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ELECTRON MICROSCOPIC INVESTIGATIONS OF THE CELL STRUCTURE IN FRESH AND PROCESSED VEGETABLES (CARROTS AND GREEN BEAN PODS)

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Abstract

The cell structure of fresh, blanched, boiled, dried and rehydrated tissues from carrot roots and green bean pods was examined in the scanning and/or transmission electron microscope. The secondary phloem of carrot roots represents a typical plant storage parenchyma characterized by a high starch and lipid content. Green bean pods show many characteristics of assimilation tissue (e.g. chloroplasts), but they also contain a considerable amount of starch. Blanching, boiling, de- and rehydration affect the texture of both vegetables in a similar way: swelling of cell walls, maceration of tissue during blanching and boiling coupled with a granular denaturation of cytoplasm. Drying leads to a shrinkage and twisting of the cells and clumping of the cytoplasm. Rehydrated tissue is characterized by strong cell wall swelling, maceration, and clumping of cytoplasm. Morphometric measurements of cell wall thicknesses after rehydration showed that various food technological process parameters may strongly influence the appearance of the rehydrated product.

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Introduction

Dried carrots and green beans are traditional ingredients of bag soups which have become more and more popular on the European market. In comparison with genuine instant products these bag soups are characterized by comparatively long cooking times which can be influenced – within certain limits – by varying process conditions during the production of such dried vegetables. The aim of industrial research now is to find suitable process conditions in order to produce dried vegetables which need extremely short cooking times and are similar to well cooked fresh vegetables as regards taste and consistency. In this sense we tried to evaluate the relationship of process conditions to cell ultrastructure during the production of dried vegetables and the ultrastructural alterations which occur during the rehydration process (i.e. cooking) by comparing them with the ultrastructural morphology of well cooked fresh products. It is hoped by understanding these interrelationships that process conditions can be optimized to obtain dried and rehydrated vegetables of high quality.

There exists large literature on the morphological structure of both vegetables, carrots and green beans. Most of them were carried out with the optical microscope, but in recent times the improved resolution power of the electron microscope was also utilized. Thus optical microscopic studies in the developmental anatomy of the root of Daucus carota were carried out by Havis (14), Esau (9) and Drake and Selstam (7, 8). Reck (18) published a detailed paper on the optical microscopical structure of the plastids in the root.

Scanning electron micrographs of raw and cooked carrot tissue are found in the studies by Davis et al. (3 - 6). Transmission electron microscopical papers on the roots of Daucus carota chiefly deal with some cytological problems such as the structure of chromoplasts (11), nuclei (15) or the in vitro culture of carrot cells and tissues (28). (For a more detailed list of literature see the above mentioned papers).

Most of the morphological studies in green bean pods that have so far been carried out were also done with the optical microscope. The first to examine the histological structure of the legumen were Kraus (16) and Steinbrink (25). Like many of the following authors, some of whom applied modern techniques such as polarisation microscopy or X-ray diffraction, they put their emphasis on the morphology of mature pericarps, especially on the structure of the vascular bundles and fibres in

order to investigate the dehiscence mechanism of the pods (10, 13, 17).

Roth (22) treats the anatomy of the legumen as part of her comprehensive work on the anatomy and histology of the fruits of angiosperms. Gassner (12), who deals with green beans within the scope of a general optical microscopical investigation of vegetable foodstuffs, also gives some histological information.

This is even more the case with the detailed studies by Reeve and Brown (21), who provide a survey of the developmental sequence and differentiation of pod tissues and their structure and composition at edible maturity.

Optical microscopic studies of the morphological changes occurring in plant tissues during preparation processes, such as blanching, cooking, and dehydration/rehydration, have been published by a number of authors.

Thus Simpson and Halliday (24) studied the disintegration of membrane material in vegetables during the cooking procedure. Reeve and Leinbach (20) and Sterling (26) examined the effect of moisture and high temperature on carrot, potato, and apple tissues. Reeve (19) published a microscopical study on textural structural changes in fresh and processed fruits and vegetables.

The effects of drying and rehydration on cellulose crystallinity of carrots were investigated by Sterling and Shimazu (27). (For more detailed literature see the above mentioned papers).

Transmission electron microscopy has so far not been employed in a systematic fine-structural investigation of prepared and processed vegetables.

Likewise, the application of morphometrical techniques in combination with transmission electron microscopy of vegetables has so far not been reported in literature.

