Changes in Thiamin Content during the Storage of Spinach

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The changes in vitamin B_1 content in fresh and frozen spinach subjected to different storage conditions with the aim of observing how this vitamin is affected in such conditions were studied. In frozen spinach stored in a display freezer and subjected to temperature fluctuations an increase was observed in vitamin B_1 content. In spinach originally frozen but stored at room temperature the vitamin B_1 content decreased after showing an initial increase. In fresh spinach stored both at room temperature and under refrigeration the content in this vitamin decreased throughout the study. Taking into account the changes and the state of deterioration of the samples observed in this study during the experimental period, it would only be possible to consider vitamin B_1 as an indicator of the quality and shelf life of this product in fresh spinach.

Knowledge of the shelf life of vegetables, i.e. the period during which they are fit for consumption, is of crucial importance in their acceptance and commercialization. Even though a given product may be acceptable according to the microbiological status, it may undergo physical and chemical changes during storage, and as a result its sensorial and nutritional qualities may be affected, even to the extent of the vegetable being unfit for consumption. Accordingly, it is of importance to have criteria to evaluate the quality of foods and, according to such criteria, determine their useful shelf life.

Among other indices that may be employed are the alteration of the sensorial characteristics and the modifications taking place in the composition of such foods. Vitamin B_1, together with vitamin C, has been studied in this sense since, as is it very water soluble, it undergoes leaching during processing; it is also highly sensitive to chemical degradation during processing and later storage. It may therefore be considered that if thiamin, like vitamin C, is well retained, so will other food nutrients (Fennema, 1977).

The lability of thiamin, together with its presence in most foods, accounts for its use as an indicator of the shelf life or of the quality of foods. Regarding this, several authors have pointed to the close relationship between the thiamin content of foods and their degradation (Kramer, 1979; Labuza, 1982). Despite this, however, there are discrepancies concerning the appropriateness of vitamin B_1 as an indicator of quality. Accordingly, the *Congressional Record* (1977), cited in Labuza (1982), recommends aroma and texture as indicators of the shelf life of a food, since in most cases these aspects deteriorate before the food has undergone important degradation. In this sense, thiamin is most widely accepted as an indicator for foods of animal origin, among other reasons because of their high content in this vitamin. In plant products, however, the most widely studied parameter is vitamin C, and there are few references relating to thiamin.

In general, during storage, the retention of thiamin in vegetables tends to decrease with time and is increasingly more affected the higher the temperature (Abou-Fadel and Miller, 1983; Aykroyd and Doughty, 1977; Tannenbaum, 1982). Normally, fruits and vegetables retain up to 90% of thiamin when stored at room temperature for 2–4 days, but if storage is further prolonged, it should be done under refrigeration to avoid vitamin losses (Kramer, 1982).

In the storage of frozen vegetables, variable losses have been observed in thiamin contents according to the products and to the conditions to which the product is subjected during this period (Dequidt et al., 1981; Fennema, 1982). Erdman and Erdman (1982) reported that the long duration of cold storage and fluctuations in temperature destroy thiamin as time progresses, though Fennema (1982) observed that a rise in temperature from −18 to −7 °C did not lead to losses in this vitamin in the storage of green beans.

The aim of the present work was to study the changes of vitamin B_1 in fresh and frozen spinach subjected to different storage conditions with a view to observing how this vitamin is affected in such conditions. A further aim was to evaluate the possible suitability of this vitamin as an indicator of the shelf life and the quality of this kind of vegetable.

EXPERIMENTAL SECTION

Samples. *Fresh Spinach (Highpack Variety).* The spinach was washed, chopped, and frozen at the factory in order to avoid spoilage during transport to the laboratory. Immediately upon arrival it was thawed at room temperature. Although strictly speaking the spinach was not "fresh", it will be referred to as such in this work in order to distinguish it from the product that has undergone the conventional freezing process.

Frozen Spinach (Highpack Variety). In the factory the spinach was selected, washed, steam-blanched, pressed, chopped, and packed in cardboard containers that were then frozen on a industrial plate freezer. The spinach was stored in a freezer at −22 °C until the time of assay.

