CARBONIZATION OF PEAS AND WHEAT – A WINDOW INTO THE PAST

A laboratory study

Freek Braadbaart

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PROEFSCHRIFT

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van de Rector Magnificus Dr. D.D. Breimer,

hoogleraar in de faculteit der Wiskunde en

Natuurwetenschappen en die der Geneeskunde,

volgens besluit van het College van Promoties

te verdedigen op donderdag 21 oktober 2004

klokke 15.15 uur

door

Frederik Braadbaart

geboren te Oisterwijk

in 1935

Promotiecommissie:

Promotoren	: Prof. Dr. C.C. Bakels Prof. Dr. J.J. Boon, FOM Instituut, Amsterdam
Co-promoter	: Dr. P.F. van Bergen, Faculteit der Geowetenschappen – Geochemie, Universiteit Utrecht
Referent	: Prof. Dr. J. Lugtenburg
Overige leden	: Prof. Dr. M.E.R.G.N. Jansen Dr. J.A. Mol Dr. P. van de Velde

Graphic design: Suzan Breedveld

Printing: drukkerij Gravé, Heemstede

ISBN: 90-808061-0-2

Seeds are the germ of live,
a beginning and an end,
the fruit of yesterday's harvest
and the promise of to-morrow's.

Orvill L. Freeman, 1961

Dedicated
to
Mieke

CONTENTS

Introduction

CHAPTER 1

ARCHAEOLOGICAL RESEARCH AND THE ROLE OF NATURAL SCIENCES

In the world of science three branches of learning are distinguished: humanities, social sciences and natural sciences. Archaeology is principally concerned with the past of humankind and as a branch of history can be considered part of the humanities (Renfrew, 1996). This discipline studies past societies primarily through their material remains – the buildings, tools, plant remains, and other artefacts that constitute what is known as the material culture left over from former societies. From these material remains the archaeologist has to reconstruct the past, for which he collects data, conducts experiments, formulates and tests hypotheses, etc. Many archaeologists are primarily interested in what these material remains can tell us about human behaviour in the distant past. Therefore archaeology could also be considered a social science. Finally, whereas not many would subscribe to the view that archaeology can be considered as part of the field of the natural sciences, the latter's contributions to archaeology are significant and increasing. This is illustrated by the dating techniques, which have become daily routine (Renfrew, 1996) or the increasing successes of molecular biology in recovering DNA from ancient materials (Brown, 1999). Thus, it is difficult to deny the importance of the natural sciences for the development of archaeology.

These observations call into question whether the division into the three branches of learning is still appropriate. Nowadays each branch of learning is linked to the other branches. How does this affect archaeological research? It might result in archaeologists becoming strict adherents to the natural sciences and accepting anything from their specialists. Alternatively, there may be those who distrust the applications and inferences of the natural scientists while unable to refute them. For fruitful interdisciplinary research it is therefore necessary that archaeologists have sufficient basic training in the natural sciences.

In most cases the remains left over from past societies have been hidden in the soil for centuries before being excavated by archaeologists. The properties of the remains themselves, the influence of the biological, physical and chemical conditions of the environment in which the remains have been hidden or the changes of these conditions during the long time between the deposition in the soil and the excavation may have all affected the remains. Thus the inferences from these remains about human behaviour in the past may be biased. The changes are usually the result of biological, physical or chemical processes and to obtain further insight into these effects basic knowledge of the natural sciences is pivotal. This thesis aims to be an example of the importance of interdisciplinary research for the archaeology and reveals that without using knowledge acquired from the natural sciences these problems cannot be solved.

CARBONIZED PROPAGULES FROM THE ARCHAEOLOGICAL RECORD

The material remains of the archaeological record comprise many items such as buildings and tools. An important part of the remains are botanical remains. Normally microorganisms degrade botanical remains exposed over time to the environment (Kirk and Cowling, 1984; Miksicek, 1987; Atlas and Bartha, 1993). In addition to decay by fungi and bacteria, physical and chemical processes deteriorate the remains. However, a number of conditions exist by which these remains survive the biological, physical and chemical attack. This may be the case in environments with extreme dry, extreme cold or waterlogged conditions. Another means of conservation is a change in the chemical or molecular properties of the remains impeding the microbial colonization or decay. The changes of these properties as a result of carbonization may allow the remains to survive the decay process. Impressions in pottery are another, but rather different, means to obtain evidence on botanical remains in the archaeological record.

Botanical evidence in the archaeological record is subdivided into three general categories i.e. macroremains, microremains and chemical evidence (Ford, 1979). This study involves the macroremains, which comprise those specimens visible to the unaided eye or requiring no more than low-power magnification for identification and taxonomic determination of the plant part.

The ability to understand the use of plants in the past through the analysis of retrieved macrobotanical remains from the archaeological record is strongly influenced by the accurate evaluation of the many depositional and post-depositional processes that transform these remains. Only then, can we begin to infer their use. Transformation processes may have degraded and distorted the macrobotanical remains morphologically or may introduce patterning of their own, may mask or exaggerate patterns in plant resource exploitation or even suggest change where none occurred (Binford, 1978). Consequently, it is of great importance to know how these remains acquired the characteristics witnessed today. To understand the significance of plants remains it is imperative to understand the past events that have affected the surviving remains.

Schiffer, a leading proponent of these investigations, has attempted to model the various processes that transform archaeological remains (Schiffer, 1972). He distinguishes between what he terms ‘c’-transforms and ‘n’-transforms, or cultural and non-cultural site formation processes, respectively. ‘C’-transforms comprise the human beliefs and actions associated with procurement, use and discard of tangible items. ‘N’-transforms comprise a wide range of non-cultural or natural agents of modification, attrition and transport. The laws of nature usually govern the latter domain. Transformations, according to Schiffer (1972, 1987), are the transition

from post-behaviours of interest to their surviving traces. To conceptualize these transformations, Schiffer makes a useful distinction between the systemic context and the archaeological context. The systemic context refers to the condition of elements such as foods, tools, fuels, etc. when they were an interactive part of an ongoing behavioural system and therefore concerns only 'c'-transforms. In contrast, the archaeological context describes those elements that have passed through a cultural system and have been affected by post-depositional processes before becoming an object of investigation by archaeologists. A simple flow model for wheat grains illustrates the movement of such an element through both contexts (Fig. 1.1). The last of the transforms of botanical remains in the systemic context results in the refuse. Refuse is divided into three categories: de facto, primary and secondary refuse (Rathje and Schiffer, 1982). These are defined as usable material abandoned at the location of use, trash discarded at the location of use and trash deposited at some other location, respectively. Subsequently, these grains may enter into the archaeological record. In the latter context post-depositional processes and a second series of cultural transforms will affect the botanical material. This series includes recovery techniques and analytical and theoretical processes that archaeologists may introduce. Thus, the transformation processes, cultural and non-cultural, create the evidence of past societies and environments that remain for the archaeobotanists and paleoethnobotanists to study. Emphasis is placed on understanding the social and behavioural character of the society that used the macrobotanical remains. The systemic contexts are understood only through inference, and the importance to identify and to take into account the transformation processes cannot be underestimated. For this purpose it will be necessary to understand the effects of the transformation processes on the botanical remains after their deposition in the archaeological record. This is only possible when the physical, chemical and molecular properties of the botanical material are known in the last phase of the systemic context and prior to their deposition.

This thesis is focussing on this last phase and more precisely on the carbonization process of the plant remains (grey area in Fig. 1.1), because macrobotanical assemblages frequently contain carbonized materials (Bakels, 1984; Miksicek, 1987). Plant parts exposed to heat under anoxic conditions at atmospheric pressure undergo a process that is frequently called carbonization, which implies an increase of the relative amount of carbon as a function of increasing temperature. Among the carbonized botanical materials, found in the archaeological record, propagules (i.e. seeds and fruits) are omnipresent. Apparently favourable conditions exist for the carbonization of propagules. The effects of the formation processes on carbonized propagules are best studied by the establishment of the physical, chemical and molecular properties of modern counterparts of the archaeological propagules after their carbonization. The following two species were selected for this study: wheat grains *(Triticum* L.) and seeds of peas *(Pisum sativum* L.). Both propagules belong to mono- and dicotyledons, respectively and are retrieved from the archaeological record.

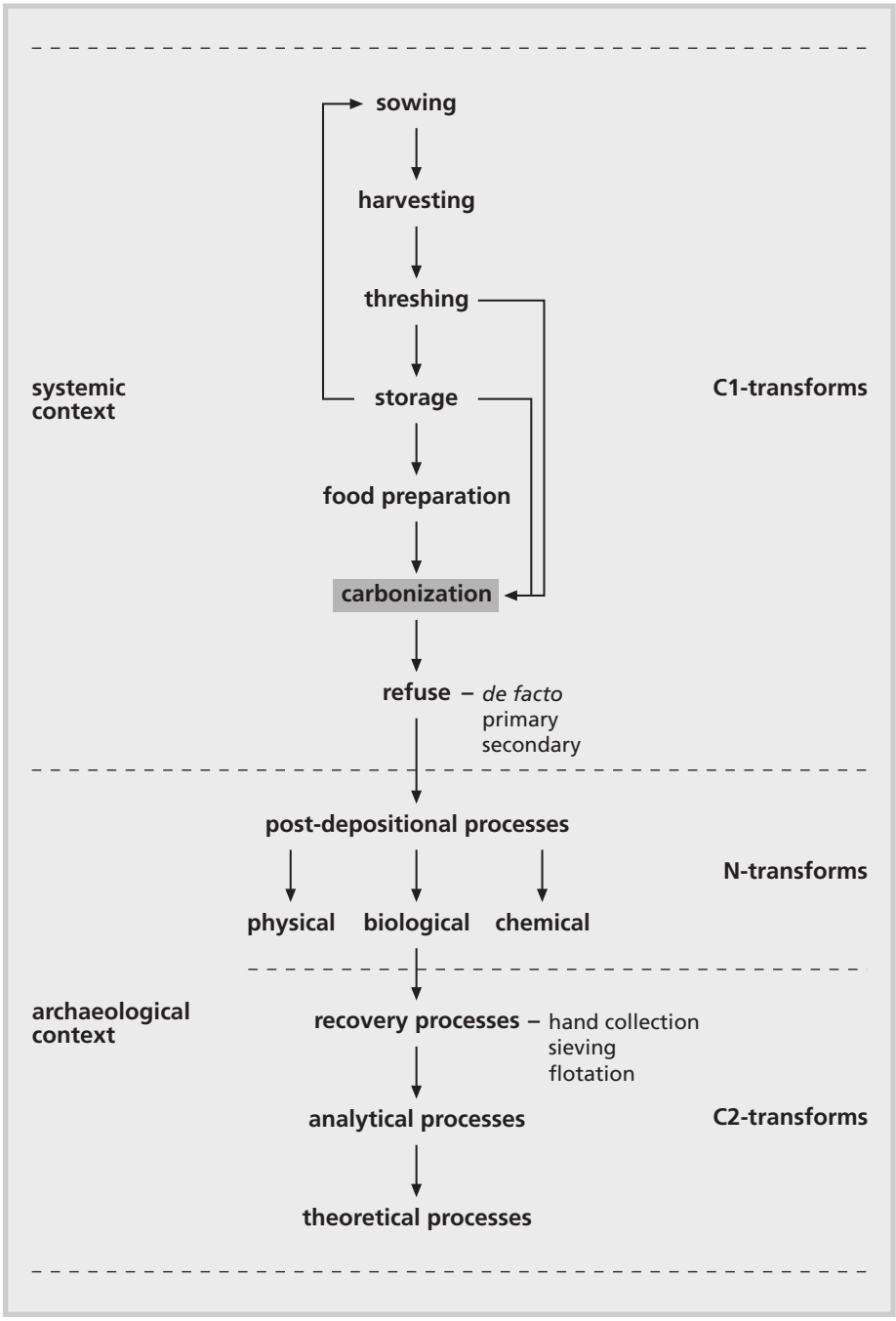

fig. 1.1
A simple flow model for viewing the live cycle of propagules and the processes that transfer them from one state to the next one (based on Schiffer, 1972). The subject of this study is shown in the grey area.

CURRENT STATE OF KNOWLEDGE OF THE SIMULATION OF THE CARBONIZATION PROCESS

Carbonized peas and wheat grains are the result of a series of reactions that affected their principal constituent components, e.g. starch and protein, when exposed to heat in the complete absence or with a limited supply of air (Shafizadeh and McGinnes, 1971). This complex of processes is also known under different names such as thermal decomposition, thermo-chemical degradation or pyrolysis (Bridgwater and Bridge, 1991; Helsen, 2000). Another term for these processes used in archaeological research is charring, derived from the production of charcoal from wood. Apparently many terms are used to describe the same process, viz. heat induced transformations of biomass. In other fields of research variations in the definition of carbonization have also become common place (Bridgwater and Bridge, 1991; Connor et al., 1994; Speight, 1994). In this thesis the term carbonization, which is widely used in archaeology, refers in general terms to black macrobotanical remains. If the conditions under which the heat treatment are emphasized, as in experiments in the laboratory, it is preferred to describe the process as exposure to heat or heating with an indication of the appropriate conditions such as rate of heat transfer, time of exposure to heat, absence or presence of air, temperature, etc.

To simulate the carbonization process propagules are usually heated in muffle furnaces (Helbaek, 1952; Hopf, 1955; Helbaek, 1970; Körber-Grohne and Piening, 1980; Wilson, 1984; Kislev and Rosenzweig, 1989; Boardman and Jones, 1990; Smith and Jones, 1990; Wright, 1998 and references therein). The conditions in these types of furnaces are not anoxic and various methods have been used to prevent the supply of air to the propagules. Mostly the propagules were placed in crucibles or other containers covered by sand in different ways and situated at a non-specified place in the furnace. The temperature of the oven and the time of exposure differed between experiments and the temperature of the propagules was not recorded.

The research on the physical properties of propagules carbonized by the described methods has focused mainly on the measurement of outside dimensions of peas and wheat grains. After removal from the furnace different classification systems were used to describe the degree of distortion and the dimensional changes. In some studies the weight loss of the samples as a function of the furnace temperature and the time of exposure are reported (Kislev and Rosenzweig, 1989; Wright, 1998). The use of microscopy and more in particular the use of scanning electron microscopy (SEM) is limited to the investigation of the internal and external

changes in anatomy and morphology in relation to the identification of different species of wheat (Körber-Grohne and Piening, 1980; Jacomet, 1987; Hillman, et al., 1993, 1996).

The chemical and molecular properties of carbonized propagules have been restricted to the discussion about the possibilities for practical applications. Infrared spectrometry (IR) and pyrolysis mass spectrometry (PyMS) have been carried out as potential tools for the identification of carbonized legumes and wheat grains retrieved from the archaeological record, but only preliminary results are given (Hillman et al., 1993). A resemblance to the chemical and molecular properties of charcoal and coal has been suggested, but only a short review of the relevant literature is given (Kislev and Rosenzweig, 1989). The literature does not provide comprehensive studies of the changes of the various properties of propagules as a result of carbonization and consequently, the processes that govern these changes are not fully understood. The different heating conditions, moreover, make it difficult to reproduce the experiments, which are carried out in muffle ovens.

Evidently there is a paucity of knowledge regarding the processes that determine the actual properties of carbonized propagules and the changes of these properties, which depend on the thermal decomposition of the main chemical components of the propagules, in this case starch and protein (Shafizadeh and McGinnes, 1971). For the purpose of this study the decomposition of starch can be compared to that of cellulose c.f. van der Kaaden et al. (1983). Heat treatment experiments using cellulose have been reported widely (For a review see e.g. Antal and Varhegyi, 1995). The processes that govern the carbonization of microcrystalline cellulose in an archaeological context have also been studied (Pastorova et al., 1993, 1994; Boon et al., 1994). The carbonization of proteins has received less attention.

This study is focussing on the investigation of the effects of the formation processes of carbonized propagules by the establishment of the physical, chemical and molecular properties of modern counterparts of those propagules that have been retrieved from the archaeological record. This implies that the modern counterparts of these propagules should be carbonized under controlled conditions in the laboratory whereby the conditions such as temperature, time of exposure, rate of the heat transfer, etc. can be adjusted and recorded. This will also make a reproduction of the experiments possible. As the conditions of the carbonization of the propagules retrieved from the archaeological record are not known and may vary widely a comprehensive set of experiments has to be carried out.

OBJECTIVES

Based on the description of the previous chapter the main objectives of the investigations are summarized.

1) To simulate the carbonization of whole modern peas and wheat grains under controlled conditions in a tube oven in the laboratory.

2) To assess the changes of the physical, chemical and molecular properties as a function of the temperature of the tube oven.

3) To characterize the processes that causes the carbonization and the subsequent changes.

The results will provide further insights into the processes that govern carbonization, which in turn will provide physical, chemical and molecular boundary conditions of carbonized propagules that in principle could enter the archaeological record. Finally the results of the experiments under controlled conditions will be compared with the results of experiments carried out under less controlled conditions such as an open fire, which is most likely the way how propagules retrieved from the archaeological record became carbonized.

THE OUTLINE OF THE THESIS

This thesis describes the carbonization of whole peas and wheat grains to study the changes of the physical, chemical and molecular properties under controlled conditions. Chapters 2, 3 and 5 describe the method of carbonization, the measurement of the properties and discussion of the processes that cause the changes of these properties. Samples of whole peas and wheat grains are heated in a tube oven under anoxic conditions at atmospheric pressure at temperatures ranging from 130 to 700 °C. The following properties of the samples are investigated as a function of temperature and time: total weight loss, internal and external morphology, vitrinite reflectance, elemental composition and molecular composition. In chapter 4 the selection of the variety of emmer wheat that is used for the experiments described in chapter 5 is discussed.

Peas and wheat grains are carbonized to study the changes of internal and external morphology as a result of heating. These changes, as a function of the temperature, are analyzed by digital imaging analysis. Three species of wheat and one species of peas are investigated. These analyses are described in chapter 6. Further experiments have been performed under less controlled conditions with respect to time of exposure and temperature to mimic the heating conditions in an archaeological context. Therefore, experiments are carried out with peas exposed for 60 minutes to different heat sources. For this purpose peas are heated in a pre-heated tube oven under anoxic conditions, on a pre-heated porcelain plate above a gas burner with a limited supply of air and on potsherds in an open fire. Additional experiments are carried out that investigate the influence of the time of exposure to a heat source. A description of these experiments and the discussion of the results is given in chapter 7. The main results and implications for archaeological research are presented in chapter 8.

CHAPTER 2

Laboratory simulations of the transformation of peas as a result of heat treatment: changes of the physical and chemical properties

ABSTRACT

The residues of heated organic remains, usually called carbonized or charred remains, are ubiquitous in the archaeological record and are often used to interpret certain aspects of ways of living in the past. This study focuses on the physical and chemical alterations, both as a function of temperature and time that occur when the transformation of a polysaccharide-rich biomass is simulated in the laboratory. Peas (*Pisum sativum* L.) are heated at temperatures ranging from 130-700 °C under anoxic conditions and atmospheric pressure, for up to two hours. Changes in weight and the relative percentages of C, N, H and O are determined alongside modifications of the internal and external morphology. Vitrinite reflectance provides an elegant tool to determine the heating temperature of the residues. The kinetics that determine the changes and modifications are discussed. The resulting solid products of the heating process can be divided into five stages, which fit the physical and chemical properties. The simulation provides a rigorous basis for a further study of the post-depositional processes, as applied in the archaeology, after the so-called "carbonization" stage.

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Published with minor adaptations in:
Journal of Archaeological Science 31 (2004) 821-833

INTRODUCTION

The most common mode of survival of archaeological plant remains is a process generally referred to as charring or carbonization, because the majority of the archaeological finds are the charred remains of wood, fruits and seeds (Van Zeist, 1970). Not all parts of a plant will survive the carbonization process, but fruits and seeds carbonize well and are often still recognizable (Boesewinkel, 1984). Generally they are morphologically well defined and thus samples of different batches can be compared while the natural variation in chemical composition is minimal. In particular, propagules of cereals, legumes, etc. are often found as carbonized entities in the archaeological record (Bakels, 1984). Previous studies on these kinds of fruits and seeds are usually restricted to the change of the dimensions as a result of carbonization (Boardman and Jones, 1990; Prior and Alvin, 1983; Smith and Jones, 1990). To date, only a few papers have addressed the change in the physical and chemical composition of fruits and seeds as a result of carbonization (Hillman et al., 1993; Kislev and Rosenzweig, 1989).

Carbonized fruits and seeds found in the archaeological record are the result of a series of processes called formation processes (Schiffer, 1983; 1987). These processes include carbonization, deposition, post-depositional alteration, excavation and analysis. Apart from the fact that carbonization is the result of heating under anoxic circumstances it is still not fully understood when and where fruits and seeds become carbonized and to what extent subsequent transformations take place. This study reports on the laboratory simulations of the transformation of recent seeds and determines the resulting physical and chemical properties.

In the different fields of thermal conversion of organic material variations in terminology have come into common use. Terms like thermal decomposition, pyrolysis and carbonization are often used interchangeably (Speight, 1994). In the context of the conversion of biomass, pyrolysis is described as thermal degradation, either in the complete absence of oxidizing agents, or with a limited supply. The pyrolysis technology has introduced terminology such as carbonization, conventional pyrolysis, fast and flash pyrolysis (Bridgwater and Bridge, 1991). The temperature range in the case of carbonization is 300-500 °C with a residence time of hours or days. In the field of coal technology pyrolysis is generally defined as the thermal decomposition of coal in the absence of air. Carbonization is applied to the production of char or coke when coal is heated at temperatures in excess of 500 °C (Speight, 1994). The term carbonization is also widely used in archaeology, but appears to refer to a black solid residue of biomass as the result of heat treatment, although the conditions under which the heat treatment took place are rarely mentioned. To date, no definitions exist for terms like char and charcoal, the solid products of conversion processes (Connor et al., 1994). In light of these

considerations the process of heating in the absence of oxygen in this paper will be called the heat treatment, and the resulting solid product, the residue.

Within the current project fruits or seeds of two species of plants are selected because of their occurrence in the archaeological record, their different chemical composition and because they include both mono- and dicotyledons. One dicotyledonous plant was chosen, namely the seeds of the pea (*Pisum sativum* L.), which contain mainly starch (56 %, by weight) and protein (24 %). The monocotyledonous plant, emmer wheat (*Triticum dicoccum* Schübl), was selected because of its high starch content (70 %) (Crocker and Barton, 1953). In the current study the results of the heat treatment on peas are presented. The seeds are composed of a seed coat (testa) enveloping two cotyledons that constitute the major portion of the seed.

A number of parameters can be directly obtained from propagules found in the archaeological record and exposed to heat. These include size and shape, morphology and anatomy, physical and chemical properties. With this in mind the objective of this study is the assessment of the changes of the physical and chemical composition of the seeds of the pea that occur during heat treatment under anoxic conditions at atmospheric pressure. The seeds are heated at temperatures ranging from 130 to 700 °C. The total weight loss, the changes of the internal and external morphology, the chemical composition and the vitrinite reflectance of the seeds are studied. Hereby the physical and chemical properties of the solid residues of the seeds will be determined, as a function of heating temperature. The vitrinite reflectance measurements provide information pertaining the anatomy and the thermal history of the propagules (Veld, 1995 and references cited therein). This means that a rigorous basis will be provided for studies regarding the effects of the formation processes on the solid residues as a function of the temperature after the “carbonization” process, which is considered as the first of the formation processes.

MATERIAL AND METHODS

Samples

Peas (*Pisum sativum* L.) were collected from the Centre for Genetic Resources, Wageningen, The Netherlands (CGN). Three Dutch varieties were selected: 'Graauwe erwt' (GE; CGN 10198), 'Noord-Hollandse Rozijnerwt' (RE; CGN 10293) and 'Wijker Vale' (WV; CGN 10312). The flowers of these varieties are red and the outside colour of their seeds is generally brown and the surface is wrinkled. Based on these features they belong to the field pea (*P. sativum* ssp. *arvense*) (Deshpande and Adsule, 1998). The dimensions are roughly 8x5x3 mm and the average weight of a single pea is about 400 mg. The three varieties were grown in the Hortus Botanicus of Leiden University and a new crop of peas was harvested at the end of the summer of 1999. About two months after harvesting, the peas were used for the carbonization experiments without any further pre-treatment. For most of the experiments variety GE was used.

A sample of peas from the archaeological record was made available by Prof. dr. C.C. Bakels from the faculty of Archaeology of Leiden University. This sample is referred to as H414 (Bakels, 1978). The peas were excavated from a site in a loess soil in Hienheim (Northern Bavaria, Germany) in pit 414. The age of the peas was determined by ^{14}C as being 5100 years cal. BC.

Heat treatment

For each experiment ten intact peas were placed into an open glass vessel and inserted in a 30 cm long glass tube (⌀ 2.3 cm) at 18 cm from the inlet. The tube was inserted in a pre-heated Carbolite tube oven (model MTF 12/38/250) and subsequently heated at one of the following temperatures: 130, 160, 190, 220, 235, 250, 270, 290, 310, 340, 370, 400, 440, 500, 600 or 700 °C under a constant flow (150 ml min^{-1}) of N_2 at atmospheric pressure. During the experiment the heating rate of the oven was set at 2 °C min^{-1}. In an additional experiment at 340 °C the heating rate of the oven was increased to 200 °C min^{-1}. The glass vessel with the peas was weighed before and after heating, in order to determine the percentage weight loss. In the initial experiments the samples were heated as a function of time at 190, 235, 250, 290, 340 and 600 °C for a period of up to120 minutes required to determine the optimal heating time. Based on these initial experiments, peas used for the subsequent analyses were heated for 60 minutes. Gases and volatiles were vented and not investigated further.

To determine the variations between internal and external temperatures experiments were undertaken with one pea in the glass vessel. A hole (⌀ 0.3 mm) was

drilled in the pea and a “K” type thermocouple was inserted in the middle of the pea. Just beside the pea a similar type of thermocouple was placed to monitor the temperature of the carrier gas. The exposed wires were insulated with glass fibre. The thermocouples were connected to a two-channel writer (Kipp en Zonen, model BD41) and the temperatures were recorded continuously versus time. The thermocouples were calibrated with a pyrometer. Similar experiments were carried out with ten peas in the glass vessel where only one of the peas in the middle was equipped with a thermocouple.

Microscopy

Visual changes in the external gross morphology of complete peas were studied using a Zeiss Axioskop incident light microscope. The internal morphology was examined on polished resin-embedded cut specimens using a Zeiss Axioskop reflected light microscope.

Chemical analyses

For the chemical analyses the testa was removed and only the cotyledons, which constitute the bulk of the peas, were ground and used. CH analyses were performed using a LECO CHN-1000 analyser. The furnace temperature was set at 1050 °C. S analyses were executed using a LECO SC-144DR. Additional C and N analyses were executed on a NA 1500 series 2 NCS analyser from Fisons Instruments. The temperature in the combustion reactor was maintained at 1020 °C, the combustion products were separated on a Porapak QS column with a length of 2 m. All values stated are based on at least two measurements corrected for H_2O and ash content, which were determined on the Thermogravimetric Analyser TGA 2950 Hi-Res. The carrier gas was air and the heating rate was set at 25 °C min^{-1}, water content was determined at 105 °C and ash content at 950 °C.

Mineral content

Five peas were ground using a Tungsten-carbide mill in an automated grinding- and pressing machine (Herzog HSM-HTP). The ground sample was pressed with wax into tablets, on which XRF-analyses were performed. The tablets were analysed for major and trace elements by X-ray microscopy, using an ARL9400 spectrometer with a Rh tube, with full matrix correction for major elements and Compton scatter method for trace elements.

Vitrinite reflectance measurements

Entire specimens of peas were heated for 60 minutes at oven temperatures ranging from 250 to 700 °C. The residues were embedded in resin blocks and polished. Mean maximum vitrinite reflectance measurements *(%Rmax)* were carried out under oil immersion at a wavelength of 546 nm using a Leitz MPV II microscope system. One hundred reflectance measurements were made on each specimen. Preparation of polished blocks and reflectance measurements were carried out according to standard methods defined in ISO 7404, part 2 (1985) and ISO 7404, part 5 (1994).

RESULTS

Heat treatment

Heat treatment at a constant temperature vs. time

The weight loss of ten peas was determined at different times (t in min) at the various constant oven temperatures (T_{oven} in °C). The conditions of the experiments did not allow for continuous measurement of the total weight loss. Therefore a separate experiment was needed for each measurement. The results indicate that for each T_{oven} the total weight loss reaches a constant level after a certain time and the higher the temperature the shorter the time after which the weight loss remains constant (Fig. 2.1). The rate of weight loss changes according to T_{oven}. At T_{oven} = 250 °C (and probably also at T_{oven} = 235 °C) two events of weight loss can be recognized, one until about 15 minutes with a weight loss of c. 20 % and a second one starting after about 25 minutes and leading to a further weight reduction of 20 %.

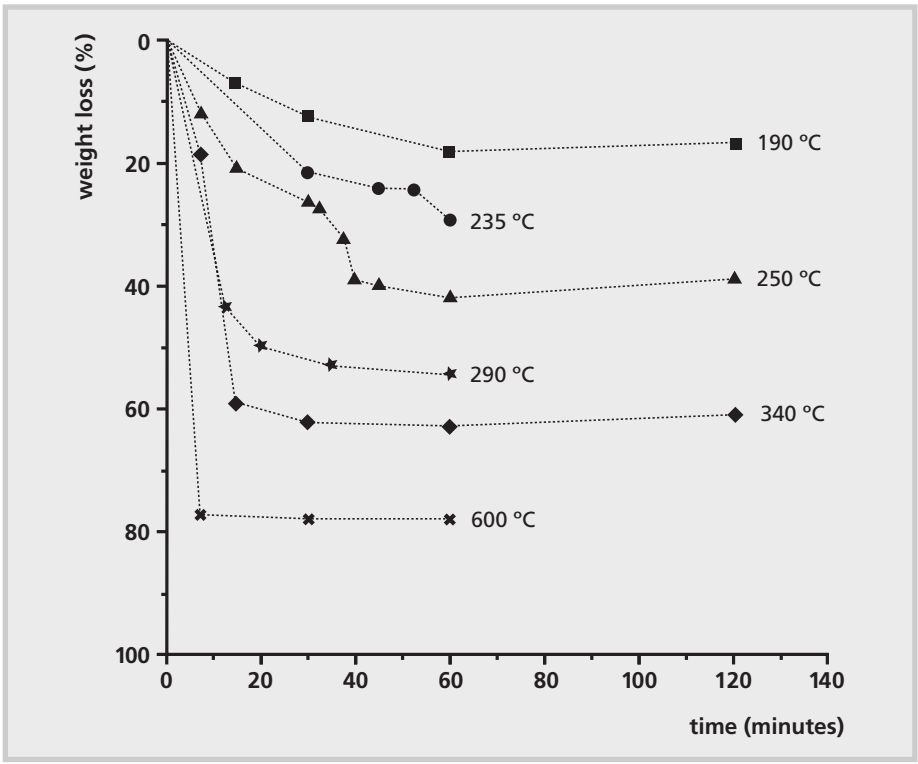


Fig. 2.1
Weight loss during heat treatment of 10 peas (P. sativum var. Graauwe erwt) with N_2 as carrier gas. Weight loss (%) vs. time (minutes) at oven temperatures of 190, 235, 250, 290, 340 and 600 °C.

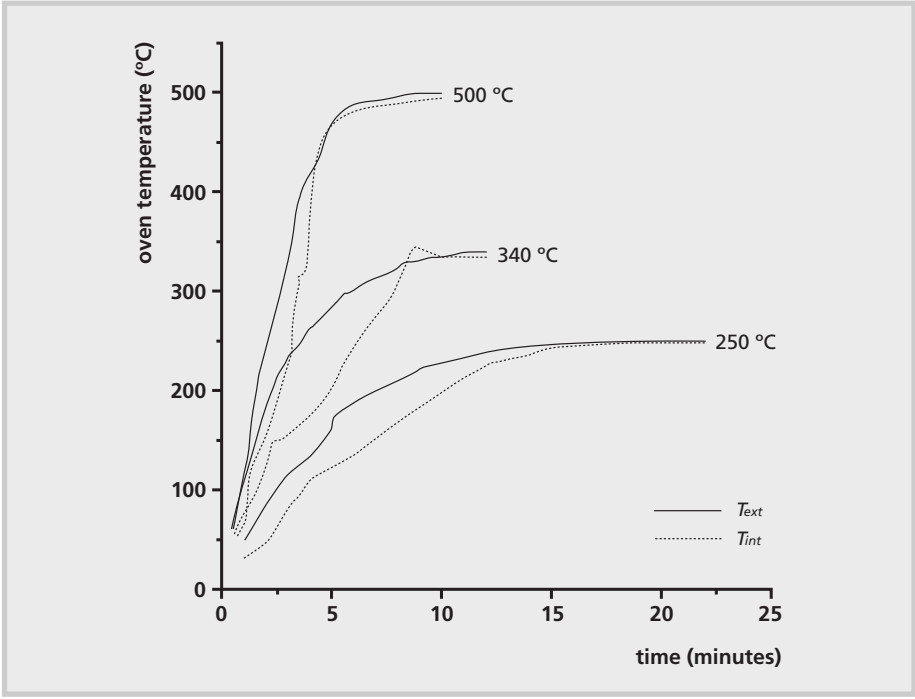


Fig. 2.2
Experiment with one pea (P. sativum var. Graauwe erwt) showing the occurrence of endotherm and exotherm reactions. Internal temperature T_{int} (°C) and external temperature T_{ext} (°C) vs. time at oven temperatures of 250, 340 and 500 °C.

To obtain additional insight into these phenomena subsequent experiments were carried out with a single pea in the glass vessel to measure the temperature as a function of time inside and outside of the pea using thermocouples. Fig. 2.2 reveals that in the first part of the curves the external temperature, T_{ext} (°C), is greater than the internal temperature, T_{int} (°C), of the peas, which indicates a competition between the heat necessary to increase the temperature of the pea and the heat necessary for the reactions. Initially this positive thermal lag ($T_{ext} - T_{int} > 0$) is simply the temperature gradient required to affect the heat transfer from the wall of the oven to the N_2 gas and to the pea, which has a poor thermal conductivity. This initial period is followed by a short period where T_{int} remains almost constant. The result is an increase of the thermal lag. After this phase T_{int} increases again, the thermal lag gets smaller as a function of time and eventually both T_{int} and T_{ext} become constant. At all oven temperatures T_{ext} becomes constant upon reaching the predefined temperature of the oven. Some minutes later T_{int} remains also constant, but at a level of about 4 °C lower than T_{ext}. At T_{oven} = 250 °C these features occur after 15 minutes and with increasing T_{oven} the necessary time decreases; at T_{oven} = 500 °C this is 4.3 minutes (Fig. 2.2).

However, from T_{oven} = 290 °C and before T_{int} and T_{ext} become constant the positive thermal lag is followed by a negative thermal lag and $T_{ext} - T_{int} < 0$. The curve, with strictly a positive thermal lag (Fig. 2.2, curves marked 250 °C), changes into a curve consisting of a positive lag, followed by a negative thermal lag (Antal and Varhegyi, 1995), as shown in Fig. 2.2 with the curves marked as 340 and 500 °C. The positive thermal lag is evidence of an endothermic or heat demanding reaction, which is present in all experiments. From T_{oven} = 290 °C the positive thermal lag is followed by a negative thermal lag, which is a signature of an exothermic or heat liberating reaction (Narayan and Antal, 1996).

For the regular experiments ten peas were used in the glass vessel. Thus to allow an appropriate comparison nine peas were added to the one pea equipped with a thermocouple. The results show a delay in reaching the final temperatures of T_{ext} and T_{int} on the order of 4 minutes in the case of T_{oven} = 250 °C and 3 minutes at T_{oven} = 340 °C. The shape of the curves did not change.

The influence of the heating rate of the oven on the results was measured by increasing the rate from 2 to 200 °C min^{-1} at T_{oven} = 340 °C. The curve of T_{ext} did not change, but the curve for T_{int} did result in a smaller thermal lag, both for the endotherm and the exotherm phase. In the endotherm phase the lag was reduced by half to 40 °C.

Heat treatment for 60 minutes at constant temperature

The results as presented in Fig. 2.1 reveal that for all T_{oven} the total weight loss after 60 minutes becomes stable. A series of experiments was executed between T_{oven} = 130 °C and 700 °C and the weight loss determined. The results for a typical pea are presented in Fig 2.3. The strongest weight loss is found in the range of T_{oven} = 220 °C to T_{oven} = 270 °C. From temperatures T_{oven} = 270 °C upwards the increase of the rate of weight loss slowly decreases until the total weight loss reaches a value of almost 80 % at T_{oven} = 700 °C. For comparative reasons the weight loss of carbonized microcrystalline cellulose after Pastorova et al. (1993b) is also shown, as the experimental conditions are almost identical. From T_{oven} > 270 °C the peas show less total weight loss compared with cellulose.

The experiments were executed at a constant flow of N_2 (150 ml min^{-1}). This means that vapours will be removed from the reaction site. To investigate the relation of weight loss versus flow, experiments were carried out with 150 and 0 ml min^{-1} flow. At T_{oven} = 250 °C for 60 minutes the total weight loss was, respectively 42.8 and 43.6; at T_{oven} = 340 °C for 30 minutes 63.4 and 62.9 %. Under the present experimental conditions the weight loss appears independent of flow rate.

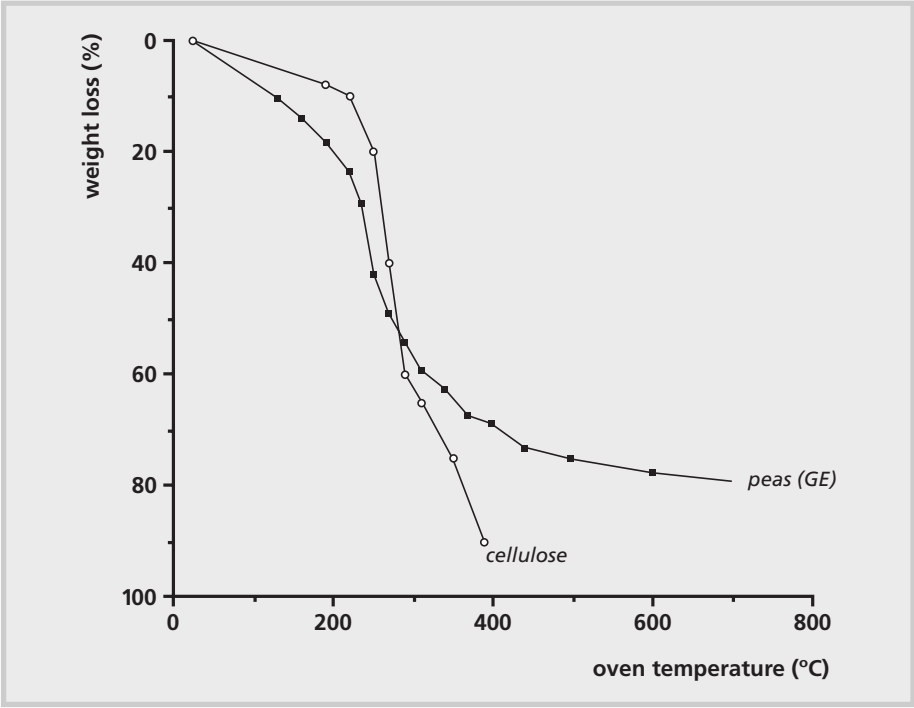


Fig. 2.3
(a) Weight loss of 10 heated peas in the absence of oxygen (P. sativum var. Graauwe erwt) vs. various oven temperatures (°C) heated for 60 minutes and (b) Microcrystalline cellulose heated for 2.5 hrs based on data by Pastorova et al. (1993b).

Morphology and Anatomy

Description of the untreated mature pea (P. sativum)

The outside of the Graauwe Erwt is wrinkled and the colour is light reddish-brown with ochre patches. The seed coat or testa is composed of 2 layers. The outside layer is called the epidermis and the inside layer the hypodermis (Reeve, 1946; Zimmermann, 1936). Elongated cells with thickened walls and a well-defined cuticle characterize the epidermis. These cells are called the palisade or Malphigian cells. The much-discussed light line of this tissue layer is observed near the apical ends of the cells. The hypodermis is composed of specialized, so-called, hourglass cells (Boesewinkel and Bouman, 1995; Esau, 1965). The chemical composition of the testa is mainly cellulose and pectin (Stein von Kamiensky-Jancke, 1957).

The hypodermis is followed by loosely organized parenchyma and the endosperm. However, these tissues are ill defined or lacking when the seed is approaching maturity. Inside the seed coat two cotyledons with the storage parenchyma cells are observed. These cells contain large starch grains, numerous protein bodies and

small deposits of fats (Bain and Mercer, 1966; Swift and Buttrose, 1973). However, the bulk of the pea is composed of starch grains. The cell walls consist mainly of non-starchy polysaccharides, which account for 80 % of the walls (Bain and Mercer, 1966).

heating time (minutes)	*outside colour (testa)*	*inside colour (cotyledon)*	*cracks in testa (%)*	*length cracks in testa (mm)*	*testa crackled*	*testa curled at cracks (%)*	*cracks in cotyledon (%)*	*colour tarry liquid in outlet tube*
untreated	lrb+op[a]	white	0	0	no	0	0	-
7,5	rd+op[b]	yellow	20	3	no	0	0	clear
15	drb+dbp[c]	brown-yellow	60	3-8	no	0	0	clear
22,5	black	brown	70	3-8	no	0	0	clear
30	black	brown	60	3-8	no	0	0	light yellow
37,5	black	brown	80	3-8	no	0	0	light-yellow
45	black	dark brown	80	3-8	no	0	0	yellow
60	black	brown-black	80	3-8	no	0	0	yellow-brown
120	black	brown-black	80	3-8	no	0	0	yellow-brown

[a] lrb+op = light reddish brown + ochre patches
[b] rd+op = red-brown + ochre patches
[c] drb+dbp = dark red-brown + dark brown patches

Table 2.1
Colour and morphology changes in peas (P. sativum var. GE) heated at T_{oven} = 250 °C for the given time (minutes) with N_2 as carrier gas

T_{oven} (°C)	*outside colour (testa)*	*inside colour (cotyledon)*	*cracks in testa (%)*	*length of cracks in testa (mm)*	*testa surface crackled*	*testa curled at cracks(%)*	*cracks in cotyledon (%)*	*colour tarry liquid in outlet tube*
untreated	lrb+op[a]	white	0	-	no	0	0	-
130	lrb+op[a]	light yellow	0	-	no	0	0	clear
160	rb+lrbp[b]	brown-yellow	0	-	no	0	0	clear
190	drb+rbp[c]	light brown	0	-	no	0	0	clear
220	black	brown	40	3-8	no	0	0	light yellow
235	black	dark brown	40	3-8	no	0	0	yellow
250	black	brown-black	80	3-8	no	0	0	yellow-brown
270	black	black	80	3-8	no	0	10	yellow-brown
290	black	black	90	3-8	no	0	0	brown
310	black	black	100	3-8	strong	60	50	brown
340	black	black	100	3-8	medium	10	80	brown
370	black	black	100	3-8	weak	20	100	brown
400	black	black	100	3-8	no	80	100	brown
440	black	black	100	3-8	no	100	100	brown
500	black	black	100	8	no	100	100	brown
600	black	black	100	8	no	100	100	brown
700	black	black	100	8	no	100	100	brown

[a] lrb+op = light reddish brown + ochre patches
[b] rb+lrbp = red-brown + light red-brown patches
[c] drb+rbp = dark red-brown + red-brown patches

Table 2.2
Colour and morphology changes in peas (P. sativum var. GE) heated for 60 minutes at the given oven temperature (T_{oven}) with N_2 as carrier gas

External changes caused by heat treatment

The external changes of the peas treated by heat were examined by light microscopy (Table 2.1 and 2.2). Table 2.1 shows the changes of the peas heated at T_{oven} = 250 °C as a function of time. The outside colour changes from light reddish-brown with ochre patches into black after 22.5 minutes of heating. The first cracks in the testa appear after 7.5 minutes in 20 % of the peas. However, even after 120 minutes not all 10 peas have cracks. Table 2.2 shows the changes during heating for 60 minutes at T_{oven} = 130 °C until 700 °C. At T_{oven} = 220 °C the external colour has changed into black and the first cracks in the testa appear in 40 % of the peas. At T_{oven} = 310 °C all peas have cracks in the testa. The cotyledons start having the cracks at T_{oven} = 270 °C and in all peas heated to 370 °C and higher. It is noteworthy that the surface of the testa becomes strongly crackled at T_{oven} = 310 °C and this feature disappears again at T_{oven} = 400 °C. At all temperatures the testa is still present, except at places where cracks have developed. Some curling occurs at the edges of the cracks.