In the present study we first want to present the basic morphological changes involved in the blanching, cooking, dehydration and rehydration of carrots and green beans without correlating them to special food technological process conditions. The ultrastructural appearance of the fresh, unprocessed cells and tissues serves as a baseline for structure.

In the second part of the present paper, we want to analyse systematically the influence of special process conditions on the degree of cell wall swelling, which – in our opinion – is the most conspicuous feature of dried and rehydrated carrots and green beans.

Materials and Methods

Two varieties of green beans ("Koralle", "Cascade") and of carrots ("Bauer's Kieler Rote", "Zino") were analysed. Blanching, drying and storing of the vegetables were carried out at the Institute of Food Technology – Fruit and Vegetables Technology – in Berlin as part of a correlated research project (AIF project No. 3664). Some of the results of this project were published by Bielig and Schwaiger (1). For drying under defined and reproducible conditions, a hot-air drying system previously designed was used (Bielig et al., 2). The dried samples were rehydrated by cooking to "doneness" according to reports from the Berlin Institute of Food Technology where the optimum cooking time for each sample had been previously determined by two different methods: by a sensory test of doneness and by measuring the shear strengths of the samples mechanically (For further details cf. Bielig and Schwaiger [1]).

For transmission electron micoscopy (TEM), from fresh, blanched and rehydrated tissues (rehydration by boiling) cubes of approx. 1 x 1 mm size (from the secondary phloem of the carrot root and the outer and inner part, respectively of the green bean pod) were cut with a sharp razor blade and fixed in 2% phosphate buffered glutaraldehyde (pH 7.3) for two hours at room temperature. After a short washing with the buffer solution, the samples were postfixed for two hours in buffered 1% OsO_4 solution. They were dehydrated in a graded series of ethanol. In 70% ethanol, block staining with phosphotungstic acid/uranyl acetate was carried out. After embedding the samples in Epon resin, ultrathin sections were cut with a diamond knife using a Reichert ultramicrotome OmU3. Sections were stained with lead citrate and examined in a Siemens Elmiskop I.

The specimens dried by hot air were briefly immersed in ethanol and then embedded in Epon resin, because it had proved impossible to obtain sections from specimens directly embedded in the resin.

For scanning electron microscopy (SEM), fixation and dehydration were carried out as described above. After the 100% ethanol stage, the samples were critical-point-dried in Freon 13 using Freon 11 as a transitional fluid. The dried specimens were mounted on specimen holders with "Leit-C", coated with gold by sputtering and observed in a STEREOSCAN Mk I.

For morphometric measurements of cell wall diameters in TEM the semi-automatic image analysis system MOP AM/03 (Kontron, Germany) was used. In each test 10 - 15 separate measurements were made on at least three parallel samples from three different experiments, and the mean values were calculated. For obtaining comparable results, the points of measurement on cell walls were defined as follows: midway between two intercellular spaces taking only those cell walls into consideration which were not (yet) separated along their middle lamella.

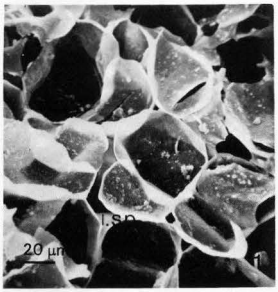
Since the morphological appearance of the boiled raw material was considered to be the "ideal" for a rehydrated vegetable, the measured cell wall thickness of the boiled raw tissue was put at 100. Cell wall alterations in dried and rehydrated samples were expressed as percentages of this value.

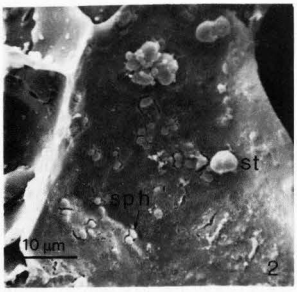
Results

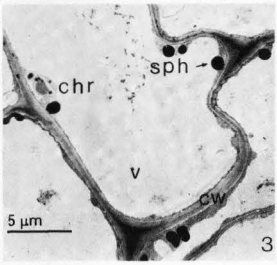
Ultrastructural aspects of fresh and processed vegetables

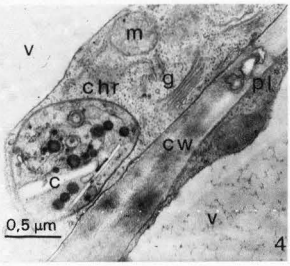
SEM of fresh carrot tissues. The critical-point-dried carrot exhibits a very good preservation of its histological structure (Fig. 1). The tissue consists of polygonal parenchynatous cells and shows many intercellular spaces.

