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TECHNICAL NOTE

Examples of direct and indirect effects of technological treatments on ascorbic acid, folate and thiamine

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The effects of sulphiting agents on thiamine and folic acid levels in treated potatoes and the effects of industrial pasteurization on ascorbic acid content of cow's milk are investigated. The results obtained appear to be particularly interesting and original. A double effect of processing, 'direct' against vitamin stability and 'indirect' against natural vitamin antagonists, is suggested to explain a similar trend in two different experiments. According to this hypothesis, in processed foods the residual levels of vitamins should depend on the equilibrium between the two effects.

INTRODUCTION

Evaluation of the chemical effects of food rocessing on nutrients is a classical subject in food chemistry. Furthermore, very often vitamin content has been utilized as an indicator of the severity of technological treatments and as a criterion of food quality.

It is generally accepted that, during processing, foods are subjected to vitamin losses because of light, heat, pH variations, etc. On the other hand, treatments may also cause enzyme inactivation, microorganism destruction, oxygen removal, resulting in improved vitamin stability. Therefore, food processing may affect vitamins both negatively by a 'direct mechanism' and positively by an 'indirect mechanism'.

This double-action hypothesis might explain the particular data obtained in two different sets of experiments and reported in the present paper.

In the first experiment, to evaluate the effect of sulphur dioxide on thiamine and folic acid level, raw potatoes were treated with different amounts of sodium bisulphite. In the second experiment, the ascorbic acid content of industrially heat-processed milk was evaluated.

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MATERIALS AND METHODS

Food samples

Raw potatoes were purchased at a local market, peeled, cubed, homogenized and divided into 200 g samples. Suitable amounts of sodium bisulphite were added to provide a concentration of sulphur dioxide ranging from 0 to 2500 ppm. The whole procedure required about two hours and was performed at room temperature.

The milk samples were processed by Italian central dairies under standardized conditions and pasteurized at 72, 75, 80, 85 and 90°C for 15 min (High temperature short time (HTST) pasteurization) or sterilized at 140°C for 3 min (ultra high temperature (UHT)). The processed milk was delivered to the laboratory in Rome and the ascorbic acid content determined.

Equipment and chemicals

A modular high performance liquid chromatograph, consisting of a Waters 6000A delivery system, a Gilson Mod 231–401 autosampling injector fitted with a 20 μl loop and a Waters Mod 490 programmable multiwavelength detector, was used. Results were elaborated by a Digital 380 Professional Computer.

The columns were: a 250 mm × 4·6 mm 5μm Supel-

cosil LC-SAX (sulphur dioxide); a 250 mm × 4·6 mm 4 μm Vydac C18 (thiamine and folic acid); and a 250 mm × 4·6 mm 4 μm Supelcosil C18 (ascorbic acid).

All reagents (Carlo Erba, Milan, Italy) were of analytical or HPLC grade, as required. Sodium hydrogen sulphite 30% (Merck) was used as sulphiting agent. L-Ascorbic acid, thiamine and folic acid (Sigma Chemical Co.) were used as the reference materials.

Analytical methods

Total sulphur dioxide, released from sulphite-treated potatoes in a boiling acid medium under a nitrogen stream, was analyzed by HPLC and evaluated by indirect photometry (Pizzoferrato *et al.*, 1990).

Folates were extracted from potato samples by the Phillips and Wright (1983) procedure using hog kidney polyglutamate hydrolase purified according to the method of Gregory (Gregory *et al.*, 1984). The samples were analyzed by a HPLC method (Pizzoferrato *et al.*, 1987).

Acid and enzymatic hydrolysis were performed to release protein-bound thiamine, to convert phosphate esters to free forms and to hydrolyze potato starch ensuring a more complete vitamin extraction and a faster sample filtration (Polesello & Rizzolo, 1986). The final solutions were injected on to an RP C18 column (Pizzoferrato *et al.*, 1987).

The ascorbic acid content was determined in milk samples stabilized with metaphosphoric acid. The centrifuged and filtered solutions were analyzed on a reversed phase C18 column (Bianchi & Rose, 1985).