Internal changes due to heat treatment

The colour of the cotyledons changes from white through yellow into black at T_{oven} = 270 °C. Polished surfaces revealed a drastic change in the internal structure. In Fig. 2.4a an example is shown of the internal structure of an untreated pea showing its distinct cells. These cells are filled with a nucleus, cytoplasm, starch grains and protein bodies (Bain and Mercer, 1966). The greater proportion consists of starch grains with a 5 to 10 times larger diameter than the protein bodies (Varner and Schidlovsky, 1963). This situation does not change until T_{oven} = 190 °C (Fig. 2.4b and c). From then on the distinct cell structure slowly disappears. At T_{oven} = 270 °C (Fig. 2.4d) there is no cell structure as such present, but one can still see some faint former cell boundaries. These are completely absent at T_{oven} = 310 °C (Fig. 2.4e). A grey matrix is visible in which light grey patches can be distinguished. These patches must have been the original starch grains, which have been converted into new thermostable entities. The patches are larger than the original grains, but from the results at T_{oven} = 270 °C and T_{oven} = 290 °C (not shown) it appears as if the grains have "fused". Another phenomenon is the formation of cavities between the cells and later in the grey matrix. Between T_{oven} = 310 and 440 °C the light grey patches become increasingly faint, but never disappear. The quantity and size of the holes increase until they constitute about 50 % of the total volume. From T_{oven} = 440 °C (not shown) a new feature becomes visible. The quantity and size of the holes increase dramatically to about 80 % of the total volume. Fig. 2.4f is an example of this stage at T_{oven} = 600 °C. Around the holes a matrix is visible in which patches can be seen that must have been the original starch grains. Streamline features are visible as if the mass has been pushed aside.

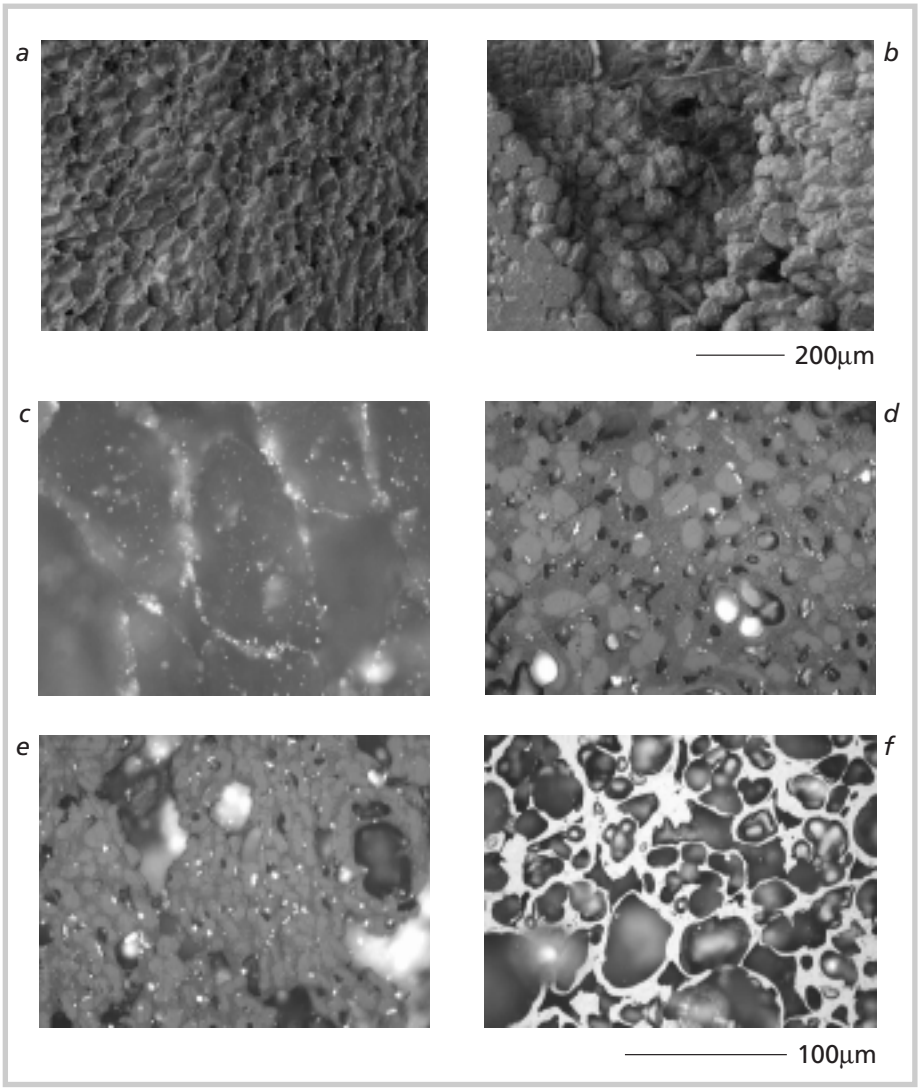


Fig. 2.4
The internal structure of peas (P. sativum var. Graauwe erwt) heated for 60 minutes with N_2 as carrier gas at various oven temperatures (T_{oven}). SEM photomicrographs: (a) Untreated pea; (b) T_{oven} = 190 °C. Reflected-light photomicrographs; (c) T_{oven} = 190 °C; (d) T_{oven} = 270 °C; (e) T_{oven} = 310 °C and (f) T_{oven} = 600 °C

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
C(%)	40.15	41.27	41.09	41.10	40.60	41.20	40.90	40.99	40.48	40.38
N(%)	3.35	3.68	4.87	5.07	2.70	3.78	3.85	4.50	2.79	3.78

Table 2.3
Weight % of C and N in ten different seeds from five plants of P. sativum var. RE

Chemical analyses and mineral composition

Chemical analyses reveal an increase in the relative percentage of carbon and a decrease of hydrogen (Fig. 2.5). The major changes occur between T_{oven} = 220 and 270 °C. Carbon increases from c. 45 % (daf) to c. 87 % (daf); hydrogen decreases from c. 7 % (daf) to c. 1.5 % (daf). Oxygen (by difference) decreases from c. 40 % (daf) to c. 6 % (daf). Nitrogen shows a strong variation between c. 2 % (daf) and c. 7 % (daf). Sulphur (not shown) varies from 0.38 % (daf) of the untreated pea to 0.27 % (daf) at T_{oven} = 700 °C. To study the natural variation of C and N in ten peas both parameters were measured and the results are shown in Table 2.3. The value of C is sufficiently constant and will not influence the results of the chemical analyses in relation to the heat treatment. This is not the case for the values of N.

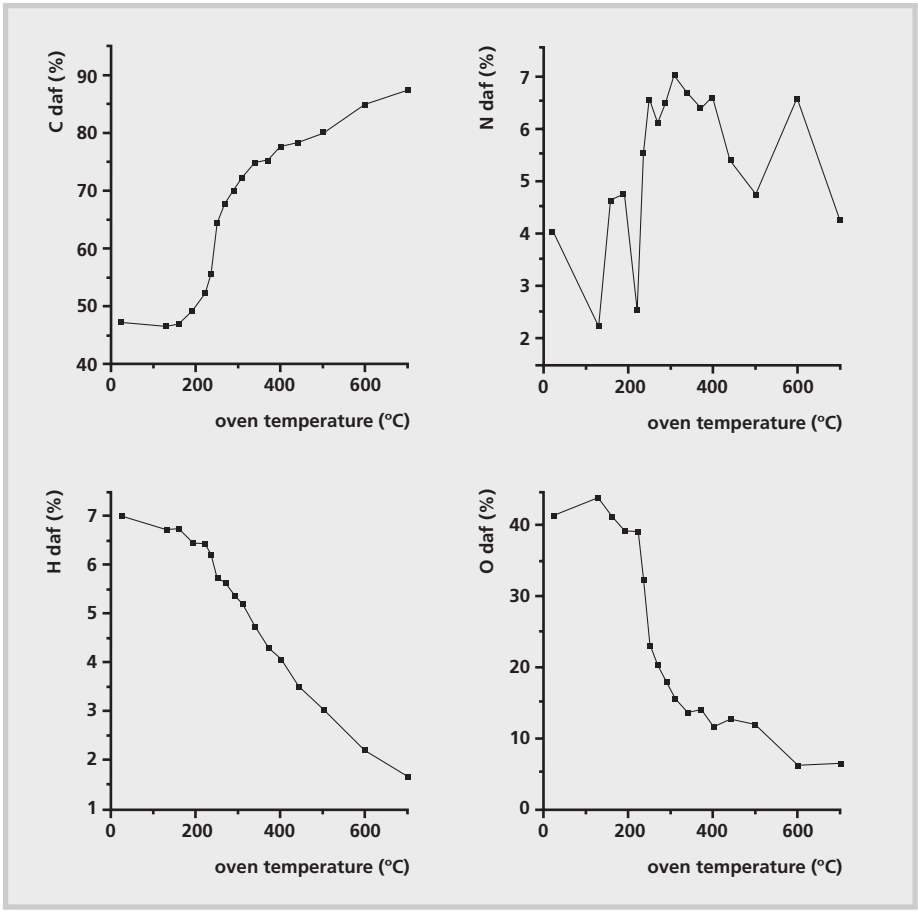


Fig. 2.5
Chemical analyses data of peas (P. sativum var. Graauwe erwt) heated for 60 minutes with N_2 as carrier gas at various oven temperatures (°C).

Plotting the atomic ratio H/C against O/C in a van Krevelen diagram (Fig. 2.6) reveals a large decrease in the H/C and O/C ratios from the untreated pea to T_{oven} = 250 °C (Van Krevelen, 1993). The mineral content in peas is shown in Table 2.4.

Vitrinite reflectance measurements

The vitrinite reflectance measured on the "starch" grains of the heated peas is presented in Fig. 2.7. Only from T_{oven} = 270 °C a true vitrinite reflectance could be measured. Two series of measurements were carried out. The reflectance of the lowest rank of vitrinites rises slowly from T_{oven} = 270 °C to 400 °C. Thereafter it rises more rapidly to the last measured reflectance at T_{oven} = 700 °C. The S.D. of the measurements increases from 0.03 at T_{oven} = 270 °C to 0.12 at T_{oven} = 600 °C and reached at T_{oven} = 700 °C a value of 0.23.

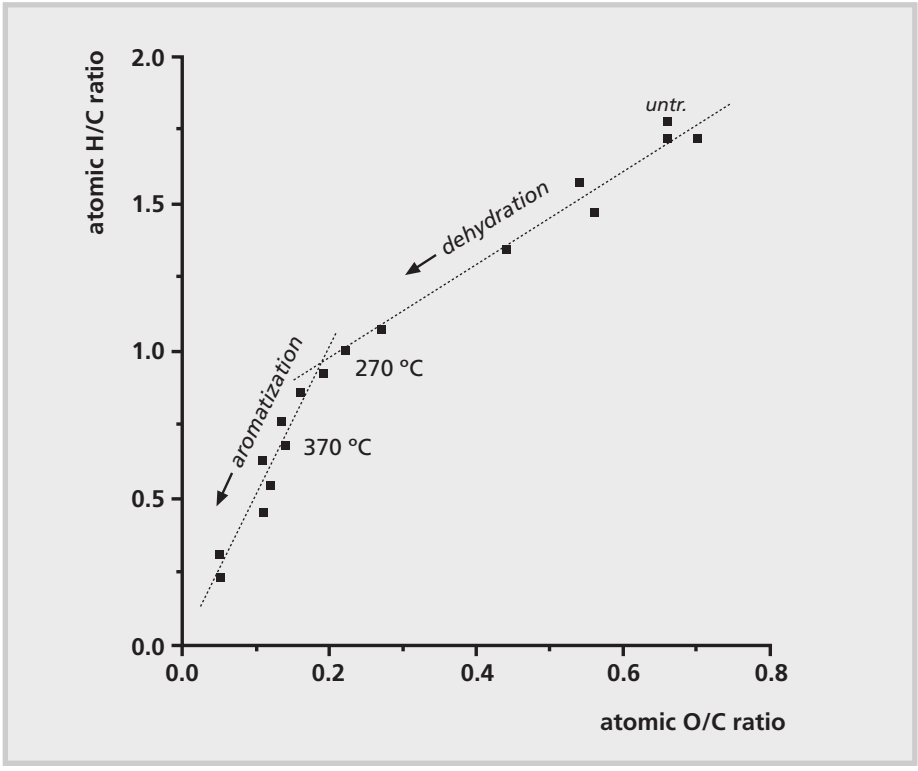


Fig. 2.6
Van Krevelen diagram for peas (P. sativum. var. Graauwe erwt) heated for 60 minutes with N_2 as carrier gas.

Archaeological peas (H414)

Chemical analyses and reflectance measurements were carried out on two different archaeological peas from the same sample. The results of the chemical analyses are: C = 68.2 % (daf), H = 5.3 % (daf) and N = 6.5 % (daf). The internal structure (not shown) does not show cell structures. A light matrix is observed with small "fused" grains and c. 20 % of the mass consists of holes. The reflectance measurements of the grains gave a value of 1.13 for *%Rmax* (fig. 2.7).

SiO_2	<10	w%	MgO	0.02	w%	Zn	43	ppm
Al_2O_3	2.20	w%	Na_2O	0.35	w%	Sr	26	ppm
TiO_2	0.131	w%	K_2O	2.99	w%	Ba	723	ppm
Fe_2O_3	<1	w%	P_2O_5	1.624	w%	Rb	49	ppm
MnO	<1	w%	Cu	<10	ppm	Zr	157	ppm
CaO	0.05	w%	Pb	45	ppm			

Table 2.4
Mineral content in peas (P. sativum var. Graauwe erwt)

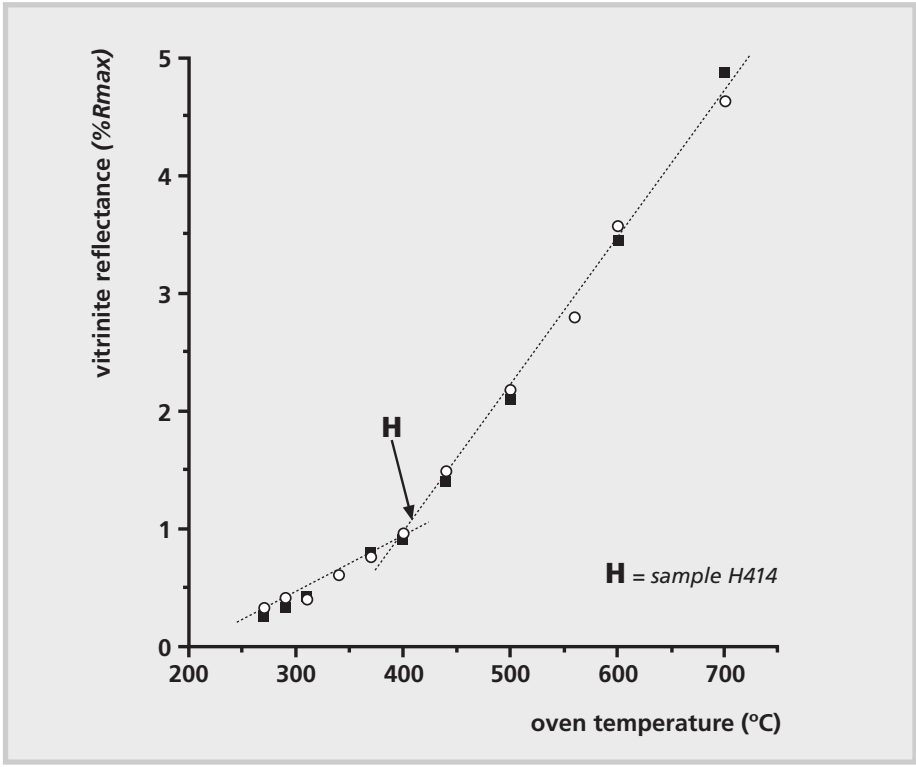


Fig. 2.7
Vitrinite reflectance measurements of heated peas (P. sativum var. Graauwe erwt) with N_2 as carrier gas vs. the oven temperature (°C). Two series (o and ■) of measurements are shown.

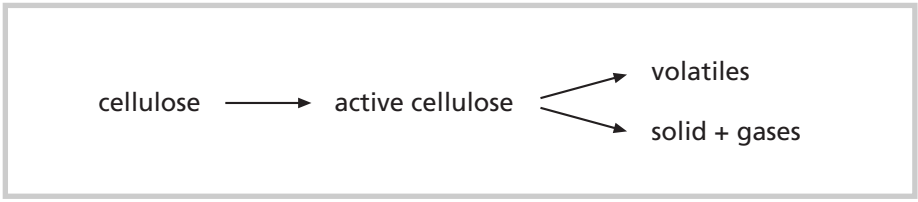
DISCUSSION

This study determined the influence of heat treatment at different heating temperatures on the seeds of *P. sativum*. These seeds contain a mixture of polysaccharides, protein, fat and minor amounts of inorganic compounds naturally present. Starch represents the bulk of the material (Daveby et al., 1993). Each compound will carbonize or degrade at different rates and by different pathways, depending on the used apparatus and the set of experimental conditions. Heat treatment of organic material always results in 3 products: gas, liquid and a solid, the latter being the focus of this study. The measured physical properties of the pea (the solid) change with increasing heating temperatures. The changes that occur correspond to several stages that will be discussed below.

The decomposition of starch can be compared with that of cellulose, because the chemical composition and structure can be considered identical for the purpose of this study (cf. van der Kaaden et al., 1983). Heat treatment of cellulose is widely reported and numerous papers have dealt in detail with this issue. (For a review see e.g. Antal and Varhegyi, 1995.)

A reaction model for solid phase heat treatment is shown in Scheme 2.1 and is usually described as two parallel reactions preceded by an initiation step, also called the Broido-Shafizadeh model (Bradbury et al., 1979; Broido and Nelson, 1975). At lower temperatures and heating rates, the path for solids and gases is preferred, but at higher temperatures and heating rates the path of the tarry volatiles will be followed.

The heat treatment of protein and the influence of starch and protein on each other through non-enzymatic browning (NEB) or Maillard reactions is a fairly unknown area of investigation as far as the solid residue is concerned (Ikan et al., 1996; Poirier et al., 2002). In a separate paper dealing with the change of the molecular properties of heated peas this feature will be discussed further (Braadbaart et al., 2004b).



Scheme 2.1
The Broido-Shafizadeh model (1979) of the "carbonization" of cellulose

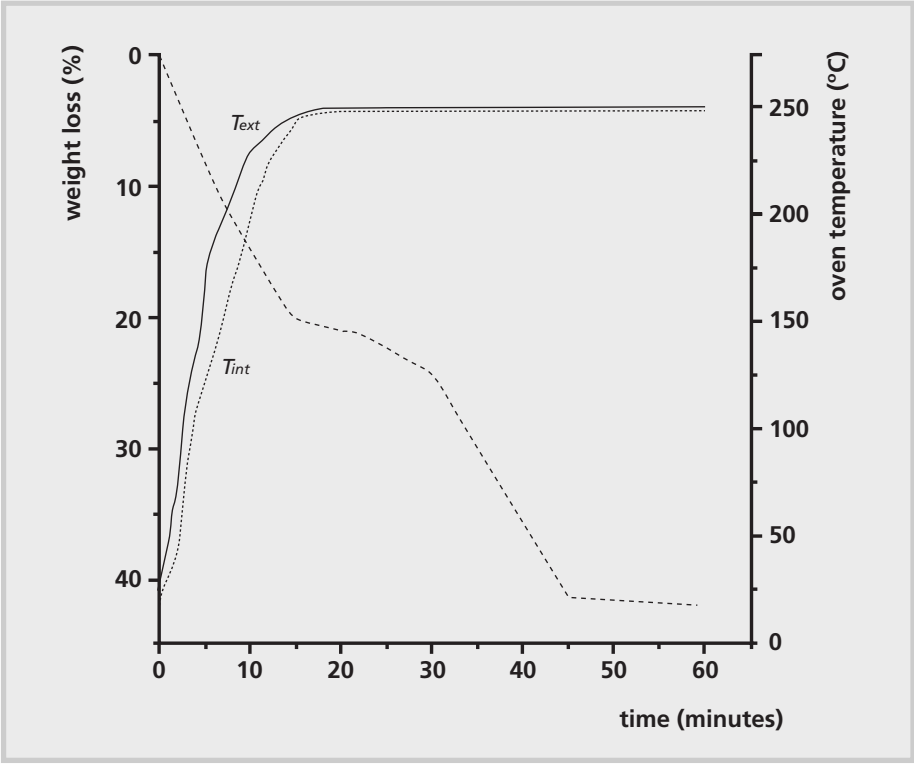


Fig. 2.8
Weight loss (%) of 10 peas heated at an oven temperature of 250 °C (P. sativum var. Graauwe erwt) with N_2 as carrier gas vs. time (minutes) as ----, the internal temperature T_{int} (°C) of one pea vs. time (minutes) and the external temperature T_{ext} (°C) next to the pea vs. time (minutes).

The results of the heat treatment of peas at T_{oven} = 250 °C is summarized in Fig. 2.8 and shows both the weight loss (%) as the internal and external temperature (°C) as a function of the time. The weight loss has two phases with a first phase up to about 20 minutes and a weight loss of 22 %. The thermal lag representing an endothermic reaction is almost completed in this phase. The colour of the inside of the pea has changed from white through yellow into brown and the colour of liquid condensate in the outlet tube is still clear (Table 2.1). The latter implies that only water has evaporated in this phase. As the amount of free water amounts to c. 11 % the other part of the weight loss must be explained by other sources. The change of the colour into brown is most likely the result of NEB reactions, which are accompanied by the release of water (Monte and Maga, 1981). The samples consist of more than one component and each component does not decompose indepently from the others, as is assumed in the case of ligno-cellulose material (Shafizadeh and McGinnes, 1971).

After 20 minutes the total weight loss remains almost constant for 10 minutes, thereupon increases again with 20 % to reach its final value of 42 % at 45 minutes, then remains constant. This last stage of weight loss is accompanied by a colouring of the condensate in the outlet tube from clear to yellow, indicating that in addition to water other compounds are volatized. Fig. 2.6 shows that at T_{oven} = 250 °C the main reaction is dehydration, so the bulk of the volatiles will be water. After 25 minutes the sample has reached the predefined oven temperature and hardly any heat is necessary to keep the sample at 250 °C. All the available heat can be used for the volatilization and no thermal lag is visible.

The heat treatment for 60 minutes at the different oven temperatures can be conveniently considered in stages as described below:

Stage A

From the untreated pea until T_{oven} = 220 °C the colour of the liquid in the outlet tube remains colourless, the inside colour of the pea changes into light brown and the weight loss is 22 % (Table 2.1 and Fig. 2.3). This is similar to the first 20 minutes as shown in Table 2.2 and Fig. 2.8. Stage A is therefore characterized by NEB reactions and dehydration.

Stage B

At T_{oven} = 220 °C the colour of the condensate turns into yellow and both the rate of the weight loss and the relative %C increases strongly until T_{oven} = 270 °C. The relative %H and %O start to decline from T_{oven} = 220 °C. Acccording to the van Krevelen diagram (Fig. 2.6) dehydration is the main process accompanied by the loss of a minor amount of other volatiles. Until T_{oven} = 270 °C only endothermic reactions are present, the distinct cell structure of the pea is still recognizable but the pea has become black.

Stage C

From T_{oven} = 270 °C the following changes occur: (i) the rate of weight loss and the rate of increase of %C are reducing (Fig. 2.3 and 2.5); (ii) both endothermic and exothermic reactions are occurring (Fig. 2.2) and (iii) the cell structure starts to disappear. The van Krevelen diagram (Fig. 2.6) shows dehydration and aromatization as the main processes. The exotherm is the result of secondary reactions between converting solids and hot vapours traversing the pea on their way to the external environment. It confirms the results of the experiments that the flow of the carrier gas did not affect the weight loss. This process will result in a destruction of the cell structure and an increase of the amount of converted solids and thus in a decrease of the rate of weight loss and. The total weight loss curve of the peas starts to deviate at T_{oven} = 270 °C from the one of microcrystalline cellulose with a higher production of converted solids (Fig. 2.3). In comparison with

cellulose, a pea has a more confined structure and will keep the volatile matter inside for further condensation (Boon et al., 1994). Another reason to be considered for a higher production of converted solids is the catalytic action of inorganic ions naturally present in peas (Richards and Zheng, 1991). For the presence of inorganic ions in the peas used in the present study see Table 2.4. The possible role of water vapours is unknown (Antal and Varhegyi, 1995).

Stage D

From T_{oven} = 310 °C the distinct cell structure is no longer present, instead a grey matrix with light grey patches and the beginning of the formation of holes is observed (Fig 2.4e). The "fused" patches are the primary conversion product of the original starch grains and the matrix is the result of secondary reactions. Other features are the cracking of the surface of the testa, the cracks in the cotyledons and a further decrease of the rate of weight loss.

Stage E

A new stage starts in the range of T_{oven} = 400 to 440 °C. The rate of heating of the pea, necessary to reach the preset oven temperature, has increased from approximately 10 °C min^{-1} at T_{oven} = 250 °C through 100 °C min^{-1} at T_{oven} = 500 °C to 200 °C min^{-1} at T_{oven} = 700 °C. The reaction rate has now become high resulting in a larger endothermic reaction (Fig. 2.2) and violent reactions are observed. The reactions took place so fast with large amounts of vapours driven off rapidly causing the matrix and grains to be pushed outwards (Fig. 2.4f). The heat demand has increased dramatically and heat transfer issues are evident enhancing the thermal lag. The total weight loss is high in a relatively short time. The endotherm is still followed by an exotherm, however smaller than at T_{oven} = 340 °C. This would point to the presence of secondary reactions, but on the other hand the vapours appear to be blown out of the pea (Fig. 2.4f) and the residence time of the vapours in the pea has become so short that gas-solid (secondary) reactions are hardly possible. Also the grey matrix of the previous stage is no longer present. As the total weight loss hardly increases anymore from T_{oven} = 440 °C no secondary reactions occur and other chemical reactions must be the cause for the exotherm (Varhegyi and Jakab, 1994). In this stage mainly volatilization reactions occur as well as primary reactions that convert starch into a new thermo stable product (Boon et al., 1994). From T_{oven} = 400 °C the maximum vitrinite reflectance *(%Rmax)* shows a rise that continues until T_{oven} = 700 °C (Fig. 2.7). The rise has been attributed to a progressive ordering of the molecular systems that comprise the converted solids of the pea (Goodarzi and Murchison, 1972; Murchison, 1978).

IMPLICATIONS FOR PEAS FROM THE ARCHAEOLOGICAL RECORD

The results of the chemical analyses and the reflectance measurements of the peas from the archaeological record (H414) are compared with the results of the heat treatment of recent peas in the laboratory as a function of the temperature. The %C (daf) of 68 corresponds to a heating temperature of 280 °C and the %H (daf) of 5.3 to 290 °C (Fig. 2.5), while the *%Rmax* shows that the pea from H414 is heated at 410 °C (Fig. 2.7). The results are encouraging considering that %C and %H is measured once on only one pea recovered from the archaeological record and the *%Rmax* on another recovered pea, moreover the morphology and the chemical composition of peas from the past are not fully known. Also, the time of exposure to a heat source is unknown and it is uncertain whether 7000 years being buried in the soil affects the physical and chemical properties of heated peas.

CONCLUSIONS

As a result of heating under controlled anoxic conditions the physical and chemical composition of the seeds of *P. sativum* (peas) change as a function of time and temperature. In spite of the complexities related to the multi-phase, multi-component aspects, the heat treatment of recent peas in a range of temperatures from 130–700 °C, after 60 minutes of heating under a flow of N_2 gas and atmospheric pressure, can be considered in five stages based on the physical and chemical properties. This implies that changes of the various properties occur at the same characteristic temperatures of 220, 270, 310 and 440 °C. The C content increases with increasing temperatures and eventually a strongly C-enriched product is formed, which will affect the molecular composition. The weight loss reaches approximately 70 % at 400 °C and results in a decrease of the dimensions of the seeds. These features suggest that the general term, carbonization, only means that the residues have been exposed to heat. The physical and chemical properties depend on the temperature, which is probably also true for the molecular composition and the dimensions. For studies regarding the effects of the formation processes on the residues the heating temperature has to be known. In particular the vitrinite reflectance measurements provide a fast and reliable tool for determining the temperature to which the specimens have be exposed to.

The seeds of peas are still recognizable even after heating at 700 °C for one hour. At all temperatures the testa remains almost intact and sticks to the cotyledons.

The simulations in the laboratory provide a rigorous basis for studies regarding the effects of the formation processes subsequent to the so-called “carbonization” process on the residues and can be described in the five stages. However, additional insights are needed to evaluate the changes of the molecular and dimensional properties as a function of the temperature.

Acknowledgements

We thank Miss K. Reimer and Mr. G. Nobbe for their help with the reflection measurements and the chemical analyses; Mr. P. Kleingeld for his assistance with the temperature measurements in- and outside the peas by means of thermocouples. The first author thanks Professor Dr C.C. Bakels for her encouraging discussions. This research is supported by FOM research program 49 funded by the Foundation for Fundamental Research on Matter (FOM), a subsidiary of the Dutch Organization of Scientific Research (NWO).

CHAPTER 3

Laboratory simulations of the transformation of peas as a result of heating: the change of the molecular composition by DTMS

ABSTRACT

Peas (*Pisum sativum* L.) consist mainly of cotyledons and their bulk material is starch and proteins. Their structure is rather confined. Peas were heated at temperatures ranging from 130-700 °C under anoxic conditions for up to two hours. For each temperature a separate experiment was carried out in a pre-heated oven. Direct Temperature-Resolved Mass Spectrometry (DTMS) under EI and CI (NH_3) conditions measured the molecular composition of each solid residue. FTIR was carried out on residues heated at higher temperatures. The resulting solid products of the heating process still show the original markers for polysaccharides and proteins up to 270 °C. Concurrently three stages can be considered, each characterized by its own products. The first stage, from 270 up to 310 °C, shows monosaccharides, protein fragments and aromatic compounds. The second stage, from 310 up to a transitional stage from 400 to 440 °C, releases various aromatic and heterocyclic compounds. The third stage at higher temperatures shows a highly C-enriched product that releases CO, CO_2, HCN, SO and SO_2. The EI and CI experiments fail to discriminate between the pyrolysis products of polysaccharides and proteins between 250 and 400 °C. FTIR shows the development of an aromatic network in this temperature range. An earlier classification based on the changes of the physical and bulk chemical properties as a function of the temperature corresponds well with the classification based on the molecular changes. Residues of heated peas, usually called carbonized peas, are found in the archaeological record. Our study suggests that peas should be heated up to at least 310 °C before their residues survive natural degradation processes.

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Published with minor adaptations in:
Journal of Analytical and Applied Pyrolysis 71 (2004) 997-1026

INTRODUCTION

Fruits and seeds are of particular interest for archaeology, as they might provide insights into the agriculture and the diet of the people who used them. Plant parts are often found in the archaeological record as charred remains of wood, fruits or seeds (van Zeist, 1970) These charred remains result from a series of processes called the formation processes (Schiffer, 1983; 1987), which include carbonization, deposition, post-depositional alteration, excavation and analysis. To understand the effect of these processes on carbonized material it is necessary to know more about the composition of the fruits and seeds just after carbonization took place and thus before they could enter a depositional stage. This study reports on laboratory simulations of the conversion of fresh to carbonized seeds as a result of progressive heating and focuses on the molecular changes that have occurred.

Because the terms carbonization and char are ill defined and have different meanings in the different fields of research (Braadbaart et al., 2004a), the process of heating has been defined as the product resulting from heat treatment: the solid residue.

The objective of this study is assessing the molecular changes of the organic matter in the seeds of peas that occur during heating under anoxic conditions at atmospheric pressure. The intact peas were heated at a range of temperatures (i.e. 130 to 700 °C). The by far largest structural components of peas are the cotyledons, which consist mainly of starch with amylose, a polyglucan, as its main component. Amylose has been studied extensively using Direct Temperature-Resolved Mass Spectrometry (DTMS) under chemical ionization conditions and the typical mass peaks and their structure are well known (Tas et al., 1989; Lomax et al., 1991a). The pyrolysis and carbonization of cellulose, which has a strong structural resemblance to amylose, has been studied extensively by DTMS, Py-GC-MS, FTIR and NMR (Pastorova et al., 1994; Boon et al., 1994; Arisz et al., 1990 and references therein). These studies provided detailed information on the processes that lead to the aromatized residual materials. Similar studies (unpublished) on amylose yielded very comparable results, although thermal dissociation reactions took place at a lower temperature (a difference of ca. 60 °C).

In the present study the assessment of the molecular changes as a result of heating of the polysaccharide/protein rich cotyledons of peas was performed with DTMS. The experiments were carried out under chemical ionization (CI) and low voltage ionization (EI) conditions. The changes of the internal and external morphology, the elemental composition and the vitrinite reflectance of the peas as a result of heating have been presented in an earlier study (Braadbaart et al., 2004a).

MATERIAL AND METHODS

Samples

The Centre for Genetic Resources in Wageningen, The Netherlands (CGN) supplied the following three Dutch varieties of pea (*Pisum sativum* L.): 'Graauwe erwt' (GE; CGN 10198), 'Noord-Hollandse Rozijnerwt' (RE; CGN 10293) and 'Wijker Vale' (WV; CGN 10312). The flowers of these varieties are red and the outside colour of their seeds is generally brown and the surface is wrinkled. Based on these features they belong to the field pea (*P. sativum* ssp. *arvense*) (Deshpande and Adsule, 1998). All these varieties were sown in the Hortis Botanicus of the University of Leiden. About 2 months after harvesting in the summer of 1999, peas of the variety GE were used for heat treatment experiments without any further pre-treatment. Inside the seed coat two cotyledons with the storage parenchyma cells are present. These cells contain large starch grains, numerous protein bodies and small deposits of fats (Swift and Buttrose, 1973; Bain and Mercer, 1966). The average chemical composition of peas is shown in Table 3.1 after Belitz and Grosch (1999). The digestible carbohydrate is mainly starch (c. 42 %) (Daveby et al., 1993). However, it has been shown that for field peas the amount of starch is reduced to c. 20 %, while the amount of hemicelluloses has increased to 30-40 % at the same time (Flinn and Pate, 1968).

Prof. Dr. C.C. Bakels from the Faculty of Archaeology of Leiden University donated a sample of peas from the archaeological record that had been exposed to heat. This sample is referred to as H414. The archaeological peas were excavated from a site in a loess soil in Hienheim (Northern Bavaria, Germany) in pit 414. The age of the peas as determined by ^{14}C was 5100 years cal. BC (Bakels, 1978).

As a reference compound a sample of a pea protein isolate with 90 % protein, Pisane HD, was kindly supplied by Cosucra SA (Belgium). The sample is referred to as Pisane HD.

	Percentage[b]
Crude protein	*25.7*
Lipid	*1.4*
Digestable carbohydrate	*53.7*
Crude fibre	*18.7*
Minerals	*3.0*

Table 3.1
Chemical composition of peas[a]
[a] *after Belitz and Grosch (1999)*
[b] *the results are average values given as wt% dry matter*

Heat treatment

For each experiment 10 intact peas were placed in an open pyrex vessel and inserted in a 30 cm long pyrex tube (⌀ 2.3 cm) at 18 cm from the inlet. The tube was placed in a pre-heated Carbolite tube oven (model MTF 12/38/250). During the experiments the rate of heating of the oven was limited by setting a ramp rate of 2 °C min^{-1}. In initial experiments the samples were heated at an oven temperature of 250 °C (T_{oven} = 250 °C) as a function of time for 7.5, 15, 22.5, 30, 37.5, 45, 60 and 120 minutes to determine the optimal heating time. Based on these initial experiments peas were heated for each experiment for 60 minutes at one of the following temperatures: 130, 160, 190, 220, 235, 250, 270, 290, 310, 340, 370, 400, 440, 500, 600 and 700 °C under a constant flow (150 ml min^{-1}) of N_2 at atmospheric pressure. The samples of the solid residues of the peas as a result of the heating for 60 minutes will be referred to as residue130, residue160, etc. The pyrex vessel with the peas or the pea residues was weighed before and after heating, in order to determine the percentage weight loss. Gases and volatiles were vented and not further investigated.

The reference compound Pisane HD was heated for 60 minutes under the same experimental conditions at 250, 310, 370, 400, 440 and 500 °C.

Direct temperature – resolved mass spectrometry (DTMS)

Chemical ionization (CI)

The samples, cotyledons of two untreated peas and residues130 to 400 °C, were measured in triplicate using a JEOL JMS-SX/SX102A tandem double focusing mass spectrometer with B/E/B/E geometry. Powered sample was deposited on the platinum/rhodium (90:10) filament of a probe, which was inserted directly into the ion source of the mass spectrometer. The filament was resistively heated by ramping the current with a rate of 0.5 A min^{-1}. Using this ramp the temperature was linearly increased from ambient to approximately 800 °C in two minutes. Desorbed and pyrolysed material was ionized by positive ammonia chemical ionization and accelerated to 8 kV. The mass spectrometer was scanned over an *m/z* range of 60-1000 using a 1 s cycle time.

Electron impact (EI)

Triplicate EI measurements of samples of cotyledons of untreated peas and residues130 to 700 were undertaken using the same set up as described above. Ions were generated using 16 eV electron ionization. The mass spectrometer was scanned over an *m/z* range of 20-1000 using a 1 s cycle time.

Multivariate analysis

Software has been developed at FOM Institute AMOLF to extract principal components from sets of DTMS spectra. In this study the FOMpyroMap multivariate program and the ChemomeTricks program were used for the calculations. As samples were analysed in triplicate, it was possible to perform discriminant analysis (DA). DTMS spectra of the samples are grouped and plotted in the DA space using scores (score maps) and loading plots recalculated as partial mass spectra of the discriminant functions. Interpretation of the chemical significance of those partial mass spectra makes it possible to evaluate the molecular significance of the distribution and grouping of the samples in the DA space (Klap et al., 1996).

FTIR

Fourier-transform infrared (FTIR) spectrometry was performed using an FTS-6000 Bio-Rad FTIR imaging system (Bio-Rad, Cambridge, Ma, USA), consisting of a Michelson interferometer (Bio-Rad FTS-6000), an IR microscope (Bio-Rad UMA-500) and a MCT narrow band detector. A sample of powdered whole grains of Pisane HD or peas was applied onto a Graseby Specac PN 2550 diamond cell (Graceby Specac, Orpington, Kent, UK) and analyzed in transmission mode at a resolution of 4 cm^{-1}. Data were processed using Win-IR 2.5 software of Bio-Rad.

DESCRIPTION OF THE MOLECULAR COMPOSITION OF UNTREATED MATERIAL

DTMS-CI-NH3

Untreated peas

Polysaccharides, proteins and lipids (Table 3.1) are the common constituents of the cotyledons of peas. The polysaccharides are represented mainly by starch and hemicelluloses (Daveby et al., 1993). The proteins legumin, vicilin and albumin are the storage proteins of peas (Belitz and Grosch, 1999). DTMS-CI-NH_3 experiments were carried out on samples of cotyledons of peas to study their chemical nature. The total ion current (TIC) of the DTMS-CI-NH_3 experiment for the sample of untreated peas shows a sharp product distribution with the apex *(Tmax)* at 52 scans. The mass spectrum of the untreated peas (Fig. 3.1) shows the polymer characteristics of starch. The main mass peak is *m/z* 180, the ammonium ion adduct of 1,6-anhydroglucose (levoglucosan) followed by the series of masses at *m/z* 342, 504, 666 and 828 corresponding to oligosaccharides with a 1,6-anhydrohexose terminal group (Tas et al., 1989). Other significant mass peaks are the dehydration products *m/z* 162 (ammonium adduct of dianhydrosugars) and 144 (ammonium adduct of trianhydrosugars). Additional series of ions are present with a starting mass of *m/z* 222 and 240. These two series, with a relative low intensity, i.e. *m/z* 222, 384, 546, 708 and *m/z* 240, 402, 564, 726, 888 represent series of oligosaccharides with attached ring-cleavage fragments (Lomax et al., 1991b).