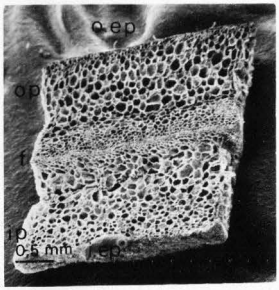
At higher magnification, details of the cytoplasmic layer become visible. The rather homogeneous cytoplasm includes particles of variable shape and size, which by comparison with transmission electron microscopic analyses can be identified as different cell organelles. Within the cells of fresh carrots, roundish-shaped inclusions of approximately 5μm diameter are seen, which have to be interpreted as starch grains or big chromoplasts. Round particles of much smaller size (1 - 2 μm

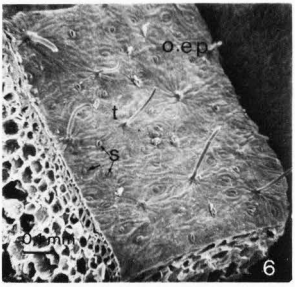


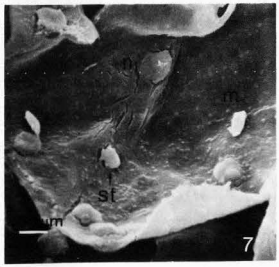












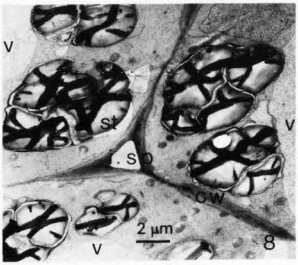


Fig. 1 – 4: Electron micrographs of fresh carrot tissue

Fig. 1: Phloem parenchyma cells at low magnification in SEM (i.sp= intercellular space)

Fig. 2: Cytoplasmic layer of a single cell in SEM (sph=spherosome, st=starch grain)

Fig. 3: Survey of phloem parenchyma tissue in TEM (sph=spherosome, chr=chromoplast, v=vacuole, cw=cell wall)

Fig. 4: Detail from cell wall and cytoplasm in TEM (v=vacuole, m=mitochondrium, g=golgi apparatus, chr=chromoplast, c=carotin pigment, cw=cell wall, pl=plasmodesm)

Fig. 5 – 8: Electron micrographs of fresh green bean pod tissue

Fig. 5: Cross section through one entire valve of the pod in SEM (o.ep=outer epidermis, i.ep=inner epidermis, op=outer parenchyma, ip=inner parenchyma, f=fibre layer)

Fig. 6: Outer epidermis of the valve with trichomes and stomata in SEM (o.ep=outer epidermis, t=trichome, s=stomata)

Fig. 7: Fracture through the outer parenchyma showing the cytoplasmic layer of a single cell in SEM (n=nucleus, m=mitochondrium, st=starch granule)

Fig. 8: Detail of parenchyma cells in TEM (v=vacuole, st=starch granule, i.sp=intercellular space, cw=cell wall)

diameter) represent lipid containing spherosomes (Fig. 2).

TEM of fresh carrot tissues. A survey of carrot phloem parenchyma tissue is shown in Figure 3. Each cell possesses a big central vacuole, whereas the cytoplasm appears as a thin layer along the walls. Numerous round particles heavily stained with osmium represent lipid droplets (spherosomes).

At higher magnification (Fig. 4), numerous plasmodesmata as plasmic connections between neighbouring cells become visible. Within the cytoplasm itself we find parts of the endoplasmic reticulum, mitochondria of the tubuli type and golgi apparatuses.

The most remarkable morphological feature of the carrot phloem parenchyma cell, however, is represented by the chromoplasts bearing the typical red or yellow pigment. On cross sections they show manifold shapes and structures. Within the fresh carrot cells, chromoplasts in most cases are round or oval and include starch grains. They often contain numerous plastoglobuli, irregularly shaped thylacoids and the outlines of pigment inclusions, whereas most of the carotin pigment itself is extracted during preparation procedures. Besides these typical chromoplasts amyloplasts also occur.

Nuclei in general show an irregular shape, with many protrusions, and two nuclear bodies within the nucleoplasm. Closely associated with the nucleolus (1.5 - 2 μm) there is another nuclear body with a size of approximately 0.6 μm.