RESULTS AND DISCUSSION

Sulphur dioxide and sulphites are widely used by the food industry for their low cost and versatility, but these substances exert some adverse effects, particularly on vitamins. Thiamine and folic acid can be taken as examples to illustrate the interactions between vitamins and sulphite. Thiamine is readily cleaved by the sulphite ion, through a nucleophilic attack on the methylene carbon, producing a pyrimidine sulphonate and a thiazole-containing product. Folate reacts reversibly with hydrogen sulphite to form a relatively unstable adduct (Wedzicha, 1984).

Thiamine and folic acid contents of potato samples treated with different amount of sulphite are presented in Table 1. The values relative to sample preparation time (2 h) suggest that, in this time interval, sulphur dioxide somehow has a stabilizing effect on thiamine. That is rather unexpected but comparable data are also observed for folate. Vitamin levels recorded after 7 days storage at 4°C are fairly homogeneous but, when compared to the corresponding values at 2 h, a significant vitamin decrease, particularly at higher sulphur dioxide levels, can be observed. As previously reported (Pizzoferrato *et al.*, 1988), after 14 days storage a substantial concentration-dependent sulphur dioxide loss is manifest for both thiamine and folic acid. However, due to the different mechanism of interaction with sulphite, the rate and magnitude of decline is more drastic for thiamine than for folic acid.

Table 1. Thiamine and folic acid retention in sulphite-treated potatoes

SO_2	Thiamine (mg/100 g)[a]			Folate (μg/100 g)[a]		
(ppm)	2 h	7 days	14 days	2 h	7 days	14 days
0	0·17	0·17	0·13	24·23	24·23	19·87
12	0·19	0·17	0·12	25·90	24·31	17·52
104	0·20	0·18	0·11	26·46	24·22	15·72
484	0·21	0·18	0·10	27·92	23·98	14·24
1060	0·22	0·18	0·09	29·10	23·05	13·90
2330	0·23	0·17	0·05	30·00	23·33	13·40

[a] Values are means of three determinations.

This particular trend is not observed in vitamin standard solutions and might be attributed to sulphite antimicrobial, antienzymatic and antioxidant activities. These properties are particularly important during sample preparation at room temperature, when the samples are very liable to degradation reactions. Folic acid and thiamine are subjected at the same time to both a 'direct' damage and an 'indirect' protection against their natural antagonists (microorganisms, enzymes, oxygen). The final vitamin content will depend on the prevailing effect.

In the second experiment, and perhaps of more practical relevance, the retention of ascorbic acid in raw, industrially pasteurized and sterilized milk was studied.

For its heat sensitivity, ascorbic acid has often been chosen as a technological marker to evaluate the severity of thermal treatments. Ascorbic acid is also a very useful parameter in shelf-life studies. In fact, in the presence of oxygen and/or enzymes, it is easily oxidized to dehydroascorbic acid, still retaining the vitamin activity, and to other degradation products. The quantitative determination of total vitamin C was not the aim of this work, but preliminary results of a nutritional study strongly suggest that the dehydroascorbic acid content cannot balance the ascorbic acid variations.

The ascorbic acid retention of milk industrially treated at 72, 75, 80, 85, 90 and 140 (UHT) °C is reported in Table 2. After milk collection, industrial treatment, transportation and sampling in the laboratory, the ascorbic acid residual amount was low at low pasteurization temperature and increased with increasing pasteurization temperature to reach a maximum at 80–85°C. At higher temperatures (90°C and UHT) the trend was reversed and the ascorbic acid content decreased. As expected, the ascorbic acid content of raw milk was very low but varied with the temperature

Table 2. Ascorbic acid retention in industrially heat treated milk from central dairies A, B, C

Temperature (°C)	Time (s)	Ascorbic acid (mg/litre)[a]		
		A	B	C
72	15	7·86	9·48	3·19
75	15	8·42	9·72	4·66
80	15	10·04a	9·96a	8·72
85	15	10·16a	10·12a	8·20
90	15	9·13	9·80a	7·72
140	3	2·47	—	5·09
Raw milk		2·35	4·91	1·81
T (°C) (after delivery)		9	7	15

[a] Values are means of three determinations.
a, Difference statistically not significant ($p > 0·05$).