The hemicelluloses are represented by anhydropentosans (xylans) in the series *m/z* 150, 282, 414 and 546 (Lomax et al., 1991a). A dehydration product is *m/z* 132. The peaks at *m/z* 146 and 164 are indicative of the presence of deoxyhexose residues. The peak at *m/z* 134 is a hexosan pyrolysis product formed due to the presence of inorganic material in the sample (Scheijen, 1991), potassium in the case of peas (Table 3.2).

	wt%
K_2O	1.00
P_2O_5	0.54
CaO	0.01
S	0.27

Table 3.2
Mineral content in peas (P. Sativum) var. GE

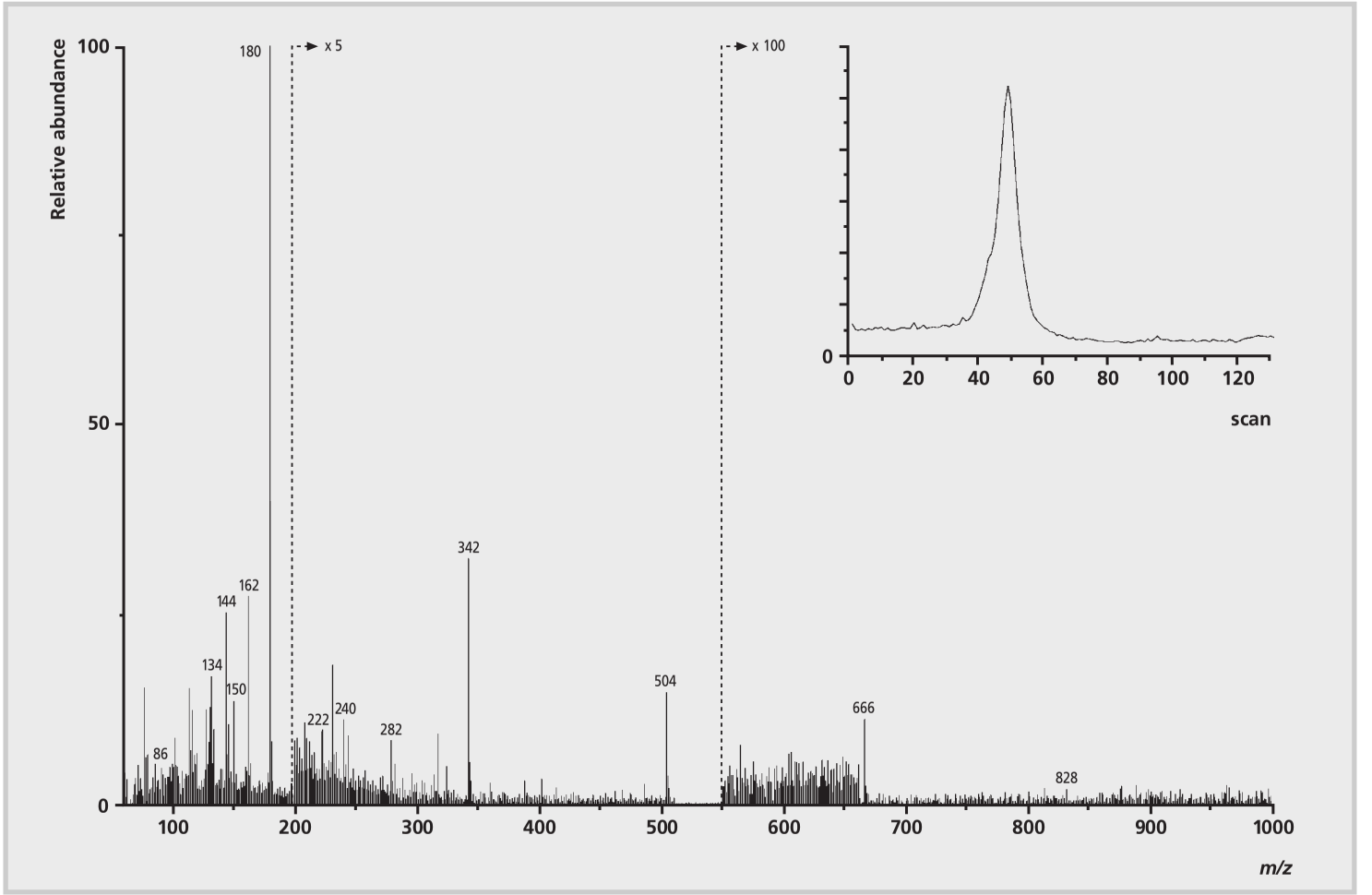


Fig. 3.1
DTMS-CI mass spectrum of untreated P. sativum (var. GE), insert: TIC

It is known that proteins could reach a content of 25 wt% or more in the cotyledons of peas (Table 3.1). However, due to the high relative abundance of polysaccharide pyrolysis products in the CI spectrum (Fig. 3.1) no clear sign of markers of protein is observed. However, on the high temperature side of the TIC trace the summated spectrum, from scan 55 to 70, shows that ions are released (not shown) attributed to proteins, like *m/z* 84, 86, 101, 115, 131 and 146 (Tas, unpublished results).

No information was obtained about the presence of lipids, as they ionize generally poorly under these conditions.

Pisane HD

The relative abundance of polysaccharide pyrolysis products did not allow for a clear recognition of protein markers. Therefore an experiment with a sample of protein isolate of peas was carried out. The CI spectrum (Fig. 3.2) of Pisane HD shows an envelope of mass peaks *m/z* 100 to 240. Many of these masses have been found previously in protein rich material (Tas, unpublished results; van de Arendonk et al., 1997), of which the most prominent are *m/z* 70, 84, 86, 101, 103, 115, 131, 139, 141, 146, 153, 155, 165, 167, 181, 186, 195, 212 and 226. These masses are considered as a fingerprint of proteins in the CI spectra of peas. Masses

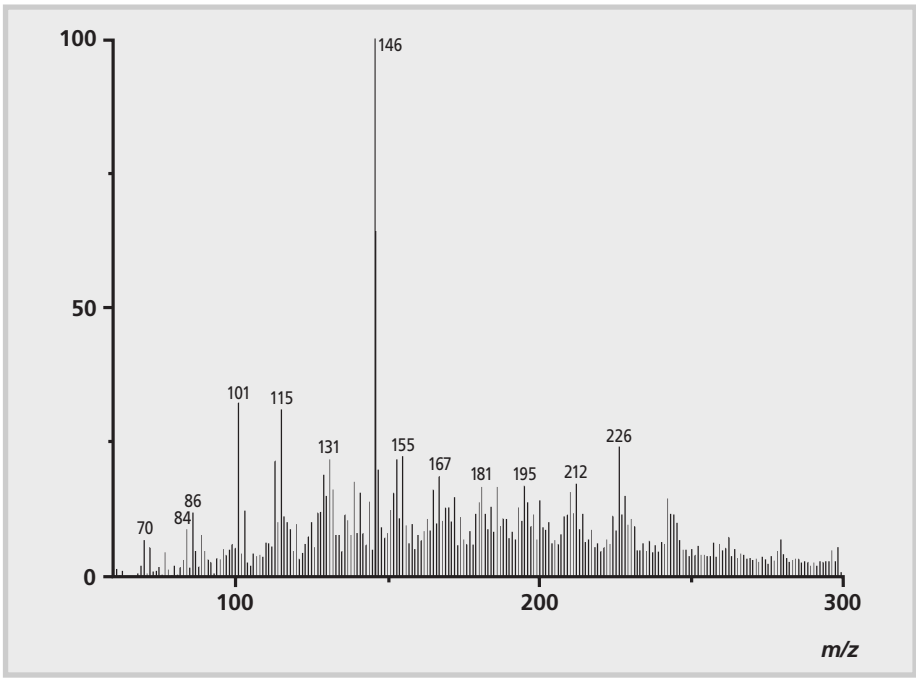


Fig. 3.2
DTMS-CI mass spectrum of Pisane HD (pea protein isolate)

m/z 84, 101, 115 and 146 have been tentatively attributed to Lys and Glu, amino acid amides (Tas, unpublished results). The ion series *m/z* 139, 153, 167, 181 and 195 have been proposed as unsaturated imidazole compounds originating from proteins (De Waard et al., 1992). As explained above the presence of proteins in cotyledons of peas is difficult to observe.

DTMS-EI

Untreated peas

DTMS under EI conditions has the potential to reveal more information on the proteins and lipids in peas, because the sugar pyrolysis products are strongly fragmented under these conditions. A lower peak at the early stage of the temperature ramp and a main peak at a higher temperature characterize the TIC. The trace shows a sharp product distribution with the apex of the main peak at 62 scans.

The EI spectrum of the untreated pea (Fig. 3.3a) shows the characteristics of polysaccharides (starch and hemicelluloses), proteins and lipids. The masses representing starch are the fragment ions *m/z* 43, 57, 60, 73, 98, 126 and 144, derived from hexosesugars (van der Kaaden et al., 1983; Ohnishi et al., 1975; Genuit and Boon, 1985; Helleur, 1987; Pouwels et al., 1989). Pentosesugars representing hemicelluloses are anhydroxyloses with *m/z* 85 and 114 (van der Kaaden et al., 1983; Pouwels and Boon, 1990).

Low voltage EI proteins markers are usually found in the range of *m/z* 130-220 (van de Arendonk et al., 1997). Typical ions in this range and the lower mass range are present in the spectrum of Fig. 3.3a, but are more prominent in the summated spectrum from the high temperature side (between scans 70 and 80) of the TIC trace (Fig. 3.3b). Visible are *m/z* 69 (Val, Leu, Lys, Gln); 70, 154, 194 (Pro, Arg, Lys); 84 (Glu, Val, Lys) 91, 92 (Phe); 94, 107, 108 (Tyr); 117, 131 (Phe); 138 (Leu, Hpro); 152, 166, 180 (Leu) and 186 (Hpro) (Scheijen, 1991; Eglinton et al., 1996; Boon and de Leeuw, 1987; Stankiewicz et al., 1996; Chiavari and Galletti, 1992). These amino acids are also present in the proteins of peas (Derbyshire et al., 1976).

The mass spectrum (Fig. 3.3c) of the low peak (scan 40-50) relates to a fraction comprising 'free' lipids such as fatty acids (m/z 228, $C_{14:0}$; 256, $C_{16:0}$; 260, M-H_2O $C_{18:3}$; 262, M-H_2O $C_{18:2}$), sitosterols (m/z 396-414, $C_{29:1}$), diglycerides (m/z 550, $C_{16:0,16:0}$; 576, $C_{16:0,18:1}$; 596, $C_{16:0,18:0}$ and 616, $C_{18:2,18:2}$) and triglycerides (m/z 854, $C_{16:0,18:2,18:2}$ and 878, $C_{18:2,18:2,18:2}$). Characteristic for triglycerides is the presence of ions in the mass spectra that correspond to the loss of a fatty acid carboxyl radical [M-RCOO] from the molecular ion as can be seen at m/z 574 and 600. Other ions that correspond to an additional 74 amu to the acylium ion, i.e. [RCO+74] are also present. These ions are present at m/z 313 ($C_{16:0}$), 337 ($C_{18:2}$) and 339 ($C_{18:1}$).

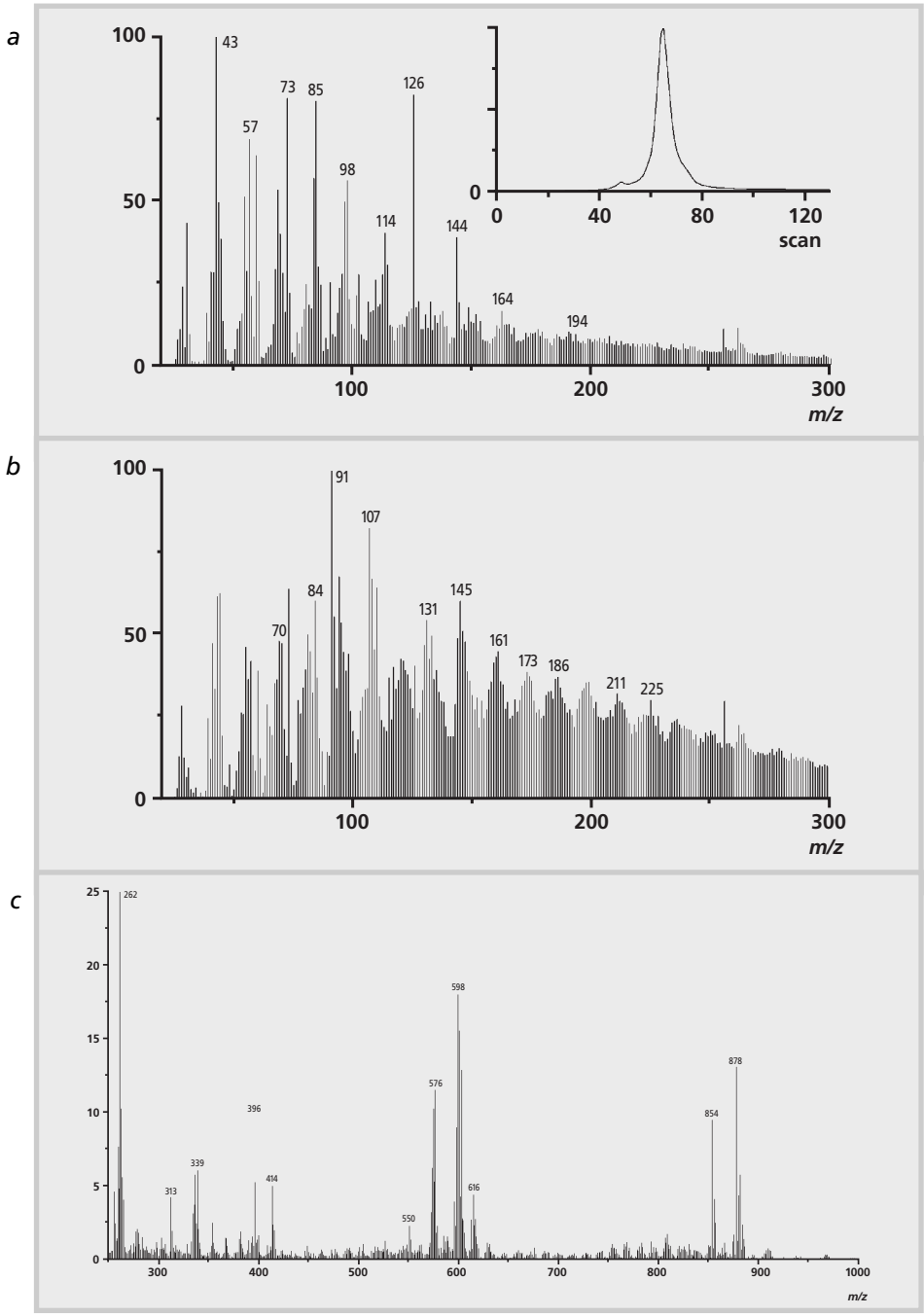


Fig. 3.3
DTMS-EI measurements of untreated P. sativum (var. GE). (a) Mass spectrum, insert: TIC; (b) mass spectrum (scan 70-80) showing protein markers and (c) mass spectrum (scan 40-55) showing lipids, note the different scale.

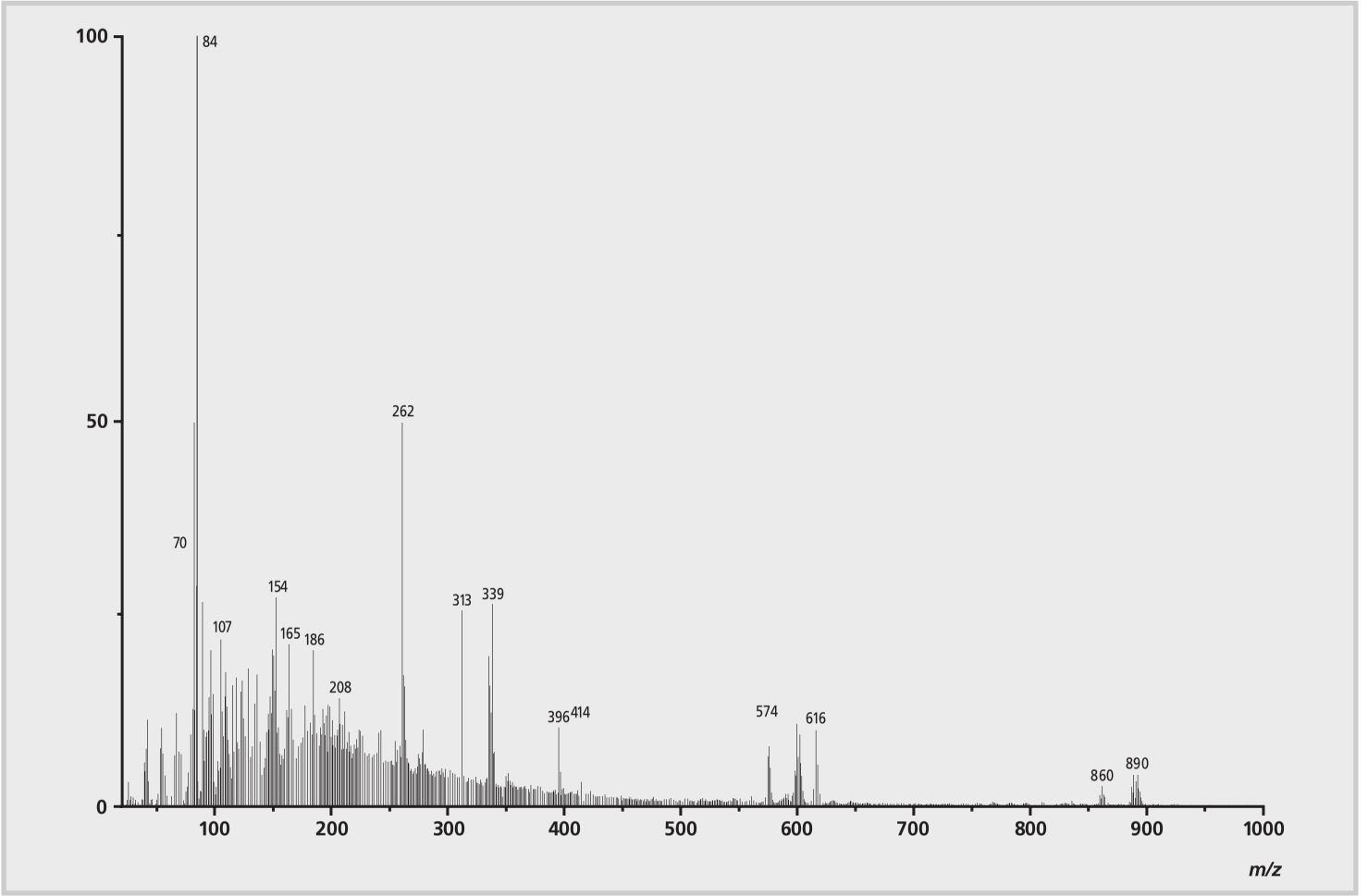


Fig. 3.4
DTMS-EI mass spectrum of Pisane HD (pea protein isolate)

Pisane HD

The EI spectrum (Fig. 3.4) of Pisane HD is characterized by the following ions: *m/z* 69, 70, 84, 86, 91, 94, 107, 108, 117, 130, 138, 152, 154, 165, 166, 168, 180, 186, 194 and 208. All these masses have been in found in the mass spectrum of untreated peas (Fig. 3.3b) and in protein rich material (van de Arendonk et al., 1997). The corresponding amino acids are common in the proteins of peas (Derbyshire et al., 1976). The masses can be considered as the characteristic protein markers for peas. The ions *m/z* 70, 84 and 154 show the highest relative intensity. The mass spectrum shows ions representing lipids: fatty acids (*m/z* 262, M-H_2O $C_{18:2}$); sitosterols (*m/z* 396-414, $C_{29:1}$); diglycerides (*m/z* 574, M-H_2O $C_{16:0,18:2}$ and 616, $C_{18:2,18:2}$) and triglycerides (*m/z* 860, M-H_2O $C_{18:2,18:2,18:2}$ and 890, $C_{18:0,18:0,18:0}$). The ions *m/z* 313 ($C_{16:0}$), and 337 ($C_{18:2}$) are present as well. This is well in accordance to the lipids found in the investigated peas.

OPTIMIZATION OF THE HEATING TIME

To determine the optimal heating time samples of peas were heated at T_{oven} = 250 °C as a function of time. A temperature of 250 °C was selected, since from this temperature the polysaccharides and proteins start their conversion into compounds that are more resistant to the natural degradation processes. The results of the physical and bulk chemical experiments allowed for a heating time of 60 minutes (Braadbaart et al., 2004a). DTMS-EI experiments were carried out on samples of residues of peas heated at 250 °C to investigate the change of the chemical nature of the cotyledons as a function of time. The apex *(Tmax)* of the TIC of the experiments shifts under these conditions to a higher temperature after 45 minutes of heating and the TIC traces start to broaden on the high temperature side in samples subjected to more than 37.5 minutes of heating. A lower peak at the early stage of the temperature ramp, due to the evaporation of more volatile components (lipids) and a dominant peak at a higher temperature as a result of the dissociation of biopolymeric constituents, characterize the TIC.

The spectrum of the sample of the residue heated for 37.5 minutes (not shown) has still an ion distribution that is dominated by masses representing starch (*m/z* 43, 57, 60, etc.), proteins (*m/z* 70 and 84) and lipids. Masses such as *m/z* 95, 96, 110 point to the presence of furans. After 45 minutes of heating the distribution has changed into an aromatic character showing alkylphenols (*m/z* 94, 108), alkylbenzenes (*m/z* 91, 105) and clusters of three mass peaks such as: *m/z* 145, 146, 147; 159, 160, 161; 173, 174, 175; etc. These clusters correspond to a homologous series of condensed aromatic compounds, of which the even numbered masses are the typical products of heat treatment of polysaccharides, such as cellulose and starch (Pastorova et al., 1993a; 1994). Typical protein markers for untreated peas like *m/z* 70, 84, 117, 131 etc. are recognized. No further change is observed in the spectra of the samples of residues heated for 60 minutes (Fig. 3.11a) and 120 minutes (not shown). Masses representing lipids (i.e. *m/z* 228, 262, 396-414, 596, 616, 854 and 878) continue to be present.

The DA of the EI spectra that are the result of heating at 250 °C as a function of time is presented in Fig. 3.5. The score plot of the first discriminant function (DF1) describes 30 % of the total variance of 82 % between the mass spectra of the samples. The higher functions are disregarded. The full mass range of 20-1000 was used. DF1 describes the changes in the molecular composition as a function of the heating time. The chemical changes accelerate up to 15 minutes to be followed by a first plateau till 30 minutes; a similar plateau is observed in the graph of the weight loss versus time (Braadbaart et al., 2004a). Next the changes accelerate again to the final plateau at 45 minutes. DF1- corresponds primarily to mass peaks characteristic for hexose and pentose polysaccharides in untreated peas that

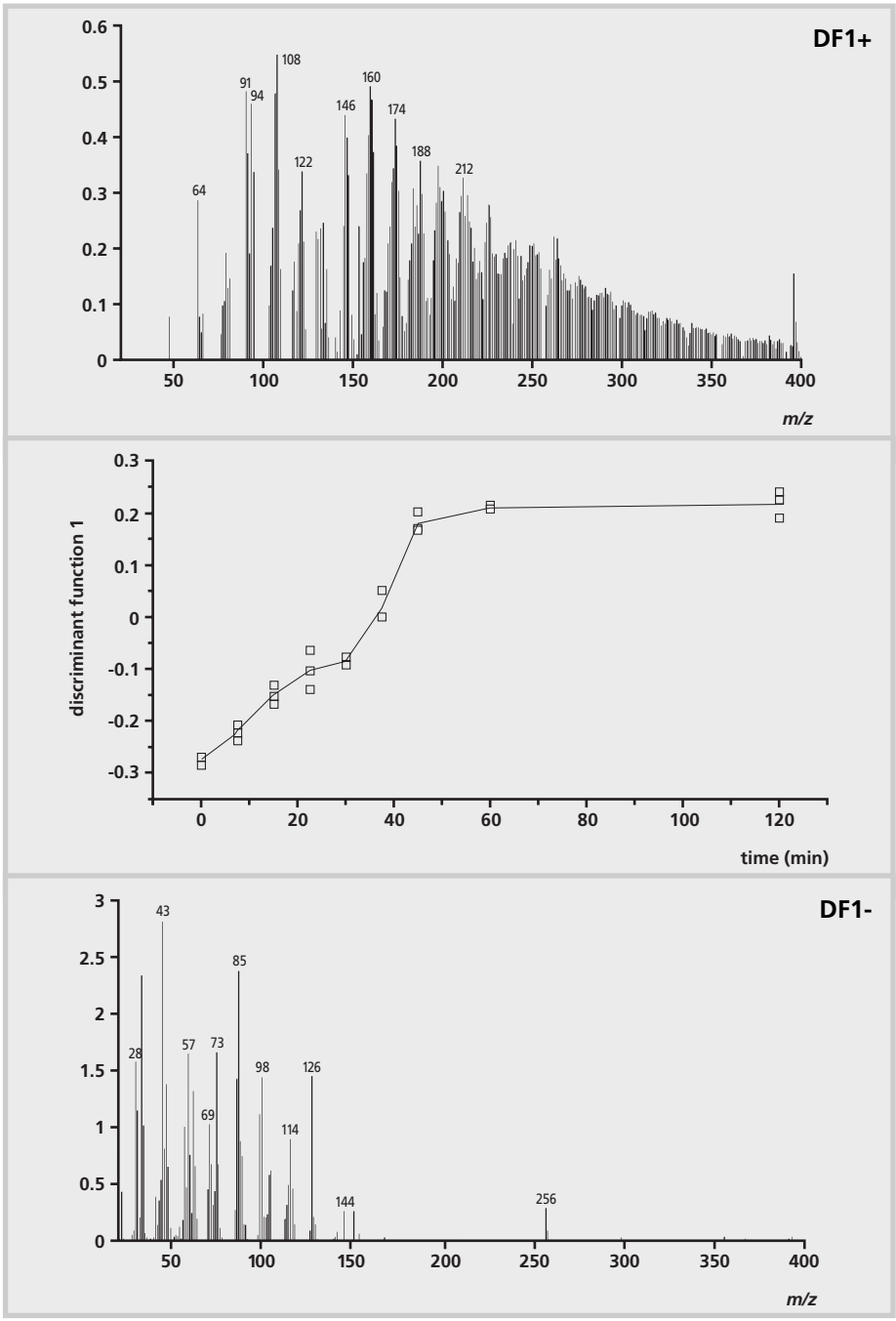


Fig. 3.5
Score plot from discriminant analyses of DTMS-EI measurements of P. sativum (var. GE) untreated and heated for 60 minutes (250 °C) as a function of time. Each sample was analysed in triplicate. DF1+ and DF1- are the "numerically extracted" mass spectra responsible for the separation into polysaccharides-rich and aromatics-rich samples, respectively.

decrease as the temperature rises. DF1+ characterises the solid residue as the result of the heat treatment. The chemical composition, as described by DF1, remains stable for heating times above 45 minutes at 250 °C. For this reason a heating time of 60 minutes is considered sufficient.

DESCRIPTION OF THE MOLECULAR COMPOSITION OF HEAT-TREATED MATERIAL

DTMS-CI-NH3

Heat-treated Pisane HD

Protein markers were not clearly recognized in untreated peas under CI conditions. In order to be able to recognize these markers in heated peas Pisane HD was heated at 310, 370, 400, 440 and 500 °C for 60 minutes. The spectrum of Pisane HD heated at T_{oven} = 310 °C (Fig. 3.6a) shows identical mass peaks as present in the spectrum of untreated Pisane HD (Fig. 3.2), but the relative intensity is substantially increased, especially *m/z* 101, 115 and 146 reaching a relative intensity of almost 100 % and *m/z* 226 reaching 75 %. In addition a new series of ions is recognized consisting of *m/z* 147, 161, 175, 189, etc. Heating experiments with microcrystalline cellulose (Pastorova et al., 1993b) attributed this series to pyrolysis products of polysaccharides. However, as the content of carbohydrates in Pisane HD is maximum 4.5 %, these ions are to be considered as indicative for the formation of N-containing heterocyclic compounds (Moldoveanu, 1998). The spectrum of Pisane HD heated at T_{oven} = 400 °C (Fig. 3.6b) shows a different mass distribution. The protein markers as present in the sample heated at 250 °C show a much lower relative intensity, but *m/z* 101 and 115 still reach a relative intensity of 30 % and 146 of 70 %. Alkylphenols (*m/z* 94, 108, 122), alkylbenzenes (*m/z* 91, 105, 119) and the ion series *m/z* 147, 161, 175, 189 are prominent. At higher temperatures these ions become less prominent and at T_{oven} = 500 °C only *m/z* 94 is recognized. Compared with the sample of Pisane HD heated at 250 °C the TIC has shifted from 60 to 90 scans for the sample heated at 440 °C pointing to a much higher degree of condensation.

Residues of heat-treated peas

The CI spectra of the samples of residues130 to 220 (not shown) are similar to that of the untreated pea (Fig. 3.1). At T_{oven} = 235 °C the relative intensity of the higher masses indicative of the polysaccharides decreases. From T_{oven} = 250 °C the TIC starts to broaden on the high temperature side. The apex is shifting to a higher temperature, but remains constant for samples of residues heated at higher oven temperatures. The shift points to the presence of an increased amount of more condensed thermally stable material. The chemical changes that accompany these features are expressed in their mass spectra.

The spectrum of the sample of residue250 (Fig. 3.7a) shows the first major changes in comparison to the spectrum of the untreated pea. Concerning the polysaccha-

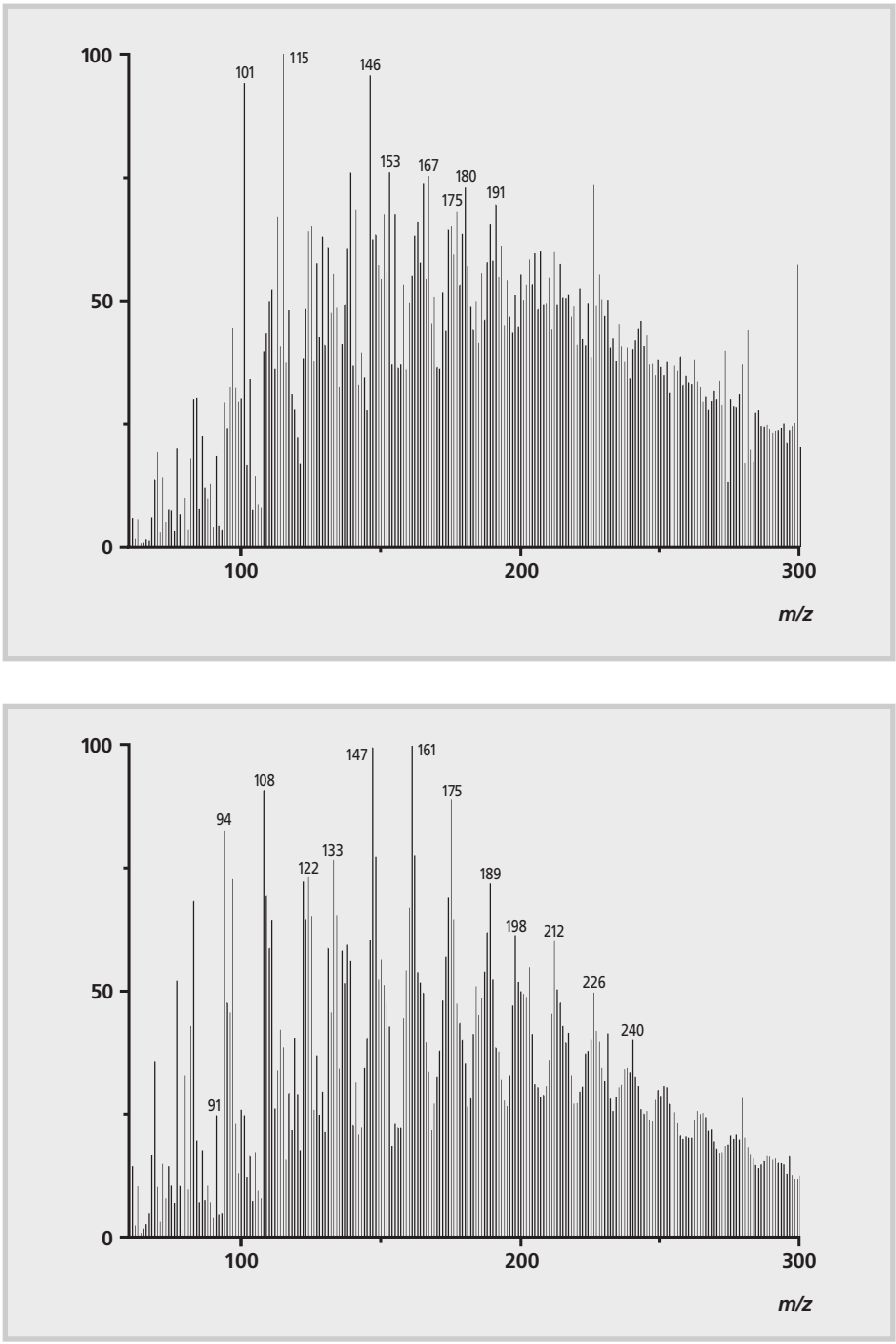


Fig. 3.6
DTMS-CI mass spectrum of Pisane HD (pea protein isolate) heated for 60 minutes at: (a) 310 °C and (b) 400 °C

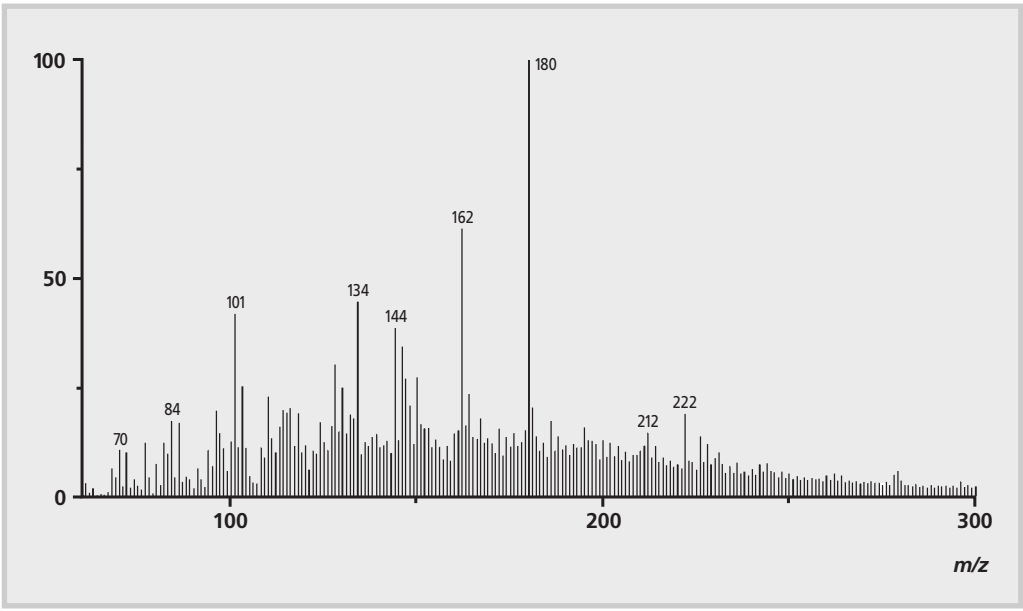
rides the oligosaccharide pyrolysis products at *m/z* 384, 402, 414, 474, 504 and higher, representing hexosan and pentosan moieties, are no longer present. On the other hand, monosaccharides with attached sugarring-cleavage fragments such as *m/z* 222 and 240 show a higher relative intensity in the solid residue. The mass spectrum also shows an increase in the relative intensity of *m/z* 128 $[M+NH4]^+$ corresponding to a compound with a molecular weight of 110. This ion (*m/z* 128) has been identified as the pseudomolecular ion of different furan moieties with the same mass (Pastorova et al., 1993b), which are known to be pyrolysis products of carbohydrates.

A new series of ions with odd numbered masses like *m/z* 147, 161, 175, 189, 201 and 213 starts to appear at T_{oven} = 270 °C in the summated spectrum from scan 90 to 115 at the high temperature side of the TIC (Fig. 3.7b). It shows the beginning of a new thermo-stable phase, which is characteristic for heat-treated polysaccharides, in this case starch and represent condensed aromatic units (Pastorova et al., 1993a). As described above this series is also present in the spectrum of the heated samples of the protein isolate Pisane HD containing 90 % protein.

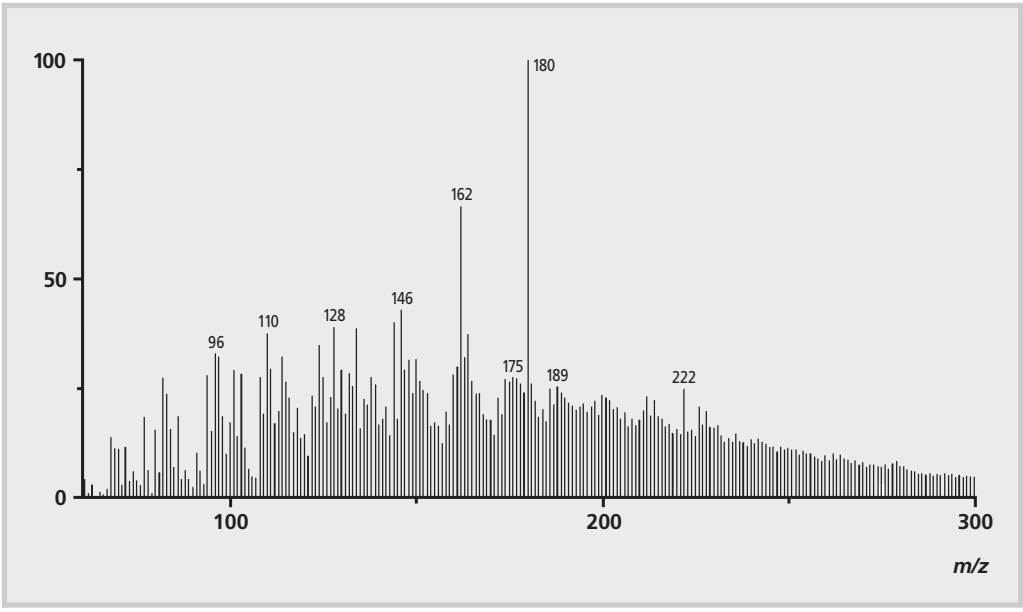
The spectrum of the samples of the residues290 (not shown) shows an increase of the odd numbered masses that are observed in the spectrum of residue270 (Fig. 3.7b). The spectrum of the sample of residue310 (Fig. 3.7c) shows that *m/z* 162 has still a relative intensity of 100 % and *m/z* 180 of 70 %, which means that structurally intact anhydromonosaccharide moieties can still be released from the residues even when they have been heated to 310 °C for 60 minutes. From T_{oven} = 250 °C the character of the spectra is changing from a number of single polymers into a three-dimensional network polymer at 310 °C.

