Technological and biological effects of sodium meta-bisulfite and ascorbic acid on solar dried sheeted tomato

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Abstract: Sodium meta-bisulphite (SMBS) and ascorbic acid (AA) were added during the processing of solar dried sheeted tomato. SMBS and AA were added to concentrated juice before drying in concentrations 0.67, 0.167and 0.335 g/L for SMBS while it was 0.110, 0.220 and 0.330g/L for AA. Colour attributes, sensory evaluation and biological evaluation were studied. The obtained results showed that both SMBS and AA improved the final product quality regarding colour and general appearance. The biological studies revealed that SMBS induced chromosomal aberrations in bone marrow and spermatocytes cells especially the concentrations of 0.335g/L. Also, ascorbic acid (0.330 g/L) induced chromosomal aberrations in bone marrow and spermatocytes more than control sample. The effect of SMBS was higher than that of ascorbic acid. Finally, it could be concluded that SMBS had adverse and undesirable effect regardless of its technological advantages.

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1. Introduction

Sodium meta-bisulphite is an organic compound. The compound has many chemical properties that make it useful for a variety of industrial purposes. However, there are many dangers of working with or investigating this compound, which make some of its uses especially that of food preservative a topic of research and controversy. Sodium meta-bisulphite is a crystalline powder. It is soluble in water. It releases sulphur dioxide gas when dissolved in water. Sodium meta-bisulfite is used as food preservative and it is usually noted as E223 (Food additives). Sodium meta-bisulphite acts as an antimicrobial and antifungal. It is a reducer. It is commonly found in fruit juices, vinegars, pickles and dried fruits.

According to Latapi and Barrett (2006), two pre-drying treatments, i.e. 1) salt and 2) sodium meta-bisulfite dips were evaluated on sun-dried tomatoes by assessing moisture content, colour, rehydration ratio, mould and yeast count, sulphur dioxide content, an/or salt content. There were significant differences in rehydration ratio, yeast count, and salt in the salt dipping pre-treatment. The most effective conditions from the salt dipping pre-treatment was using a concentration of either 10% or 15% salt for 5 minutes. There were significant differences in rehydration ratio, yeast count, colour and sulphur dioxide in the sodium meta-bisulfite dipping pre-treatment. Dipping tomatoes in either 6 or 8% sodium meta-bisulfite for 5 minutes resulted the best red colour. Pre-drying treatments have been found to improve the quality of stored sun-dried tomatoes. Specific pre-treatments were chosen for effects on nutritional value, sensory quality, and safety before and after 3 months of storage (25 °C and 30% to 34% relative humidity). These pre-treatments included (1) direct gas sulphuring with 2.3 kg (5 lb) SO_2; (2) with 3.6 kg (8 lb) SO_2; (3) dipping in 10% salt for 5 min; (4) 8% sodium meta-bisulfite for 5 min; and (5) 8% sodium meta-bisulfite and 10% salt for 5 min. The use of SO_2 improved colour, rehydration ratio, and minimized the loss of ascorbic acid and lycopene. Sodium meta-bisulfite dipped tomatoes had better rehydration ratio and colour than gas sulphured sun-dried tomatoes. Untrained consumers ranked gas sulphured tomatoes higher than sun-dried tomatoes produced by dipping in either sodium meta-bisulfite alone or sodium meta-bisulfite plus salt.

Sulphites are extensively used in the food and drinks industry. Their toxicity has been previously evaluated by addition to the diet or drinking water of laboratory animals. Because interactions between sulphites and food constituents occur (Ribera, *et al.*,

2001). The results revealed that, these anionic sulphur compounds interact with DNA possibly by changing the topology of this macromolecule. Effects may be due to interactions of these sulphur compounds at higher concentrations with DNA, with resulting ligand-DNA super-coiling. This process could protect against HD intoxication, which is caused in part by the uncoiling of DNA (Baskin, etal., 2000).