SEM of fresh green bean tissues. Cross sections through one entire valve of the pod show an outer and inner surface tissue (epidermis) and a bisection of the enclosed parenchyma tissue into an outer and inner parenchyma ("seed-cushion") by a middle layer of cells with very small lumina (Fig. 5). The outward epidermis reveals a pattern of trichomes, stomata and a characteristically ridged cuticle (Fig. 6), whilst the inner surface tissue adjoining the seed consists of small papillate cells.

Fracturing of the parenchyma tissue allows the observation of the cytoplasmic layer inside the single cells. Especially conspicuous are round particles of approximately 10μm diameter, which are preferably located within the cells of the outer parenchyma. These organelles may occur in large numbers within the cells.

As a comparison with transmission electron microscopical observations shows, they represent starch grains or chloroplasts with extensive starch inclusions. Flat-roundish bodies of approximately 15μm diameter might represent nuclei whereas the numerous very small particles within the cytoplasm (approximately 0.6 x 0.8 μm) must be regarded as mitochondria (Fig. 7).

TEM of fresh green bean tissues. In contrast to the inner parenchyma, the outer parenchyma tissue of the immature pod (Fig. 8) shows many intercellular spaces. The single cells possess big central vacuoles and parietal cytoplasmic layers of varied thicknesses. The cell walls themselves are frequently traversed by plasmodesmata. A detailed analysis of the cytoplasm shows many small vacuoles, flatshaped nuclei, mitochondria of the tubuli-type, golgi apparatus and frequently a richly developed rough endoplasmic reticulum.

The morphological appearance of the plastids is remarkably heterogeneous. Besides well developed starch grains (amyloplasts) we find chloroplasts with small starch granules and chloro-amyloplasts with considerable starch inclusions, but there are still clearly visible stroma and grana thylacoids. Whereas these types of plastids occur in the outer parenchyma tissue, the inner parenchyma possesses only small lens-shaped chloroplasts with apparently little or no starch grains.

TEM of processed carrot tissues. After blanching, carrot parenchyma cells show a slight swelling of their cell walls. They tend to separate along their middle lamellae. The cytoplasmic compounds still adhere to the inner side of the walls in the form of small granules (Fig. 9). Boiled tissue (Fig. 10) exhibits still thicker cell walls. The cytoplasmic granules are coarser, but on the whole they still retain their marginal position. Always lipid droplets of various sizes can be observed in blanched and boiled tissues. Blanched and dried carrot root tissue is characterized by an enormous shrinkage and twisting of the cells, but no rupture occurs. The cytoplasm fills the remaining lumina of the cells as a dense, clumpy material enclosing large lipid droplets (spherosomes) which seem to stem from the fusion of several small ones (Fig. 11).

The morphological appearance of blanched, dried and rehydrated cells and tissues (Fig. 12) strongly depends on the drying conditions (temperature, atmospheric moisture, drying time etc., [see below]) but, as a rule, cell walls are thicker after blanching, drying and rehydration than they are after cooking alone. Intercellular spaces seem to be enlarged and increased in number after drying and rehydration. The cytoplasm is more strongly condensed and clumped after rehydration and often does not regain its original marginal site, but remains in the centre of the cells. The degree of cytoplasm clumping and the localization of the cytoplasm within the cell can also be strongly influenced by the kind of dehydration employed.

TEM of processed green bean tissues (inner parenchyma). Blanched pod cells show cell walls which are slightly thickened. Cell wall junctions are slightly enlarged, their electron density being decreased at the same time. The cytoplasm being denatured by heat forms a marginal layer of fine granules still closely lining the cell walls (Fig. 13).

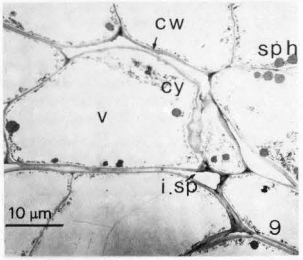
After boiling the tissue, cell walls appear increased in thickness. Sometimes larger intercellular spaces are formed. The cytoplasm consists of granules coarser than those in blanched cells. The strictly marginal arrangement of the cytoplasmic layer is disturbed but, on the whole, the cytoplasmic granules are still assembled along the cell walls (Fig. 14).