As shown in the spectrum of untreated Pisane HD (Fig. 3.2) masses such as *m/z* 101, 115, 146 and the series *m/z* 153, 167, etc. can be considered as protein markers. In the spectra of untreated peas and those heated up to 235 °C protein markers are not very well recognized under CI conditions. This changes from 250 °C when the relative intensities of these ions become stronger. For example, at T_{oven} = 310 °C (Fig. 3.7c) the relative peak intensities reach 95 % for *m/z* 101, 70 % for *m/z* 146 and 50 % for the series *m/z* 153, 167, etc. At 400 °C (Fig. 3.7d) these relative intensities reduced considerably and at 440 °C are not observed anymore. From T_{oven} = 310 °C the alkylphenols and alkylbenzenes, as recognized in heated Pisane HD, are present in the samples heated up to 440 °C. The odd numbered series *m/z* 147, 161, 175, etc. has a strong presence at 400 °C (Fig. 3.7d) and is still present at 440 °C. The series can be attributed to pyrolysis products from polysaccharides (condensed aromatic compounds) as well as from proteins (N-containing heterocyclic compounds). High resolution data could resolve this ambiguity, but such experiments are not possible under DTMS conditions on our instrument. The heating experiments with microcrystalline cellulose (Pastorova et al., 1993a) do not allow for a comparison, as the highest temperature during these experiments was 310 °C only.

a


100
50
0
180
162
134
144
101
84
70
212
222
100
200
300
m/z

b


100
50
0
180
162
146
128
110
96
175
189
222
100
200
300
m/z

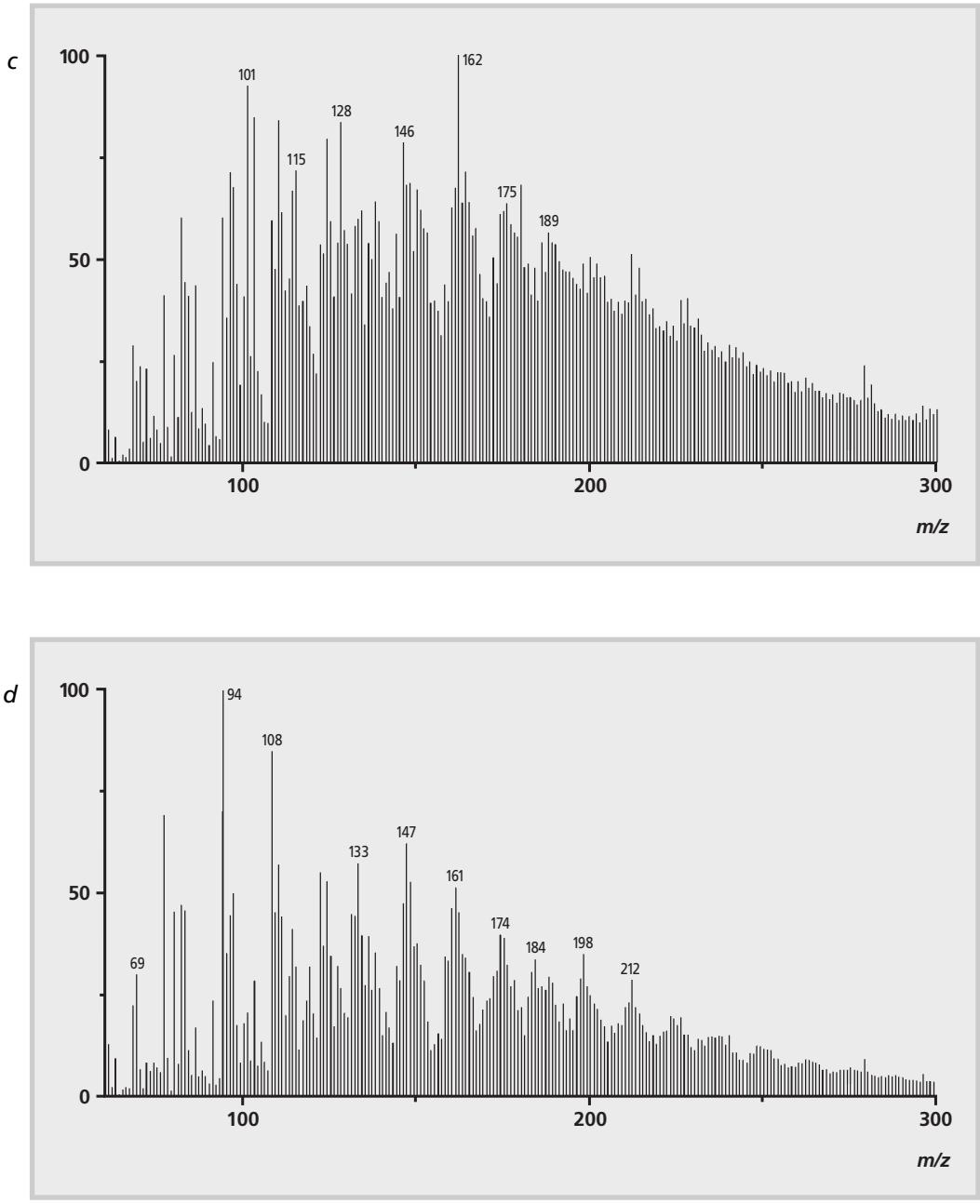


Fig. 3.7
DTMS-CI measurements of P. sativum (var. GE) heated for 60 minutes: (a) mass spectrum of residue250; (b) mass spectrum of residue270 (scan 90-100) showing ions representing condensed aromatic compounds; (c) mass spectrum of residue310 and (d) mass spectrum of residue400

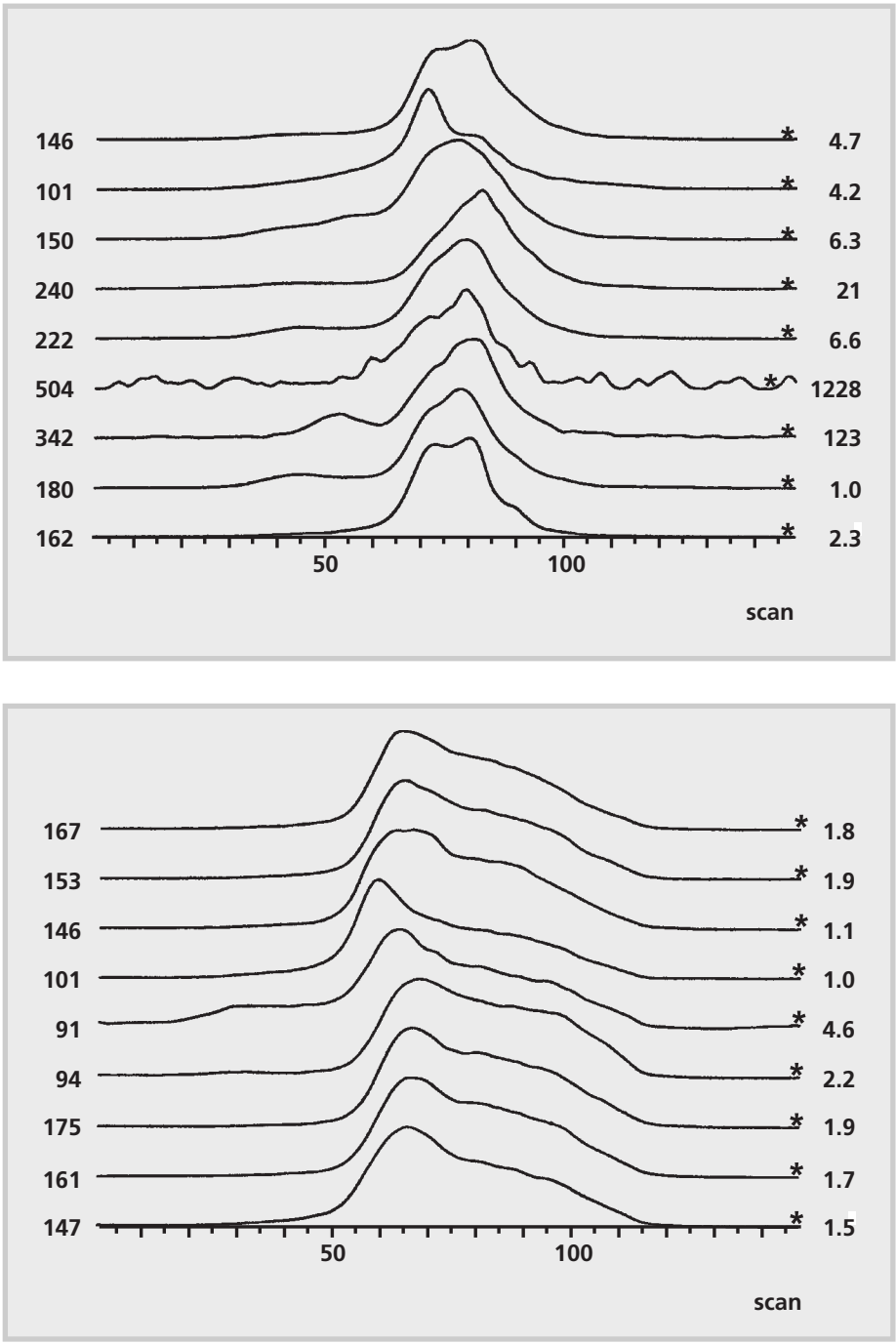


Fig. 3.8
DTMS-CI mass chromatographs of P. sativum (var. GE) heated for 60 minutes: (a) for the indicated compounds of residue250 and (b) for the indicated compounds of residue290

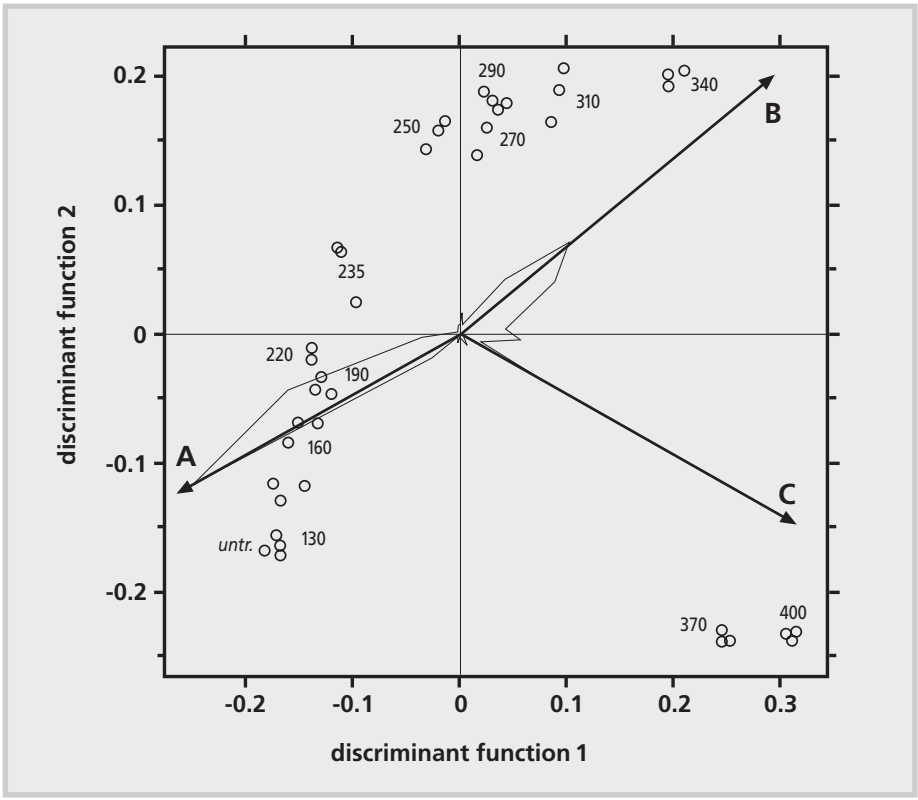
The TIC of residue250 (Fig. 3.8a) was studied by the time-resolved mass chromatograms (MC's) of the following ions: *m/z* 162, 180, 342, 504, 222, 240 and 150 (hexoses and pentoses) and 101 and 146 (protein markers). The apex of the trace has shifted to a higher temperature compared with the trace of the untreated pea (Fig 3.1). Protein markers *m/z* 101 and 146 appear at a low temperature together with anhydro-oligosaccharides like *m/z* 180, 162, 150, 222 and 240. Both the *m/z* 146 and the anhydromonosaccharides *m/z* 222 and 240 have a second peak at higher temperatures. It means that ion *m/z* 146 represents a protein marker as well as a deoxysugar. At T_{oven} = 290 °C (Fig. 3.8b) the shape of the trace of the protein markers (*m/z* 101 and 146) has hardly changed, but the relative intensity has increased. The odd-numbered masses *m/z* 147, 161, 175, etc. show two peaks, one at 65 and a second one at 85 scans. This indicates the presence of two identical series of masses, which are pyrolysis products of either polysaccharides or proteins.

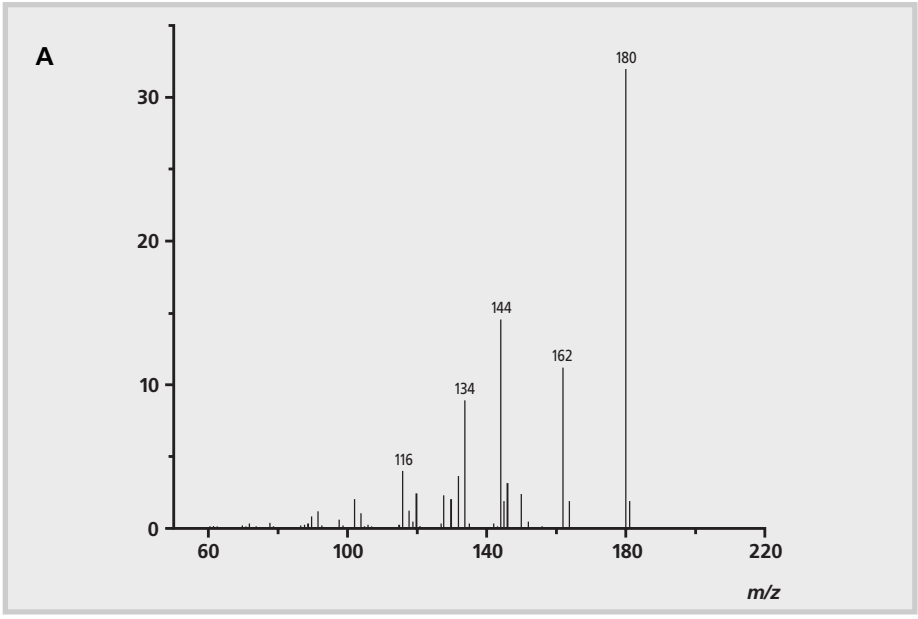
DTMS-NH3-CI spectra of the samples of untreated peas and residues130 to 400 were subjected to discriminant analysis (DA). The first discriminant function (DF1) describes 46 % and the second discriminant function (DF2) 21 % of the characteristic variance. The higher functions were disregarded. The full mass range of 60-1000 was used. The score map (Fig. 3.9) describes the relative differences in the molecular composition of the samples plotted as discriminant scores as a function of the oven temperature. DF1 describes the conversion of the polysaccharides into aromatic compounds. DF2 shows the presence of protein markers at 250 °C and the conversion of these into heterocyclic compounds, alkylphenols and alkylbenzenes at 440 °C. This is shown in the product axes A, B and C and their "numerically extracted" spectra, which are incorporated in the score map of Fig. 3.9. It shows the presence of protein markers up to 340 °C and related aromatic and heterocyclic compounds up to 400 °C. Thus proteinaceous marker moieties can be considered as relatively thermally stable.

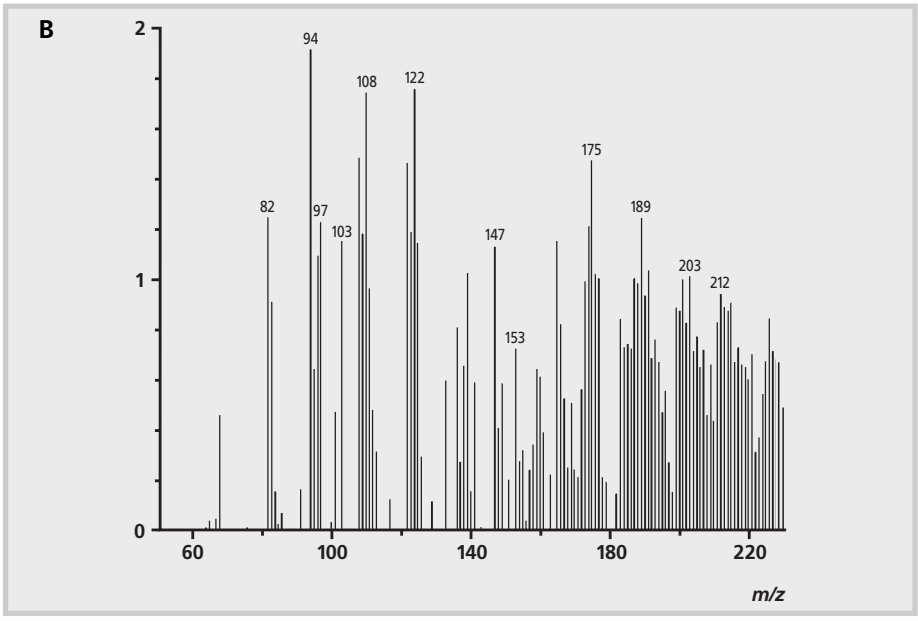
DTMS-EI

Heat-treated Pisane HD

DTMS-EI experiments were carried out on samples of Pisane HD heated for 60 minutes at the various oven temperatures. The EI spectrum of the sample heated at 310 °C (Fig. 3.10a) is characterized by a prominent presence of ions, which are related to alkylated phenols and benzenes such as *m/z* 91, 92, 94, 105, 107, 108, 119 and 122. Next a homologous series of odd numbered masses (*m/z* 117, 131, 133, 147, 161, 175, 189, 199, 213, 227, etc.) is observed, which have been tentatively attributed to N-containing heterocyclic compounds (Moldoveanu, 1998). An identical series is recognized in the CI spectrum (Fig. 3.7d). The ions *m/z* 70 and 84 are still present. The spectrum also shows masses related to lipids (i.e. *m/z* 396-414 from sitosterol $C_{29:1}$). The spectrum of the sample heated at 400 °C (Fig. 3.10b) is


discriminant function 2
discriminant function 1
0.2
0.1
0
-0.1
-0.2
-0.2
-0.1
0
0.1
0.2
0.3
290
310
340
250
270
235
220
190
160
130
untr.
370
400
A
B
C


A
180
144
162
134
116
30
20
10
0
60
100
140
180
220
m/z



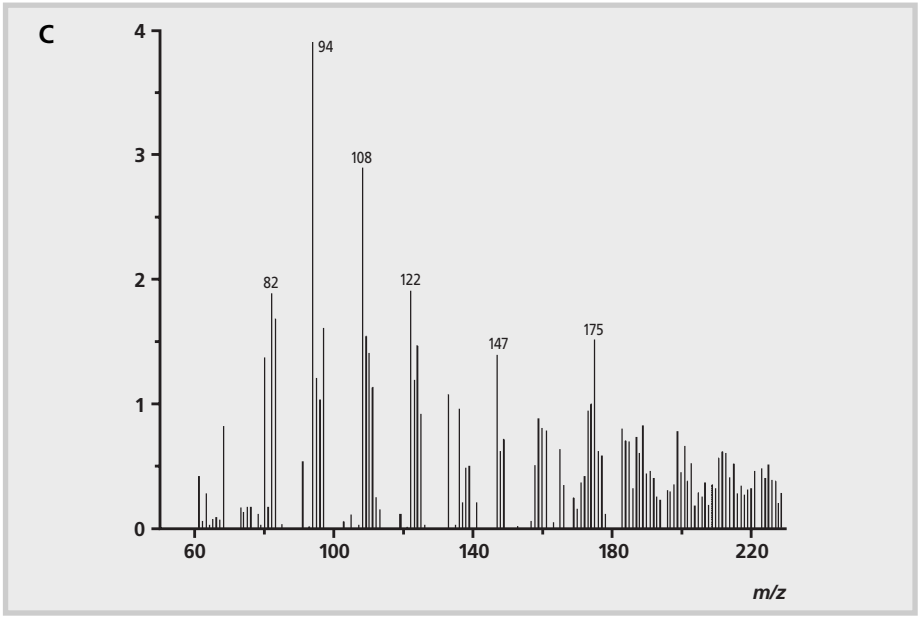


Fig. 3.9
Score plot from discriminant analyses of DTMS-CI measurements of P. sativum (var. GE) untreated and heated for 60 minutes. Each sample was analysed in triplicate. The heating temperatures (in °C) are indicated in the figure. Product axes A, B and C are superimposed and the "numerically extracted" mass spectra for each axis are shown.

practically identical to that of 310 °C, but the series of odd numbered masses is decreasing. Now the dominant peaks are even numbered (*m/z* 146, 160, 174, etc.). This phenomenon becomes stronger at higher temperatures. At 440 °C the spectrum (Fig. 3.10c) is characterized by a strong presence of the ions *m/z* 27, 41 (N containing fragments representing HCN and CH_3CN), 28 (CO), 78 (benzene) and 91, 92, 94, 107, 108, 117, 122 and 131. The series of even numbered masses is still present. In the spectrum of 500 °C the only ions present are *m/z* 27 (HCN), 28 (CO), 44 (CO_2), 64 (SO_2), 78 (benzene) and 92 (toluene).

Pea residues heated for 60 minutes at a constant temperature

DTMS-EI measurements were also carried out on samples of the residues of peas heated at the various oven temperatures. The TIC traces show a sharp product distribution with temperature until T_{oven} = 235 °C. The traces start to broaden at the high temperature side from T_{oven} = 250 °C. The apex of the traces remains constant until T_{oven} = 235 °C, than rises until T_{oven} = 290 °C to remain constant until T_{oven} = 440 °C. At temperatures above T_{oven} = 440 °C the TIC becomes very irregular indicative of instrumental noise due to very low amounts of detectable compounds.

The EI spectra of the samples of residues130 until 220 have a similar mass distribution as the untreated pea (Fig. 3.3b). The spectrum of residue235 (not shown) points to changes in the distribution of the individual masses compared with the previous spectra and new mass peaks are observed. These phenomena are enhanced in the spectra of residue250 (Fig. 3.11a). The masses show the presence of furans (*m/z* 95, 96, 110), alkylphenols (*m/z* 94, 107, 108, 121, 122), alkylbenzenes (*m/z* 91, 105, 119) and clusters of three mass peaks with (CH_2) mass increments such as: *m/z* 146, 147, 148; 160, 161, 162; 174, 175, 176; 186, 187, 188; 198, 199, 200; 212, 213, 214; etc. The clusters correspond to a homologues series of condensed aromatic compounds, of which the even numbered masses are the typical products of heat treatment of polysaccharides, such as cellulose and starch (Pastorova et al., 1993a; 1994). In the spectra of residue270 (not shown) and higher the characteristic masses of polysaccharides are no longer observed. The masses representing furans are still present. New are the masses for (alkyl)phenols, (alkyl)catechols, (alkyl)benzenes and condensed aromatics as shown above. The mass distribution does not change from residue270 until residue310 (not shown), when furans are no longer present. From residue340 (Fig. 3.11b) to residue400 the spectra show a new mass distribution characterized by the presence of the masses *m/z* 78 (benzene) and 64 (SO_2 from sulfates), while at the same time the masses that represent the (alkyl)phenols, (alkyl)catechols and the condensed aromatics are still present. The amount of pyrolysis products in the spectra of the residues440 (Fig. 3.11c), 500, 600 and 700 is very low and decreases as a function of temperature. The spectrum of residue700 (not shown) shows mass *m/z* 44 (CO_2) and a homologous series of masses *m/z* 83, 97, 111 etc., fragment ions from aliphatic

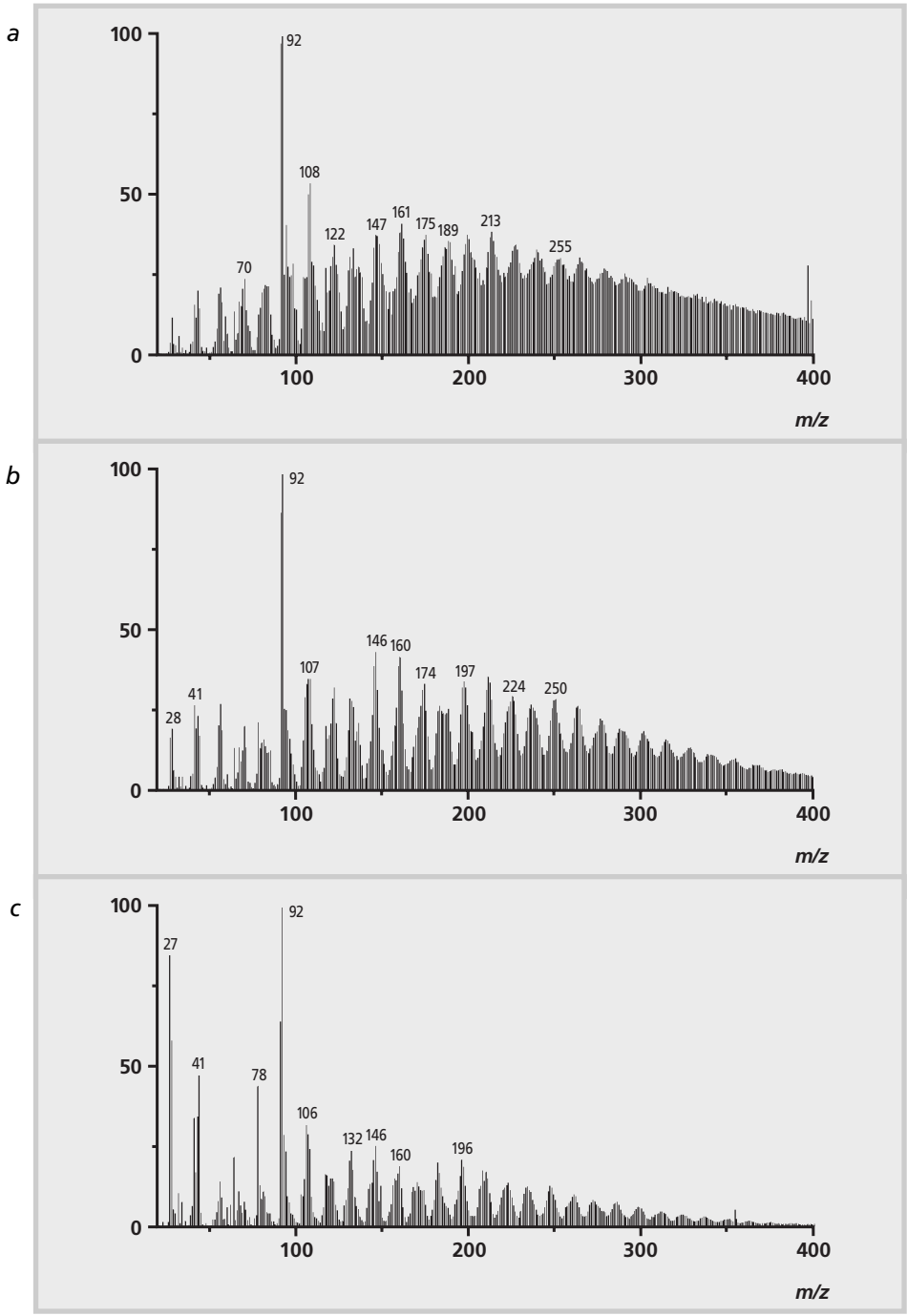


Fig. 3.10
DTMS-EI mass spectra of Pisane HD (pea protein isolate) heated for 60 minutes (a) at 310 °C; (b) at 400 °C and (c) at 440 °C

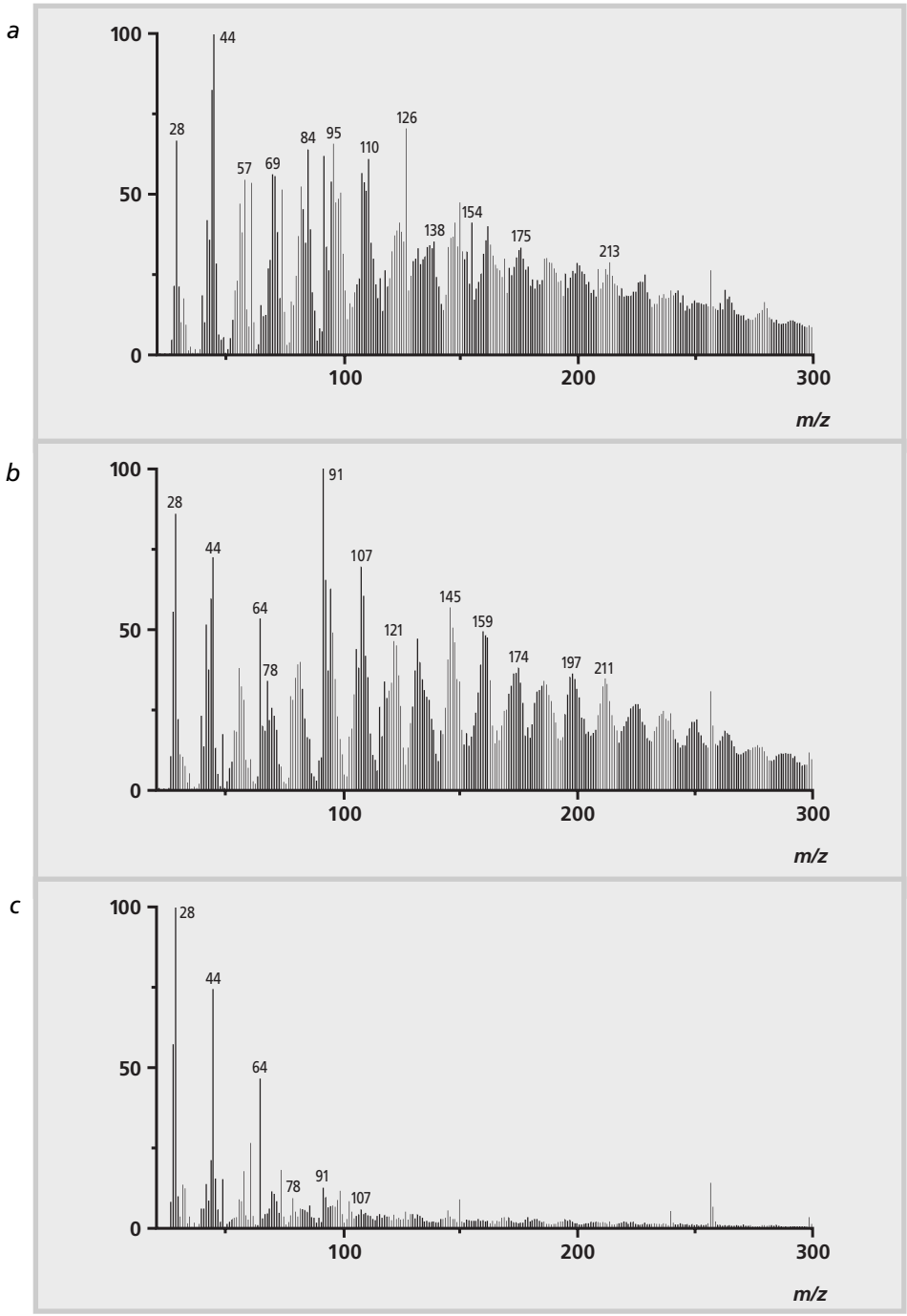


Fig. 3.11
DTMS-EI measurements of P. sativum (var. GE) heated for 60 minutes: (a) Mass spectra of residue250; (b) residue340 and (c) residue440

moieties. This latter series is considered to be background noise, as they are present in the blanks and in the all the summated spectra from the low temperature side of the TIC traces.

The protein markers (*m/z* 69, 70, 81, 84, 138, 152, 154, 166, etc.), as observed in the spectra of the samples of untreated Pisane HD (Fig. 3.4) and peas (Fig. 3.3b), are prominent up to residue290. From residue250 in addition to the ions *m/z* 117 and 131 more odd-numbered ions are observed. Two series can be distinguished, firstly *m/z* 117, 131, 145, etc., (alkyl)indole from Try and secondly *m/z* 147, 161, 175, etc. Both series are attributed to N-containing heterocyclic compounds and are recognized up to residue400 (Moldoveanu, 1998). From residue440 (Fig. 3.11c) the spectra are characterized by the presence of ions that represent N-containing compounds like *m/z* 27 (HCN) and 41 (CH_3CN), both pyrolysis products from amino acids (Simmonds et al., 1972), while CO is still released. It shows that proteins and/or residues of amide structures are present in the residues440 and higher. Proteinaceous moieties are clearly a rather thermally stable material.

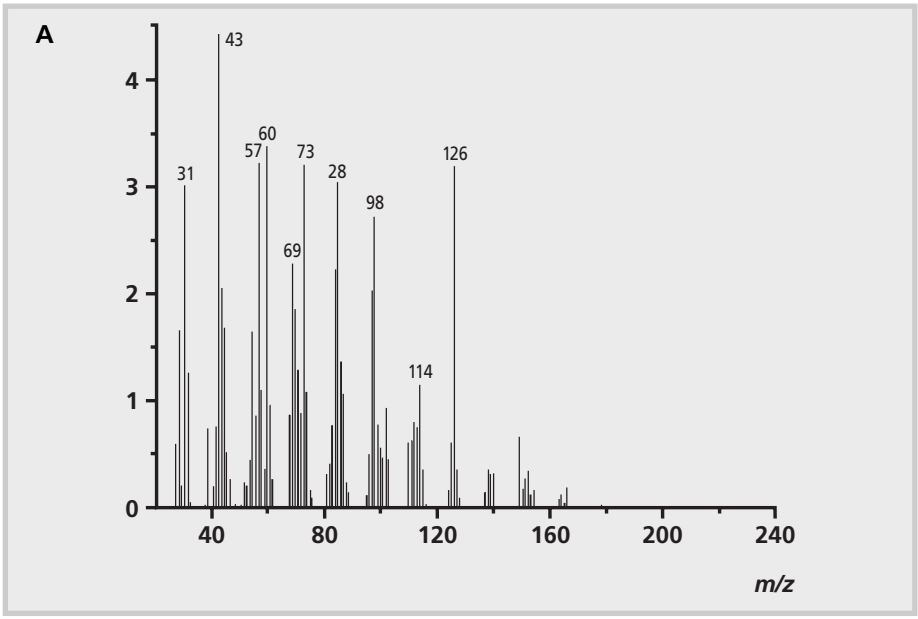
The volatile fraction of lipids is still present in residue310, but they are no longer observed in residue340.

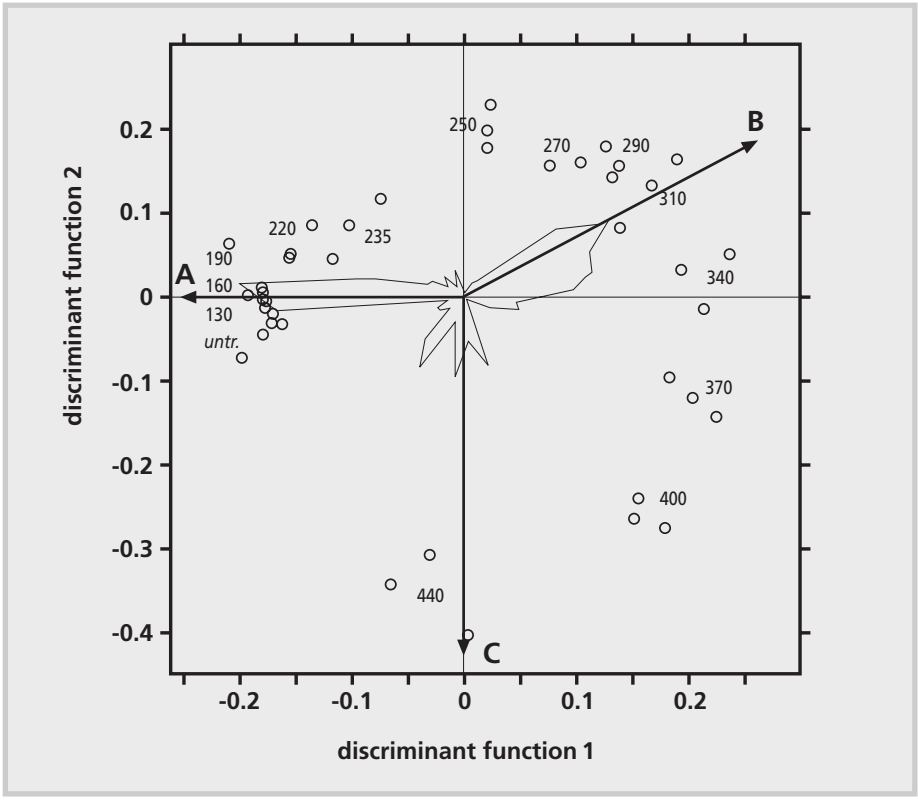
The phenomena that accompany the changes as a function of temperature are studied using DA for samples of untreated peas and the residues130 to 440. Residues from temperatures higher than 440 °C were ignored because not enough detectable pyrolysable material is present. The scores of the samples on the first (DF1) and the second (DF2) discriminant function are presented in a score map (Fig. 3.12). The first two functions describe, respectively 29 and 20 % of the total variance. The higher functions are disregarded. The full mass range of 20-1000 was used.

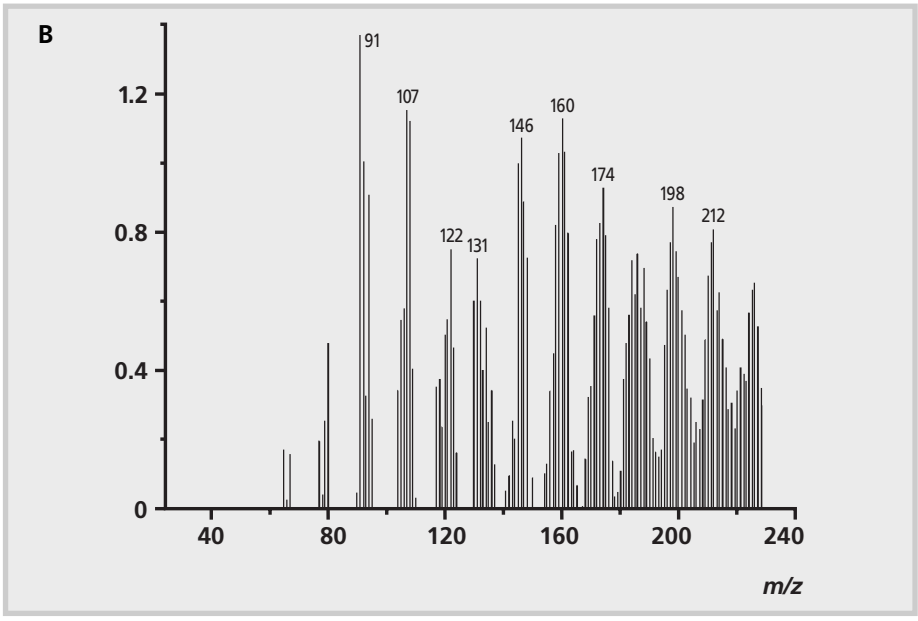
Each discriminant function, plotted as a discriminant spectrum, is characterized by a number of specific masses that are the pyrolysis products of the relevant samples of the residues. These products originate from the three main components of the cotyledons of peas: polysaccharides, proteins and lipids. The variance diagram, incorporated in Fig. 3.12, shows three directions in which the mass variables of several classes in the discriminant space tend to cluster. The chemical nature of each axis is visualized by their 'numerically extracted' spectra A, B and C, each showing the masses that are typical.

In spectrum A masses are visible that represent polysaccharides, proteins and lipids as described above. The centre of the cluster represents the samples of the untreated pea (the reactant) and the low temperature residues130, 160 and 190.

Spectrum B, corresponding to residue310, shows the masses representing (alkyl) phenols (*m/z* 94 and 108), (alkyl)benzenes (*m/z* 78, 91, 92, 105), condensed aromatic and heterocyclic compounds. This shows the conversion of the reactant


A
43
60
57
73
31
28
126
98
69
114
0
1
2
3
4
40
80
120
160
200
240
m/z


B
A
C
250
270
290
310
220
235
190
160
130
untr.
340
370
400
440
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.2
-0.1
0
0.1
0.2
discriminant function 2
discriminant function 1



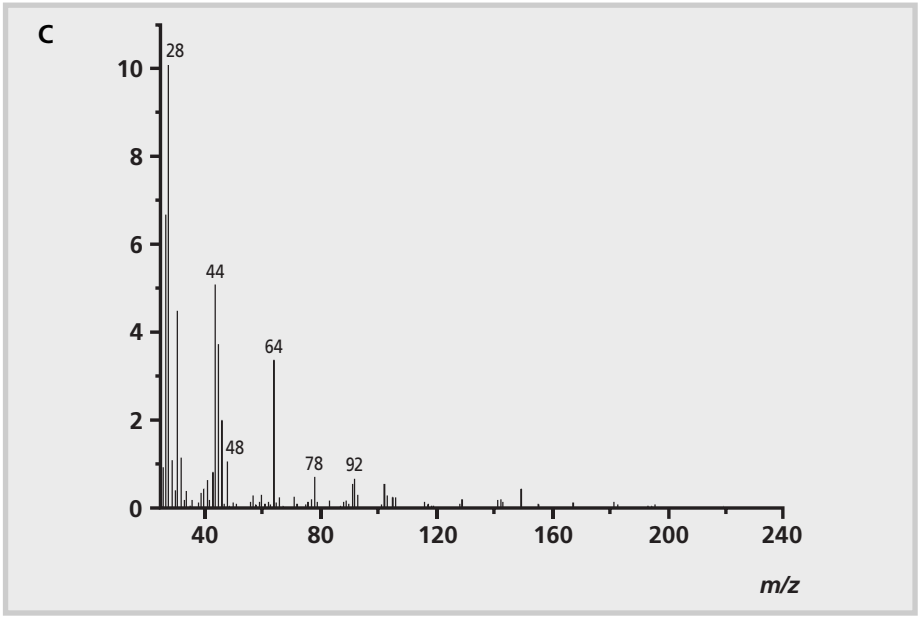


Fig. 3.12
Score plot from discriminant analyses of DTMS-EI measurements of P. sativum (var. GE) untreated and heated for 60 minutes. Each sample was analysed in triplicate and the heating temperatures (in °C) are indicated in the figure. Product axes A, B and C are superimposed and the "numerically extracted" mass spectra for each axis is shown.

into a thermally more stable material that is characterized by the masses in the extracted discriminant spectrum.

Spectrum C (residue440) is characterized by the presence of masses that are attributed to HCN (*m/z* 27), CO (*m/z* 28), CO_2 (*m/z* 44), SO (*m/z* 48), SO_2 (*m/z* 64), benzene (*m/z* 78), and alkylbenzenes (*m/z* 91 and 105). This extracted mass spectrum is also the result of the conversion of the reactant, but in this case into a tertiary material that differs from the one characterized by spectrum B.

DTMS-EI EXPERIMENTS ON SAMPLE H414 FROM THE ARCHAEOLOGICAL RECORD

The yield of detectable masses in the spectrum of sample H414 (Fig. 3.13) is low. The series of masses *m/z* 83, 97, 111 etc. is considered as background, as explained above. Ions indicating the release of CO, CO_2 and HCN are observed. The H414 spectrum is identical to spectra of the residues of recent peas heated for 60 minutes at 440 °C and higher.