On the other hand, **(**Kayraldiz and Topaktas. 2007) investigated the genotoxic effect of sodium meta-bisulphite (SMB), which is used as an antimicrobial substance in foods on bone marrow cells of rats and found that intra-peritoneal implement of SMB generally more effective increasing the percentage of abnormal cells and CA/cell in all concentrations and treatment period. In addition, mitotic index (MI) data of intra-peritoneal injection are lower than gavages. It can be concluded that potential genotoxic effects of SMB by IP injection is higher than GV injection. The ability of sodium meta-bisulfite which is used as an antimicrobial substance in food, to induce chromosome aberrations (CA) and sister chromatid exchanges (SCE) in human lymphocytes was investigated by Rencüzogullari et al., (2001). SMBS-induced CAs and SCEs at all concentrations (75, 150 and 300 micro-g/ml) and treatment periods (24 and 48h) dose-dependently. However, SMBS decreased the replication index (RI) and the mitotic index (MI) at the concentrations of 150 and 300 micro-g /ml for 24 and 48h treatment periods. This decrease was dose-dependent as well.

The chemical properties of ascorbic acid provide a wide range of industrial applications. The use of ascorbic acid or vitamin (C) depend on its chemical properties as an antioxidant or on its health-related properties. About one-third of total production is used for vitamin preparations in the pharmaceutical industries ((vitamin C). the rest is mainly applied as an additive to food (E300) and feed to enhance product quality and stability. Ascorbic acid technological functions include antioxidant in aqueous systems, retardation of oxidative rancidity and protection from enzymatic browning in processed fruits and vegetables.

Vitamin C is a wide spectrum antioxidant essential for humans, which are unable to synthesize the vitamin and must obtain it from dietary sources. There are two biologically important forms of vitamin C, the reduced form, ascorbic acid, and the oxidized form, dehydro-ascorbic acid. Vitamin C exerts most of its intra-cellular biological functions and is acquired by cells with the participation of specific membrane transporters. This is a central issue because even in those species capable of synthesizing vitamin C, synthesis is restricted to the liver and pancreas from which is distributed to the organism. Most cells express two different transporter systems for vitamin C; a transporter system with absolute specificity for ascorbic acid and a second system that shows absolute specificity for dehydro-ascorbic acid. In humans, the maintenance of a low daily requirement of vitamin C is attained through an efficient system for the recycling of the vitamin involving the two families of vitamin C transporters, Rivas et al. (2008).

Hesta et al. (2009) investigated the ability of vitamin C to increase the anti-oxidative and immunomodulating potential in healthy dogs. They found that, there was no clear evidence for an effect of dietary vitamin C on antioxidative capacity in healthy dogs fed a diet with vitamin E concentrations well above the recommendations. Yet, a limited number of immunological parameters were slightly affected.

Some biochemical functions of vitamin C make it an essential component of parenteral nutrition (PN) and an important therapeutic supplement in other acute conditions. Ascorbic acid is a strong aqueous antioxidant and is a cofactor for several enzymes. The average body pool of vitamin C is 1.5 g, of which 3%-4% (40-60 mg) is used daily. Steady state is maintained with 60 mg/d in non-smokers and 140 mg/d in smokers, Berger (2009). Vitamin C is a powerful antioxidant and its levels are decreased in Alzheimer's patients. Even sub-clinical vitamin C deficiency could impact disease development. The results indicated an interaction between the cholinergic system and vitamin C that could be important given the cholinergic degeneration associated with Alzheimer's disease, Harrison et al., (2009).

This study was carried out to investigate the technological and biological effects of adding SMBS and AA during the processing of solar dried tomato sheets especially colour quality and sensory evaluation as well as bone marrow and spermatocytes aberrations.

2. Material and methods

2.1.Materials:

2.1.1.Tomato fruits (*Lycopersicon esculentum*) used for the present study were obtained from local market, Cairo, Egypt.

2.1.2.Sodium metabisulphite and ascorbic acid were obtained from Merck Company, Germany.