Both the fresh and frozen spinach samples were supplied by Hispareco (Badajoz, Spain), were from the same cultivar, and were collected at the same time.

Reagents. Thiamin dichloride and Diastase 500 E/g were obtained from Merck (Darmstadt, West Germany). Biorex 70 (50–100 mesh, sodium form) was obtained from Bio-Rad Laboratories (Richmond, CA).

Storage Conditions. *Fresh Spinach.* This was divided into portions of 200 g and wrapped in strong wrapping paper. One of these portions was employed for the initial determination of vitamin B_1, and of the remaining samples, half was stored at room temperature (18–22 °C) and the other half under refrigeration (4 °C). One sample of each type was periodically removed, homogenized, and analyzed for thiamin content.

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Frozen Spinach. The containers were placed in a display freezer (−18 °C). On the first day vitamin B_1 content was analyzed in the spinach of one of the containers, taking the amount found as a reference value. Later, periodic samples were taken, homogenizing the whole content of the container and analyzing their thiamin content.

In the storage study simulating temperature fluctuations the containers were placed in a freezer (−22 °C) for 14 h, after which they were transferred to a refrigerator (4 °C) for 10 h; following this they were returned to the freezer. This operation was performed daily throughout the study period. Samples were taken periodically and analyzed for their thiamin content.

Finally, in the assays examining improper storage conditions, four containers were kept at room temperature, collecting and analyzing samples as described above.

The duration of the different experiments was established as a function of what was considered to be a normal storage time and was limited by the sensorial characteristics acquired by the samples during the period.

Analytical Method. *Determination of Thiamin.* The method employed is based on the criteria of Edijala (1979), Schulte et al. (1983), and the AOAC (1984) with certain modifications to adapt it to the samples and aims of the work. All of the analyses for each sample were carried out in duplicate. To 10-g portions of the previously homogenized sample was added 60 mL of 0.1 N HCl, heating in a water bath to 100 °C for 30 min. Following this, pH was adjusted to 4–4.5; 5 mL of 10% diastase was added, and the sample was incubated at 45 °C for 15 h. After this time, it was centrifuged at 11 000 rpm for 20 min, filtered with a No. 2 porous plate, and brought to 100 mL with acetate buffer. Twenty milliliters of this solution was passed through a column packed with 2 g of Biorex-70 after which the thiamin was eluted with acidic KCl, bringing volume to 25 mL.

Oxidation and extraction of the thiochrome were carried out according to Edijala (1979), performing spectrofluorometric measurement on an Aminco SPF 125 apparatus. The reaction was carried out in triplicate for each purified extract. Quantitation was performed with respect to a calibration straight line prepared on solutions of thiamin in acidic KCl from 0.01 to 0.50 μg/mL, which were subjected to the same formation and thichrome extraction procedures as the samples. A thiamin-free blank was prepared to adjust the fluorescence intensity to zero. In order to eliminate the fluorescence contributed by other compounds, it was necessary to prepare another blank for each sample to which the oxidizing reagent was not added. The fluorescence emitted by this blank was subtracted from that emitted by the sample, and this difference was compared with the calibration straight line. Absence of interference was checked by the excitation and emission spectra of the thiochrome.

Determination of Moisture. The product was dried until constant weight according to the method described by Casares (1967).

RESULTS AND DISCUSSION

Precision and Accuracy of the Method. To evaluate precision, the method for the determination of thiamin was applied to eight portions of a sample and a variation coefficient of 3.67% was observed, which is satisfactory when taking into account the complexity of the samples and the low concentrations of the vitamin to be determined.

In order to check the accuracy of the method different amounts of thiamin (0.25, 0.50, 0.75, 1.00 μg/g) were added to a sample, carrying out determination in triplicate for each amount added. Mean recovery proved to be 89.72%.