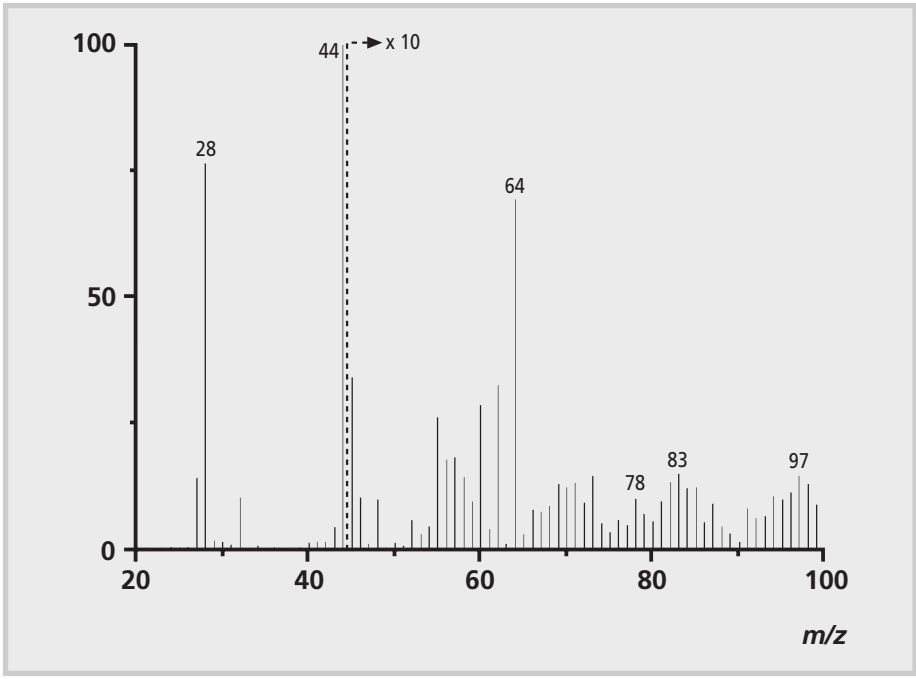
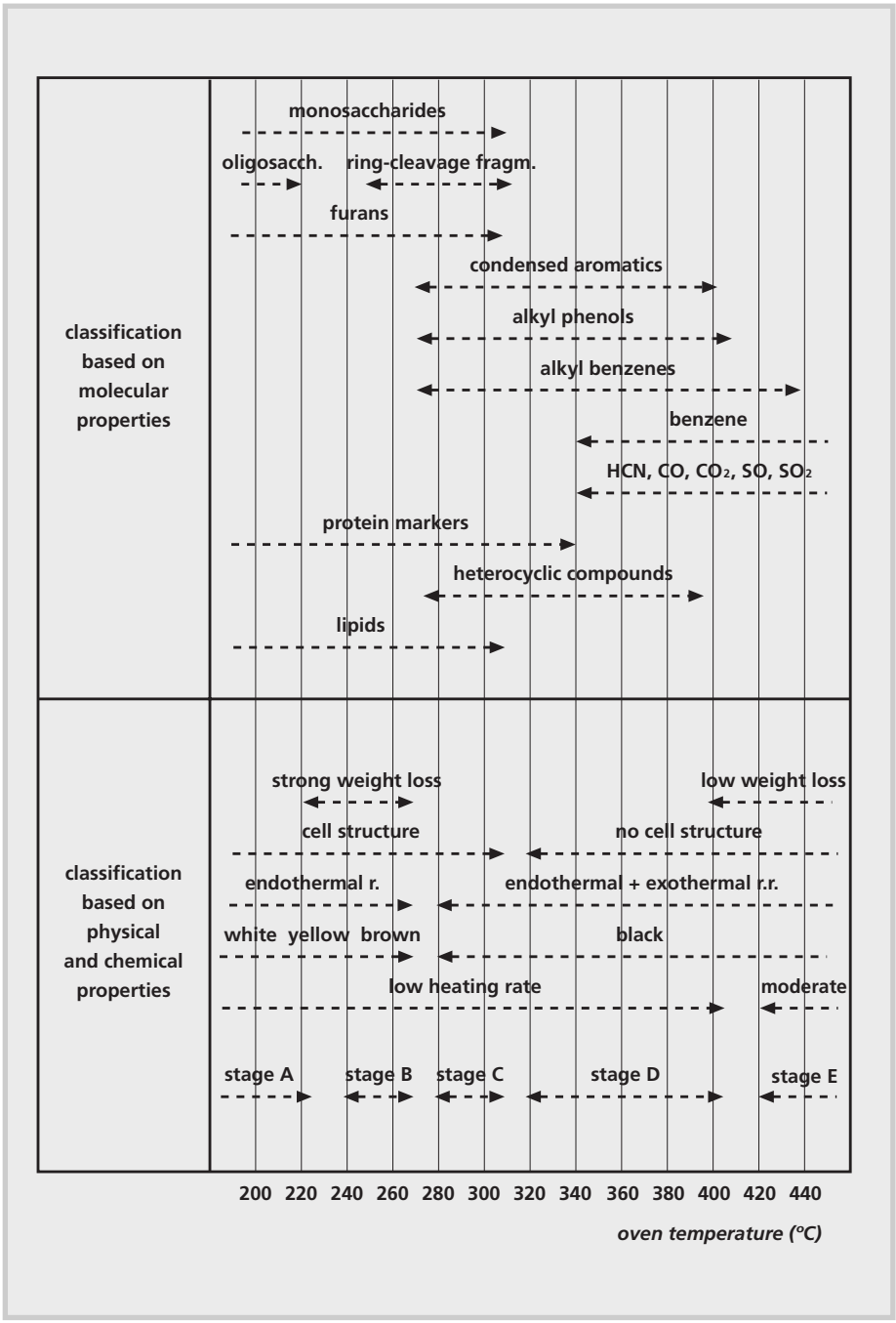


Fig. 3.13
DTMS-EI mass spectrum of the archaeological sample H414



Scheme 3.1

Classification of P. sativum (var. GE) untreated and heated for 60 minutes based on the molecular properties compared with the physical and bulk elemental properties from Braadbaart et al. (2004a)

DISCUSSION

Pea seeds contain a mixture of polysaccharides, proteins and minor amounts of fat and inorganic compounds that are present naturally. Starch is the bulk material; protein can reach 25 wt% (Daveby et al., 1993). By heat treatment both compounds will convert at different rates and by different pathways, depending on the experimental conditions. The conversion of starch can be compared with that of cellulose, because the chemical composition and structure can be considered identical for this purpose c.f. van der Kaaden et al. (1983). Heat treatment of cellulose is widely reported and numerous papers have dealt in detail with this issue (Shafizadeh and Bradbury, 1979; Antal and Varhegyi, 1995). The conversion of proteins and the interaction between starch and proteins is a lesser studied issue. In an earlier paper the changes of the physical and bulk chemical properties of pea seeds as a result of heat treatment has been reported (Braadbaart et al., 2004a). The changes that occur correspond to a classification in five stages (Scheme 3.1). Results, such as shown in the score plot of Fig. 3.12, could be interpreted as if the physical and molecular changes, resulting from heating, are recorded continuously as a function of the oven temperature, similar to a thermo gravimetric technique. This latter technique can be considered as one process with a constant heating rate resulting in one residue. However, in the experimental set-up used in this case each residue is obtained through a separate process and in total 15 experiments were performed to obtain the 15 residues. This set-up was used to simulate the drop of peas into an open fire, where the temperature will differ as a function of the place in the fire. Thus each experiment took place in a pre-heated oven and has a different heating rate, increasing as a function of the oven temperature. The physical data of the heating of peas at T_{oven} = 250 °C as a function of time shows, that the weight loss remains constant after 40 minutes of heating (Braadbaart et al., 2004a). The DA of the DTMS-EI experiments (Fig. 3.5) shows that from 40 minutes the molecular composition does not change either (for 250 °C). The time to reach the stage of constant weight loss and the constant molecular composition decreases considerably with increasing T_{oven}. Consequently, the average heating rate increases from a slow 4 °C min^{-1} at T_{oven} = 235 °C to a moderate 175 °C min^{-1} at T_{oven} = 700 °C (Braadbaart et al., 2004a). The oven temperature and accompanying heating rate of each experiment determines the mechanism and pathway that will take place. The result will be a residue typical for the temperature of each experiment and its accompanying regimen.

Polysaccharides

The DTMS-CI-NH3 spectrum of a sample of untreated peas is dominated by *m/z* 180, a cluster ion of ammonia with the molecular ion of levoglucosan. This ion is

followed by a series of anhydro-oligosaccharides with and without attached ring-cleavage fragments (Fig. 3.1). These products point to respectively a transglycosidation and a (2+2+2) cycloreversion mechanism (Lomax et al., 1991b). Both mechanisms are operating at the same time and will reduce the initial polymer size by in-chain cleavages. The spectra of residue130 up to residue235 still contain domains with oligosaccharides, but in residue250 the relative amount is reduced and mainly monosaccharidic constituents appear to be present as witnessed by the MCs (Fig. 3.8a). Monosaccharidic moieties are still present in residue310, although the relative amount of sugar units with attached ring-cleavage fragments is increased in the spectra. Concurrently, from residue270 new elements, such as furans, phenols and other aromatic compounds become increasingly more important. It should be noted that the aromatic compounds could be pyrolysis products from polysaccharides as well as from proteins. The corresponding solid matter is thermally more stable as is shown by the corresponding TIC traces. Not only the apex of the pyrolysis events has shifted to a higher temperature, but the traces have also broadened towards the higher temperature side. The compounds that are released at the higher temperatures are the compounds released from new, more aromatic, networks. This indicates that the thermally labile polysaccharide rich material has changed into a thermally more stable material enriched in an aromatic substructure. The results show that the proposed model as presented by Boon et al. (1994), on the formation of a thermostable polymer network at oven temperatures higher than 270 °C in the residues of pyrolyzed microcrystalline cellulose is also applicable to a thermally modified polysaccharide/protein rich biomass such as whole peas. In this model residual non-volatile glucose cores with attached ring-cleavage fragments as a result of (2+2+2) cycloreversion reactions act, increasingly from T_{oven} = 250 °C, as aldol-condensation sites for volatized compounds that will react with larger non-volatile residual structural elements. In the spectra of heated peas this can be observed by the increasing presence of *m/z* 222 and 240 in the spectra, which occurs simultaneously with the appearance of the aromatic compounds characteristic for the residues up to 310 °C. The polymer network will change from a carbohydrate-dominated reactant into a material with mainly aromatic compounds in residue310. The linear polysaccharide polymer has changed into a growing network polymer. It is clear that the performed CI experiments provide detailed insight into the conversion of polysaccharides, the bulk fraction of peas, as a result of heating.

Apart from the CI studies DTMS-EI experiments were carried out on samples of untreated peas and the set of thermal residues of peas. DA was performed on samples up to residue440. The distribution of the samples in the discriminant score plot based on EI-data (Fig. 3.12) is governed by the progressive heating of the samples in the set of residues. The evolution of the various classes of compounds, which are represented by specific masses, is a function of the oven

temperature and thus the heating rate of each experiment. It shows that until residue235 polysaccharides, proteins and lipids are observed with a distribution of the mass peaks in the spectra that remains unchanged. From residue250 the polysaccharide rich material changes progressively into an aromatic material which is most pronounced in residue310. The lower temperatures and slower heating rates result in a preference for the path that produces solids and gases. The exothermal reaction (Scheme 3.1) is the result of secondary reactions between converting solids and the gases traversing the residue on the way to the external environment. The confined structure of the pea enhances the production of secondary products. The linear polymer has thus changed into a growing network polymer, which is thermally more stable. This newly formed thermally stable polymer system dissociates further at higher temperatures and a disproportionation occurs by loss of CO and CO_2 leading to a highly condensed aromatic polymer. From residue340 the mass spectra show a considerable increase of the relative intensities of the corresponding masses *m/z* 28 and 44, respectively. Simultaneously the following masses show a higher relative intensity in sequence of their relative abundance: *m/z* 78 (benzene), 27 (HCN), 28, 44, 48 (SO) and 64 (SO_2). The presence of HCN indicates that nitrogen is built in the residue; SO and SO_2 are attributed pyrolysis products of sulfates or sulphonates or from compounds like $CaSO_4$, formed during the heating process. The sample of residue440 shows that functionalized hetero aromatic compounds are not present anymore and only (alkyl)benzenes are released. The masses *m/z* 27, 28, 44, 48, 64 and 78 are still observed. At higher oven temperatures the relative intensity of these masses progressively decreases and in the spectrum of residue700 *m/z* 44 is present only. The process results in a carbon rich material in residue500 with no signs of secondary reactions suggesting a primary product through volatization reactions. The path to form volatiles at these high oven temperatures is clearly stronger than the formation of secondary products (Piskorz et al., 1986). The higher heating rates contribute to this result. The pyrolysis of high-ranking coals (anthracite) shows that the same ions are released as in residue500 and higher (Tromp et al., 1988), suggesting a high similarity of high ranking coals and the residues formed in this study.

Proteins

Processes are different for the proteins present in peas. Proteins are heteropolymers that dissociate thermally into a large number of compounds. DTMS of proteins therefore have a tendency to show unresolved envelopes of mass peaks. The CI experiments of Pisane HD, however, do show a number of characteristic protein markers (Fig. 3.2). This mass distribution does not show a strong difference with the spectrum of the sample heated at 310 °C (Fig. 3.6a). The main differences are the increase of the relative intensities of the protein markers and the

beginning of a homologous series of odd-numbered masses: *m/z* 147, 161, 175, etc. At higher temperatures the mass distribution changes. New mass peaks representing alkylphenols and alkylbenzenes are recognized and become stronger up to a heating temperature of 400 °C (Fig. 3.6b). Moreover the relative intensity of the protein markers decreases and the series of odd-numbered masses increase in relative intensity. This implies that CI experiments, for samples heated up to 310 °C, are not very useful for discriminating between untreated and heat-treated samples of a protein isolate, i.e. Pisane HD, from peas. However, the mass peaks of the compounds generated from untreated and heated Pisane HD are not present in the spectra of untreated peas and residue130 up to 250. From residue250 the protein markers are present in all the spectra of the residues of peas and can be considered as a fingerprint of protein markers. Thus the method is a very useful tool to indicate the presence of proteins in residues of peas heated at temperatures higher than 250 °C.

The reference spectra of Pisane HD under DTMS-EI show the relevant markers for the presence of proteins and for N-containing compounds (odd mass peaks). The spectra reveal that protein mass markers are present up to the sample heated at 310 °C (Fig. 3.10a). The homologous series of masses present in the spectra of Pisane HD (from the sample heated at 250 °C) are almost identical to those that are generated from polysaccharides (Pastorova et al., 1994). Also the masses representing alkylphenols and alkylbenzenes are present in both heated polysaccharides and proteins. This suggests that thermal treatment of proteins and polysaccharides leads to solid residues that are increasingly similar. EI and CI experiments, under nominal mass measuring conditions, fail to discriminate between pyrolysis products of polysaccharides and proteins, as present in peas, at heating temperatures between 250 and 400 °C. At higher temperatures N-containing compounds, such as HCN and CH_3CN, are still observed up to a heating temperature of 440 °C. The presence of HCN in the spectra up to this temperature is considered as a marker for the presence of such N-containing compounds, although their chemical structure is unknown. FTIR shows that CN moieties as such are not present, but there are indications for in chain nitrogen in an aromatic main structure. All (CH_2) moieties have completely disappeared.

Untreated peas and residue130 up to 340, under EI conditions, show the relevant protein markers. From residue270 up to 400 alkylphenols, alkylbenzenes and the homologous series are observed. These masses are pyrolysis products of starch and proteins. Small molecules, such as HCN and CH_3CN can be recognized up to residue700. This suggests that under EI conditions protein derived structural moieties can be observed up to heating temperatures of 340 °C. Proteinaceous moieties also show a clear difference between the pyrolysis products of the residues heated at temperatures lower and higher than 440 °C. At temperatures lower than 440 °C the residues show volatile matter with a recognizable chemical

relationship to the native polymer and as such no structural resemblance is lost. At higher temperatures and heating rates more highly condensed solid material is formed that releases CO, CO_2, HCN, SO and SO_2. More hetero atoms are built in into the aromatic network. From elemental analyses it is known that nitrogen (5 wt%, daf) and sulphur (0.27 wt%, daf) are still present in residue700 (Braadbaart et al., 2004a).

Lipids

The masses representing lipids are recognizable in the EI experiments of the sample of Pisane HD as well as in the samples of peas and their pyrolytic residues up to residue310. Although this is surprising at first hand it is postulated that those lipids are chemically bound or strongly adsorbed inside the residues.

Implications for the archaeology

The results of the experiments show that after 60 minutes exposure up to a temperature of 270 °C the molecular composition of the residues of recent peas mainly consist of polysaccharides and proteins. When these residues are deposited into the soil they will be degraded microbially, as untreated peas will be. The stage between 270 and 310 °C consists of polysaccharides, proteins, heterocyclic and aromatic compounds and it is not sure if these residues or parts of the residues will survive the various degradation processes. The residues heated at temperatures higher than 310 °C will have a better chance to be found in the archaeological record, however proteinaceous marker compounds seem to be present up to 340 °C and their residues at much higher temperatures.

The results of the EI experiments on sample H414 shows that its spectrum has an identical mass distribution as the spectra of residue440 and higher, which suggests that this pea residue has been exposed to at least 440 °C. The vitrinite reflection measurements on polished specimens support this (Braadbaart et al., 2004a).

SUMMARY: CLASSIFICATION OF HEAT-TREATED PEA SOLIDS

The original product (peas) and the new products, resulting from the heating process, are visualized together with the physical and bulk chemical changes (Braadbaart et al., 2004a) (Scheme 3.1). It shows that both the latter and molecular changes can be conveniently considered into identical stages. Cotyledons are the bulk material of peas and are enclosed by the seed coat. The cells in the cotyledons contain large starch grains, protein bodies and small deposits of fats. The cell walls consist mainly of non-starchy polysaccharides. Thus peas have a rather confined structure. Based on analytical mass spectrometry (DTMS-CI and –EI) the conversion of the intact polysaccharide/protein rich cotyledons is described as a function of the temperature. The actual experimental conditions allow for a heating rate that increases from a slow 4 °C min^{-1} at a heating temperature of 235 °C to a moderate 175 °C min^{-1} at 700 °C. This results in different paths of conversion.

Polysaccharides and/or proteins show a strong dehydration and produce a brown product up to 270 °C, the result of Maillard reactions. In residue270 the polysaccharidic and proteinaceous material is still dominating. At higher temperatures a path that produces thermally more stable products determines the conversion. Three types of products can be distinguished as a function of the heating temperature. The first one, from 270 to 310 °C, is characterized by the presence of monosaccharides, protein fragments and aromatic compounds. From 270 °C the reactions show also an exotherm, explaining the presence of secondary products. This second type, from 310 °C up to a transitional stage from 400 to 440 °C, consists of various aromatic and heterocyclic compounds. The third type, at higher temperatures, is a tertiary product that releases (alkyl)benzenes, CO, CO_2, HCN, SO and SO_2. In this type of products a path can be distinguished, characterized by a strongly C-enriched material with some remaining hetero atoms in the network structure.

Thus the simulation in the laboratory of the heating provides a rigorous base for studies regarding the effect of the various formation processes, as used in the field of archaeology, on the residues corresponding to the five stages. It suggests that peas should have been heated up to at least 310 °C before their residues survive natural degradation processes.

Acknowledgements

The authors are grateful to Dr. A.C. Tas of TNO Nutrition and Food Research, Zeist, The Netherlands for his suggestions regarding the pyrolysis of proteins. This research is supported by FOM research program 49 funded by the Foundation for Fundamental Research on Matter (FOM), a subsidiary of the Dutch Organisation for Scientific Research (NWO).

CHAPTER 4

Heating experiments under anoxic conditions on varieties of wheat

ABSTRACT

Species of wheat were used to study the effects of post-depositional processes on carbonized grains. For this purpose grains of recent wheat were heated under controlled anoxic conditions. These experiments revealed that the grains of the various varieties of wheat are not homogeneous in physical and chemical properties. Seven varieties of emmer wheat, two varieties of bread wheat and one variety of macaroni wheat were used for experiments, in order to select a variety of wheat. The grains, both mealy and vitreous ones, were heated to 270 °C for 60 minutes under anoxic conditions. Bulk elemental data showed that the carbon content is similar for all the untreated varieties, but that the N content varies. The results of DTMS-EI experiments show that the molecular composition of the untreated grains of the ten varieties is similar, which applies also for the heated grains of the ten varieties. The morphology of the heated grains showed a wide variation between the grains of the tested varieties. The grains become swollen and show protrusions, open creases or remain intact. The reasons for this variation are the mechanical properties of the pericarp and the proteinaceous matrix of the endosperm. Based on the results one variety of emmer wheat was selected for further experiments.

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An adapted version is accepted for publication in
Archaeometry

INTRODUCTION

In the archaeological record carbonized fruits and seeds are found in abundance. The carbonization process they have undergone can be considered as one of the formation processes as described by Schiffer (1972). Subsequently, deposition, post-depositional processes and excavation will affect the condition of carbonized seeds. In order to understand the effects of the latter processes on these seeds one has to know the initial properties of these carbonized seeds. Within the context of a much larger research project, a variety of recent fruits and seeds are been carbonized at different temperatures in the laboratory and the physical and molecular properties are studied as a function of temperature. One of the selected taxa is domesticated wheat, which has been of great importance as staple food for large portions of the world population throughout the ages.

The various species of the genus wheat (*Triticum* L.) can be distinguished by different characteristics. Each species contains a multiple of the basic haploid set of seven chromosomes per reproductive cell (Feldman and Sears, 1981). The species with the lowest chromosome number are diploid; a typical diploid species is einkorn (*Triticum monococcum* L.). In contrast, emmer wheat (*Triticum dicoccum* Schübl) and macaroni wheat (*Triticum durum* Desf.) are tetraploid. Bread wheat (*Triticum aestivum* L.), which is nowadays economically by far the most important, is the species with the highest chromosome number and is hexaploid. The genus can also be divided in hulled wheat and free threshing wheat (Zohary and Hopf, 2001). Einkorn and emmer wheat are hulled wheats and are characterized by a persistent enclosing hull, the palea, lemna and glumes, which makes threshing difficult. Macaroni- and bread wheat are free threshing. Wheat grains are not uniform. Variations in structure and composition occur between the different parts of grains of different varieties of one species. Even between grains of the same variety differences occur. These variations can occur as a result of genetic differences between varieties, different growing conditions, the location of the grain on the plant or through other natural variations (Whitworth, 2000). Within each variety of wheat compact, translucent grains can be found as well as floury, opaque grains (Mabille et al., 2000). The former are called vitreous and the latter mealy; vitreousness is an optical property. A separate property is the hardness, which is defined as a measure of the resistance against deformation. Other differences are the flour and dough properties.

Hulled wheat was among the earliest domesticated plants, but is now overtaken by free threshing wheat. Both types of wheat are found in the archaeological record and the most frequent means of preservation is carbonization. It is often mentioned that carbonized plant remains retain their shape and anatomical features and can be compared with modern reference material and thus identified

(Hillman et al., 1996). This is true for wheat as long as whole spikelets are found or when the constituent parts of the spikelet are still present. However, when only wheat grains are available this poses difficulties. The carbonization process affects the morphology of the wheat grain and it is difficult to distinguish between the varieties of the same species and even between the different species (Nesbitt and Samuel, 1996; Hillman et al., 1996). This heterogeneity and the differences between the species may be the reason for different physical and bulk chemical properties as well as variations in molecular composition.

These considerations and the results of an earlier study of other propagules i.e. peas (Braadbaart et al., 2004a; b) led to the conclusion that a research project concerning the initial properties of carbonized wheat grains should start with an appropriate selection of one variety of wheat, before conducting tests on the grains to study the physical and molecular properties of carbonized grains as a function of the temperature. This paper focuses on the selection of a variety of wheat. A series of tests was carried out with grains of seven varieties of emmer wheat (a tetraploid, hulled wheat), of two varieties of bread wheat (a hexaploid, free threshing wheat) and of one variety of macaroni wheat (a tetraploid, free threshing wheat). From each variety mealy and vitreous grains, if available, were tested separately. The physical and molecular properties of untreated grains of these varieties were studied. Grains of each variety were heated for 60 minutes under anoxic conditions at 270 °C in the laboratory and the properties of the grains of the varieties studied. The temperature of 270 °C was chosen as the conversion of the polysaccharides, the bulk material of the grains, into aromatic compounds takes place from c. 250 °C (Pastorova et al., 1993a). The experiments are similar to those executed for an earlier study of other propagules i.e. peas (Braadbaart et al., 2004a).

MORPHOLOGY OF WHEAT

For a detailed description of the wheat morphology and anatomy the reader is referred to the work of Bradbury and co-workers (1956), Evers and Bechtel (1988) and Evers and Millar (2002). Briefly, wheat grains are single seeded monocot fruits. These are about 4-10 mm long and 2.5-4.5 mm wide. The outside colour varies and can be light yellow, yellow-grey or reddish. Clearly visible structures are a crease or furrow on the ventral side of the grain, the embryo on the dorsal surface at one end of the grain and the brush hairs at the other end. The crease runs nearly the entire length of the grain and its depth extends almost to its centre. The seed consists of the embryo and endosperm, enclosed from the outside to the inside by the seed coat and nucellar tissue.

The pericarp surrounds the seed and is part of the fruit and not the seed. The pericarp, which makes 4-6 % of the total weight of the grain, is composed of several layers of cells. In order, from the outside inwards these are: epidermis, hypodermis, remains of thin-walled cells, intermediate cells, cross-cells and tube cells. The seed coat forms a nearly complete covering over the embryo and the endosperm. At the bottom of the crease the pericarp is followed by the pigment strand and together with the seed coat it forms the complete covering of the seed. The seed coat is firmly united with the innermost tube cells of the pericarp. Between the seed coat and the endosperm a single row of compressed cells is found, the nucellar tissue. The shape and size of wheat grains varies with species and its varieties.

The endosperm, the food storage tissue, constitutes about 90-92 % of the total weight of the grain. It consists of two tissues, starchy endosperm and aleurone. The latter are an outermost row of thick-walled cells surrounding the endosperm and account for 6-7 % of the whole grain. The major part of the endosperm is composed of cells that contain many starch granules embedded in a matrix, consisting of protein bodies compressed together in mature grains. In the crease area the endosperm contains a cavity, which is called the endosperm cavity. The endosperm is sometimes compact and translucent, which is called vitreous. Endosperm can also be floury and opaque, which is called mealy. The cells of vitreous endosperm are completely filled with starch and protein packed together in a solid mass. The cells of mealy endosperm have air spaces, which act to scatter reflected light and this causes a mealy appearance. Vitreousness and mealyness are thus optical properties. The presence of air spaces makes the mealy grains less dense. Both vitreous as well as mealy regions are sometimes present in the same grain. The hardness of wheat is usually represented by the texture or the relative hardness of the endosperm, because of the greater bulk of the endosperm. It can be defined as a measure of the resistance to deformation. The deformation resistance may result from the cohesion between the cells itself or the cohesion between

the starch granules and the protein matrix in the cells. Hardness is a highly heritable trait that can be affected by the environmental conditions. The physical and chemical bases however, are still a matter of discussion (Mabille et al., 2000; Turnbull and Rahman, 2002). In summary hardness and vitreousness should be considered as separate properties and thus the wheat endosperm may vary both in texture (hardness) and appearance (vitreousness).

The embryo, about 2-3 % of the total weight of the grain, is partly embedded in the endosperm at the base of the grain. It is composed of two major parts, the embryonic axis and the scutellum.

MATERIALS AND METHODS

Samples

Four varieties of emmer wheat were obtained from the Centre of Genetic Resources, Wageningen, The Netherlands (CGN): CGN 08340 (Germany), CGN 11482 (Germany), CGN 11485 (France) and CGN 11486 (Austria). Two varieties of emmer wheat are from the collection of the Faculty of Archaeology, Leiden University, The Netherlands: nrs. 4855 (Belgium) and 5801 (Switzerland). One variety of emmer wheat (AR) was grown at Archeon, Alphen a/d Rijn, The Netherlands. Two varieties of bread wheat (i) scipion (S), a soft wheat and (ii) qualital (Q), a hard wheat and one variety of macaroni wheat, ardente (A), were donated by the Unité de Technology des Céréales et des Agropolymères, ENSAM-INRA, Montpellier, France. If possible, the samples of each variety were divided into mealy and vitreous grains and were used separately for the experiments.

Elemental analyses

For the elemental analysis seven whole wheat grains of each variety were powdered, mixed and analyzed. If available, mealy and vitreous were studied separately. Carbon and nitrogen analyses were executed in duplicate on a NA 1500 series 2 NCS analyser from Fisons Instruments. The temperature in the combustion reactor was maintained at 1020 °C, the combustion products were separated on a Porapak QS column with a length of 2 m.

Microscopy

Visual changes in the external gross morphology of whole wheat grains were studied using a Zeiss incident light stereomicroscope. The visual changes in the internal gross morphology and anatomy were studied in cross sections by Scanning Electron Microscopy (SEM). The specimens were cut by a razor blade.

Heat treatment

For the experiments a Carbolite tube oven (model MTF 12/38/250) was used, the rate of heating was limited by setting a ramp rate of 2 °C min^{-1}. An open pyrex vessel was inserted in a 30 cm long pyrex tube (∅ 2.3 cm) at 18 cm from the inlet. In each experiment 40 whole wheat grains of each of the mentioned varieties of wheat were placed in the pyrex vessel and heated at an oven temperature of 270 °C (T_{oven} = 270 °C) for 60 minutes under a constant flow (150 ml min^{-1}) of N_2 at atmospheric pressure. The oven was pre-heated. The pyrex vessel with the

wheat grains was weighed before and the residues were weighted after heating, in order to determine the percentage weight loss. Gases and volatiles were vented and were not investigated further.

TGA experiments

TGA tests were executed on the Thermo gravimetric Analyser TGA 2950 Hi-Rees. The carrier gas was N_2 and the heating rate was set at 2 °C min^{-1}. After initial tests up to 950 °C the final temperature was set at 600 °C. For each test one whole grain of wheat was powdered. Vitreous and mealy grains of three varieties of emmer wheat (5801, AR and 08340), a mealy grain of bread wheat (S) and a vitreous grain of emmer wheat (11482) were used. The water content of each grain was measured at 105 °C.

Direct temperature – resolved mass spectrometry (DTMS)

The mass spectrometer used was a JEOL SX-102A double focusing mass spectrometer (B/E), using a direct insertion probe equipped with a Pt/Rh (9/1) filament for electron impact (EI) ionization. Two varieties of emmer wheat (08340 and 5801), two varieties of bread wheat (S and Q) and one variety of macaroni wheat (A) were measured in triplicate. A sample of seven powdered and mixed grains of each variety was deposited on the filament of the DCI probe and inserted directly into the ion source of the mass spectrometer. Ions were generated by electron impact (16eV) in an ionization chamber kept at 180 °C and accelerated to 10 kV. The scan range was *m/z* 20-1000 with one second cycle time and mass resolution of 1000. Data were acquired using a JEOL MP-7000 data system. Data acquisition and processing was performed on-line. The results of the DTMS experiments were subjected to multivariate analysis. In this study discriminant analysis was performed using the FOMpyroMap multivariate program and the Chemotricks computer program package (Klap et al., 1996).

Behaviour of heated wheat grains under static load

A compression testing machine was used to measure the load at failure of whole heat-treated vitreous wheat grains. The experiment was executed by applying a load in Newton (N) on one grain, placed on its ventral side, and the total load (N) at failure was measured. Five whole grains of each variety were tested and the mean load was calculated.

RESULTS

Samples of untreated wheat grains

Elemental analyses

The relative weight% of C for the various samples ranges between 39.4 and 40.8 for mealy grains and between 40.4 and 41.5 for vitreous grains of the examined varieties of wheat (Table 4.1). There is a slight tendency that vitreous grains have a higher C content of 0.5–2 %. The relative weight% of N ranges between 1.4 and 2 for mealy grains and between 2 and 3.2 for vitreous grains. The latter have always a higher N content. The N content is a measure for the protein content and by multiplying the weight% of N by the factor F = 6.25 the protein content can be calculated (Belitz and Grosch, 1999). The protein content in of bread wheat and macaroni wheat grains is lower than in emmer wheat grains.

DTMS-EI experiments

DTMS-EI experiments were carried out on samples of powdered mealy as well as vitreous grains of five varieties of wheat to study the molecular composition. Polysaccharides, proteins and lipids are the common compounds in wheat grains. The polysaccharides are represented mainly by starch and hemicelluloses (Belitz and Grosch, 1999), proteins by gliadins/glutenins (glutamic acid and proline) and albumins/globulins (lysine) (MacRitcie and Lafiandra, 1997). Fig. 4.1 presents the data of the total ion current (TIC) of the DTMS-EI experiments of a sample of vitreous 5801 and the spectrum of the same sample. The latter shows the characteristics of polysaccharides, in this case mainly starch. The mass ions representing starch are *m/z* 43, 57, 60, 73, 98, 126 and 144, being indicative of hexose sugars

Species	*Variety*	*C(%)**		*N(%)**	
		mealy	*vitreous*	*mealy*	*vitreous*
Emmer wheat	*08340*	*40.4*	*40.6*	*1.9*	*2.3*
	11482	*- ***	*41.5*	*-*	*3.0*
	11485	*-*	*41.2*	*-*	*3.2*
	11486	*-*	*41.1*	*-*	*2.8*
	4855	*-*	*41.5*	*-*	*2.5*
	5801	*40.1*	*41.1*	*2.0*	*2.6*
	AR	*40.2*	*40.6*	*1.8*	*2.6*
Bread wheat	*Qualital*	*40.8*	*40.8*	*1.8*	*2.2*
	Scipion	*39.4*	*40.4*	*1.4*	*2.0*
Macaroni wheat	*Ardente*	*40.2*	*40.6*	*1.6*	*2.2*

** not corrected for water and ash*
*** no mealy grains present in the sample*

Table 4.1
Results of chemical analyses of untreated whole grains of ten varieties of wheat

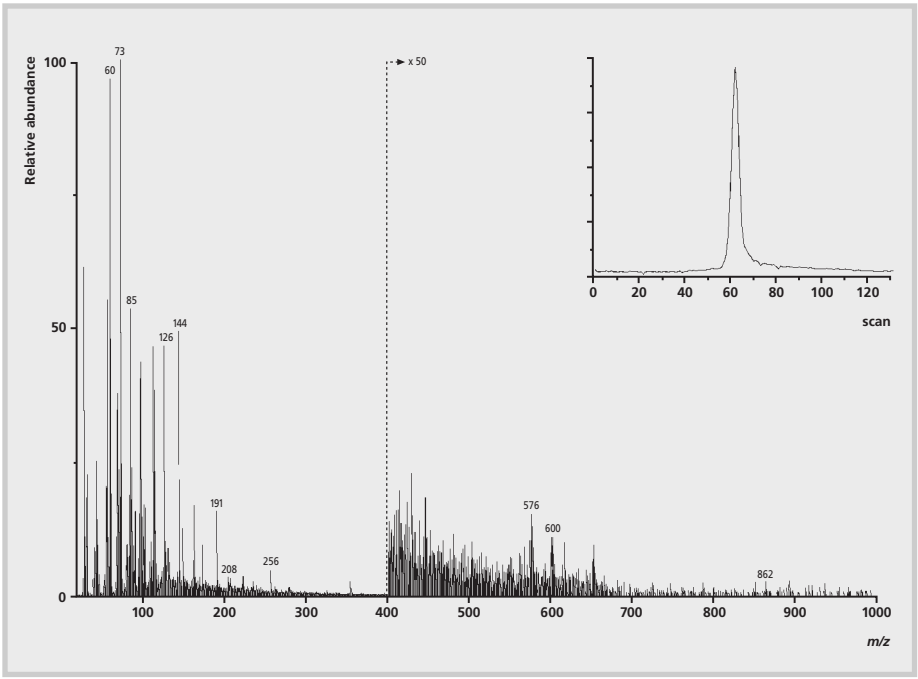

Fig. 4.1

DTMS-EI mass spectrum of an untreated sample of vitreous grains of a selected variety (5801) of emmer wheat, insert: TIC

Species	Variety	Weight loss (%)	
		mealy	vitreous
Emmer wheat	08340	48.1	46.8
	11482	-*	44.0
	11485	-	43.0
	11486	-	44.0
	4855	-	43.5
	5801	48.4	46.8
	AR	45.5	45.3
Bread wheat	Qualital	48.7	47.3
	Scipion	50.9	50.2
Macaroni wheat	Ardente	47.8	45.5

* no mealy grains present in the sample

Table 4.2
Weight loss of wheat grains heated at 270 ºC for 60 minutes under anoxic conditions

(van der Kaaden et al., 1983). Pentosesugars, representing hemicelluloses, are xylans (*m/z* 85 and 114) (Pouwels and Boon, 1990). The peaks at *m/z* 163 and 191, fragments of disaccharides, are also present in a reference sample of wheat starch (not shown). The spectrum of the reference sample of wheat protein (not shown) has peaks at *m/z* 70, 84, 91, 131, 138, 152, 154, 166, 180, 194, 208, 244 and 262. In the spectrum of the measured samples (Fig. 4.1) *m/z* 70, 84, 91, 131 and 208 are recognized. Characteristic for lipids are the presence of fatty acids (*m/z* 256, $C_{16:0}$), sitosterols (*m/z* 396-414, $C_{29:1}$), diglycerides (*m/z* 576, $C_{16:0,18:1}$; 600, M-H_2O $C_{18:1,18:2}$; 616, $C_{18:2,18:2}$) and triglycerides (*m/z* 862, $C_{16:0,18:0,18:0}$; 890, $C_{18:0,18:0,18:0}$).

The TIC traces of all tested wheat samples show the same apex (Tmax) and sharp product distribution. The spectra are similar too, showing the same masses with the same relative intensity. Principal component analyses followed by discriminant analyses were applied on the results of the DTMS-EI experiments. The first two discriminant functions describe together 42 % out of the total variance. The ratio of the between-group variance and the within-group variance (BW^{-1}) is <10 for all the discriminant functions indicating that the discrimination between the varieties is caused by the sampling and instrumental noise. These results suggest that the molecular composition of untreated mealy and vitreous grains of the examined three species of wheat and its varieties are qualitatively identical as the spectra showed.

Samples of residues of wheat grains heated at 270 °C for 60 minutes

Weight loss of the residues

The weight loss of vitreous and mealy grains of each variety of wheat was determined after 60 minutes of heating at T_{oven} = 270 °C under anoxic conditions and at atmospheric pressure. The results show that the weight loss of the mealy grains is between 45.5 and 50.9 %, while the vitreous grains have a weight loss between 43 and 50.2 % (Table 4.2). The weight loss of the mealy grains of each variety is always higher than the weight loss of the vitreous grains of the same variety.

Morphology of the residues

Light microscopy and SEM examined the external changes of the wheat grains. The outside colour has changed into black or black-brown. The external shape of the heat-treated grains of the three species shows in general two phenomena. First, the external shape of the untreated grains (Fig. 4.2a) has become rounder and more compact (Fig. 4.2b), as if the grains are swollen. Second, three types of grain have developed. The first type consists of grains, which show, apart from the swollen character, their original shape (Fig. 4.2b). These will be referred to as the intact grains. The second type is burst open at the crease (Fig. 4.2c). The third type shows a bulge of black material fixed at the pericarp. This material is apparently originated from the endosperm and will be referred to as the protrusion. Three

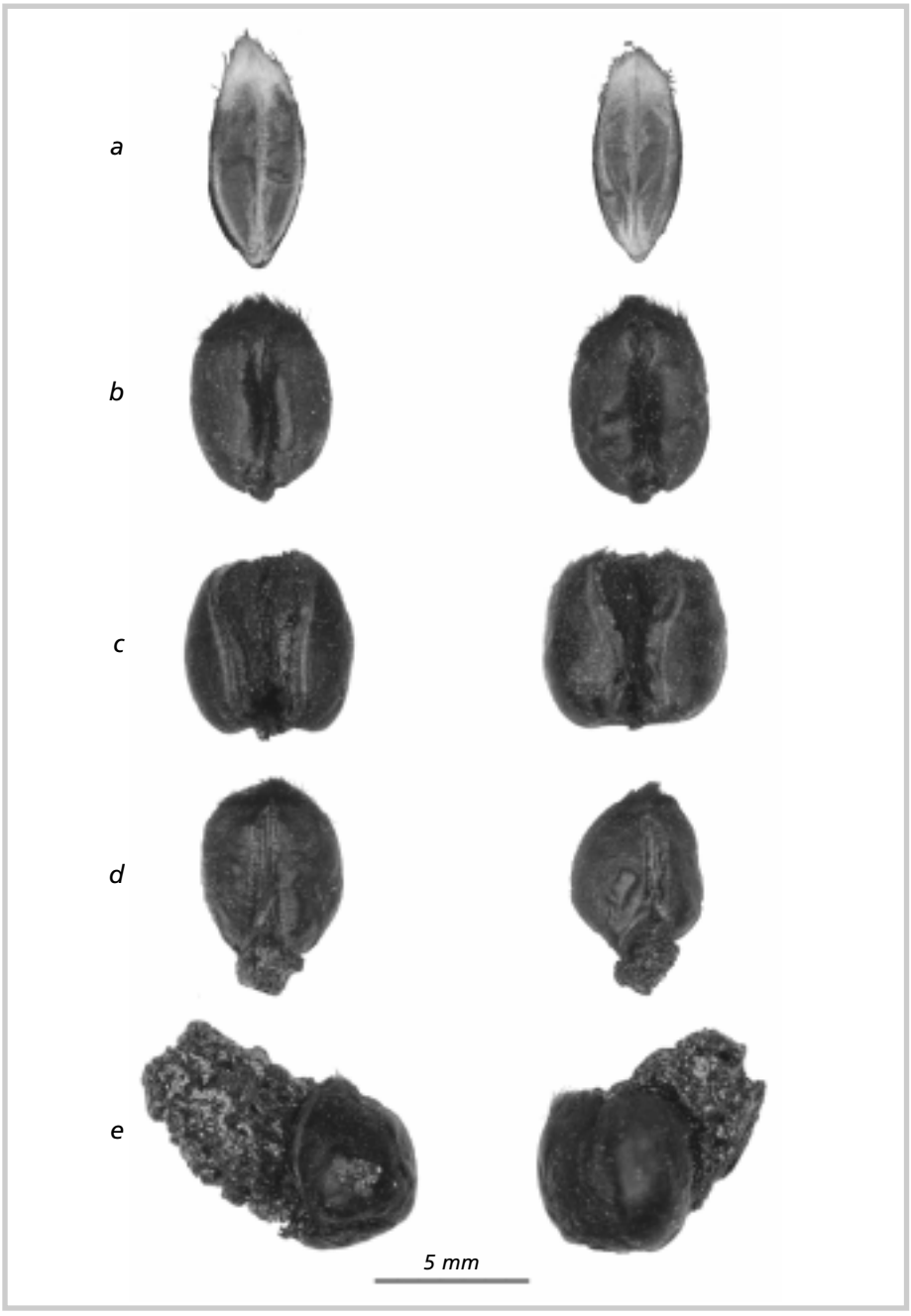

Fig. 4.2
External shape of emmer wheat grains: (a) untreated; (b) intact grains heated at 270 °C; (c) grains heated at 270 °C with open crease; (d) grains heated at 270 °C with small protrusion near embryo; (e) grains heated at 270 °C with large protrusion through crease. The heating time was 60 minutes.

types of protrusion can be distinguished. 1. A spherical bulge with a diameter of at least 1 mm. It originates in the area between the attachment region and embryo (Fig. 4.2d). 2. The protrusion seems to appear from the crease after it has burst open, these are larger and can reach a size, which is as big as the grain itself (Fig. 4.2e). 3. A protrusion through the pericarp at a random place (not shown). A section of a heat-treated grain with a protrusion shows that the structure of the endosperm and the protrusion appears to be identical on the SEM microphotograph (Fig. 4.3a and b).

The percentage of each type of heat-treated grains of the examined varieties is shown in Table 4.3. It shows that 0-50 % of the mealy and vitreous grains remain intact. The grains that do not remain intact show either an open crease or a protrusion and the two types are never encountered together in one grain. The percentage of grains of the varieties of emmer wheat with an open crease varies from 0 to 35 %, thus the percentage of grains with a protrusion is higher and varies from 35 to 95 %. It is striking that variety S, which is a soft wheat, shows the same behaviour as the variety of macaroni wheat, which is considered as hard. In all the varieties the embryo is still present.