2.2. Methods

2.2.1.Experimental design

The fresh fruits were washed with spray washer. Hot break tomato juice was concentrated under vacuum at 60 °C till the total soluble solids were 12-13% (pureé). Sodium meta-bisulphite in concentrations (0.67, 0.167 and 0.335 g/L) and ascorbic acid in concentrations (0.110, 0.220 and 330 g/L) were added before drying process, as well as, control sample was produced for comparison.

Drying process

Dried tomato sheets were prepared from tomato pureé (12-13% T.S.S.) by spreading on stainless trays and left under solar energy drier designed and equipped with thermostat for temperature control, National Research Centre, Egypt.

2.2.2. Sensory evaluation

The acceptability were carried out according to Gould (1974). 14 judge gave degrees to odour, taste acceptability and colour.

2.2.3.Color Attributes

The colour of tomato sheets samples was measured using a spectro-colorimeter with the CIE colour scale (Hunter, Lab scan XE). This instrument was standardized against the white tile of Hunter Lab colour standard (LX No.16379): X= 77.26, Y= 81.94 and Z= 88.14. The L, a and b values were reported. Total colour difference (ΔE) was calculated as:

$$[(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

2.2.4.Biological studies

Male Swiss mice aged 8-10 weeks and weighting 25-30 grams, obtained from a closed random-bred colony at National Research Centre, Cairo, Egypt were used. Food and water were provided libitum.

Tomato sheets containing sodium meta-bisulfite (0.067, 0.167 and 0.335 g/L) and tomato sheets containing vitamin C (0.110, 0.220 and 0.330 g/L) suspended in distilled water, were ingested orally by a dose level 2.5mg/kg body weight. The animals were divided into 7 groups (each of 5mice). The first group was kept as a control and the other 3 groups were orally ingested tomato sheets containing sodium meta-bisulfite with concentration 3 groups and 3 groups were orally ingested tomato sheets containing vitamin C with 3 concentration. The animals were killed by decapitation at the end of experimental period 30days. The animals were injected intra-peritoneal with Colchicine (0.05%). They were sacrificed 2hr. later to prepare the chromosome of bone marrow and spermatocyte cells according to Yosida and Amamo (1975). Slides were prepared and 50 well-superadded metaphases were examined for each animal at each concentration for chromosomal aberrations in bone marrow or spermatocyte cells. The results of chromosomal aberrations were analyzed using analysis of variance (ANOVA).

3. Results and discussion

3.1. Color attributes of tomato sheets

Data presented in Table (1) show the effect of SMBS and AA treatments on colour characteristics of tomato sheets. As shown in the table, (b values) increased as a result of sing SMBS in all tested levels. That effect because SMBS inhibit the oxidative enzymes resulting red colour and improved tomato sheets quality regarding colour characteristics. Slight darkness (a value) was observed as a result of using SMBS and dehydration process. Regarding a values the treatment of tomato sheets by SMBS slightly affected tomato sheets resulting yellow colour. SMBS is known as anti-browning agent because its inhibitory effect of oxidative enzymes which negatively affected of some food colours. The same trend was also observed regarding ascorbic treatment. AA acid known as antioxidant agent. The effect of SMBS was more effective than AA. The effect was the same on upper of tomato sheets.

Such findings were observed by Latapi and Barrett (2006) as he reported that, the use of SO_2 improved colour, rehydration ratio, and minimized the loss of ascorbic acid and lycopene. Sodium meta-bisulfite dipped tomatoes had better rehydration ratio and colour than gas sulphured sun-dried tomatoes. Untrained consumers ranked gas sulphured tomatoes higher than sun-dried tomatoes produced by dipping in either sodium meta-bisulfite alone or sodium meta-bisulfite plus salt.