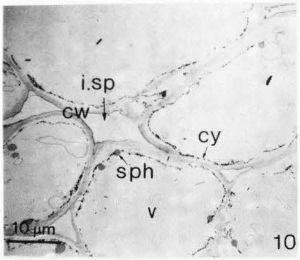
As in carrot tissue, blanched and dehydrated pod tissue is built up of shrunk and twisted, but still intact and clearly distinguishable individual cells. The small lumina of the cells are more or less filled up with clumped cytoplasmic material (Fig. 15).

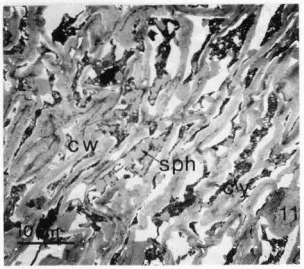
As to the morphological picture of the blanched, dried and rehydrated tissue (Fig. 16), many of the characteristics observed in the rehydrated carrots are found in the rehydrated beans as well. Here, too, we observe a striking dependence of the morphological appearance on the influence of the preceding food technological processing (see below).

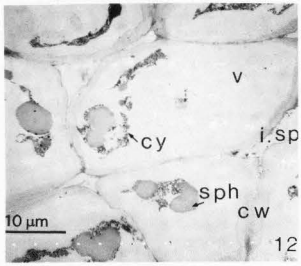
Morphometric measurements of cell wall swelling in processed vegetables

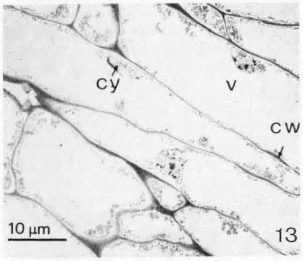
Since swelling of the cell walls seemed to be one of the most striking phenomena occurring in rehydrated tissues (see above), we carried out systematic measurements of cell wall thicknesses in rehydrated carrots and green beans in order to

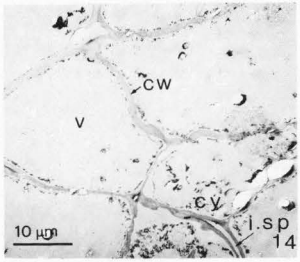












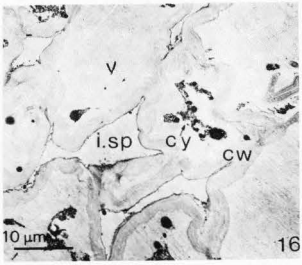


Fig. 9 – 12: TEM micrographs of processed carrot phloem parenchyma tissue

Fig. 9: Blanched tissue (v=vacuole, cy=cytoplasm, cw=cell wall, sph=spherosome,i.sp=intercellular space)

Fig. 10: Boiled tissue (v=vacuole, cw=cell wall, i.sp=intercellular space, sph=spherosome, cy=cytoplasm)

Fig. 11: Dried tissue (cw=cell wall, sph=spherosome, cy=cytoplasm)

Fig. 12: Rehydrated tissue (v=vacuole, cy=cytoplasm, i.sp=intercellular space, cw=cell wall, sph=spherosome)

Fig. 13 – 16: TEM micrographs of processed green bean pod tissue

Fig. 13: Blanched tissue (cy=cytoplasm, v=vacuole, cw=cell wall)

Fig. 14: Boiled tissue (v=vacuole, cw=cell wall, cy=cytoplasm, i.sp=intercellular space)

Fig. 15: Dried tissue (cw=cell wall, cy=cytoplasm)

Fig. 16: Rehydrated tissue (v=vacuole, cy=cytoplasm, i.sp=intercellular space, cw= cell wall)

Table 1. Influence of different blanching periods upon the degree of cell wall swelling in rehydrated green beans (var. "Cascade")

Sample No.	Blanching time min	Cell wall swelling %
B1	3	135
B2	6	167
B3	9	190
B4	12	192

Table 2. Influence of different blanching periods upon the degree of cell wall swelling in rehydrated carrots (var. "Zino")

Sample No.	Blanching time min	Cell wall swelling %
C5	6	99
C6	10	119

Table 3. Influence of blanching in different blanching media upon cell wall swelling in carrots (var. "Bauer's Kieler Rote") after drying and rehydration

Sample No.	Blanching time min	Blanching medium	Cell wall swelling %
C1	2	H_2O	212
C2	5	H_2O+3%NaCl	287
C3	10	H_2O	244
C4	10	H_2O+5%NaCl	293

Table 4. Influence of the relative humidity of the drying air upon cell wall swelling in rehydrated green beans (var. "Koralle")