DTMS-EI experiments on the residues

The spectrum of a sample of emmer wheat (AR) heated at 270 °C is characterized by the presence of aromatic compounds (Fig. 4.4). The main masses observed are *m/z* 95, 96 (furans); 94, 108 (alkylphenols); 91, 105 (alkylbenzenes) and 146, 162, 176, etc. (condensed aromatics). These masses are the typical products from the conversion of polysaccharides as a result of heat treatment (Pastorova et al., 1994). At this temperature monosaccharides with ring-cleavage fragments are still present (Boon et al., 1994). The protein markers *m/z* 70 and 84 are still recognized,

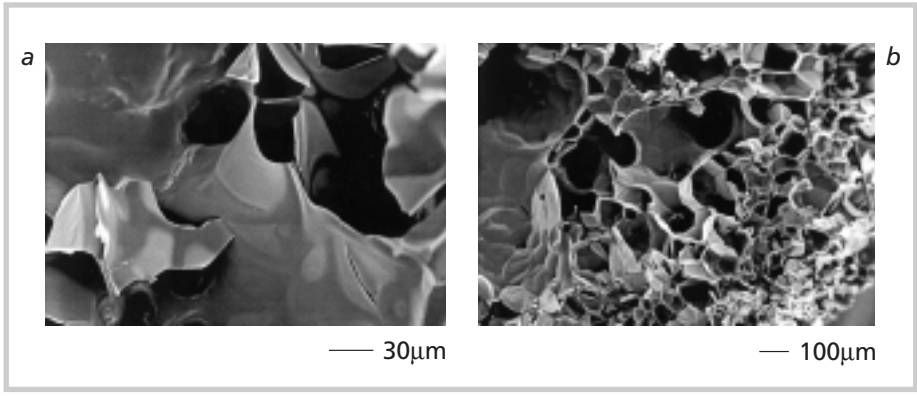

Fig. 4.3
SEM microphotographs of internal structure of grain of emmerwheat heated at 270 °C for 60 minutes: (a) inside of the grain and (b) inside of the protrusion

Species	Variety	Open crease (%)		Protrusion (%)		Intact (%)		Static load at failure (N)
		mealy	vitreous	mealy	vitreous	mealy	vitreous	vitreous
Emmer wheat	08340	4	0	88	95	8	5	11
	11482	- *	32	-	48	-	20	27
	11485	-	25	-	57	-	18	28
	11486	-	15	-	35	-	50	35
	4855	-	21	-	71	-	8	11
	5801	21	9	32	45	47	46	12
	AR	12	12	70	47	18	41	31
Bread wheat	Qualital	0	20	92	72	8	8	31
	Scipion	37	95	56	5	7	0	50
Macaroni wheat	Ardente	31	73	63	7	6	20	48

* no mealy grains present in the sample

Table 4.3
Mealy and vitreous wheat grains heated at 270 °C for 60 minutes, percentage of intact grains and grains with open crease or protrusion; strength of vitreous grains under static load (N)

other masses like *m/z* 81, 117, 131, 147, 161, etc. are representing N containing heterocyclic compounds (Moldoveanu, 1998). The mass distribution of the spectra of the carbonized grains of the tested varieties, as mentioned in Table 4.1, is similar indicating an identical chemical composition.

The DTMS-EI experiments on a sample from the endosperm and one from the protrusion of the same grain of one variety yield the same spectrum (not shown) and thus the molecular composition appears identical.

Residues of wheat grains under static load

If mechanical properties of wheat grains are measured they are usually restricted to the properties of the two main constituent parts: the pericarp and the endosperm (Mabille et al., 2000; Peyron et al., 2000). The reason is that much research on wheat grains is related to the separation of the pericarp and the aleurone layer from the endosperm and the grinding of the latter into wheat flour. In the archaeological record mostly whole, heat-treated wheat grains are found. The mechanical properties of whole grains are thus more of interest for this study. Vitreous wheat grains heated at 270 °C were used for the experiment. The mean static load, in N, at failure for each variety is summarized in Table 4.3. It shows that three groups of varieties of wheat can be distinguished. Group 1 with a load at failure of c. 50 N, consists of the variety of bread wheat S and macaroni wheat A. Group 2 with a load at failure between 27-35 N, encloses four varieties of emmer wheat (AR, 11482, 11485 and 11486) and the variety of bread wheat Q. Group 3 with a load at failure of c. 10 N, thus the group with the weakest grains, shows three varieties of emmer wheat (5801, 4855 and 08340).

TGA experiments

TGA experiments were carried out on one powdered grain of each variety to simulate the heat treatment of the grain without the presence of the enclosing pericarp, so volatiles are removed freely. The results of the experiments on the various grains are identical; the curves for the vitreous grain of emmer wheat 5801 are shown as an example (Fig. 4.5). The water content of the mealy grains is c. 10 % and c. 7 % for the vitreous grains.

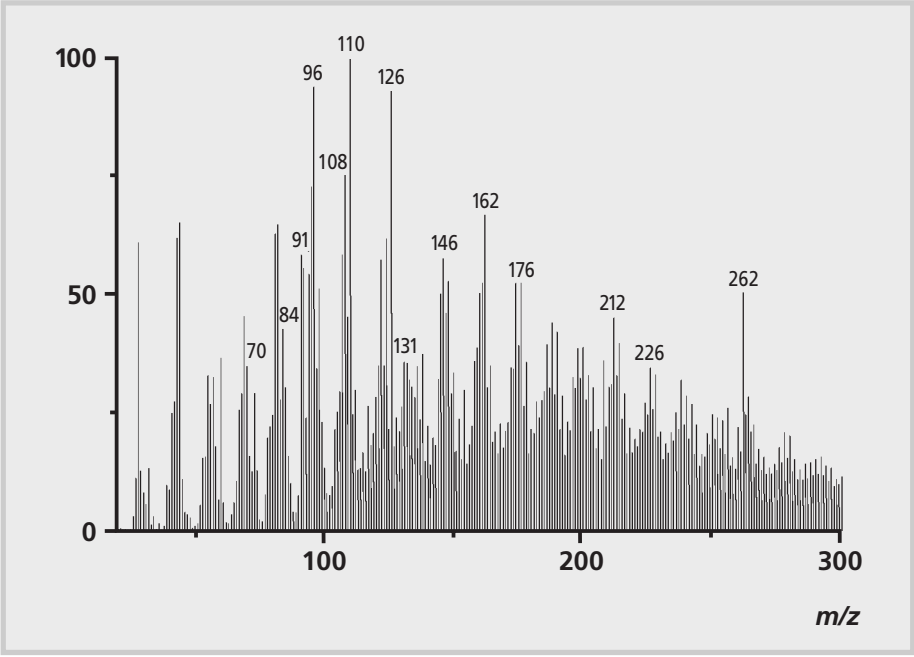

Fig. 4.4

DTMS-EI mass spectrum of a sample of vitreous grains of a selected variety (AR) of emmer wheat heated at 270 °C for 60 minutes

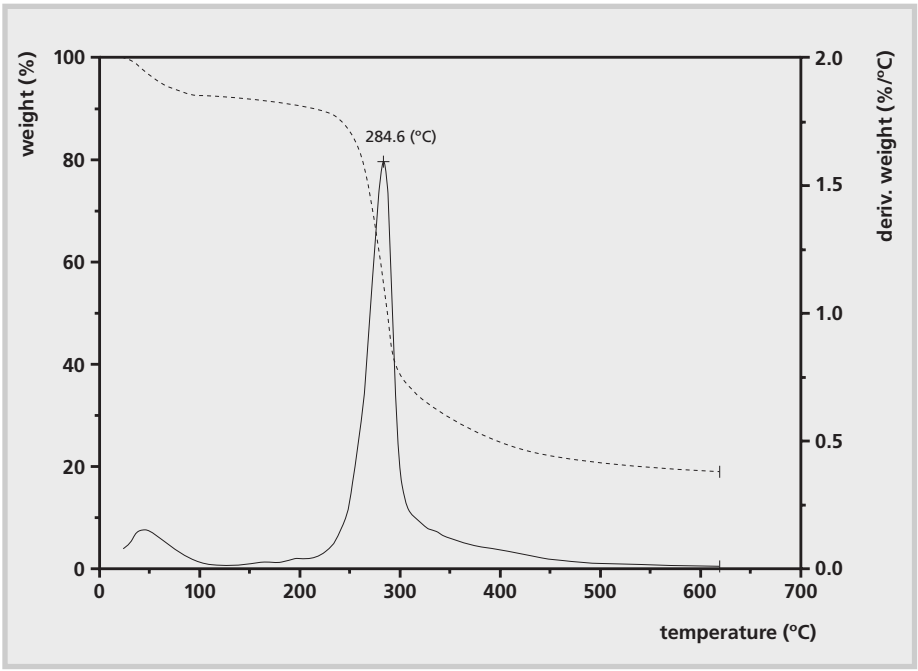

Fig. 4.5

TGA measurement of a selected powdered whole vitreous grain of a variety (5801) of emmer wheat. ----- = weight

DISCUSSION

Whitworth (2000) showed that the grains of varieties within a species of wheat are not homogeneous. The purpose of this study was to select a representative variety of wheat that will be used in the future to investigate the physical and chemical properties of the grains after heating as a function of the temperature. Seven varieties of emmer wheat were tested. Two varieties of bread wheat and one variety of macaroni wheat were included for comparative reasons.

Untreated grains were tested to investigate if any differences in the bulk chemical and molecular properties might be related to the various properties of heated grains. The results of the experiments show that the mealy and vitreous grains of the ten varieties have an identical molecular composition. No significant differences are measured between the C content of the grains of the ten varieties, however, there is a tendency observable that mealy grains have a smaller C content of max 2.5 %. The N content, which is a measure for the protein content, varies. In the mealy emmer wheat grains it ranges from 1.8 to 2.0 % and in the vitreous grains from 2.3 to 3.2 %. The protein content of the grains of bread and macaroni wheat is lower and ranges from 2.0 to 2.2 % for the vitreous grains. Mealy grains have always a lower protein content (Table 4.1) than vitreous grains, which corresponds to the presence of air spaces in the proteinaceous matrix of the endosperm.

As a result of heat treatment of whole grains at 270 °C for 60 minutes under anoxic conditions the grains show a considerable weight loss of about 45 %. Mealy grains have a greater weight loss (from 45.5 to 50.9 %) compared with the vitreous ones (from 43.0 to 50.2 %), which is most probably caused by the higher water content of the mealy grains. The weight loss of the vitreous grains of the seven investigated varieties of emmer wheat ranges from 43 to 46.8 %.

The molecular composition changes from a mainly polysaccharide character into a mixture of aromatic compounds and monosaccharides (Boon et al., 1994). The aromatic compounds consist of furans, (alkyl)phenols, (alkyl)benzenes and condensed compounds as well as N containing heterocyclic compounds at this temperature. This conversion of starch present in the endosperm and cellulose in the pericarp is accompanied by the release of volatiles (Braadbaart et al., 2004b). The volatiles have apparently built up a pressure inside the pericarp, which acts as a pressure vessel, as the external shape shows. The heated grains are swollen, bigger and more compact (Fig. 4.2b). This will cause a tensile force in the pericarp and may result in a collapse of the pericarp at weak spots. Results show that weak spots are the crease area and the area between the attachment region and the embryo. Other weak spots may occur at random in the pericarp, probably resulting from damage during harvest and/or other activities. After the collapse of the pericarp the volatiles are now able to escape from the inside, but at places where a

sudden drop of pressure occurs the gas phase will change into the solid phase. The latter is the protrusion and the former will result in the open crease. Not all the grains show these two phenomena, but some retain their original, however swollen, shape. A similar study with peas, which mainly consists of two starchy cotyledons enclosed by a seed coat, does not show these phenomena (Braadbaart et al., 2004a). Thus the presence of the pericarp, which encloses the seed, may be the reason for the build-up of pressure inside the wheat grains.

To further understand this mechanism TGA experiments were carried out. The results show similar curves for all the tested grains and the powdered wheat grains react as a biomass containing cellulose, hemicelluloses and proteins (Shafizadeh and McGinnes, 1971). This suggests that the mechanical properties of the pericarp determine the percentage of grains that remain intact or have protrusions and open creases as a result of heat treatment. An effort was made to measure the deformation by applying a static load at failure on the heat-treated grains. The results show that the grains as a function of this load can be divided into three groups (Table 4.3). The vitreous emmer wheat grains are included in the two groups with the lowest static load at failure, viz. 11N and 30N. The group of 11N comprises the three varieties with a low percentage of grains with an open crease and the group of 30N the varieties with a higher percentage with an open crease. The protein content of untreated emmer wheat grains varies also and shows a positive correlation (R = 0.80) with the percentage of heated grains with an open crease (Fig. 4.6). This figure shows also that the three varieties with a load at failure of 11N have lower protein content than those with a load at failure of 30N. It implies that the percentage of grains with an open crease is not only determined by the mechanical properties of the pericarp but also by those of the proteinaceous matrix of the endosperm. The higher the protein content the higher the resistance against deformation and the higher the percentage of heated grains with an open crease. Exceptions are the varieties S (soft bread wheat) and A (hard macaroni wheat), but these are not varieties of emmer wheat. Both show a high static load at failure (50N) and a much higher percentage of grains with an open crease. The percentage of both the intact grains and the grains with a protrusion do not correlate with the protein content of the untreated grains. When both percentages are added they evidently show the same, but negative correlation as shown in Fig 4.6. It suggests that intact grains are in fact grains with zero percent protrusion.

Mealy and vitreous grains of one variety have different optical properties, which cause a different appearance. But this feature does not show major differences in the investigated properties of the heated grains of both types. Therefore it is not necessary to separate both types in the sample of the selected variety of emmer wheat.

The C content (Table 4.1) of the untreated grains of the seven varieties of emmer wheat is identical. After heating the measured weight loss (Table 4.2) of the grains of these varieties is almost identical. The molecular composition has changed as a result of the heating process. But the molecular composition, as shown by their DTMS-EI spectra, of the seven investigated varieties are identical for the untreated grains as well as for the heated grains. This implies that from the point of molecular composition any variety of emmer wheat can be selected for further experiments. Based on the results as visualized in Fig. 4.6 and its ready availability in sufficient quantities led to the selection of the variety of emmer wheat AR for further experiments (Braadbaart et al., 2004d), although it is not a representative variety as far as the protein content and the morphological changes are concerned. There is no reason to carry out separate experiments on mealy and vitreous grains.

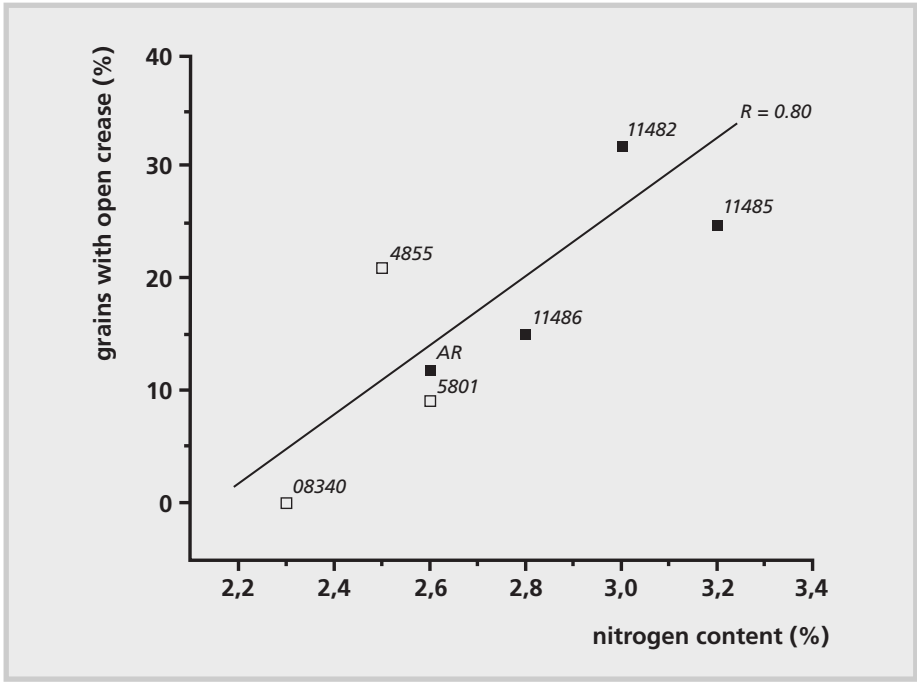

Fig. 4.6

Correlation of N content of untreated grains and grains with an open crease heated at 270 °C for 60 minutes. (□) Variety of emmer wheat with a load at failure of c. 10N; (■) variety of emmer wheat with a load at failure of c. 30N. The varieties of emmer wheat are indicated in the figure.

SUMMARY

In summary the untreated grains of the examined species of emmer wheat and its varieties are very homogeneous as far as the endosperm is concerned, except for the protein content. But the pericarp, together with the protein content, determines the percentage and shape of the deformations. It is apparently very heterogeneous, as the morphological changes show. The selection of the variety of emmer wheat AR for further heating experiments is based on the correlation between the protein content and the percentage of grains with an open crease.

Acknowledgements

We thank Mr. P. Kleingeld of the Faculty of Earth Sciences of Utrecht University for his assistance in general, but especially for the help with the static load experiments. Regarding the DTMS experiments the help of Mr. J van der Horst of the FOM Institute, is kindly acknowledged. This research is supported by FOM research program 49 funded by the Foundation for Fundamental Research on Matter (FOM), a subsidiary of the Dutch Organisation of Scientific Research (NWO).

CHAPTER 5

Laboratory simulations of the transformation of emmer wheat as a result of heating

ABSTRACT

Emmer wheat grains, variety AR, were heated at temperatures ranging from 130-700 °C under controlled anoxic conditions for maximum 280 minutes. For each temperature a separate experiment was carried out in a pre-heated tube oven. In this way the drop of wheat grains into an open fire was simulated to answer the question on how grains could get carbonized and eventually become part of the archaeological record. For each solid residue physical properties, bulk chemical analyses and chemical composition were measured and changes as a function of the temperature were determined. The bulk composition of emmer wheat grains *(Triticum dicoccum* Schübl), which consist mainly of an endosperm, is starch and protein. Physical properties determined during and after heating were the weight loss, thermal lag, external and internal morphological changes and the vitrinite reflectance. The C and N content of the residues were analyzed. Direct Temperature-Resolved Mass Spectrometry (DTMS) under CI (NH_3) and EI conditions was used to monitor changes in the chemical composition as a function of the temperature. The results show remaining starch and protein rich material up to 250°C. Microscopically the original starch granules in a proteinaceous matrix disappear. Starch and protein gradually decrease and the relative amount of aromatic compounds increases. At 310 °C a secondary, thermally stable, product is formed consisting of a condensed aromatic network. The composition does not change up to 400 °C, but at higher temperatures a strongly C-enriched tertiary product is formed. This study suggests that residues of heated wheat grains, found in the archaeological record, must have been heated up to at least 310 °C before they can survive the natural degradation processes. This statement could be verified by the measurement of the vitrinite reflectance, a fast and cheap method to determine the temperature at which archaeological residues have been heated.

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An adapted version is accepted for publication in
Journal of Thermal Analyses and Calorimetry 77 (2004) in press

INTRODUCTION

Carbonized remains of wood, fruits and seeds are often found in the archaeological record (van Zeist, 1970). The carbonization process can be considered as one from a series of formation processes that may have affected these remains, prior to the description by the archaeologists (Schiffer, 1983; 1987). The effects of the various formation processes on the carbonized material will depend on its physical and chemical properties. To understand these properties modern fruits and seeds of two different species of plants were selected. Samples of these fruits and seeds were heated under controlled anoxic conditions at atmospheric pressure to simulate a carbonization process. The two species selected are the dicotyledon peas *(Pisum sativum* L.) and the monocotyledon emmer wheat *(Triticum dicoccum* Schübl), a monocotyledon. The term carbonization, which is usually used in a rather general way, is not further used in this study and replaced by heat treatment plus the temperature at which the heating took place.

The current study assesses the changes as a function of temperature, of the physical properties, the bulk chemical composition and the molecular composition of emmer wheat grains. A grain of wheat is a single seeded fruit, composed of an endosperm and an embryo enclosed by a multi-layered pericarp or fruit coat. The presence of this pericarp will affect the way that the morphology changes as a result of heating (Braadbaart et al., 2004c). The morphology of wheat is species dependent and even on variety level differences occur (Whitworth, 2000; Mabille et al., 2000). Moreover grains in each variety may appear translucent or opaque, so-called vitreous and mealy, respectively (Mabille et al., 2000). The morphological differences that exist between species allow for an easy distinction between for example bread wheat *(Triticum aestivum* L.) and emmer wheat. However, after heat treatment the grains of both species become more similar (Hillman et al., 1996).

An appropriate selection of a variety of emmer wheat was needed considering the heterogeneous character of wheat grain varieties. For this purpose heating experiments at a temperature of 270 °C were carried out on seven varieties of emmer wheat, two varieties of bread wheat and one variety of durum wheat *(Triticum durum* Desf.) (Braadbaart et al., 2004c). The results showed a wide range of physical and bulk chemical differences between the grains of the ten varieties. Most conspicuous were the variations in the mechanical properties of the pericarp and the varying content of proteins. The molecular composition as determined by DTMS however, showed no differences between the untreated grains neither between the heated residues of the ten varieties (Braadbaart et al., 2004c). Based on these results the variety of emmer wheat AR, as one of the first species of wheat used in agriculture, was selected for the experiments. Experiments were carried out to investigate the changes as a function of the temperature, of weight loss, morphology, bulk chemical properties, molecular composition and vitrinite reflectance.

MATERIAL AND METHODS

Samples

Emmer wheat was grown at ARCHEON, Alphen aan de Rijn, The Netherlands and will be referred to as AR (Braadbaart et al., 2004c). The wheat was harvested in the summer of 2002. For the experiments the chaff was removed from the grains. No selection took place between mealy and vitreous grains and the sample contained the grains as naturally present. Meneba Meel B.V., Rotterdam, The Netherlands kindly supplied reference samples (technical grade) of wheat starch and wheat protein isolate.

Heat treatment

Wheat grains were heated in a preheated Carbolite tube oven (model MTF 12/38/250) whereby the rate of heating was limited by setting a ramp rate of 2 °C min^{-1}. An open pyrex vessel was inserted in a 30 cm long pyrex tube (∅ 2.3 cm) at 18 cm from the inlet. The experiments were carried out under a constant flow (150 ml min^{-1}) of N_2 at atmospheric pressure. In initial experiments 40 grains of AR were placed in the pyrex vessel and heated in the preheated oven at temperatures (T_{oven}) of 220, 250, 270, 290, 310, 340, 440 and 600 °C as a function of time for 7.5 up to 280 minutes to determine the optimal heating time. Based on these initial results grains of AR were heated for 120 minutes at one of the following temperatures: 130, 160, 190, 220, 235, 250, 270, 290, 310, 340, 370, 400, 440, 500, 600 and 700 °C. Forty grains were used up to an T_{oven} = 370 °C, at higher temperatures 60 grains were used. The pyrex vessel with the grains of AR was weighed before and after heating, in order to determine the percentage weight loss. Gases and volatiles were vented and not further investigated.

The variation, as a function of the time, between the internal temperature of the grains and external gas temperature was measured with one grain of AR in the pyrex vessel. A hole (∅ 0.3 mm) was drilled in the grain and a "K" type thermocouple was inserted in the centre of it. A similar type of thermocouple placed just beside the grain monitored the temperature of the carrier gas. The exposed wires were insulated with glass fibre. The thermocouples were connected to a two-channel writer (Kipp en Zonen, model BD41) and the temperatures were recorded continuously versus time. The thermocouples were calibrated with a pyrometer.

Microscopy

Visual changes in the external gross morphology of whole untreated wheat and the residues of the grains of AR as a function of the oven temperature were studied

using a Zeiss incident light stereomicroscope. Scanning electron microscopy (SEM) was used to study the internal changes on fresh cross sections of specimens, heated up to 270 °C, cut in half using a razor blade. At higher temperatures the vitrinite reflection was measured and the internal morphology was examined on polished resin-embedded specimens using a Zeiss Axioskop reflected light microscope (International Standard, 1985).

Elemental analyses

Seven whole untreated grains of AR or residues thereof were used after grinding and mixing. C and N analyses were executed on a NA 1500 series 2 NCS analyser from Fisons Instruments. The temperature in the combustion reactor was maintained at 1020 °C. The combustion products were separated on a Porapak QS column with a length of 2 m. All values stated are based on at least two measurements corrected for H_2O and ash content (daf: dry and ash free). H_2O and ash content were determined on a Thermogravimetric Analyser TGA 2950 Hi-Res. The carrier gas was air and the heating rate was set at 25 °C min^{-1}, water content was determined at 105 °C and ash content at 950 °C.

Vitrinite reflectance measurements

The residues of whole grains of AR, heated for 120 minutes at oven temperatures ranging from 270 to 700 °C, were used. For each oven temperature three specimens were embedded in resin blocks and polished. Maximum vitrinite reflectance measurements *(%Rmax)* were carried out under oil immersion at a wavelength of 546 mm using a Leitz MPV II microscope system. Thirty reflectance measurements were made on each specimen and at least two residues heated at the same temperature were used to calculate the mean reflectance. Preparation of polished blocks and reflectance measurements were carried out according to standard methods defined in ISO 7404, part 2 (1985) and ISO 7404, part 5 (1994).

Direct temperature-resolved mass spectrometry (DTMS)

The mass spectrometer used was a JEOL SX-102A double focusing mass spectrometer (B/E), using a direct insertion probe equipped with a Pt/Rh (9/1) filament for analysis under both chemical ionization (CI) and electron ionization (EI) conditions. The sample consisting of the powdered mixture of seven whole grains or residues thereof was deposited on the filament of the filament and inserted directly into the ion source of the mass spectrometer. Previous measurements on residues of peas showed that the sampling and experimental variance was very small (Braadbaart et al., 2004a). Therefore only single measurements were carried out. For CI, ions were generated under positive ammonia chemical ionization

conditions. The accelerating voltage was 2.2 kV, the scan range was *m/z* 60-1000 and the mass resolution was set at 1000. Under EI conditions ions were generated by low voltage electron ionization (16eV) in an ionization chamber kept at 180 °C and accelerated to 10 kV. The scan range was *m/z* 20-1000 with one second cycle time and mass resolution of 1000. For both methods data were acquired using a JEOL MP-7000 data system. Data acquisition and processing was performed on-line. The results of the DTMS experiments were subjected to multivariate analysis. In this study Principal Components Analyses (PCA), in combination with graphical rotation (Windig et al., 1983) was performed on the results of the DTMS experiments, using the FOMpyroMap multivariate program and the ChemomeTricks program for the calculations (Klap et al., 1996).

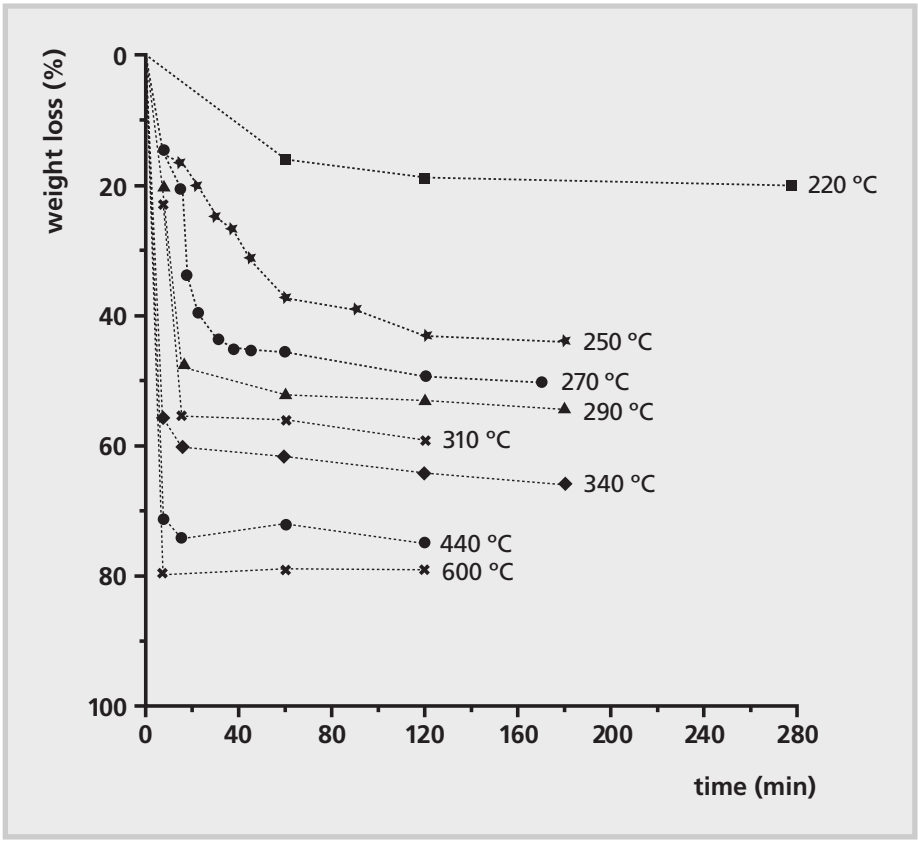


Fig. 5.1
Weight loss (%) of heated emmer wheat grains (var. AR) as a function of the heating time in minutes. The oven temperature is indicated in the figure in °C.

Weight loss and optimization of the heating time

The weight loss of the wheat grains was determined as a function of time in minutes at the various oven temperatures (T_{oven}). The experimental conditions did not allow continuous measurement of the weight loss during the entire time range of 280 minutes. A separate experiment was necessary for each time interval. The results show that for each T_{oven} the weight loss approaches a constant level after a certain time (Fig. 5.1). The higher the T_{oven} the shorter the time to reach this level, for example it takes c. 120 minutes at T_{oven} = 250°C, c. 20 minutes at T_{oven} = 290 °C, but it only takes 5 minutes at T_{oven} = 600 °C. It was shown in any case for oven

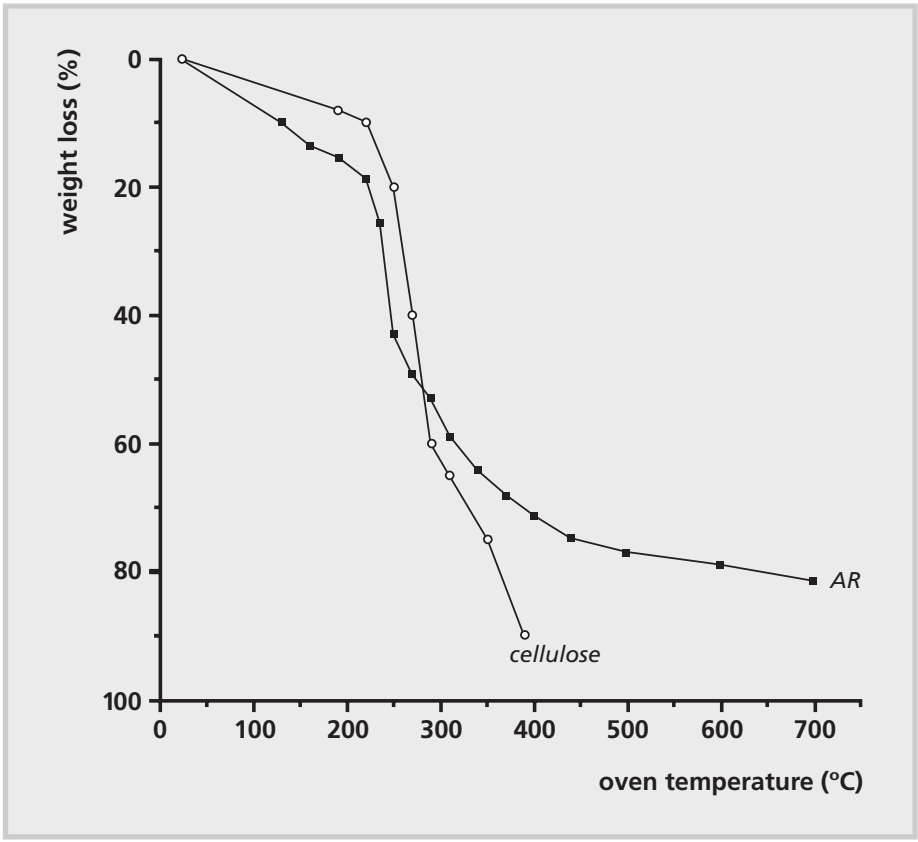


Fig. 5.2 Weight loss (%) of emmer wheat grains (var. AR) heated for 120 minutes as a function of the oven temperature in °C. Microcrystalline cellulose is heated for 2.5 hours after Pastorova et al. (1993).

temperatures from 250 °C upwards that the molecular composition remains constant after the total weight loss has become constant (Braadbaart et al., 2004b). Following these results a heating time of 120 minutes for each heating experiment is considered sufficient.

Heating experiments were carried out at oven temperatures in the range of 130 to 700 °C for 120 minutes. A strong rate of weight loss was observed in the range of T_{oven} = 220 to 250 °C, after which the rate of weight loss slowly decreased with temperature (Fig. 5.2). The total weight loss does not become constant. For comparative reasons the weight loss of microcrystalline cellulose heated for 2.5 hours under almost identical conditions (Pastorova et al., 1993b) is shown. Cellulose shows a higher weight loss from T_{oven} > 270 °C and is almost completely converted to volatile matter at 400 °C.

One grain of AR was heated in a pre-heated oven to determine the thermal regime inside the grains (Braadbaart et al., 2004a). The temperature, as a function of the time, inside and outside the grain was measured using thermocouples. The results show that the external temperature T_{ext} (°C) in the first stage was greater than the internal temperature T_{int} (°C) of the grains. Thus a positive thermal lag (i.e. $T_{ext} - T_{int} > 0$) was observed. However after a certain time period T_{ext} becomes equal to T_{int}. This period depends on the temperature set for the experiment. At T_{oven} = 250 °C T_{int} becomes equal to T_{ext} after 10 minutes. At higher oven temperature this time period decreases: at T_{oven} = 310 °C 7 minutes and at T_{oven} = 400 °C 3.5 minutes. From T_{oven} = 310 °C upwards the $T_{ext} = T_{int}$ stage is followed by a negative thermal lag ($T_{ext} - T_{int} < 0$) for a maximum of two minutes. Subsequently the negative lag becomes again a positive lag at higher temperatures. At all oven temperatures T_{ext} becomes constant upon reaching the predefined oven temperature of each experiment. Some minutes later T_{int} also becomes constant, but at about 4 °C lower than T_{ext}. The positive thermal lag is a signature of an endothermic or heat demanding reaction, which is present over the entire range of oven temperatures. From T_{oven} = 310 °C upwards the positive thermal lag is followed by a negative thermal lag, which signifies an exothermic reaction (Narayan and Antal, 1996). The most important changes are summarized in Scheme 5.1.

Morphology

The morphology and anatomy of wheat grains in general has been described in an earlier paper (Braadbaart et al., 2004c) and the description of the morphology of grains of AR is therefore confined to its particulars. The study by light microscopy shows an outside colour of greyish yellow. The length of the grains varies between 5.8-8.4 mm and the width from 1.7-3.2 mm. The microphotographs of the SEM work in Fig. 5.3a on the untreated grains show from the outside inwards the various cell layers of the pericarp (P), followed by the round

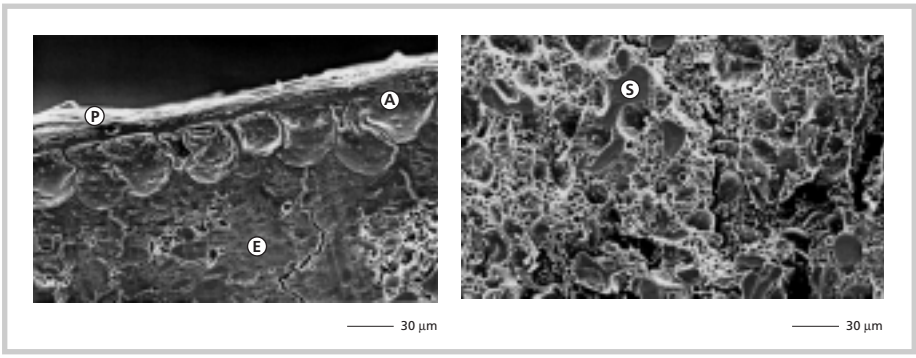


Fig. 5.3
SEM microphotographs of the internal structure of untreated emmer wheat grains (var. AR). (a) Outer side of the grain with pericarp (P), aleurone cell (A) and endosperm (E) and (b) loosely packed structure in the middle of a grain with starch granules (S).

cells of the aleurone layer (A), the outermost layer of the endosperm (Bradbury et al., 1956; Evers and Millar, 2002). The latter is followed by the endosperm (E) without a distinct cell structure, probably as a result of the preparation method. The endosperm shows a densely packed structure with starch granules (S) embedded in a proteinaceous matrix, followed by a less densely packed structure and finally in the middle of the grain the endosperm structure can be described as loosely packed (Fig. 5.3b) (Evers and Millar, 2002).

The external changes as a result of heating of the grains of AR as determined by light microscope show a change in the outside colour from greyish yellow through brown to black at T_{oven} = 290 °C (Table 5.1). The formation of grains with an open crease or solid protrusions on the grains as a result of heating has been described in an earlier paper (Braadbaart et al., 2004c). Up to T_{oven} = 250 °C no grains are observed with a protrusion. At T_{oven} = 270 °C 64 % of the grains have a protrusion. This increased to 88 % at 290 °C (Table 5.1). This percentage does not change until T_{oven} = 370 °C; subsequently it decreases to 21 % at T_{oven} = 700 °C (Scheme 5.1). As a result of heating of the grains the size and/or shape change, resulting in swollen grains at 250 °C. Subsequently the size decreases and the grains get rounder. The details of these dimensional changes as a result of heating will be discussed in a separate paper. At T_{oven} = 340 °C the pericarp shows vesicles and this feature is present until T_{oven} = 400 °C. The colour of the endosperm changes from white through brown to black at T_{oven} = 270 °C (Table 5.1). The SEM photomicrographs of the residues of grains heated at T_{oven} = 190 (Fig. 5.4a) and 235 °C (Fig. 5.4b) show no change in the structure compared with the SEM photomicrograph of the untreated grain (Fig. 5.3). The starch granules (S) in the matrix material remain visible and have not changed in size. Towards the middle of the grain the matrix becomes more loosely packed. The SEM microphotograph of the residue heated at 270 °C reveals a drastic change in the internal structure with cavities (C)

and converted endosperm (CE) (Fig. 5.4c). From 270 °C upwards the internal structure was further studied as polished surfaces of the resin-embedded grains The photomicrographs show a still intact pericarp (P) and aleurone layer (A), but the endosperm consists now of cavities (C) in a matrix of grey solid material, which is chemically converted endosperm material (CE). This general feature does not change anymore at the higher temperatures, as is shown in the polished surfaces of the resin-embedded residues heated at 340, 370 and 600 °C (Fig. 5.5). The changes of the morphology with temperature are shown in Scheme 5.1.

Elemental analyses

The elemental analyses of the untreated grains of AR show a C content of 46.3 wt%, daf and an N content of 2.1 wt%, daf (Fig. 5.6). The results of the carbon (solid line) and nitrogen (dotted line) analyses of heat-treated samples reveal a relative increase of the content of both elements as a function of oven temperature (Fig. 5.6). Starting at T_{oven} = 220 °C the C content increases sharply almost from 50 wt% (daf) at 220 °C to 65 % at T_{oven} = 250 °C. Beyond the latter temperature it increases more slowly to stabilize at almost 90 % (daf) at T_{oven} = 700 °C. At the same time the N content increases from 2.7 wt% (daf) at T_{oven} = 220 °C to about 5 % (daf) at T_{oven} = 370 °C and afterwards remains more or less constant until the highest oven temperature (Scheme 5.1).

Oven temperature (°C)	*Colour pericarp*	*Colour endosperm*	*Grains treated (nr)*	*Grains with protrusion (%)*	*Colour tarry liquid in outlet tube*
untreated	*greyish yellow*	*white*	-	-	-
130	*light brown*	*blackish white*	*40*	*0*	*clear*
160	*grey-brown*	*light brown*	*40*	*0*	*clear*
190	*brown-grey*	*brown*	*40*	*0*	*clear*
220	*brown*	*dark brown*	*40*	*0*	*clear*
235	*dark brown*	*brown-black*	*40*	*0*	*clear*
250	*brown-black*	*black-brown*	*40*	*0*	*light yellow*
270	*black-brown*	*black*	*40*	*64*	*yellow*
290	*black*	*black*	*40*	*88*	*yellow*
310	*black*	*black*	*40*	*83*	*dark yellow*
340	*black*	*black*	*40*	*78*	*yellow-brown*
370	*black*	*black*	*40*	*83*	*yellow-brown*
400	*black*	*black*	*60*	*58*	*brown*
440	*black*	*black*	*60*	*40*	*brown*
500	*black*	*black*	*40*	*42*	*brown*
600	*black*	*black*	*60*	*24*	*brown*
700	*black*	*black*	*60*	*21*	*brown*

Table 5.1
Colour and morphology changes in emmer wheat grains (Triticum dicoccum L.) variety AR heated for 120 minutes at the given oven temperature with N_2 as carrier gas

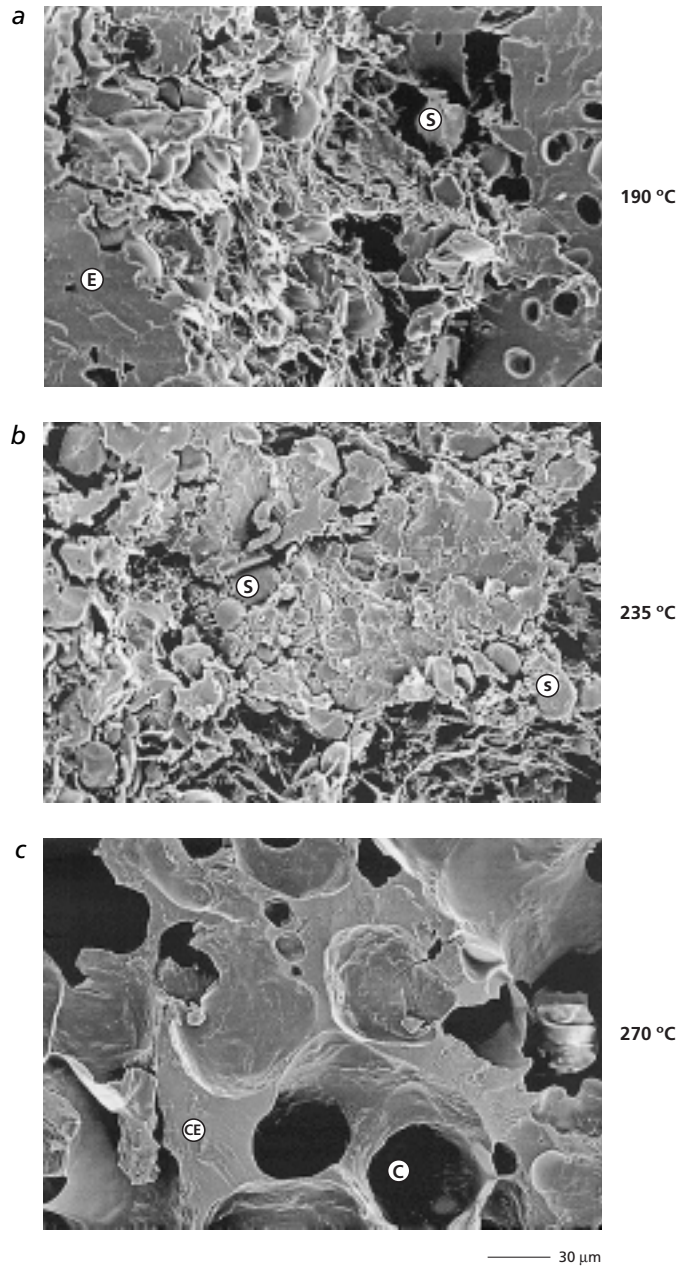


Fig. 5.4
SEM microphotographs of the internal structure of emmer wheat grains (var. AR). (a) Heated at 190 °C; (b) heated at 235 °C and (c) heated at 250 °C. S = starch granule, E = endosperm, C = cavity and CE = converted endosperm. Heating time: 120 minutes

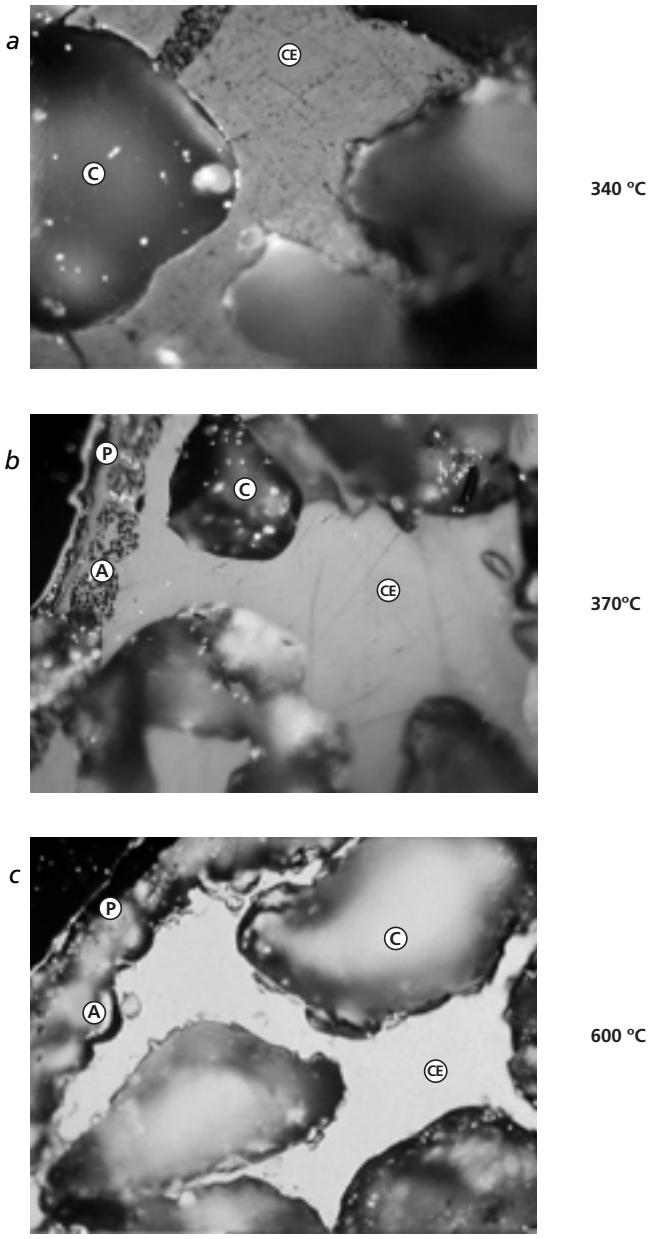


Fig. 5.5 Polished cross sections of resin-embedded heated emmer wheat grains (var. AR) showing the internal structure. The oven temperatures (°C) are indicated in the figure. C = cavity, CE = converted endosperm, A = aleurone layer and P = pericarp. The thickness of the aleurone layer is on the order of 50 µm. Heating time: 120 minutes

Molecular composition by DTMS

DTMS-CI-NH3

Polysaccharides, protein and lipids are the common constituents of the endosperm of wheat grains; the bulk material of the grains is polysaccharides represented mainly by starch (Table 5.2). The CI data of the total ion current (TIC) of the sample of untreated grains show a very sharp product distribution with the apex (Tmax) at 78 scans. The known polymer characteristics of polysaccharides, in this case starch, are present in the mass spectrum (not shown) (Braadbaart et al., 2004a). The protein content is calculated as 13.1 wt% by applying the factor N = 6,25 (Belitz and Grosch, 1999) to the wt% of the N content. However, no indicative markers of proteins are observed, due to the high relative abundance of polysaccharide pyrolysis products in the CI spectrum (Braadbaart et al., 2004b). No information was obtained about lipids, as they ionise poorly under these conditions.

