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Soluble Proteins in Developing Peach Mesocarp

By

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With 1 Figure

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Summary

GALLESCHI L., REPICCIOLI R. & SCHIANO E. 1992. Soluble proteins in developing peach mesocarp. – Phyton (Horn, Austria) 32 (2): 201–207, 1 figure – English with German summary.

The changes in protein content and the pattern of soluble proteins during peach mesocarp development were determined. Protein content was high in early maturation, it later decreased rapidly and then slowly, it subsequently increased again and decreased during ripening. SDS-PAGE of soluble proteins showed a complex pattern characterized by the appearance, disappearance and quantitative changes of protein bands. A comparision of these results with the proteolytic activity, which had previously been determined, indicates that proteogenesis and proteolysis may substantially contribute to fruit development.

Zusammenfassung

GALLESCHI L., REPICCIOLI R. & SCHIANO E. 1992. Lösliche Proteine im Pfirsichmesocarp während seiner Entwicklung. – Phyton (Horn, Austrian) 32 (2): 201–207, 1 Abbildung – Englisch mit deutscher Zusammenfassung.

Es werden die Veränderungen im Proteingehalt und die Muster löslicher Proteine während der Entwicklung des Pfirsichmesocarps untersucht. Der Proteingehalt ist hoch während des frühen Reifestadiums, später nimmt er sehr stark und dann langsamer ab, steigt neuerlich etwas an und nimmt wieder während der Reifung ab. SDS-PAGE von löslichen Proteinen zeigt ein sehr komplexes Muster, welches durch das Auftreten, das Verschwinden und quantitative Veränderungen in den Proteinbanden charakterisiert wird. Ein Vergleich dieser Ergebnisse mit der proteolytischen Aktivität, welche bereits früher untersucht wurde, deutet darauf hin, daß Proteinsynthese und Proteolyse wesentlich zur Fruchtentwicklung beitragen.

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Introduction

The development of fleshy fruits is a complex process of growth, which produces several morphological and biochemical changes in the developing fruit tissues. These events are responsible for the acquisition of the edible characteristics of the majority of fruits, and culminate in ripeness. While there have been many studies in ripe fruits (LILL & al. 1989), few papers have been published on fruit maturation (COOMBE 1976).

Proteins are the least characterized compounds in fruits, both by the presence of high concentrations of phenols and co-extracted pectins, which can make proteins insoluble or trap them during the extraction procedures. Bearing this in mind, we have developed a method which can extract the proteins, by minimizing the problems of the interfering substances.

In a previous paper (GALLESCHI & al. 1991) we preliminarly characterized some protease activities during peach mesocarp development. Here, we report the changes in soluble proteins during the development of peach mesocarp.

Materials and Methods

Plant material

Developing fruits of *Prunus persica* Batsch (c.v. Redhaven) were collected and treated as reported in GALLESCHI & al. (1991).

Protein extraction

Lyophilized mesocarp (1.5 g) was resuspended in 15 ml of phosphate buffer, 0.2 M pH 7.0 containing 5 mM diethyldithiocarbamic acid, 0.1 M ascorbate, 1 mM EDTA, 0.5 mM phenylmethanesulfonyl fluoride in the presence of Polyclar AT (0.9 g). The mixture was stirred at 4° C for 30 min, then centrifuged at 35,000 g (4° C, 10 min), the supernatant was then collected. The extraction was repeated and the two supernatants were pooled. One half was then precipitated with ammonium sulphate (80%), dialyzed against distilled water (4° C, overnight) and used, after lyophilization, for SDS-PAGE. The remaining supernatant was utilized for protein content determination.

Protein determination

The BENSADOUN & WEINSTEIN (1976) method was used.

Gel electrophoresis

This was performed by utilizing 12% polyacrylamide gel slabs. The samples were resuspended in 0.0625 M tris-HCl buffer (pH 6.8) containing 2% SDS and 5% 2-mercaptoethanol and boiled for 3 min at 100° C. The amount of protein applied for each sample onto the gel was 80 µg. Low calibration kit proteins (Pharmacia) were utilized for molecular weight (M_r) determinations. The electrophoresis was performed at 20° C in a tris-HCl running buffer pH 8.3 containing 1% SDS and by applying 35 mA per slab. The gels were stained with 0.2% Coomassie and destained with acetic acid/isopropanol/water 7.5/20/72.5. The optical density of the electrophoretic bands was measured with an UltroScan XL (Pharmacia). The instrument was equipped with GelScan software which automatically determined the M_{rs} of the protein bands and gave a simultaneous comparison of the readings.

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Results

Changes in proteins

Table 1 summarizes the variations in soluble proteins during the development of *Prunus persica* mesocarp. The protein content of this tissue was high in the early developmental phase (49 days after flowering, DAF), then decreased rapidly by about five times and then slowly at 100 DAF. In the final stages, the protein concentration increased (113 DAF) and decreased notably during the ripening on the tree (122 DAF), reaching the levels which are characteristic of the ripe fruit.