Sample No.	Blanching time min	Relative humidity %	Cell wall swelling %
B5	6	21	189
B6	6	3	125

Table 5. Influence of the residual water content of dried carrots (var. "Bauer's Kieler Rote") upon cell wall swelling after rehydration

Sample No.	Residual water content %	Cell wall swelling %
C7	6	122
C8	13	200
C9	18	173
C10	22	184
C11	6	126

Table 6. Influence of the drying temperature upon cell wall swelling in rehydrated green beans (var. "Cascade")

Sample No.	Blanching time min	Drying temperature °C	Cell wall swelling %
B7	6	50	160
B2	6	70	167
B8	6	90	178

Table 7. Influence of the drying temperature upon cell wall swelling in rehydrated carrots (var. "Zino")

Sample No.	Blanching time min	Drying temperature °C	Cell wall swelling %
C12	6	70	99
C13	6	90	90
C14	10	80	104
C15	10	90	119

Table 8. Influence of the storing temperature upon cell wall swelling in carrots (var. "Bauer's Kieler Rote") after rehydration

Sample No.	Storing temperature °C	Cell wall swelling %
C16	4	217
C17	20	273
C18	4	198
C19	20	261

more closely analyze the influence of some process parameters. All samples were blanched before drying and rehydration (The rehydration periods are not shown in the tables).

Influence of different blanching times. Blanching in water for different periods led to different degrees of cell wall swelling after rehydration (Tables 1 and 2). The values in these tables show that cell wall swelling in the rehydrated tissue is correlated with different blanching times: the longer the blanching time, the greater the cell wall swelling. Different rehydration periods do not seem to influence much the alterations caused by different blanching periods. This means that samples with short blanching periods which require long rehydration times for reaching the point of doneness do not show the same morphological appearance (expressed by the degree of cell wall swelling) as those with long blanching times and – consequently – shorter rehydration periods.

Influence of the addition of NaCl to the blanching medium. Blanching with variable concentrations of NaCl led to a general increase in cell wall thickness and maceration of the tissue in the rehydrated carrots as compared with blanching in pure water (Table 3). It is remarkable that the addition of 3% NaCl causes almost the same amount of cell wall swelling in 5 min as the addition of 5% in 10 min. Blanching with NaCl reduced the cooking times of the samples considerably.

Dehydration in dried air. If the relative humidity of the drying air was reduced to 3%, the samples exhibited less cell wall swelling than parallel samples dried in air with a relative humidity of 21% (Table 4). Lowering the relative humidity also led to a reduction of the cooking times and a better conservation of chlorophyll.

Residual water content of the dried vegetables. As Table 5 shows, the morphological structure of the rehydrated carrots is adversely affected by a comparatively high water content (> 10%) of the dried product. Reducing the residual water content to 6% by prolonged drying reduces cell wall swelling considerably. There were no significant differences with regard to the cooking times.

Drying temperature. If the blanching time is kept constant, whereas the drying temperature is varied, morphological differences in the rehydrated sample were observed (Tables 6 and 7). As the figures from Table 6 show, in green beans less cell wall swelling was observed at low drying temperatures. In carrots, however, (Table 7), this is only true for samples blanched for 10 min. The short blanching time (6 min) gives better results with the high drying temperature. In green beans, drying at 90° C reduced the cooking time considerably. Reduction of cooking time was also observed in carrots after blanching for 6 min and drying at 90° C. Carrots after blanching for 10 min, however, showed shorter cooking times at low temperatures.

Influence of storing. Dried carrots were stored at different temperatures (4° C, 20° C) for 5 weeks. As the figures in Table 8 show there is less cell wall swelling in rehydrated samples after storing the dried vegetables at the lower temperature. Apart from a general prolongation of the cooking time due to the storing process itself, no difference between the two storing temperatures as regards the cooking time could be observed. However, there was a tendency to a better conservation of pigments in carrots stored at 4° C.

Discussion

The SEM and TEM micrographs show that fresh carrot root parenchyma is a typical plant storage parenchyma, characterized by a high starch content and numerous lipid droplets. Green bean pod parenchyma, on the other hand, represents another type of plant tissue, i.e. that of assimilating tissue although the relatively high starch content within the chloroplasts and the occurrence of intermediate forms between typical chloroplasts and typical amyloplasts point out that it has storage functions as well.

As expected, structural changes involved in the processing of both vegetables affect cytoplasm and cell wall in a specific way.