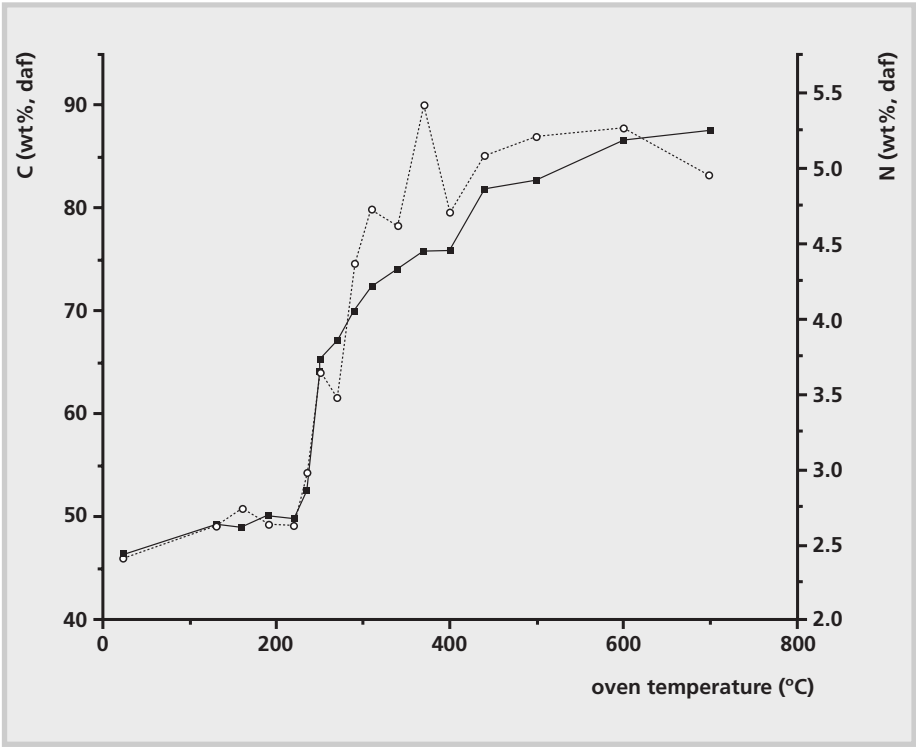


Fig. 5.6
The results of the bulk elemental analyses of untreated emmer wheat grains (var. AR) and grains heated for 120 minutes as a function of the oven temperature in °C. ■ = C content in wt%, daf and o = N content in wt%, daf.

Measurements under CI conditions were carried out on powdered solid residues of wheat grains to evaluate the impact of heating on their molecular composition at various oven temperatures. The TIC traces of the samples of grains heated at T_{oven} = 130 up to 235 °C show very sharp product distributions with the apices (Tmax) of the profiles remaining constant. From T_{oven} = 250 °C the profile broadens at the high temperature side and the apex shifts to higher scans. The shift points to the presence of an increasing amount of more condensed thermally stable material. The chemical changes that accompany these features are expressed in the mass spectra.

The mass spectra of AR samples heated from T_{oven} = 130 to 235 °C (not shown) are virtually identical to that of the untreated sample being dominated by polysaccharides. The mass spectra of samples heated at T_{oven} = 250, 270 (Fig. 5.7a) and 290 °C have changed in comparison with the mass spectrum of the sample heated at T_{oven} = 235°C. In the mass spectrum of the sample heated at T_{oven} = 310 °C (Fig. 5.7b) the relative abundance of mass peaks (*m/z* 162 and 180) characteristic for starch has substantially decreased. However, these ions still show a relative intensity of 60 %, indicating that structurally intact anhydromonosaccharidic moieties can still be released from the residues even when they have been heated to 310 °C for 120 minutes (Braadbaart et al., 2004b; Pastorova et al., 1993b). From T_{oven} = 310 °C a series of odd numbered masses is becoming more prominent giving the spectrum a different mass peak pattern appearance. These are characteristic for thermally modified polysaccharides, in this case starch (Braadbaart et al., 2004b; Pastorova et al., 1993b).

Although the protein markers at oven temperatures up to 235 °C under CI conditions are not very recognizable it is shown that from T_{oven} = 250 up to 370 °C this method is very useful in determining the presence of proteinaceous material (Braadbaart et al., 2004b). In the mass spectrum of the sample heated at 250 °C masses that are characteristic for proteins (*m/z* 84, 86, 101, 103, 146, 212 and 226) are observed (Fig. 5.7a and b) (Braadbaart et al., 2004b; de Waart et al., 1992; Tas,

	Wheat (wt%)
Moisture	*13.2*
*Protein (N * 6.25)*	*11.7*
Lipids	*2.2*
Starch	*59.2*
Other carbohydrates	*10.1*
Crude fibre	*2.0*
Minerals	*1.5*

Table 5.2
Chemical composition of wheat after Belitz and Gross, 1999

unpublished work). In order to study the change, as a function of the temperature, of the molecular composition the spectra of the samples of untreated grains and grains heated at T_{oven} = 130 up to 370 °C were analysed by principal component analyses (PCA) over a mass range of 60-400. The PCA shows that 69 % of the total variance is described by the first principal component. Higher components were ignored. The first component (PC1) describes the conversion of a polysaccharide rich material into an aromatic material as function of the increasing oven temperature. The score plot in Fig. 5.8 shows negative scores representing the mass peaks characteristic for polysaccharides (PC1-). The positive scores represent the heat-treated samples at $T_{oven} \geq 250$ °C (PC1+) and show the presence of the protein markers and the pyrolysis products from polysaccharides. From T_{oven} = 250 °C the character of the mass spectra starts to change from a number of single polymers into a three-dimensional network polymer at 310 °C. Apparently the proteinaceous material can be considered as relatively thermally stable. The main changes of the molecular composition as a function of the temperature are visualized in Scheme 5.1.

DTMS-EI

DTMS-EI has the potential to reveal more information, in comparison with DTMS-CI, on the proteins and lipids of AR in the lower temperature range and on the molecular composition of the residues heated from 250 °C upwards. Thus, EI experiments were carried out on untreated grains and on the residues heated from 250 °C.

Low voltage EI experiments on untreated biological material show usually protein markers in the range of *m/z* 130-200 (van de Arendonk et al., 1997). This protein "hump" is not present in the mass spectrum of the sample of untreated grains of AR. Only mass ions *m/z* 69 (Val, Lys, Arg, Cys); 70, 154 (Arg, Pro, Lys); 84 (Lys, Val, Gln) and 91 (Phe) are observed (Scheijen, 1991; Chiavari and Galletti, 1992; Stankiewicz et al., 1996; Boon and de Leeuw, 1987) as typical protein markers in the low mass range. Lipids are recognized as fatty acids, sterols, diglycerides and triglycerides. Fatty acids are represented by *m/z* 256, ($C_{16:0}$) and 262, M-H_2O $C_{18:2}$; diglycerides by *m/z* 574, M-H_2O $C_{16:2,18:2}$ and 616, $C_{18:2,18:2}$ and triglycerides by *m/z* 862, $C_{16:0,18:0,18:0}$ and 890, $C_{18:0,18:0,18:0}$. Also present are the ions *m/z* 396-414 of sitosterol ($C_{29:1}$) and *m/z* 430 of tocopherol.

The mass spectrum of the samples of wheat grains heated from 250 °C upwards show the mass peaks that describe the conversion of a polysaccharide and protein rich material at 250 °C into a material that with increasing temperature becomes dominated by aromatic compounds and finally is a strongly C-enriched material (Braadbaart et al., 2004b; Pastorova et al., 1994). These molecular changes, as a function of the temperature, were studied by principal component analyses (PCA), over a mass range from 20-400. The scores of the first (PC1) and the second (PC2) principal component are presented in a score plot (Fig. 5.9). These components

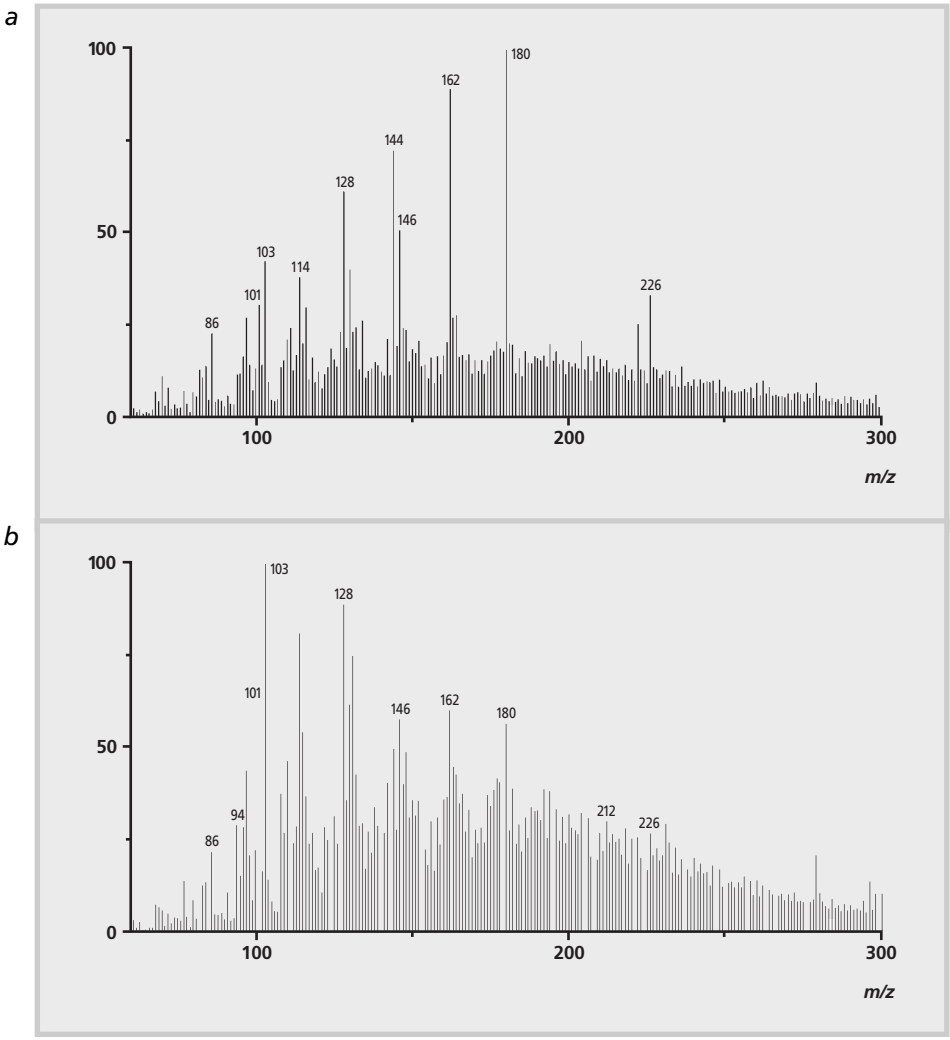


Fig. 5.7
DTMS-CI mass spectra of samples of emmer wheat grains heated for 120 minutes (var. AR). (a) Toven = 270 °C and (b) Toven = 310 °C.

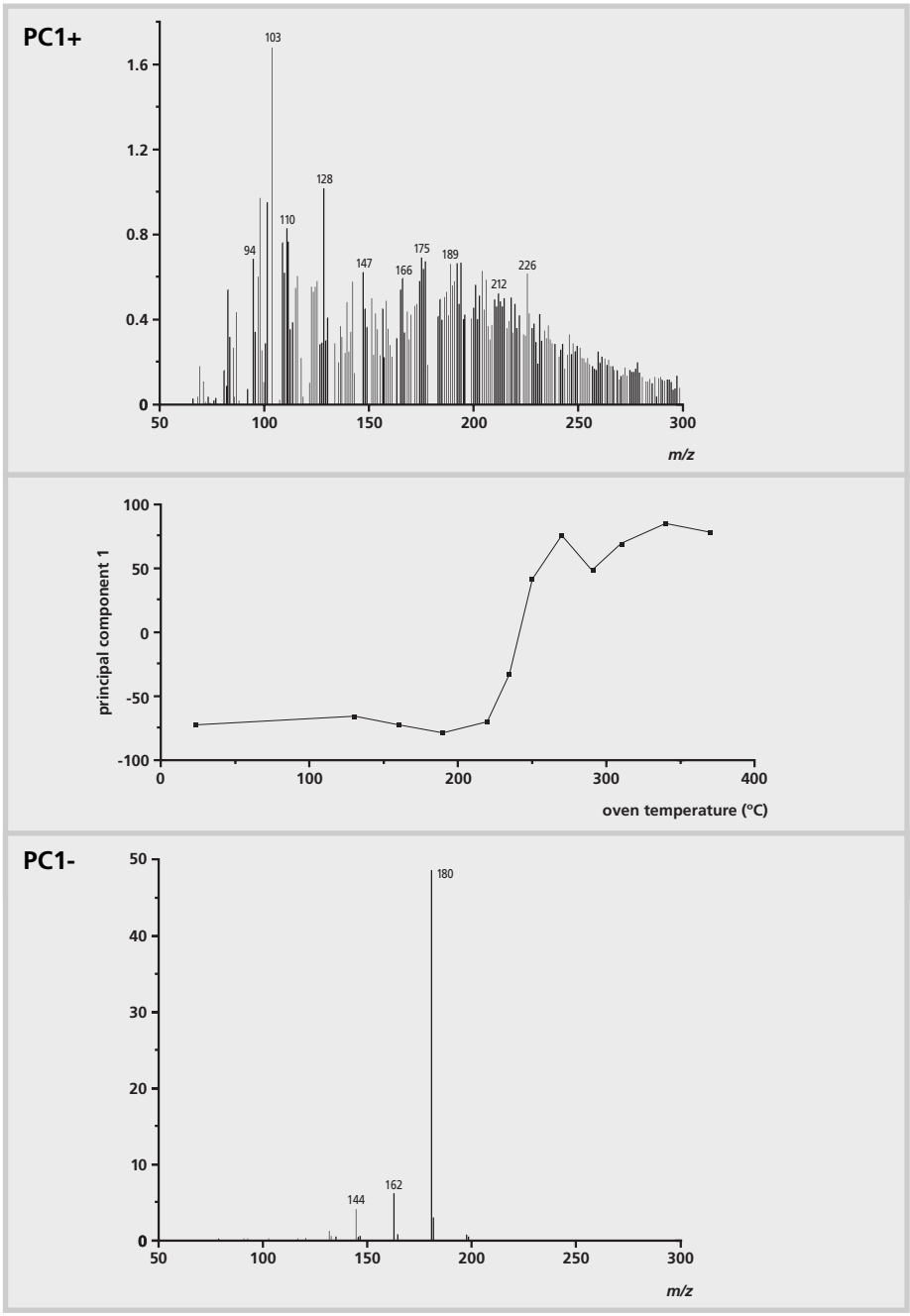
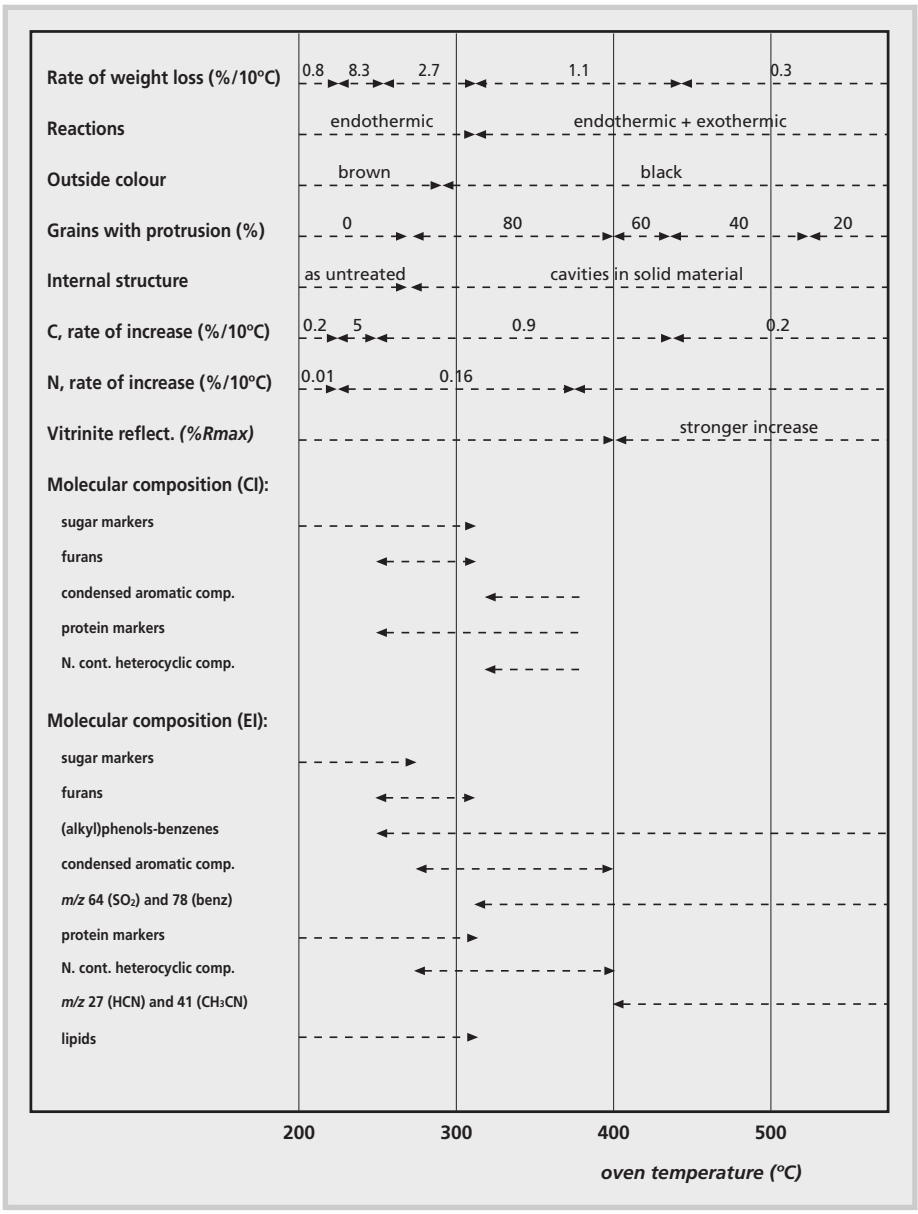


Fig. 5.8
Score plot of the principal component analysis of DTMS-CI measurements of untreated emmer wheat grains (var. AR) and grains heated for 120 minutes as a function of the oven temperature in °C. PC1- and PC1+ are the "numerically extracted" mass spectra responsible for the separation into polysaccharide-rich and aromatic-rich samples, respectively.



Scheme 5.1
Classification of the changes of the physical properties, the bulk chemical analyses and the molecular composition of emmer wheat grains (var. AR) heated for 120 minutes as a function of the oven temperature in °C.

describe, respectively 51 and 27 % of the total variance of 100 %. The higher components are ignored. The PCA is used in combination with graphical rotation (Windig et al., 1983) to adjust the PCA mass spectra to specific thermal regimes. The axes are plotted in Fig. 5.9, which also show the loading plot. The chemical nature of each axis is visualized by its "numerically extracted" mass spectrum A, B and C (Fig. 5.9).

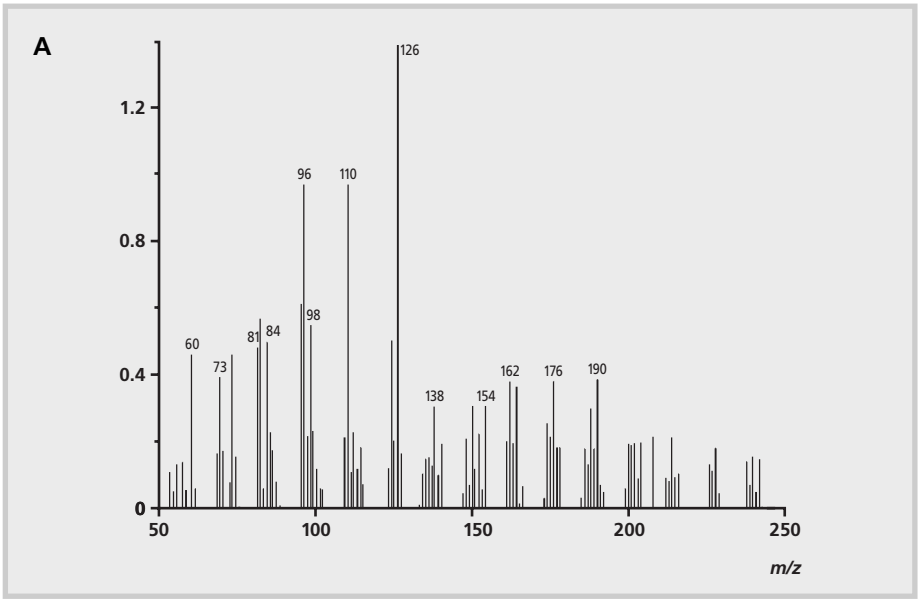
Spectrum A, corresponding to the samples heated at 250 and 270 °C, shows ions indicative of polysaccharides (*m/z* 60, 73, 98, 126), proteins (*m/z* 70, 84, 138, 154) and lipids (*m/z* 262) similarly to the mass spectrum of untreated AR (Braadbaart et al., 2004c). Ions *m/z* 67 and 81 are tentatively attributed to (alkyl)pyrroles as the result of Maillard or Strecker reactions between sugars and amino acids (Vernin and Párkányi, 1982). The presence of furans is recognized by the release of the ions *m/z* 95, 96 and 110 (Pastorova et al., 1994). The series of masses *m/z* 148, 162, 176, 190, etc., which are tentatively attributed to condensed aromatic compounds (Pastorova et al., 1993a), show the beginning of the conversion of the original biomaterial into an aromatic rich condensed material.

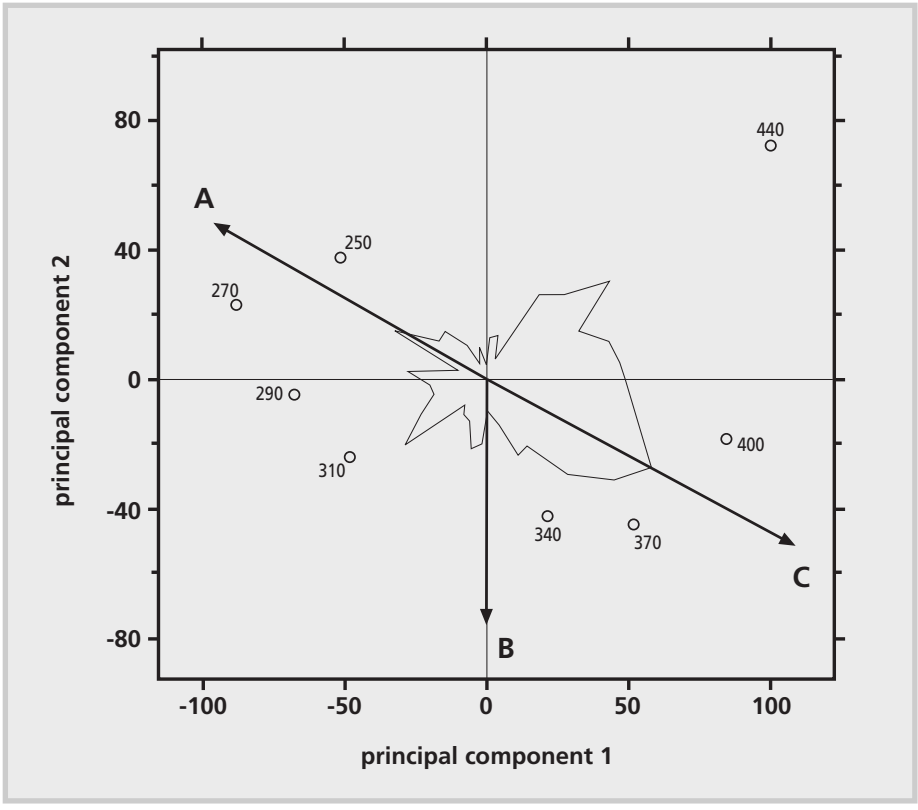
Spectrum B (DF2-) represents a heating temperature, which is just higher than 310 °C. The markers indicative for the presence of polysaccharides, proteins, lipids and furans are no longer present. The spectrum is now characterized by masses released from aromatic compounds. The following functional groups can be recognized: (alkyl)phenols (*m/z* 108, 122), (alkyl)benzenes (*m/z* 91, 92, 105, 106) and condensed aromatic compounds (*m/z* 146, 160, 162, 172, 174, 186, 198, etc.) (Pastorova et al., 1993a). Two series of odd numbered masses are observed. Firstly by the series *m/z* 117, 131, 145, etc., identified as (alkyl)indoles derived from Tryptophane in proteins and secondly by the series *m/z* 147, 161, 175, etc. Both series are attributed to N containing heterocyclic compounds (Moldoveanu, 1998).

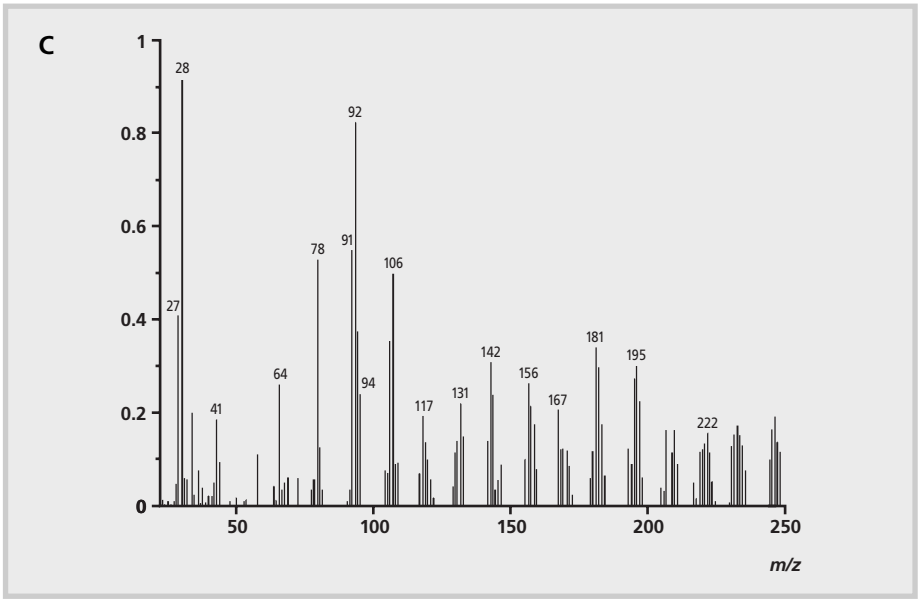
Spectrum C corresponds to a heating temperature of 400 °C. The characteristic ions indicative of condensed aromatic compounds in the sample are no longer present at this temperature. The main ions released from this sample are phenol (*m/z* 94), (alkyl)benzenes (*m/z* 78, 91, 92), SO_2 from sulfates (*m/z* 64) and the N containing compounds HCN (*m/z* 27) and CH_3CN (*m/z* 41). The series *m/z* 167, 181, 195, etc. could point to the formation of alkylidene-imidazole compounds at this temperature (de Waart et al., 1992).

Vitrinite reflectance measurements

A vitrinite reflectance could be measured on samples from T_{oven} = 270 °C upwards. The reflectance of the lowest temperature samples rises slowly from T_{oven} = 270 °C to 400 °C (Fig. 5.10). Thereafter the reflectance rises more rapidly. The S.D. of the measurements increases from 0.03 at T_{oven} = 270 °C to 0.15 at 600 °C.


A
126
1.2
96
110
0.8
98
60
81
84
73
0.4
162
176
190
138
154
0
50
100
150
200
250
m/z


80
440
A
250
40
270
0
290
400
310
-40
340
370
C
B
-80
-100
-50
0
50
100
principal component 2
principal component 1



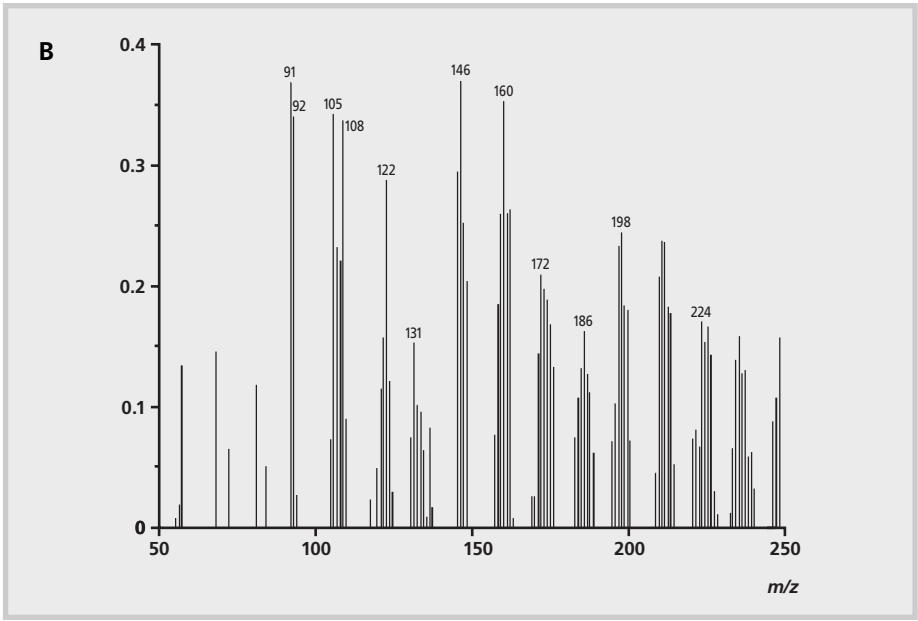


Fig. 5.9
Score plot of principal components analysis of DTMS-EI measurements of untreated emmer wheat grains (var. AR) and grains heated for 120 minutes as a function of the oven temperature. The oven temperatures of the samples are indicated in the figure in °C. Product axes A, B and C are superimposed and the "numerically extracted" mass spectrum of each axis is shown.

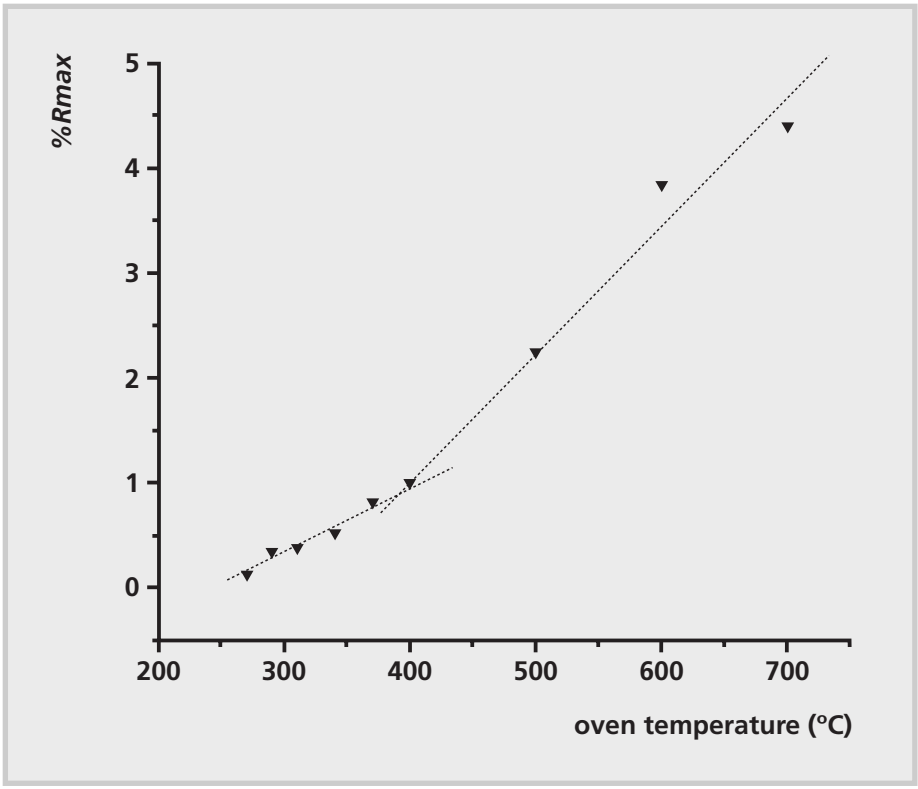


Fig. 5.10
The results of the vitrinite reflectance (%Rmax) measurements of grains of emmer wheat (var. AR) heated for 120 minutes as a function of the oven temperature in °C.
▼ = samples of emmer wheat.

DISCUSSION

Fruits of wheat consist of a fruit coat (pericarp) enclosing the endosperm and the embryo. The endosperm is the bulk material of a grain of wheat and consists for about 60 % of starch and 10-15 % of proteins (Table 5.1). It has been shown that the presence of the pericarp and the varying content of protein affects the conversion of the untreated wheat grains as a result of heat treatment (Braadbaart et al., 2004c). The changes of the physical properties, the bulk elemental contents and the molecular composition of grains of AR as a function of the oven temperature are summarized in Scheme 5.1 and will be discussed accordingly. The experimental set-up simulates for example the dropping of wheat grains into an open fire, where the temperature differs as a function of the place where they end up. Thus for each temperature a separate experiment was carried out in a pre-heated oven. The time to approach the constant weight loss decreases as a function of the oven temperature (Fig. 5.1). Consequently the average heating rate inside the grains increases from a slow 30 °C min^{-1} at T_{oven} = 340 °C to a moderate 175 °C min^{-1} at 700 °C. The oven temperature and the accompanying heating rate of each experiment determines the mechanism and compositional pathway that will occur. The result will be a typical residue for each temperature.

The strong weight loss rate (Fig. 5.2) in the range of T_{oven} = 220 up to 250 °C is the result of dehydration, which is confirmed by the clear liquid in the outlet tube (Table 5.1). This liquid was not further analysed, but most likely only water is released at these temperatures and pressures (Pastorova et al., 1993b; Braadbaart et al., 2004a). The C content rises strongly from 220 up to 250 °C, indicating that relatively less C containing compounds were removed from the residues in this temperature range. The molecular composition did not change significantly according to the mass spectra obtained from the samples under DTMS-CI and EI conditions. However the colour of the grains did change from their original colour, through brown into brown-black. This is indicative for the occurrence of Maillard reactions of sugars and amino acids. At T_{oven} = 250 °C, in spite of the strong dehydration, a material is present that is still characterized by the presence of DTMS features of polysaccharides and proteins. Related to the swollen character of the grains at this temperature it is noted that the starch granules in the endosperm cells did not change in size (Fig. 5.4). The dimensional changes will be discussed elsewhere in a separate study (Braadbaart et al., 2004e).

From T_{oven} = 250 up to 310 °C many changes are observed. The polysaccharide rich material changes gradually into a material richer in aromatic compounds. However even the material heated at 310 °C for 120 minutes still contains sugar markers (Fig. 5.7b). The conversion of polysaccharides into the various aromatic compounds is accompanied by the release of volatiles (Shafizadeh, 1975). Many

of these volatiles are apparently not able to pass the pericarp on their way to the external environment and thus a pressure is build up inside the grain resulting in a swelling of the grains and/or collapse of the pericarp between T_{oven} = 250 and 270 °C (Braadbaart et al., 2004c). Hence, the number of grains with a protrusion increases sharply at T_{oven} = 270 °C. It needs remarking that the molecular composition of the pericarp, which mostly consists of polysaccharides, has also changed. The sudden release of the volatiles is also expressed in the change of the internal structure as the volatiles apparently are channelled through pipe like structures to the outside (Fig. 5.4c). Compounds that were formed as a result of the dehydration, such as anhydrohexoses, decompose further to form furans (Pastorova et al., 1994).

Between T_{oven} = 290 and 310 °C the volatile producing endothermic reactions are accompanied by exothermic reactions. The latter are the result of secondary reactions between the converting solids of the residues in the reaction zone and the hot volatiles transferring the residues on their way to the external environment. This suggests that the condensed aromatic compounds at this oven temperature are secondary products, which is also expressed in the increase of the total weight of the residues in comparison to the residue of microcrystalline cellulose (Fig. 5.2). The volatiles that are released from the residues and condensate in the cold outlet tube are now water and a tarry product, which colours the liquid light to dark yellow (Table 5.1). At this oven temperature and after heating for 120 minutes sugar markers are still released from the residues under CI conditions, i.e. mass ions *m/z* 162, 180, 222 and 240 (Fig. 5.7b). This agrees with the proposed model as presented by Boon and co-workers (1994) regarding the formation of a thermostable polymer network at oven temperatures higher than c. 270 °C in the residues of heated microcrystalline cellulose, a polysaccharide with a glucose repeating unit almost similar to starch. In their model residual non-volatile glucose cores with attached ring-cleavage fragments, as a result of (2+2+2) cycloreversion reactions, act increasingly from T_{oven} = 250 °C, as aldolcondensation sites for volatized compounds that will react with larger non-volatized residual structural elements. The polymer network has thus changed from a carbohydrate-dominated reactant into a material with mainly aromatic moieties, which are thermally more stable. The performed DTMS-CI experiments provide detailed insights into the conversion of polysaccharides, the bulk material of wheat grains, as a result of heating.

From T_{oven} = 310 up to 400 °C no major changes in the molecular composition occur. At higher oven temperatures the condensed aromatic compounds are no longer observed. The rate of heating has now strongly increased from a low 30 °C min^{-1} at 340 °C to a moderate 175 °C at 700 °C. The high reaction rate from T_{oven} = 440 °C is causing the release of large quantities of volatiles that appear to be removed from the reaction zone in the grains so fast that no reactions can take

place between the volatiles and the converting material inside the grain. Therefore the suggested secondary products such as the condensed aromatic compounds are no longer formed from T_{oven} = 440 °C upwards. On the other hand the exothermic reaction is still present at these temperatures and other chemical reactions must be the cause for the exotherm (Varhegyi and Jakab, 1994). The C content has increased to 82 % (daf) at 440 °C and reaches 88 % (daf) at 700 °C. The N content remains almost constant at c. 5 % (daf), however ions like *m/z* 27 (HCN) and 41 (CH_3CN) are still released from the residues under DTMS-EI conditions. Thus a tertiary product is formed characterized by a strong C-enrichment and the presence of N containing moieties. From T_{oven} = 400 °C the maximum vitrinite reflectance *(%Rmax)* shows a stronger rise that continues up to 700 °C (Fig. 5.9). This rise has been attributed to a progressive ordering of the molecular system that comprises the C-enriched residues of the grains of AR (Goodarzi and Murchison, 1972; Murchison, 1978).