Table 1

Soluble proteins during the course of mesocarp development. The values of protein content are the averages of two extractions and eight determinations for each extraction. The values are expressed as mean ± S. E.. n = 16.

Days after Flowering	Protein content (mg g^{-1} DW)
49	25.3 ± 0.6
65	5.4 ± 0.2
100	4.9 ± 0.2
113	6.3 ± 0.1
122	2.4 ± 0.1

SDS-PAGE

Figure 1 shows the electrophoretic patterns of soluble proteins extracted from peach mesocarp during its development. They were analysed emphasizing the appearance, the deletion and the persistence of the most abundant components during the course of maturation and ripening of *Prunus persica* mesocarp. The densitometric readings highlighted that the greatest qualitative changes are present at 49, 65, and 122 DAF, while the other developmental periods only showed quantitative variations. For this reason and for an easier interpretation of the results, in Fig. 1 we only show the densitometric readings which refer to the developmental stages reported above. Most of the proteins which appear during the development are absent in early maturation, but by 65 DAF they can be detected. They persist, with some quantitative variations, throughout the remaining developmental period. These had M_{rs} 30.0, 31.4, 40.3, 63.0 and 67.4 kDa. Only one component, M_r 74.8 kDa, appears during ripening (122 DAF) but is not present in other stages.

Another set of proteins disappears during the development in a different way. One band, with the highest M_r (86.3 kDa) of those detected by densitometric readings, is present precociously (49 DAF) and disappears

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later. However, most of the deleted proteins are already present early (49 DAF) and disappear during the ripening period. These have M_{rs} 17.5, 29.0, 35.7, 36.9 and 51.3 kDa.

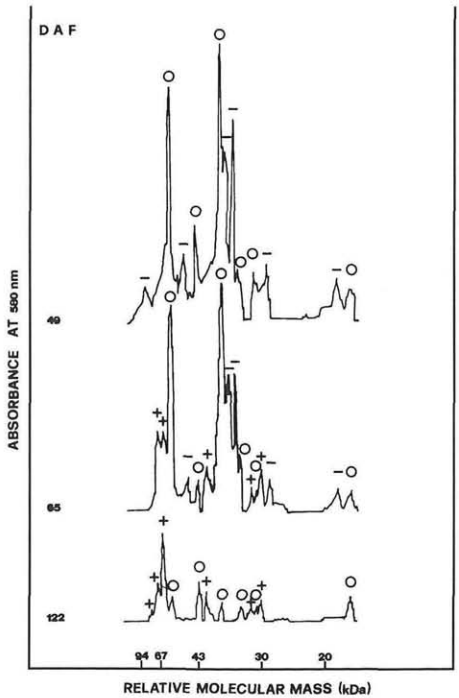

Fig. 1. Electrophoretic analysis of peach mesocarp proteins. (+), peak appearance; (–), peak deletion; (o), peak persistence.

Finally, some proteins persisted during overall development. There were two components with M_{rs} 60.5 and 37.8 kDa respectively, which were the most abundant among the proteins examined. Other minor components had M_{rs} 17.7, 31.0, 34.0 and 43.0 kDa.

Discussion

Our study considered fruits by 49 DAF, not at an earlier stage because of the difficulties encountered during the dissection of fruit pericarp, which could not be separated into its components at this point of the developmental process. An alternative could have been to use whole fruits during precocious maturation stages, but this, in our opinion, would have prevented a good comparison of the soluble proteins being made from

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different stages, because of the presence of the growing seed. In fact, this organ could contribute to masking the protein pattern of the mesocarp.

Studying proteins during the development of fruits is made difficult by the presence of high concentrations of interfering substances, particularly phenols and pectins, as already emphasized by other authors (CLEMENTS 1965, PECH & al. 1970). Several attempts were performed to obtain the extraction method which is reported in the Materials and Methods section, and which minimizes the problem of the interference by the above compounds. It also offers a valid protection against the proteolysis by co-extracted proteases, as reported in our previous studies (GALLESCHI & al. 1991).

Our results show a dramatic variation in the protein content during development. It is surprisingly high initially, decreases suddenly and then slowly, increases again and decreases deeply during the ripening on the tree. The densitometric analyses show corresponding variations in the M_r of some components. In particular, the most abundant bands (M_{rs} 37.8 and 60.5 kDa) in early development undergo a dramatic decrease in the following stages and are only just detectable during ripening. Likewise the change is also important in those components which are present precociously and disappear later, i. e. the heavy band with M_r 86.3 kDa, or of other ones which are present during the most of the development and disappear tardively, when ripening starts. Finally, the appearance of many protein bands mainly happens at the second stage and only for one component at the last stage. This pattern suggests that the deepest changes are manifested in early development (the first and second stages), or at the end of the maturation (the fifth stage). Unfortunately, similar studies have not been performed on other fruits and only one paper on fruit proteins is available in literature; however, examines the protein evolution of the pear in too short a period, i. e. the ripening (PECH & al. 1970).