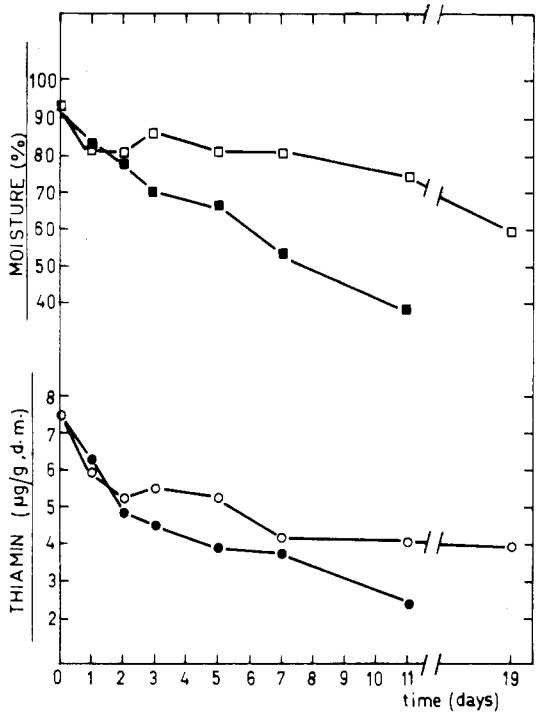

Figure 1. Changes in thiamin content (expressed with respect to dry matter) and moisture in fresh spinach, stored at room temperature (●, ■) and temperature of refrigeration (○, □).

Vitamin B_1 Content in Fresh Spinach. In fresh spinach stored at room temperature a gradual loss was observed in thiamin content. Indeed, it may been seen (Figure 1) that after 11 days of the study, losses were 67% expressed with respect to dry weight. From the third day of the study onward the appearance of improper smells, color, and texture could be observed. At this time, the losses in thiamin were already 37.9% compared with initial values.

When the spinach was stored under refrigerated conditions (Figure 1), these signs of deterioration were not observed until 11 days of storage, when losses in thiamin were 41.9%. In a new determination performed at 19 days, when the samples were evidently deteriorated, vitamin B_1 losses were 45.0%.

In view of the changes followed by the fresh spinach in both kinds of storage, refrigeration temperatures, as expected, may be said to delay both the onset of deterioration and the increase in the losses of vitamin B_1. However, in spite of the fact that this kind of storage is the most suitable, the loss of thiamin after 2 days is similar for both temperatures.

It should be taken into account that strictly one was not dealing with fresh spinach but rather with the plant after previous washing and freezing, which could affect the results.

Vitamin B_1 Content in Frozen Spinach. In frozen spinach stored in a display freezer (Figure 2) an increase (25.4%) in thiamin content during the 40 days after the start of the study could be observed. This increase should be considered as statistically significant and in no case can be attributed to possible deviations derived from the analytical method employed according to the method's variation coefficient.

Increases such as those observed in this study have sometimes been reported, though no satisfactory explanations have been provided to account for the phenomenon. Evidence exists to suggest that in some vegetables there are enzymes able to carry out condensation of the

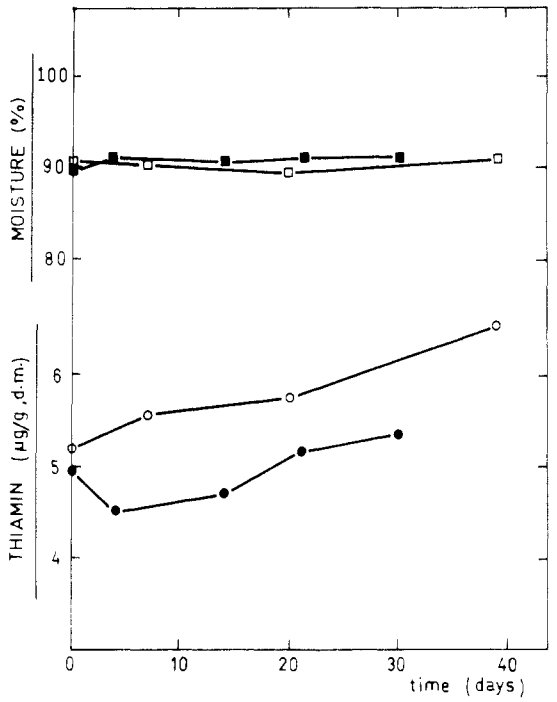

Figure 2. Changes in thiamin content (expressed with respect to dry matter) and moisture in frozen spinach, stored in a display freezer (○, □) and with temperature fluctuations (●, ■).