3.2. Sensory evaluation of tomato sheets

Data presented in Table (2) showed that the effect of addition of sodium meta-bisulphite and ascorbic acid on the sensory evaluation of dried tomato sheet. As shown in the table there was no significant differences between control and treated samples regarding taste, odour and general appearance. But there was significant effect regarding to colour. Both SMBS and AA do as antioxidant and anti-browning agents. They inhibited enzyme activities resulting

desirable colour. The deterioration of colour may be because the oxidation process occurring during dehydration while the presence of SMBS and AA minimized the oxidation process resulting desirable colour. Latapi and Barrett (2006) reported that pre-drying treatments have been found to improve the quality of stored sun-dried tomatoes. Specific pre-treatments were chosen for effects on nutritional value, sensory quality, and safety before and after 3 months of storage (25 °C and 30% to 34% relative humidity). These pre-treatments included (1) direct gas sulphuring with 2.3 kg (5 lb) SO_2; (2) with 3.6 kg (8 lb) SO_2; (3) dipping in 10% salt for 5 min; (4) 8% sodium meta-bisulphite for 5 min; and (5) 8% sodium meta-bisulfite and 10% salt for 5 min.

Table 1: Effect of treatment on colour attributes

Treatment	Surface			Back		
	L 92.43	a 0.84	b 0.16	L 92.43	a -0.84	b -0.16
Control	25.26	4.51	3.46	26.11	9.34	8.14
SMBS (0.067g/l)	25.86	7.74	4.63	27.97	12.13	11.66
SMBS (0.167g/l)	27.87	4.63	3.47	25.61	8.13	7.71
SMBS (0.335g/l)	26.07	8.05	4.93	28.64	14.17	13.55
AA (0.110g/l)	26.24	7.40	4.84	27.36	12.94	10.78
AA (0.220g/l)	25.25	8.45	4.91	29.23	14.23	13.25
AA (0.330g/l)	25.32	6.30	4.53	25.14	7.51	6.90

SMBS = sodium metabisulphite; AA = ascorbic acid

Table 2: Statistical parameters of sensory evaluation of treated sheets

Treatment	Color (10)	Taste (10)	Odor (10)	General appearance (10)
Control	8.27	8.18	8.36	8.26
SMBS (0.067g/l)	8.26	7.90	8.18	8.18
SMBS (0.167g/l)	8.27	7.54	8.00	7.90
SMBS (0.335g/l)	8.09	7.81	7.54	7.81
AA (0.110g/l)	8.18	7.90	7.72	8.09
AA (0.220g/l)	8.00	7.63	7.18	8.18
AA (0.330g/l)	8.81	8.36	8.27	8.63
LSD	N.S	N.S	N.S	N.S

SMBS = sodium metabisulphite; AA = ascorbic acid

3.3. Biological studies

The results of the cytological examination of bone marrow and spermatocytes cells of mice, ingested orally with tomato sheets containing sodium meta-bisulfite (2.5mg/kg b.w) and tomato sheets containing vitamin C were listed in tables (3and 4). The structural aberrations induced in both types of cells were highly significant ($p< 0.05$) in the case of the tomato sheets containing sodium meta-bisulfite and tomato sheets containing vitamin C. they were represented by gap, deletion, fragment, centric function and polyploidy and spermatocytes were types autosomal, x-y univalent and polyploidy.

As the results show, tomato sheets containing sodium meta-bisulfite concentrations (0.335g/L) caused a highly significant increase in the mean value of chromosome aberrations in both bone marrow and spermatocytes cells. While tomato sheets containing sodium bisulfite concentrations (0.067g, 0.168g/L) were lower than those caused by the control. Tomato sheets containing vitamin C concentrations (0.330g/L) caused a highly significant increase in chromosome aberrations in both bone marrow and spermatocytes cells, while the concentrations (0.10g, 0.220g/L) were lower than those control, sub acute treatment caused high percentage of aberrant cells due to the accumulation effect of the tomato sheets containing sodium meta-bisulfite and vitamin C. centric fraction is the main type of chromosomal aberrations in both types of examined cells and the main type of chromosomal aberrations x-y univalent in spermatocytes were the most common chromosomal abnormalities in table (3and 4).