Cytoplasmic changes are expressed by a variable degree of clumping and by translocation of cytoplasmic substance from its marginal site into the vacuole space.

After being transformed into a fine (blanched) or coarse (boiled) granular substance the cytoplasm keeps its place even in boiled tissue. The strong clumping and translocation of cytoplasmic substance into the cell lumen which can be observed in dried specimens is in general not compensated by water absorption during rehydration. So within the cytoplasm, dehydration seems to bring with it physical and chemical changes that can only partially be made reversible during rehydration.

As far as the cell wall is concerned two aspects seem important: (1) the degree of swelling and (2) the separation of the single cells along the middle lamellae.

As the electron micrographs of raw, blanched and boiled tissues show, the effects of moisture and heat lead to a swelling of the walls and to a beginning of separation of the individual cells. This "thermal maceration" has long been observed in prepared tissues by optical microscopists, and has been attributed to the extraction of protopectin from the middle lamella.

In the present results, however, it is remarkable that not only short blanching, but also boiling to "doneness" preserves the histological structure of the tissue so well. Apart from moderate cell wall swelling, only occasional cell separation and genuine "maceration" take place.

Like Davis and Gordon (3) we found no submicroscopic evidence that any rupture of cell walls ("holes") occurs during cooking or dehydration and rehydration, as some authors reported (23, 24). This would indicate a degradation of the cellulose skeletal network. The observed swelling of the cell walls, which is accompanied by a loss of electron density, surely indicates a loosening of the cellulose fibril network. If this loosening is caused by a breakdown of matrix material (pectin, hemicelluloses, etc.) and/or by a loosening of chemical bonds between the cellulose fibrils or by some other factor must be decided by cytochemical analysis.

The observed shrinkage of dehydrated cell is compensated by water absorption during rehydration by boiling. Rehydrated samples often show more voluminous cell walls than boiled raw tissue. As other experiments demonstrated, this swelling may lead to cell walls being five to ten times thicker than those in raw tissue. In most cases these tissue samples also exhibited a higher degree of maceration. It is obvious that such enormous structural deviations from the appearance of the boiled raw tissue must be regarded as undesirable from the morphologist's

point of view.

Another structural alteration occurring during dehydration which was observed by Sterling and Shimazu (27) is the increase in cellulose crystallinity in dehydrated samples. According to these authors this phenomenon may account for the often observed toughness of rehydrated vegetables and the prolongation of their cooking times, because a high amount of crystalline cellulose would prevent the cell wall from taking up water during swelling.

The present results clearly demonstrated the tendency of an increased swelling of cell walls during dehydration/rehydration so that cellulose crystallinity might seem to be rather decreased.

The results of our morphometrical measurements of cell wall swelling in processed vegetables show that in the production of dried food the process parameters may exert a decisive influence on the ultrastructural appearance of the rehydrated product.

Some of these parameters (short blanching without NaCl, low relative humidity of the drying air, low residual water content of the dried product and storage at low temperatures) produced samples that after rehydration showed a morphological status more like that of the boiled raw tissue than samples treated in the opposite way. Whereas the influence of blanching upon cell wall swelling was remarkably distinct, even in the rehydrated product, the choice of the drying temperature did not seem to be very critical within the above limits.

In some cases (e.g. relative humidity of the drying air) favourable morphological and favourable food technology results (e.g. short cooking times) agreed. In other cases (most prominent: long blanching times) a discrepancy was observed between the morphologist's claim for a good preservation of structural features and the food technologist's claim for short cooking times.

Some parameters (e.g. storage temperature) did not have any influence upon the cooking times of the dried samples, whereas – from the morphological point of view – distinct differences in cell wall swelling were observed. There was, however, a better conservation of pigments in carrots at low storage temperatures.

Conclusion

Although the present investigation answers some questions dealing with the morphological consequences of food technological procedures, the goal pursued by both the morphologist and the food technologist ("The rehydrated product should be similar in all aspects to the boiled raw tissue") has not yet been fully reached. For the food scientist this means further improvement of process engineering in close correlation with morphological investigations.

Beside cell wall swelling, these future morphological studies should examine further characteristics of cell wall alterations, e.g. changes in the degree of crystallinity of the cellulose fibres (Sterling and Shimazu (27)),which seems to be quite significant for a deeper understanding of the process involved in the drying and rehydration of plant tissues.