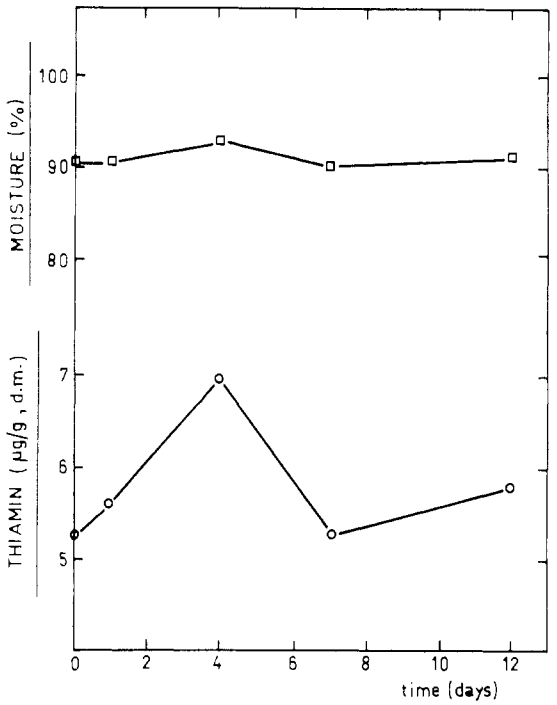

Figure 3. Changes in thiamin content (expressed with respect to dry matter) and moisture in spinach originally frozen but stored at room temperature (○, □).

preformed pyrimidine and thiazole rings to give rise to the thiamin molecule (Aurand and Woods, 1973). Moreover, it has been suggested that the freezing process releases bound, biologically inactive forms of the vitamins or converts inactive precursors to the active vitamins (Fennema, 1982). Taking into account the lability of vitamin B_1, one might assume that a drop in thiamin content would be more logical. This latter idea has been described by several authors (Dequidt et al., 1981), even in spinach, though the storage times were much longer than in our study and temperatures were lower.

The results obtained with spinach subjected to temperature fluctuations (Figure 2) show that although there is an initial decrease in thiamin content, a gradual increase later takes place that is maintained until the end of the assay (6.7% with respect to the initial value). Also, an increase is observed in moisture content throughout the assay, which could be accounted for by the partial thawings and refreezings to which the samples were subjected.

When the initial thiamin content of frozen spinach was compared with that of the fresh samples, a loss of approximately 30% may be observed. This may be attributed to the influence of the blanching procedure, since it is the treatment that differentiates the processing of both types of samples until arrival at the laboratory. Similarly, blanching may condition the later evolution of vitamin B_1.

In the spinach originally frozen but stored at room temperature, as expected, from the very first day of the study it was possible to observe improper smells, color, and texture in the samples, thus making it unfit for consumption and finally reaching a state of putrefaction at the end of the study. In spite of this, the aim of this part of the study was to observe the changes in vitamin B_1 content rapidly, to see whether they followed a regular evolution or not. Owing to the advanced state of deterioration reached, the moisture values determined are heterogeneous and the variations in the thiamin content when referred to dry weight are irregular (Figure 3). The increase observed in the first few days of the assay did take place although the samples were increasingly disagreeable in aspect. Later, a decrease is observed such that it is not possible to establish a general norm concerning the behavior of thiamin in the conditions examined, and in this case one could not consider vitamin B_1 as a possible indicator of shelf life.

The vitamin B_1 content in frozen spinach stored both in the display freezer and with temperature fluctuations (Figure 2) shows a trend to increase, which throws doubt on the usefulness of vitamin B_1 as an indicator for these kinds of storage conditions. It should be noted that the samples did not show any appreciable deterioration in their sensorial characteristics.

However, in fresh spinach, when stored both under refrigeration and at room temperature (Figure 1), evident deterioration takes place coinciding with a marked decrease in the vitamin B_1 content. Accordingly, in these conditions, thiamin could be employed as an indicator of quality, although further and more detailed studies are necessary to fully evaluate this point.