The results obtained showed that, tomato sheets containing sodium meta-bisulfite concentrations (0.335g/L) caused significant increase in chromosome aberrations than the tomato sheets containing vitamin C concentrations (0.330g/L). The potency of sodium meta-bisulfite on the induction of chromosomal aberrations were highly significant than those caused by by the vitamin C compared with control. In this study tomato sheets containing sodium meta-bisulfite concentration (0.335g/L) significantly induced chromosomal aberrations. This suggestion is in agreement with those found by Ashby and Ishidate (1986) who found that sodium salt was clastogenic to Chinese hamster lung (CHL) fibroblast cells in vitro.

Bhanot and Chambers (1977), and Chen and Shaw (1994), found that sodium metabisulfite converts to sodium bisulfite and sulpherdioxide when dissolved in water. Bisulfite (HSO_3) causes the deamination of

cytosine in both double-stranded and single-stranded DNA and in RNA. Also Meng and Zhang, (1999) reported that bisulfite caused gene mutation under acidic pH and Na salt caused chromosomal aberrations. In addition bisulfite gave cytosine 5-methyleselfomate at pH 6-7. However, in this study sodium meta-bisulfite did not changed the medium pH (pH = 6.8-7.2). it was found that bisulfite induced the GPT mutations in CHO-AS52 cells. Also Meng and Zahang (1992), reported that bisulfite induced chromosomal aberrations, sister chromatid exchange and formation of micronuclei in human lymphocytes. As seen for the results of Pagano and Zeiger, (1987), bisulfite is a weak mutagen in s. typhimurium TA 97 and TA 1535 strains.

Popescu and Dipaolo, (1988) showed that sodium bisulfite induced a significant, but minimal increase in the sister chromatid exchange, however did not cause to the chromosomal aberrations in Syrian hamster cells. Eyyup, et al., (2001a) reported that sodium metabisulfite induced the chromosomal aberrations and the sister chromatid exchanges, decreased the replication index and the mitotic index in human peripheral lymphocytes in a dose dependent manner. Eyyup, et al., (2001b) found that the effect of sodium meta-bisulfite (SMB) on mitosis was investigated in Allium cepal. The roots of A. cepa were treated. With SMB concentrations of 7.5mg/lt, 15mg/lt and 30mg/lt for 10-and 20-hour treatment periods. SMb significant decreased the mitotic index (MI) at all concentrations and treatment periods. While the decreasing of the MI was dose dependent at 10hours treatment time, SMB increased the mitotic abnormalities dependently. In this study tomato sheets containing vitamin C concentrations (0.330g/L) caused highly significant increase in chromosomal aberration in bone marrow and speramatocyte cells.

Table (3): Mean and standard error (frequencies) of chromosomal aberrations induced by tomato sheets containing sodium meta-bisulfite and vitamin C in bone marrow cells of mice.

Treatment	No. of animal	No. of metaph examined	Structural aberrations					
			Chromatid gap (M±S.E)	Dilation (M±S.E)	Fragment (M±S.E)	Centric fuction (M±S.E)	Polyploidy (M±S.E)	Total aberration (M±S.E)
Control	5	250	0.20 ± 0.20^{E}	0.20±0.20	0.40±0.24	0.20±0.20	0.00±0.00	1.00 ± 0.63^{D}
SMBS (0.067g/l)	5	250	0.60 ± 0.22^{E}	0.40±0.16	0.80±0.24	0.30±0.15	0.60±0.16	3.10 ± 0.50^{C}
SMBS (0.167g/l)	5	250	4.20 ± 0.66^{B}	1.20 ± 0.20^{B}	5.80 ± 0.72^{B}	0.80 ± 0.24^{B}	2.20 ± 0.53^{B}	14.80 ± 0.85^{B}
SMBS (0.335g/l)	5	250	8.60 ± 1.66^{A}	10.20 ± 1.02^{A}	9.80 ± 1.46^{A}	12.40 ± 1.50^{A}	7.40 ± 0.67^{A}	48.60 ± 3.85^{A}
AA (0.110g/l)	5	250	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
AA (0.220g/l)	5	250	0.20 ± 0.20^{D}	0.00±0.0	0.40 ± 0.24^{D}	0.20±0.20	0.00±0.00	0.60 ± 0.24^{C}
AA (0.330g/l)	5	250	0.00±0.00	0.00±0.00	0.40 ± 0.16^{C}	1.20 ± 0.29^{C}	20 ± 0.20^{C}	3.20 ± 0.42^{C}