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Discussion with Reviewers

S. Jones: One of your basic assumptions is that the dried, rehydrated product should resemble as closely as possible the fresh cooked vegetable with respect to morphology of the cell wall. Has this assumption been tested?

Authors: In most cases foodstuffs during preservation (drying, canning, freezing) lose some of their qualities which they had in the fresh or fresh cooked state . In this sense preservation can be regarded as a kind of expedient, which is dictated by seasonal or commercial needs. The aim of food scientists is to keep these negative influences of preservation as minimal as possible as regards taste, consistency and chemical composition of the preserved food. Our morphological investigations must be regarded as part of these efforts in minimizing deviations from the original material which occur during preservation. In this sense the morphology of fresh or freshly cooked, i.e., unpreserved and unaltered tissue, seems to be the reasonable standard for estimating the influences of processing conditions.

S. Jones: What are the processing parameters?

Authors: Detailed information of the processing parameters are given in literature references 1,2.

S. Jones: How reproducible are your swelling data, given that a subjective estimation of doneness is (probably) used? Are your data from single or multiple runs? What are the standard errors?

Authors: Since sensory as well as shearing tests were used for the estimation of doneness, the reproducibility of our morphometric results must be considered as good. Our data are from multiple runs extending over a total period of three years. This means that the influences of three different annual growth conditions on the structure of the raw material are included. Nevertheless, standard deviations ranged only between 10 – 15 %.

S. Jones: Are all the dried, rehydrated samples first blanched? What is blanching, specifically? If the vegetable is dried and rehydrated without blanching, is the only effect a longer cooking time?

Authors: All samples were blanched before drying, only blanching periods and blanching media were varied (s. Tables 1 – 3). Drying of vegetables without previous blanching is – from the view of food conservation – undesirable, because blanching inhibits enzyme activity and therefore leads to a better conservation of the colour, taste, and chemical composition of vegetables. We did not check whether drying without previous blanching leads to a prolongation of the cooking time although this might well be the case.

K. Saio: Many starch grains are observed in fresh green bean tissue (Fig. 8), but not in processed green bean tissues (Figs. 13 to 16). The authors do not refer to the changes in starch grains during processing. It is also considerable in carrot tissue, although the number of starch grains are fewer than in green beans. Please explain.

Authors: Micrograph No. 8 was taken from the outer part of the green bean pod (see text) which is characterized by a high starch content within the cells. All micrographs of processed green beans were taken from the inner part where there are only few amyloplasts occurring in the cytoplasm. We did not particularly focus on the fate of starch grains during processing in this study since the degradation of starch seems to be a complex phenomenon which surely requires separate investigations. But as far as we noticed there did not seem to be big differences between carrots and green beans with regard to these organelles.

K. Saio: To resolve the "hard-to-cook" phenomenon of dried beans, soaking in salt solutions prior to cooking (quick-cooking) have been reported by many researches (Rockland and Jones. J. Food Sci. 39, 342, 1974, Sefa-Dedeh et al., J. Food Sci., 43, 1832, 1978, Jackson and Variano-Mortston, J. Food Sci., 46, 799, 1981 etc.). They pointed out that the quick-cooking was rendered by the mechanism of ion exchange and possibly by chelation, as the middle lamella which cements the individual cells together consists of a calcium salt of polymers of galacturonic acid. The cell wall swelling in these experiments seems to be essentially the same as the phenomenon above, in view of the acceleration of swelling with NaCl. The TEM micrographs show that after processing the microstructure is largely unchanged

except for enlarged intercellular spaces and lower density. But, what happens to the actual texture of the vegetables on processing? Aren't the lowering of the crispness and hardness dependent on the treatments? And how is the degree of cell wall swelling related to such textural properties?

Authors: We agree with you that cell wall swelling and maceration of tissue during processing is surely connected with reactions taking place within the pectin/protopectin component of the cell wall, especially the middle lamella. Dissolution of pectin/protopectin is not only promoted by salt addition, but also by the action of hot water which might explain the swelling and maceration of cell wall material in the experiments without the addition of salts. We suppose that the lowering of the density of cell walls which is visible in our TEM micrographs is at least partially due to the extraction of matrix material such as pectin resulting in a loosening and broadening of the remaining cellulose fibre network. The actual composition and texture of the walls (e.g. crystallinity of cellulose fibres) cannot be elucidated by purely morphological methods, but their analysis seems to be the next step towards a deepened understanding of the effects of processing.