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Determination of Sulfhydryl Groups and Disulfide Bonds in Heat-Induced Gels of Soy Protein Isolate

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Thermally induced gels were prepared from dispersions of soy protein isolate at varying protein concentrations, pH, and heating intensity. Increasing protein concentration (0.9–18.2%), pH (7–10), or temperature (80–120 °C) caused (1) a decrease in the content of free SH groups (from an initial 8 μmol/g of protein) and in the protein solubility of gels (in Tris–glycine buffer), (2) no change in the half-cystine content (~100 μmol/g of protein), and (3) an increase in gel firmness. Heating at 130 °C caused (1) an increase in the content of free SH groups and in the protein solubility of gels, (2) a decrease in the half-cystine content, and (3) a decrease in gel firmness. These data and the severely reduced firmness of gels formed in the presence of *N*-ethylmaleimide suggest that newly formed additional S–S bonds and/or SH-induced S–S interchange reactions contribute markedly to the strengthening of gels observed at alkaline pHs or at high temperatures (115–120 °C). At or above 130 °C, the partial breakdown of S–S bonds (with half-cystine losses) may contribute to gel softening.

Sulfhydryl (SH) groups and disulfide (S–S) bonds influence significantly the functional properties of food proteins and play an important role in the formation of relatively rigid structures such as protein gels or doughs. Heat-induced changes in SH group and S–S bond contents of food proteins have been reported by Saio et al. (1971), Hashizume and Watanabe (1979), and Yamagishi et al. (1984) for soy proteins, by Watanabe and Klostermeyer (1976) for β-lactoglobulin, by Patrick and Swaisgood (1976) for skim milk, by Li-Chan (1983) for whey protein concentrate, by Beveridge and Arntfield (1979) for egg white, and by Opstvedt et al. (1984) for fish protein. However, most of these studies have been carried out with dilute protein solutions or with protein powders, and little is still known concerning the changes occurring in SH groups and S–S bonds when heating is performed at concentrations leading to protein gelation. The firmness of tofu gel (Ca^{2+} coagulated after heating—a traditional Japanese food) made from soy 11S globulin increased with increasing SH group content before heating (Saio et al., 1971). Minced and washed fish muscle (surimi) with a high content of SH groups led to kamaboko gels of better quality (Jiang et al., 1986). The content of SH groups in protein gels has been determined with whey protein concentrate and egg white (Beveridge et al., 1984) and with ovalbumin (Hayakawa and Nakai, 1985). These authors have observed a correlation between increased gel firmness and decreased SH group content as a result of gelation. These results suggest that S–S bonds form during heat gelation through oxidation of SH groups (and not only through SH, S–S interchange), and markedly influence the gel network structure and mechanical strength. Voutsinas et al. (1983) found that gel strength was significantly correlated with both the initial SH group content and the hydrophobicity of several globular proteins. In contrast, Hegg (1982) suggests that there is no obvious correlation between the S–S bond or SH group content and the gel-forming ability of conalbumin, serum albumin, and β-lactoglobulin. Such a contradiction may reflect differences in the reactivity of SH groups and in the respective roles of hydrogen bonds, electrostatic interactions, S–S bonds, and hydrophobic interactions for the gelation or different proteins (Shimada and Matsushita, 1980).

Various investigators have recently reported that S–S bonds play an important role in the gelation of soy proteins. Evidence for this role came from the effects of the presence or absence of *N*-ethylmaleimide (NEM) or of 2-mercaptoethanol (2-ME) on the formation of protein aggregates and from gel solubilization by 2-ME (Mori et al., 1982; Utsumi and Kinsella, 1985a,b; Mori et al., 1986). However, the changes in SH group and S–S bond contents were not directly determined.

In the present study, we have attempted to determine the changes in SH group and S–S bond contents in soy protein isolate (SPI) as a result of heat-induced gelation. These changes have been studied as a function of protein concentration, pH, and intensity of heat processing. Correlations have been sought between S–S bond formation and gel firmness. This necessitated a preliminary study of conditions leading to an extensive solubilization of protein gels into transparent solutions where SH groups

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