SMBS = sodium metabisulphite AA = ascorbic acid

Gruff et al., (1995) ; Jacob, (1999), found that the saturate able kinetics of vitamin C make toxicity more likely when multiple large doses (1g) are consumed throughout a day versus one single dose. A common symptom of unabsorbed vitamin C left in the gastrointestinal tract is osmotic diarrhea. Vitamin C can be transformed in the body to oxalate, which is a common constituent of kidney stones. Doses up to 10grams have shown to be associated with a higher prevalence oxalate excretion, but the level does not fall outside of the normal range. As a precaution, people who are prone to kidney stones may want to avoid large doses 10timed the Dietary References Index (DRI) or greater of vitamin C. people who lack the control to regulate iron uptake should also avoid large doses of the vitamin. As stated earlier vitamin C enhances iron absorption which can lead to toxicity of iron on some people. Furthermore, excess ascorbate in the urine and faces can falsify lab tests such as glucose in the urine and faecal occult blood test.

A number of possible problems with very large doses of vitamin C have been suggested, mainly based on in vitro experiments or isolated case reports, including: genetic mutations, birth defects, cancer, atherosclerosis, kidney stones, rebound scurvy, increased oxidative stress, excess iron absorption, vitamin B12 deficiency and erosion of dental enamel. However, none of these adverse health effects have been confirmed, and there is no reliable scientific evidence that large amounts of vitamin C (up to 10grams/day in adults) are toxic or detrimental to health. With the latest recommended dietary allowance (RDA) published in 2000, a tolerable upper intake level in (ul) for vitamin C was set for the first time. A ul of 2grams (2, 000 milligrams) daily was recommended in order to prevent most adults from experiencing diarrhea and gastrointestinal disturbances. Such symptoms are not generally serious, especially if they resolve with temporary discontinuation or reduction of high-dose vitamin C supplementation.

According to these results, it can be concluded that sodium meta-bisulfite most probably posses a genotoxic risk. For this reason it is necessary to be careful when using it in food as antimicrobial substance and it is necessary to find new safe substances alternative to sodium meta-bisulfite.

Table (4): Frequencies of chromosomal aberrations induced by tomato sheets containing sodium meta-bisulfite and vitamin C in spermatocytes cells in mice.

Treatment	No. of animal	No. of metaph examined	Structural aberrations			
			Autosomal (M±S.E)	x-y univalent (M±S.E)	Polyploidy (M±S.E)	Total aberration (M±S.E)
Control	5	250	0.20 ± 0.20^{e}	0.40 ± 0.24^{e}	0.00±0.00	0.60 ± 0.24^{f}
SMBS (0.067g/l)	5	250	0.20 ± 0.13^{e}	0.80 ± 0.24^{e}	1.40±0.22	2.40 ± 0.22^{e}
SMBS (0.167g/l)	5	250	3.20 ± 0.20^{b}	5.40 ± 0.16^{b}	3.20 ± 0.24^{b}	10.80 ± 0.29^{b}
SMBS (0.335g/l)	5	250	8.00 ± 1.58^{a}	11.20 ± 1.11^{a}	6.60 ± 1.03^{a}	25.80 ± 1.53^{a}
AA (0.110g/l)	5	250	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}
AA (0.220g/l)	5	250	0.20 ± 0.20^{d}	0.00 ± 0.00^{d}	0.40 ± 0.20^{c}	0.60 ± 0.25^{d}
AA (0.330g/l)	5	250	0.00 ± 0.00^{e}	2.20 ± 0.20^{e}	1.80 ± 0.24^{c}	3.40 ± 0.25^{c}

SMBS = sodium metabisulphite

AA = ascorbic acid

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