Other studies performed during fruit ripening emphasize that proteogenesis must happen during this period, particularly early in the climateric (SACHER 1973). Probably the same process is also very important during maturation, but at the moment this is only a speculation. Our observations, which show an increase in protein content at the onset of ripening, can be explained with a protein synthesis, in a moment particularly important for the fruit, when the synthesis of the "ripening enzymes" starts, as demonstrated for many fruits (SACHER 1973, LILL & al. 1989). Among these enzymes, polygalacturonase activity is detectable when peach fruit begins to soften and increases during ripening. (PRESSEY & al. 1971); cellulase activity is absent in unripe peach fruits and increases when ripening starts, just before fruit firmness is significantly altered (HINTON & PRESSEY 1974). On the other hand pectin methylesterase activity has been found at all stages of peach fruit development and ripening (SHEWFELT

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1965). The increase in the protease activity detected just before ripening starts (100 DAF; GALLESCHI & al. 1991) could so substain the synthesis of polygalacturonase and cellulase by providing amino acids which have derived from the hydrolysis of fruit proteins. The protein component with M_r 74.8 kDa appearing in the ripe fruit could represent a "ripening enzyme", probably polygalacturonase which has a key-role in the ripening process (PRESSEY & AVANTS 1973). However, our analysis performed only on the ripe fruit and not during all phases of the ripening, and the absence in literature of detailed informations about the M_{rs} of the "ripening enzymes" in denatured state (PRESSEY & AVANTS 1973, HINTON & PRESSEY 1974) need further experimental work to support this hypothesis. After the synthesis of the "ripening enzymes", the senescence process of fruit mesocarp is irreversible and the protein content and the proteolytic activities decrease or disappear (GALLESCHI & al. 1991). Probably the other modifications of the protein pattern detected by SDS-PAGE during maturation are due to proteolysis and to proteogenesis, but more data on the localization of proteins and enzymes are needed.

In conclusion, our data suggest that during peach mesocarp development soluble proteins are subjected to many modifications, including deletion, appearance and quantitative variations of several components. These changes could be important for the synthesis and the modification of proteins during maturation and for the production of "ripening enzymes" during final stages of fruit development.

Acknowledgements

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Recensiones

MENGEL, K., PILBEAM, D. J., 1992. Nitrogen Metabolism of Plants. – Proceedings of the Phytochemical Society of Europe 33.

Pflanzen spielen die wesentliche Rolle bei der Umwandlung von anorganischem Stickstoff in organisch gebundenen. Der Ertrag landwirtschaftlich genutzter Flächen hängt aber darüber hinaus vom Ausmaß der Stickstoffdüngung ab. Heute meint man, daß ohne Düngung mit Stickstoff die Weltbevölkerung nicht mehr ernährt werden kann.

Das vorliegende Buch ist in 16 Beiträge verschiedener Autoren gegliedert, dabei werden die neuen Ergebnisse aus dem Gebiet der Physiologie, der Biochemie und der Molekularbiologie präsentiert. Der Bogen spannt sich von der landwirtschaftlichen Produktivität bis zur Pathogenabwehr, der gemeinsame Nenner ist der Stickstoff.

Neues über die Knöllchenentwicklung und die Fixierung von Stickstoff im Rhizobium/Bradyrhizobium-System nimmt breiten Raum ein. Die Beziehung zwischen der Synthese organischer Säuren und der Assimilation von Stickstoff in der Pflanze wird auch in Zukunft bearbeitet werden. Für die Untersuchung der Assimilation von NO_3 scheint das Wurzelsystem von Zea mays besonders geeignet. Der Transport von Ammonium- und Nitrationen durch pflanzliche Membranen wird durch einen Regelkreis gesteuert, in den Nitrat- und Nitratreduktion einbezogen ist. Die Untersuchung des Transportes von Aminosäuren durch die Plasmamembran wird an Protoplasten oder Zellsuspensionen durchgeführt.

Die Synthese von Speicherproteinen im Samen von Getreide nimmt wegen seiner Bedeutung für die Ernährung auch von Mensch und Tier breiten Raum ein; die Gentechnik arbeitet auf diesem Gebiet. Ein Kapitel über stickstoffhaltige Inhibitoren von Glykosidasen aus Pflanzen, Bakterien und Pilzen, die auch antivirale oder insektizide Eigenschaften besitzen, beschließt das Buch.

Es ist mit einem Sachregister und einem Register der erwähnten Organismen ausgestattet. Der am Stickstoffhaushalt interessierte Leser wird mit diesem Buch auf den neuen Wissensstand gebracht.

M. GAILHOFER

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