a 4% dose causes a significant decrease in red blood cells and haemoglobin levels and a significant increase in white blood cells in the experimental rats compared to the controls. These results are in agreement with the work of Til [36], which showed the appearance of anaemia at 2% and a leucocytosis to 6% of NaMBS in Wistar rats. According to Gunnison [40], rats fed a diet containing 6% of NaMBS Joglur for 21 days became severely anaemic. The results showed a significant increase in the concentration of serum calcium in the experimental group receiving 4% of NaMBS Joglur. Sulphites act on the excretion of the calcium through sulphate, to which they give rise *in vivo* [41].

Uric acid is the final product of the catabolism of purine [42]. The levels of uric acid in most mammals are lower than in humans due to the presence of uricase, a liver enzyme that degrades uric acid into allantoin [43]. Our results illustrate hyperuricemia exclusively in the group treated with the highest dose (4%). The observed increase in serum levels of uric acid may be the result of reduced urinary excretion of the metabolite. Creatinine and urea are excellent markers of renal function, and their increase or decrease reflects a dysfunction of the renal function [44]. Transaminases are enzymes with important metabolic activity inside cells. Their increase in serum reflects cell damage, in particular at the hepatic level [45].

The results indicate that doses of 4% cause an increase in serum urea, creatinine and transaminases marking abnormal renal and hepatic function. Degradation of hepatic protein compounds may explain the increase in urea and serum creatinine in the treated rats, where proteins can be degraded into amino acids and then into urea and creatinine. Thus, these amino acids can be transformed by the action of the serum transaminase in carboxylic compounds such as pyruvic acid [46] which requires the high enzymatic activity of transaminases in rats treated with 4% of NaMBS. In this case, we may suggest that the high enzyme activity of the aspartate ami-

notransferase (ASAT) and alanine aminotransferase(ALAT) is linked to the hepatotoxic effect of the xenobiotic, given that the liver is the principal organ of detoxification because it contains most of the enzymes of metabolism.

The assessment of the relative weight of the various bodies reveals that the doses of 4% cause an enlarged liver, spleen, kidney, stomach. The increase in the hepato-body and reno-body report has been highlighted indirectly by a disruption of the renal and hepatic enzyme system (increase serum in urea, creatinine, uric acid and transaminases in the animals treated with the highest dose). The increase of thespleen weight is the result ofan increased extra-medullary splenic hematopoietic activity and increased leucocyte counts. This result is fully consistent with the work of Til *et al*. [36], which showed the presence of splenomegaly in rats receiving doses of 4% or more of NaMBS. In a follow-up study, they showed an enlarged liver, kidney and heart of pigs treated with 0.83% and 1.72% of NaMBS [36].

## 5. Conclusions

Females Wistar rats oral exposed to NaMBS, at levels up to 4% in the water, during 12 weeks, shown alterations in immunochemical, biochemical (calcium, uric acid, urea, creatinine, transaminases), hematological (hemoglobin, red blood cell, and white blood cells) parameters in relation to the control group. Similarly, the oral administration of 4% of NaMBS for 12 weeks causes hypertrophy of kidneys, liver, spleen and stomach. These results lead to considerations of the development of other research focused on the determination of biomarkers of oxidative stress induced by sulphite (glutathione, superoxide dismutase, cytochrome P450, catalase, substances reacting with thiobarbituric acid, malondialdehyde, vitamins, etc.) for a better analysis of potential toxic effects of this synthetic preservative. Our recommendations relate to the control of their unauthorized presence in foods or their presence at unauthorized rates:

- remove or replace their use in foodstuffs for which their use is not required;
- reduce the use of these additives when technologically possible;
- increase the legibility and visibility of labelling (this is particularly useful for asthmatics).

In addition, sulphites do not meet the current demand for consumer which is turning more and more towards a natural diet free from synthetic additives. Natural additives such as vitamin C, citric acid and tocopherols can also be used as alternative preservatives to be able to propose products to consumers without hazards, natural, good for health and practices.

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