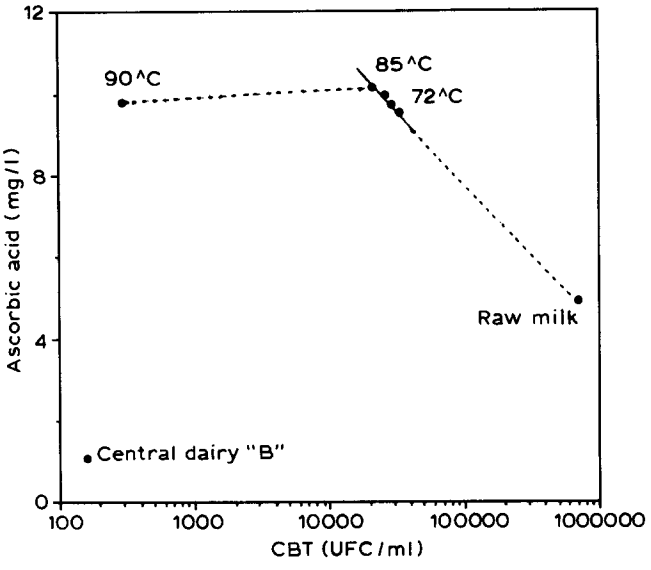

Fig. 1. Correlation between bacterial population and ascorbic acid retention in pasteurized milk.

recorded in the laboratory after delivery. Recovery of the vitamin in the central dairy C, with a higher delivery temperature (15°C), was significantly lower than the others and the ascorbic acid level in the processed samples varied widely, ranging from 3·19 to 8·72 mg/litre.

On the other hand, central dairy B showed a better retention in the raw milk and a more homogeneous trend (from 9·48 to 10·12 mg/litre) in the processed milk. Differences between samples treated at 80, 85 and 90°C are statistically not significant ($p > 0·05$). It is evident that the history of the milk from the time of processing to the time of analysis plays a key role. On the basis of the time–temperature history of the product in all distribution channels, intrinsic milk factors, endogenous enzymes, microbial population or oxygen content after industrial treatment can all affect the vitamin content.

High pasteurization temperatures have already been reported (Lavigne *et al.*, 1989) to stabilize ascorbic acid, especially close to 80°C, when, by exclusion of oxygen from milk during heat processing, the protective action can take place. At the same temperature (80–85°C) peroxidase, active in the samples up to 75°C, is completely inactivated and consequently it is quite unlikely that other enzymes might have survived.

Heat processes can also reduce the microbial population of the product; ascorbic acid content versus the residual bacterial population is reported in Fig. 1. For temperatures ranging from 72 to 85°C, a good correlation ($r = 0·989$) between these two parameters can be observed. The values for raw milk and milk treated at 90°C are out of the linearity range. The former is obviously not comparable to the treated samples while the latter is past the temperature threshold at which the indirect protective effect of heat treatment prevails.

As a conclusion, if this hypothesis were true, in the samples stored in the laboratory, oxygen, enzymes and microorganisms should continue to decrease vitamin contents. The ascorbic acid retention in milk analyzed immediately after delivery and after a few hours of storage at room temperature is reported in Table 3. The drastic effects of the experiment on ascorbic acid retention in raw milk and the increase of the vitamin recovery when raising the temperature from 72 to 85°C are evident.

CONCLUSIONS

During processing, foods are subjected, to some extent, to vitamin losses. Such losses are unavoidable, regardless of the type of processing, either industrial or domestic. On the other hand, treatments allow inactivation of undesirable enzymes and destruction of microorganisms, and so result in improved shelf-life. Since vitamins can also be degraded by endogenous enzymes and/or microorganisms, it is not surprising that industrial practices may induce substantial fluctuations in retention, either positive or negative, depending on the prevailing effect.

Table 3. Ascorbic acid recovery (mg/litre) in industrially heat-treated milk stored at room temperature for 5 h

Storage (h)	Raw milk	Pasteurized milk			
		72°C	75°C	80°C	85°C
0	2·35	7·86	8·42	10·04	10·16
5	1·01	6·76	7·49	9·93	10·06
Recovery (%)	43	86	89	99	99

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