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Influence of Heat Treatment of Spinach at Temperatures up to 100°C on Important Constituents

VI. Total Lipids and Glycolipids

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Heat treatment of vegetables (e.g. blanching before freezing) influences the contents of nutritive components in the food. The grade of variation (e.g. dissolution of mineral salts, denaturation of proteins, inactivation of enzymes) depends on time and temperature of the treatment. Whether and how lipid compounds are affected by this procedure has not been proved so far. Within the frame-work of our systematical work concerning influences of heat treatment of vegetables, particularly spinach, we investigated the effect of heating fresh spinach leaves for 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 minutes at 70, 80, 90 and 100 centigrades, resp., on the lipid constituents of spinach. The experiments have shown that with increasing temperature and time the amounts of substances soluble in methanol-chloroform, the so-called lipids, were increasing. A clear relationship between the time-temperature product was observed. The composition of the total lipids also depended on that product: the quantity of monogalactosyl diglycerides increased with higher time-temperature product, whereas the quantity of digalactosyl diglycerides decreased. It is discussed whether this fact is due only to a simple hydrolytic cleavage of 1 mol galactose or whether the galactosyl diglycerides become easier soluble from the cell wall under the influence of heat treatment.

1.0 Introduction

Before undergoing any industrial processing such as deep freezing or heat sterilization, vegetables are usually treated by heat. For this pretreatment called blanching the vegetable material is immersed in hot water for as long as is necessary to inactivate enzymes which may cause spoilage. Numerous studies are known on the effect of this heat treatment, using temperatures of 80° to 100°C. There have not yet been any systematic investigations, however, covering a wide range of temperatures and times which could serve as basis for a true optimization of processes. In a series of studies we have therefore treated spinach at temperatures of 70°, 80°, 90° and 100 °C over various lengths of time ranging from 0.5 to 32 min (1, 2, 3, 4, 5). Spinach has been chosen because it represents an important product for both the deep freezing and canning industries. Within the frame-work of these studies which have been mainly concerned with enzymes, vitamins and protein fractions, it seemed of interest also to learn whether the small amounts of lipids, approximately 0.2 to 0.4% of the fresh plant material, are influenced in a characteristic manner under varying conditions of temperature and time.

2.0 Material and Methods

2.1 Thermal treatment

Freshly harvested spinach was exposed to heat by immersing the vegetable in water over certain lengths of time (ref. 1). After immediate cooling and draining, the vegetable was frozen at a high freezing rate. The frozen material was ground at −20 °C and freeze-dried.

2.2 Determination of the total lipid content

Lipids were extracted essentially according to FOLCH *et al.* (6), modified as described by FRICKER (7). After evaporation of the solvents in a stream of nitrogen the residue was weighed.

2.3 Determination of fatty acids

The methyl esters of fatty acids were analysed by means of conventional gas-chromatographic methods (Hewlett-Packard model 5750, column 6 m x 0.4 cm, 5% Carbowax 20 M on Chromosorb W 80–100 mesh, temperature programme 160–200 °C, 2 °/min, 200 °C isothermal).

2.4 Preparative separation and quantitative determination of glycolipids

Total lipid extracts were fractionated by thin-layer chromatography on silica gel G., layer thickness 0.5–1 mm, using the solvent mixture: chloroform-methanol-water 75 : 25 : 4 in a saturated chamber. The various zones were detected by means of iodine. The fractions of monogalactosyl diglycerides and digalactosyl diglycerides were scraped off, placed in columns and eluted with chloroform-methanol 1 : 1. The purity of the eluted fractions was checked by means of analytical thin-layer chromatography; quantitative determinations were carried out by measuring of galactose (Duden, unpublished method).

3. Results

3.1 Dependence of extractable total lipids on thermal treatment

Tab.1 clearly demonstrates that the amounts of extractable total lipids depend on the intensity of the heat applied.

Tab. 1 Total lipids extracted from thermal treated spinach

Thermal treatment °C/min	% of dry matter
unblanched	9.4
70°/1 min	10.5
70°/32 min	13.4
90°/0.5 min	10.8
100°/8 min	14.4

Whereas only about 9.4% of dry matter could be extracted from fresh, non-blanched spinach, higher values were obtained throughout with heat-treated spinach. These values clearly reflect the influence of temperature and time. After heating to 70°C for 32 min, 13.4% of lipids were obtained; but if the material was heated to 90°C for 0.5 min only 10.8% lipid material are extracted. The highest value was observed after heating the vegetable for 8 min to 100°C. It should be noted that utmost care was applied to ensure that the extraction was carried out consistently in all cases.

3.2 Fatty acid composition of total lipids

Linolenic acid provides the greatest share of the constituent fatty acids, whereas palmitic acid and linoleic acid are present at levels of more than 10%. The fatty acid present at a level of 5–7% which, according to the retention time in the gas chromatogram, could either be $C_{17:0}$ or $C_{16:3}$ acid, was shown by mass-spectrometry to be definitely $C_{16:3}$ **(Tab. 2).**

Tab. 2 Fatty acid composition of total lipids from spinach (% of identified fatty acids)

Fatty acid	%
$C_{16:0}$	12
$C_{16:1}$	0.2
$C_{16:3}$	6
$C_{18:2}$	13
$C_{18:3}$	62
Σ	99.7

3.3 Dependence of the ratio of monogalactosyl diglycerides to digalactosyl diglycerides on thermal treatment

Thin-layer chromatographic analysis of the total lipids extracted from spinach treated in different ways showed that there were remarkable differences between extracts from blanched and unblanched spinach. There is particularly an increase in monogalactosyl diglycerides and a decrease in digalactosyl diglycerides depending on the time and temperature of treatment. These results are confirmed by quantitative determinations of the two classes of galactolipids in the total lipid extracts.

The ratio of the two galactolipids changed, with increasing heat intensity, from 1:1 over 1.4:1 to 1.6:1. It is necessary to emphasise, however, that the methods used in the quantitative determination of the two lipid classes are relatively inexact. Therefore the values given should be assessed with caution. Undoubtedly, however, there is a clear tendency towards an increase of monogalactosyl diglycerides and a decrease of digalactosyl diglycerides in dependence on the heat treatment applied **(Tab. 3).**

Tab. 3 Dependency of the monogalactosyl-digalactosyl diglyceride ratio from the thermal treatment

Mode of treatment	MGDG (% of total lipids)	DGDG (% of total lipids)	MGDG : DGDG ratio
unblanched	22.6	22.2	1:1
70°/1 min	28.6	20.6	1.4 : 1
100°/8 min	33.4	20.8	1.6 : 1

MGDG = Monogalactosyl diglycerides; DGDG = Digalactosyl diglycerides

4.0 Discussion

That lipid fractions in spinach are influenced by heat has, as far as we are informed, not been known so far. The results reported here demonstrate, however, that these substances are also affected by heat treatment. This is demonstrated by the fact that the amount of extractable lipids is dependent on temperature and duration of the heat treatment applied. One reason for this may be that influence of heat changes the plant cells and their membranes in such a way that lipids which in the fresh product are not "accessible" to the solvent become extractable.

Of interest seems our finding of a shift in the ratio of monogalactosyl diglycerides to digalactosyl diglycerides depending on the intensity of heat applied. This apparently is not stoichiometric in so far as to allow the computation of a stoichiometric increase of monogalactosyl diglycerides from the decrease of digalactosyl diglycerides. It is well possible that further reactions lead to some hitherto unidentified products.

5.0 Acknowledgement

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6.0 Literature

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