IMPLICATIONS FOR THE ARCHAEOLOGY

The results of the experiments with recent emmer wheat grains (AR) show that during a 120 minutes exposure up to a temperature of 250 °C, the molecular composition of the residues still consists of dehydrated polysaccharides and of proteins. These residues when deposited into the soil may be microbially degraded similar to the degradation of untreated wheat grains. If the heating takes place between 250 and 310 °C, the residues consist of a decreasing amount of polysaccharides and an increasing amount of aromatic compounds. Whether these residues or parts of it will survive the various degradation processes is not sure. The residues heated at temperatures higher than 310 °C might have a better chance to survive the degradation processes and thus may be found in the archaeological record. A vitrinite reflectance measurement is a fast and cheap method to determine the temperature at which fruits and seeds have been heated under the present experimental conditions. Application of this method will make it possible to verify the earlier statement about the residues heated at temperatures higher than 310 °C.

Acknowledgements

Technical assistance with scanning electron microscopy by Dr. W. de Priester (Clusius Laboratory, Leiden University, The Netherlands) was gratefully acknowledged. We thank Miss K. Reimer (Geo-Environment, TNO-NITG, The Netherlands) for her assistance with the vitrinite reflectance measurements. This research is supported by FOM research program 49 funded by the Foundation for Fundamental Research on Matter (FOM), a subsidiary of the Dutch Organisation for Scientific Research (NWO).

CHAPTER 6

Digital imaging analysis of size and shape of wheat and pea as a result of heating under anoxic conditions as a function of the temperature

ABSTRACT

Three types of experiments were carried out. In the first series of experiments emmer wheat grains *(Triticum dicoccum* Schübl), var. AR, and peas *(Pisum sativum* L.), var. RE, were heated at temperatures ranging from 130-700 °C under controlled anoxic conditions for maximum 120 minutes. For each temperature a separate experiment was carried out in a pre-heated tube oven. Image acquisition is carried out on a flat bed scanner with a transparency adapter; for the analysis the image program ImageJ 1.27 was used. Various size and shape factors are discussed and the definitions of the selected size and shape factors are given. From thirty specimens of the untreated specimens and the solid residues heated at each temperature size and shape were measured and the mean calculated. The results show a change of size and shape as a function of the temperature. The changes for wheat grains and peas are not identical. It is suggested that the presence of the pericarp enclosing the wheat grains causes this difference. In a second series of experiments it was shown that untreated grains of emmer, bread and macaroni wheat can be separated by measuring the shape, but size varies too much within each species, probably the result of different growing conditions, to be useful for separation purposes. As a result of heating at temperatures higher than 290 °C the shape of the three species becomes identical. This implies that the three species cannot be separated anymore after being heated, based on size and shape solely. Finally both emmer wheat grains enclosed by chaff and without chaff were heated under similar conditions. After removal of the chaff from the former size and shape of the grains were measured. The results show no significant difference in size and shape between both types.

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Accepted for publication in
Vegetation History and Archaeobotany

INTRODUCTION

Macrobotanical remains of fruits and seeds found in the archaeological record are of importance for our understanding of the way ancient people lived. One of the processes by which these remains may survive biological, physical and chemical decomposition in the soil is carbonization. This process is usually described as heating under anoxic conditions at a specific temperature. The physical and molecular properties change as a function of the heating temperature and thus their chance for survival (Braadbaart et al., 2004a; b; d). Heating under anoxic conditions causes also distortions of the fruits and seeds (Hopf, 1955; van Zeist, 1970; Kislev and Rosenzweig, 1989; Smith and Jones, 1990; Wright, 1998). Usually the length, width and thickness of the fruits and seeds are measured and the ratios length/width and thickness/width calculated, but measurement or calculation of the size was not possible.

Digital imaging analysis offers an objective and quantitative method to extract digital images from fruits and seeds and their heated residues. These images can be used to measure the dimensions of individual specimens and mathematically extract size and shape related information. In the cereal industry several imaging systems have been developed for the characterization and classification of wheat varieties (Sapirstein, 1994). Also in other fields of biology digital imaging systems are applied (Bannur et al., 1999). Most systems employ video cameras or CCD cameras for digitizing the images, but this has posed problems in the repeatability of the results (Shouche et al., 2001). In this study a digital scanner was used and the imaging was performed in transparency mode.

Differentiating species and varieties of the carbonized fruits and seeds has been the subject of many studies (van Zeist, 1970; Jacomet, 1987; Hillman et al., 1993; Zohary and Hopf, 2001). For grains of a species of wheat *(Triticum* L.), the presence of carbonized parts of the chaff in the assemblage is usually necessary and additional characters of the grains are used to separate species such as embryo angle, embryo roundness, cross section, apex bluntness, etc. The identification of grains based solely on the measured dimensions poses problems (Hillman et al., 1996). For the separation of varieties of pea *(Pisum sativum* L.) it is suggested that for example the length and the proportions of the hilum is used (Hubbard, 1992).

This study assesses the extent and the possible causes for the change in size and shape of heated modern fruits and seeds as a function of the heating temperature. In addition the relationship between these morphological changes and the changes in physical and molecular parameters will be evaluated. Also the possibility to separate three species of heated wheat grains based on size and shape is investigated. Wheat grains and pea seeds were selected as they frequently occur

in the archaeological record and because of the morphological differences between them. Wheat is a one seeded fruit with the pericarp enclosing the seed and pea is a seed with two cotyledons enclosed by a seed coat. It has been shown that the presence of the pericarp in wheat grains affect the morphology differently as a result of heating when compared with peas (Braadbaart et al., 2004a; c). For each temperature a separate heating experiment was carried out. The dimensions were always extracted from the images with the fruit and seed or their heated residues in the same orientation and measured by digital image analysis.

MATERIALS AND METHODS

Samples

Four varieties of emmer wheat *(Triticum dicoccum* Schübl) were obtained from the Centre of Genetic Resources, Wageningen, The Netherlands (CGN): CGN 08340 (Germany), CGN 11482 (Germany), CGN 11485 (France) and CGN 11486 (Austria). Two varieties of emmer wheat are from the collection of the Faculty of Archaeology, Leiden University, The Netherlands: nrs. 4855 (Belgium) and 5801 (Switzerland). One variety of emmer wheat (AR) was grown at Archeon, Alphen a/d Rijn, The Netherlands. Two varieties of bread wheat *(Triticum aestivum* L.) are CGN 04263 (USSR) and 10417 (India). One variety was obtained from a harvest in the summer of 2003 in the department Aisne (France). One variety of bread wheat, qualital (Q, a hard bread wheat) and one variety of macaroni wheat *(Triticum durum* Desf.), ardente (A), were donated by the Unité de Technology des Céréales et des Agropolymères, ENSAM-INRA, Montpellier, France. Two varieties of macaroni wheat are from the collection of the Faculty of Archaeology: nr. 125 (Italy) and 1476 (The Netherlands). The peas used: the variety 'Noord-Hollandse Rozijnerwt' (RE; CGN 10293) was obtained from CGN.

Heat treatment

Heating experiments were undertaken using a Carbolite tube oven (model MTF 12/38/250); the rate of heating was set at 2 °C min^{-1}. The wheat grains and peas were placed in the pyrex vessel. The vessel was inserted in a 30 cm long pyrex tube (∅ 2.3 cm) at 18 cm from the inlet and heated. Separate experiments were carried out at oven temperatures ranging from 130 up to 700 °C. A constant flow (150 ml min^{-1}) of N_2 at atmospheric pressure was maintained to assure anoxic conditions. Peas were heated for 60 minutes and the wheat grains for 120 minutes. The oven was pre-heated and for each experiment the temperature was fixed. Gases and volatiles were vented and not further investigated.

Earlier investigations have shown that heat treatment up to a temperature of 250 °C causes wheat grains to swell and at higher temperatures the pericarp collapses with or without the formation of protrusions on the outside of the pericarp (Braadbaart et al., 2004c). The shape and size analysis took place after the protrusions were removed. In peas these features were not observed (Braadbaart et al., 2004a).

Imaging hardware and software

A flat bed scanner (Umax Mirage IIse) with transparency adapter and Umax majic scan software was used for image acquisition. Adobe Photoshop 7.0 software was

used to ensure an identical orientation of the specimens. The digital image analysis was carried out by the standalone version of ImageJ 1.27, a program developed at the National Institutes of Health of the USA. A standard personal computer was used for image analysis.

Image grabbing and analysis

The wheat grains were placed on the scanner bed with the ventral side down with about one cm space between the specimens to avoid grain-to-grain contact. Grains were arranged in rows with the apical brush oriented upwards and the embryo downwards. The peas were placed in rows with hilum and chalaza oriented downwards (Fig. 6.1). Grey images of the specimens were grabbed with the scanner software. The scanner resolution was set at 300 dpi and for all images the identical settings of illumination were used. Images were stored in .tiff format and exported to the Photoshop program for possible corrections regarding the orientation. Subsequently the ImageJ program was used for feature extraction. For the calculation of size and shape related features of individual specimens it is important to detect each specimen in the image. For this purpose thresholding (Binary Contrast Enhancement) is commonly used. A greyscale image is converted to binary by defining a greyscale cut-off point, the threshold. Greyscale values below the threshold become black and those above become white. The threshold value was set so embryo and brush are still recognized in the image. It was kept constant for the analysis of all images.

Feature extraction

Geometric features of each specimen in the described position, including length and width, were measured from the binary images (Fig. 6.1). The size of the specimens can be characterized by the size factors: area and perimeter. The area is measured as the surface (in mm^2) of the longitudinal section at plane A of each specimen. This section is thus situated parallel to the scanner bed and can also be described as the space "seen" from above. The perimeter (in mm) is the length of the outer boundary of this section (Fig. 6.1). Area and perimeter are mathematically extracted from the geometric features, but in this study only the area was selected. The shape of the specimens can be expressed in a shape factor, calculated from the geometric features, which is determined by the morphology of the investigated fruits and seeds. In the cereal industry various shape factors are in use (Symons and Fulcher, 1988). One is the compactness of a grain, which is calculated as (perimeter*perimeter)/(4*π*area). This factor can be compared with the slenderness of a grain or 100 Length/Width ($100LW^{-1}$), a shape factor frequently used in archaeology (van Zeist, 1970; Hubbart, 1992). Both factors were calculated. Although the absolute numbers differ, the variation in shape as a function

of variety or temperature is identical. In this study $100LW^{-1}$ is used as the shape factor. In the archaeology the thickness of a specimen is often measured in addition to length and width. Additional measurements, with the specimens positioned on their side, showed that the variation of the width and the thickness are proportional. Thus measurements with wheat grains in the ventral side down position were considered sufficient. The peas were measured in an identical manner (Fig. 6.1).

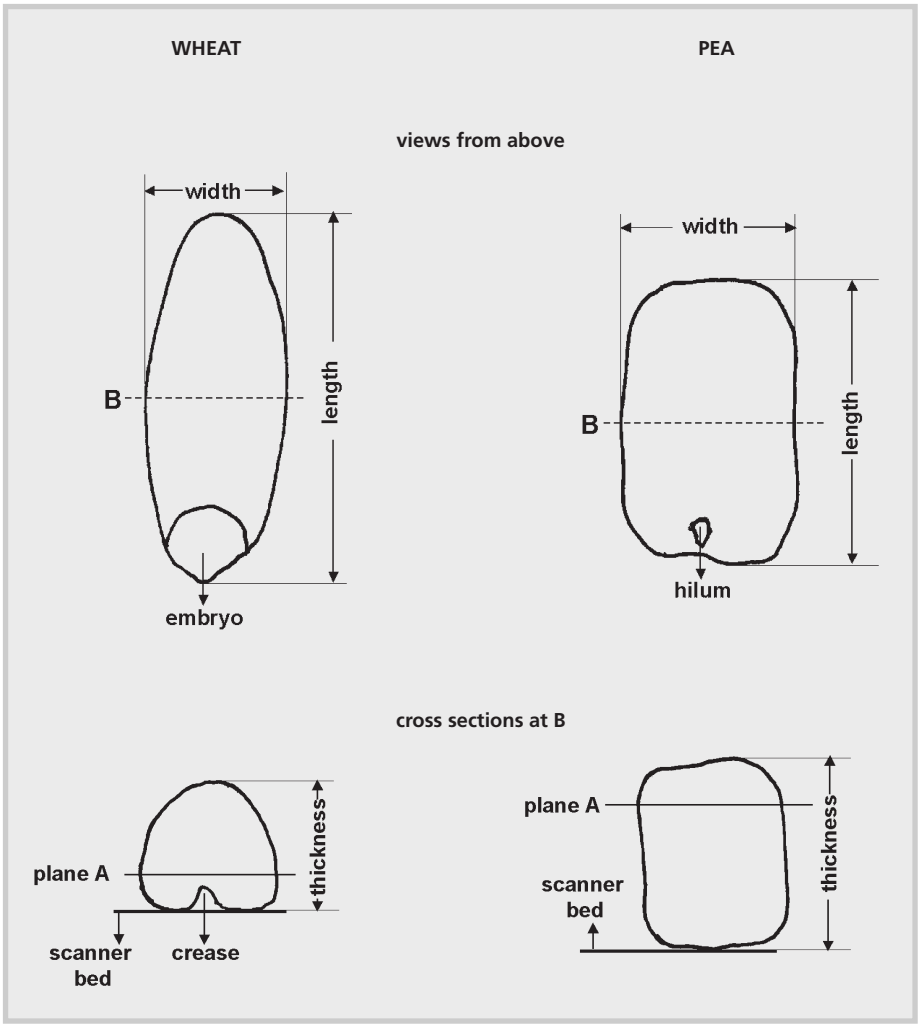


Fig. 6.1
Key to the measured dimensional features and the orientation of wheat grains and pea seeds on the scanner bed.

Sample size

It was argued that for the determination of the changes in size and shape as a result of heating length, width and area of each untreated and heated specimen in a sample was measured. In order to achieve this the mean of each of these features of the specimens in a sample is calculated. The precision of the mean, based on independent measurements, depends upon its standard error, $\sqrt{(\sigma^2 n^{-1})}$, in which σ^2 is the variation of the population and n the sample size or in this case the number of measurements of each of the three features. The former is determined by the population and therefore fixed. An increase of n will reduce the standard error, but should not be increased unnecessarily. Therefore confidence intervals can be calculated for the mean of the features of the population with a 95 % chance that the mean of the features of a random sample is situated within the calculated interval. The population of the emmer wheat grains (var. AR) has a normal distribution with parameters mean (μ) and standard deviation (σ) as shown in Table 6.1. The 95 % intervals for the mean of each feature are calculated from $\mu \pm 1.96 * \sigma * (\sqrt{n})^{-1}$. The results show that for emmer wheat grains a random sample of n = 30 is sufficient to fit the confidence interval of each feature. For peas (var. RE) the same procedure was followed and it shows that a sample of 10 peas will be sufficient (Table 6.2).

	Total population			*Sample of 30 grains*
	mean (μ)	*S.D.* (σ)	*95 % conf. interval (n = 30)*	*mean* (μ)
Area (mm²)	*12.4*	*2.1*	*11.17 – 13.25*	*13.1*
Width (mm)	*2.37*	*0.31*	*2.37 – 2.59*	*2.59*
Length (mm)	*7.14*	*0.51*	*6.96 – 7.32*	*7.20*

Table 6.1
Statistical parameters of emmer wheat grains (var. AR)

	Total population			*Sample of 10 seeds*
	mean (μ)	*S.D.* (σ)	*95 % conf. interval (n = 10)*	*mean* (μ)
Area (mm²)	*65.0*	*8.2*	*59.90 – 70.10*	*68.8*
Width (mm)	*7.24*	*8.0*	*6.74 – 7.74*	*7.72*
Length (mm)	*11.59*	*6.3*	*11.19 – 11.99*	*11.54*

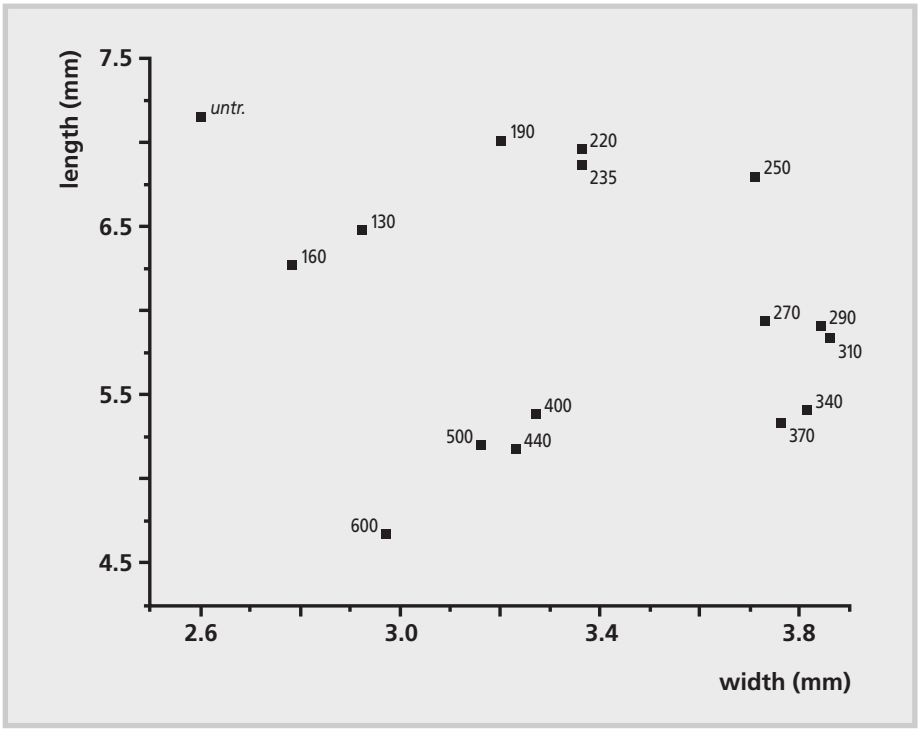
Table 6.2
Statistical parameters of seeds of peas (var. RE)

HEATING AND THE CHANGE OF SIZE AND SHAPE, AS A FUNCTION OF TEMPERATURE

Emmer wheat grains

Samples of 30 grains of the variety AR of emmer wheat, after removal of the chaff, were heated at 130, 160, 190, 220, 235, 250, 270, 290, 310, 340, 370, 400, 440, 500 and 600 °C for 120 minutes in separate experiments. Length, width and area of each specimen in the samples at each temperature were measured. The mean value of each feature at each temperature was calculated. The length was plotted versus the width (Fig. 6.2a) and the area versus the temperature (Fig. 6.2b). To obtain more insight into the changes of the shape, the shape factor, $100LW^{-1}$, is plotted as a function of the area at the various temperatures (Fig. 6.2c). The results show that the grains become rounder ($100LW^{-1}$ decreases) at increasing temperatures up to 250 °C, with an increase of the area. From 270 °C upwards the shape factor remains constant with a decreasing area. The samples of emmer wheat (var. AR) heated at 130 and 160 °C have the same area as the untreated grains, but the length is decreased and the width increased. This is the beginning of a swelling process, whereby only changes in shape are observed, resulting in a rounder grain. At 190 °C the area has increased and it continues to do so up to 250 °C. A reason for the swelling could be the actual swelling of the individual starch granules in the cells of the endosperm, being the bulk material of wheat grains. Swelling of starch occurs when heated in the presence of water and is called gelatinization (Singh et al., 2003). However, it has been shown through SEM microphotographs of the internal structure that as a result of heating of whole wheat grains the starch granules do not swell (Braadbaart et al., 2004c; d). The main process in these whole grains as a result of heating up to 250 °C is dehydration. Evidently the water vapour is not able to pass the pericarp directly causing an increase of the pressure inside resulting in the swelling of the grain. Simultaneously the molecular and/or physical properties of the pericarp have changed too and this suggests that from 190 °C the pericarp is able to stretch, resulting in a larger area. Heating at temperatures higher than 250 °C results in a gradual conversion of starch into aromatic compounds and from 310 °C starch is no longer present. This conversion will result in the release of many volatiles and the pressure inside the grains will increase in such a way that eventually the pericarp will collapse. Hereafter no more swelling occurs and the size no longer increases, but decreases as the total weight decreases with increasing temperature (Braadbaart et al., 2004c). In this range of temperatures (270–310 °C) similar molecular reactions take place in the grains and the heated residues of 270, 290

a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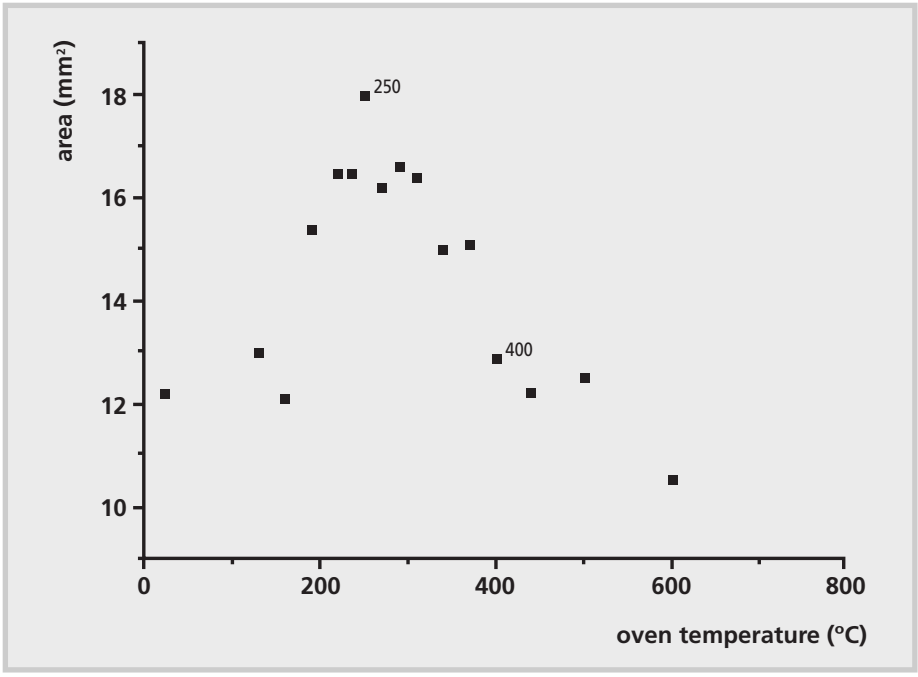
370
600

b


area (mm²)
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14
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oven temperature (°C)
250
400

c

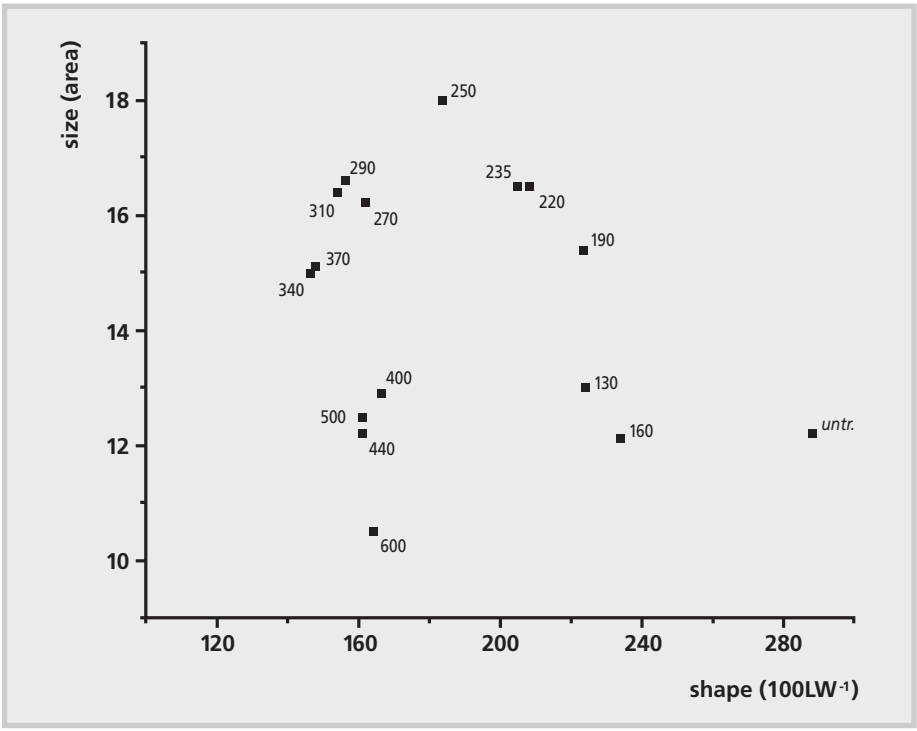


Fig. 6.2
Dimensional changes of emmer wheat grains (var. AR) as a result of heating for 120 minutes: (a) length (mm) as a function of width (mm) heated at the indicated temperatures (°C); (b) area (mm^2) as a function of the oven temperature (°C) and (c) size (area) as a function of the shape ($100LW^{-1}$) heated at the indicated temperatures. Sample size 30 grains.

and 310 °C have the same characteristic size and shape. The heated residues of 340 and 370 °C show the presence of exothermic reactions and thus the formation of secondary compounds (Braadbaart et al., 2004d). A further decrease of the size for both residues is observed, but the shape has not changed. From 400 °C upwards a different type of conversion takes place, mainly because the heating rate has increased and no secondary reactions can occur as the volatiles are removed too fast from the reaction zone. Thus the residues at 400, 440 and 500 °C show the same size, however as the weight still continues to decrease the size has become smaller compared with the residues heated at 340 and 370 °C. The shape did not change. Untreated grains and grains heated at 290, 340 and 440 °C are visualized and show the differences in size and shape (Fig. 6.3).

The physical and molecular changes that take place from 250 °C upwards distinguishes three stages as a function of the temperature i.e. the stage from 250-310 °C, the stage from 310-400 °C and a stage at temperatures higher than 400 °C. These stages correspond well to the changes in size and shape.

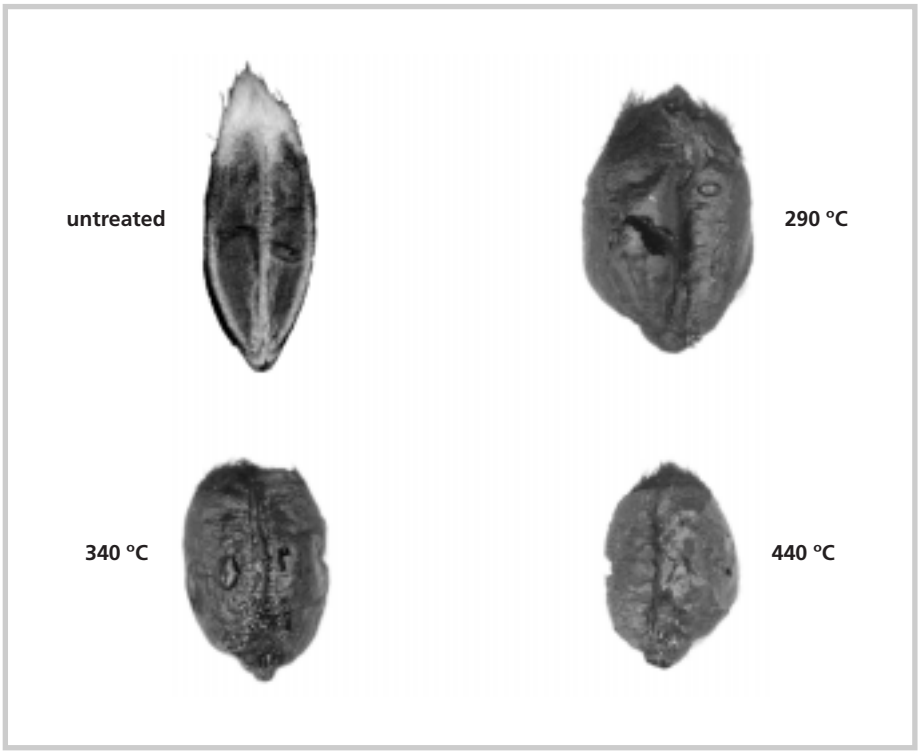


Fig. 6.3
Samples of emmer wheat grains (var. AR) heated at the indicated temperatures for 120 minutes (°C).

Seeds of peas

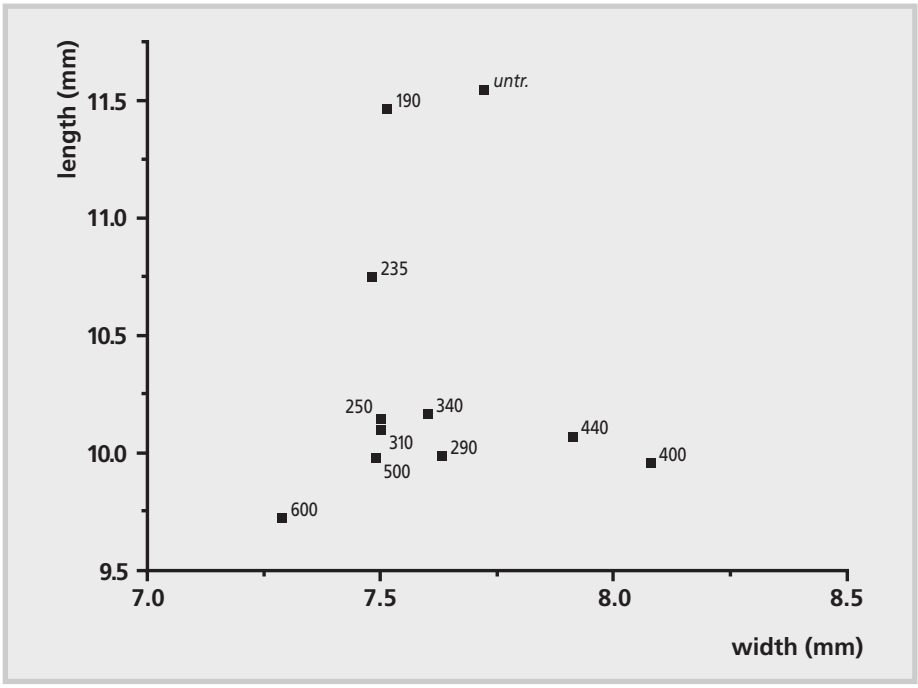
Samples of 10 peas were heated at the same steps under similar conditions in separate experiments for each temperature. The mean values of width, length and area of the specimens in each samples of untreated peas and of the residues at each temperature were calculated. (i) The width as a function of the length (Fig. 6.4a), (ii) the area as a function of the oven temperature (Fig. 6.4b) and the size factor, represented by the area, as function of the shape factor, $100LW^{-1}$ (Fig. 6.4c) are shown. Until 190 °C no major changes in size and shape are observed. At higher temperatures up to 250 °C a decrease of the size is shown and the residues become rounder. The dehydration of the starch, being the bulk material of peas, is the main process at these temperatures (Braadbaart et al., 2004a; b). The resulting water vapour is freely removed from the seeds. The residues heated from 250 up to 340 °C do not show changes in size and shape. This implies that the physical and molecular changes that occur at 310 °C do not show their effects on the size and shape. Subsequently at 400 °C the size increases and the shape factor, $100LW^{-1}$, decreases resulting in rounder seeds. The physical and molecular properties change at this temperature, but no explanation of the changes in size and shape is possible. From 400 °C upwards the size gradually decreases, which corresponds to the gradual decrease of the weight (Braadbaart et al., 2004a). Simultaneously the seeds become less round.

In the case of peas the stages, based on the physical and molecular changes, are from 270–310, from 310–400 and higher than 400 °C. The changes in size and shape, as a function of the temperature, show only two stages one stage from 250–370 °C and a second stage from 400 °C upwards.

Comparison between wheat grains and peas

The size and shape of wheat grains and peas as a function of the temperature change in a different way. The bulk material of both is starch and the same conversion reactions will take place as a result of heating. However, the morphology is different and the endosperm of wheat grains is enclosed by the pericarp, while the cotyledons of the peas are surrounded by the seed coat. The volatiles are apparently freely removed through the seed coat of the peas and no pressure is build up inside. In contrast in wheat grains a pressure is build up and a swelling of the grains occur. The change in size and shape of both the wheat grains as the peas corresponds well to the changes in physical and molecular properties. There is one exception in the case of peas where at 310 °C no change in size and shape is observed.

a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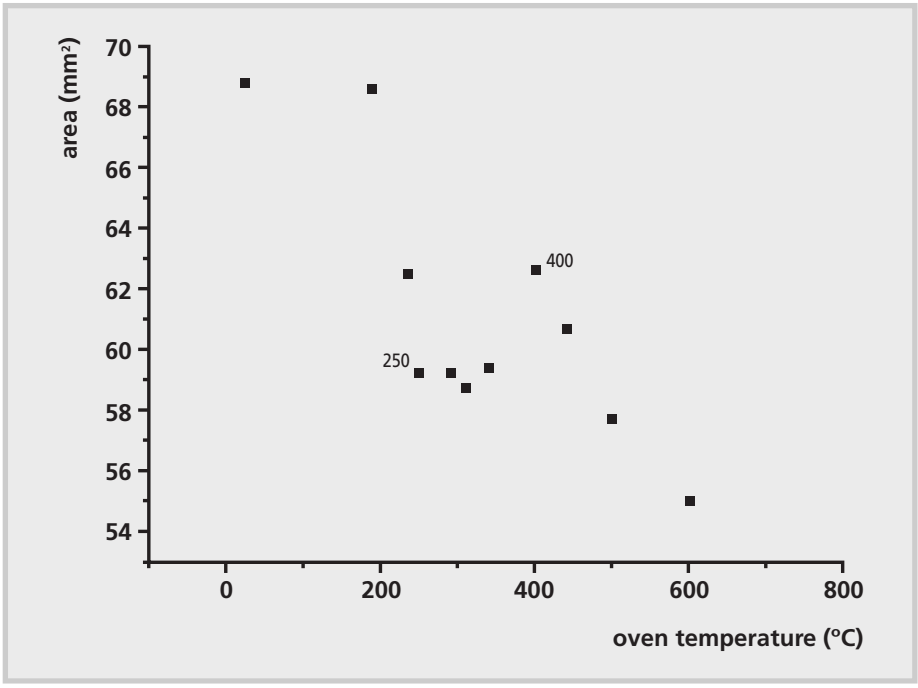
b


area (mm²)
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68
66
64
62
60
58
56
54
0
200
400
600
800
oven temperature (°C)
400
250

c

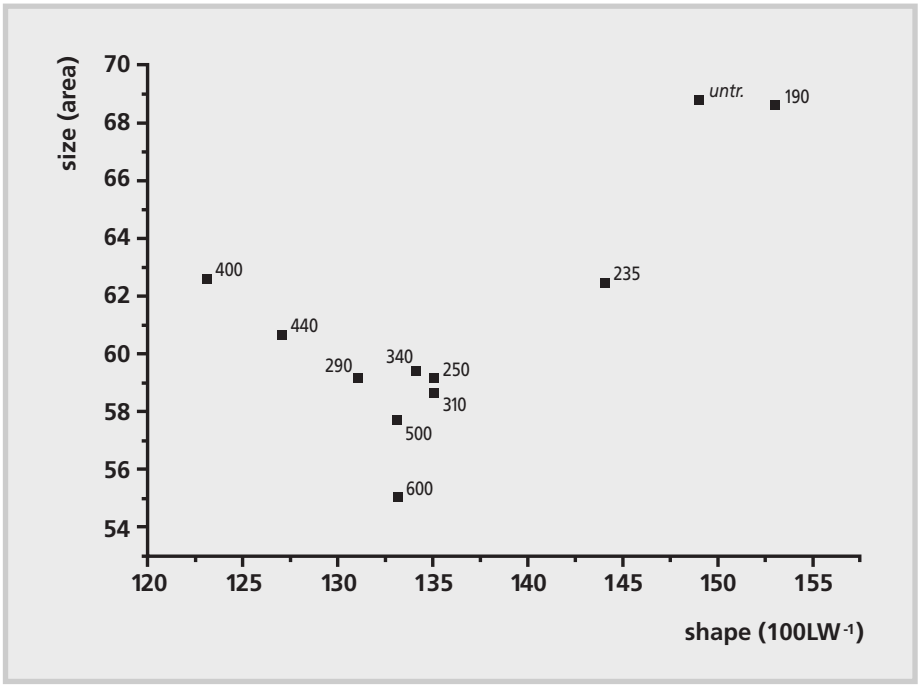

Fig. 6.4
Dimensional changes of pea seeds (var. RE) as a result of heating for 60 minutes; (a) length (mm) as a function of the width (mm) at the indicated temperatures (°C); (b) area (mm2) as a function of the oven temperature (°C) and (c) size (area) as a function of the shape ($100LW^{-1}$) heated at the indicated temperature. Sample size 10 seeds.

SEPARATION OF THREE SPECIES OF WHEAT

Untreated emmer, bread and macaroni wheat grains

The length, width and area of the specimens in the samples of untreated grains of seven varieties of emmer wheat, four varieties of bread wheat and three varieties of macaroni wheat were measured and their mean values calculated. The size factor, in this case the area (mm^2) is plotted as a function of the calculated shape factor $100LW^{-1}$ (Fig. 6.5). It shows that the grains of each of the three species have a characteristic shape factor. For emmer wheat it varies between 265 and 290, for bread wheat between 215 and 230 and for macaroni wheat between 170 and 195. The area of the grains of the three species varies greatly, which can be explained by a variation in climate, local soil conditions, weather, position in the ear, etc. (Whitworth, 2000). Nevertheless grains of the three species can be easily separated on the basis of the applied shape factor, $100LW^{-1}$.

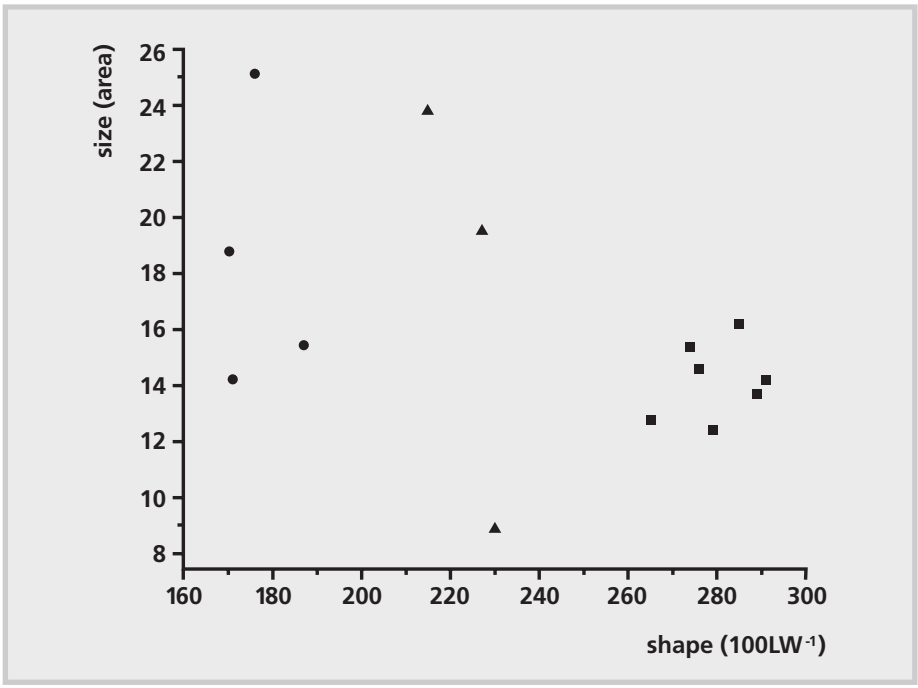

Fig. 6.5
Size factor (area in mm^2) as a function of the shape factor ($100LW^{-1}$) of selected varieties of wheat. (■) Seven varieties of emmer wheat; (●) four varieties of bread wheat and (▲) three varieties of macaroni wheat.

Heated emmer, bread and macaroni wheat grains

The changes in area and shape of the grains of each of the three species as a result of heating were measured and calculated (Fig. 6.6). Samples of 100 grains of the species AR (emmer wheat), Q (bread wheat) and A (macaroni wheat) were heated at 290, 340 and 440 °C. These temperatures were chosen because each belongs to one of the three groups with similar changes of size and shape as explained in a previous section. The observed changes are basically identical for the three species. At 290 °C the area has increased and the grains have become rounder. From 290 °C upwards the shape does not change, but the size gradually decreases. The variety Q of bread wheat shows a different behaviour at 340 °C. The size of this residue does not decrease, but remains more or less similar to the residue of 290 °C. The residues at 440 °C of the three species show a size that is almost identical to the size of the untreated grains. Thus at this temperature the differences in size of the residues between the three species remain, but the differences in shape observed in the untreated grains no longer exists in the residues. The change in shape of the three species is visualized by one untreated grain and one heated at 440 °C of each species (Fig. 6.7). It shows that the shape of each grain

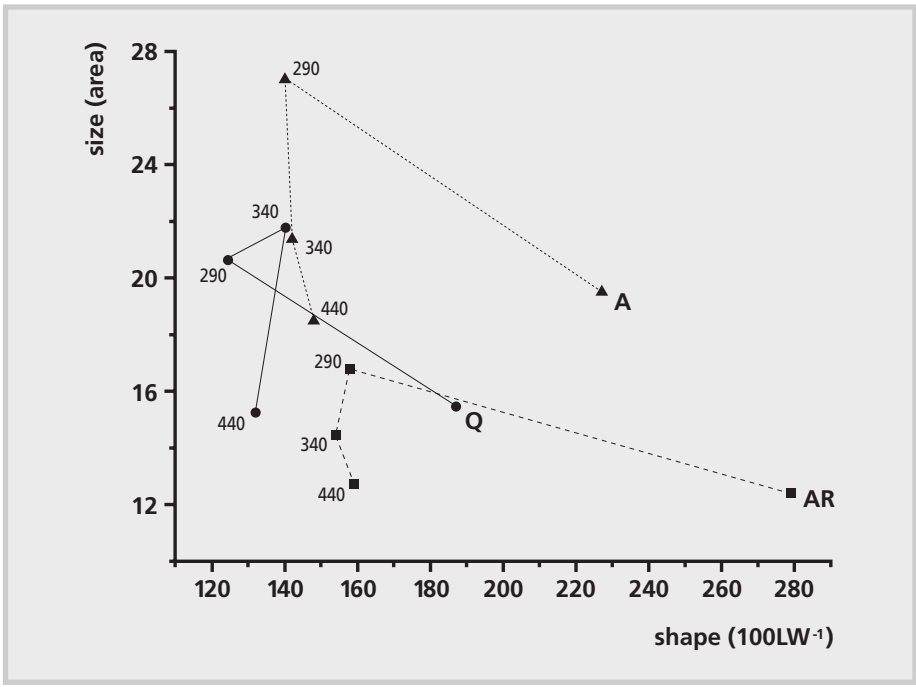

Fig. 6.6
Size (area) as a function as function of the shape (100LW^{-1}) of varieties of three species of wheat heated at the indicated temperatures (°C) for 120 minutes: (■) emmer wheat (var. AR); (●) bread wheat (var. Q) and (▲) macaroni wheat (var. A). Sample size 100 grains. Dotted lines added to aid the reader.

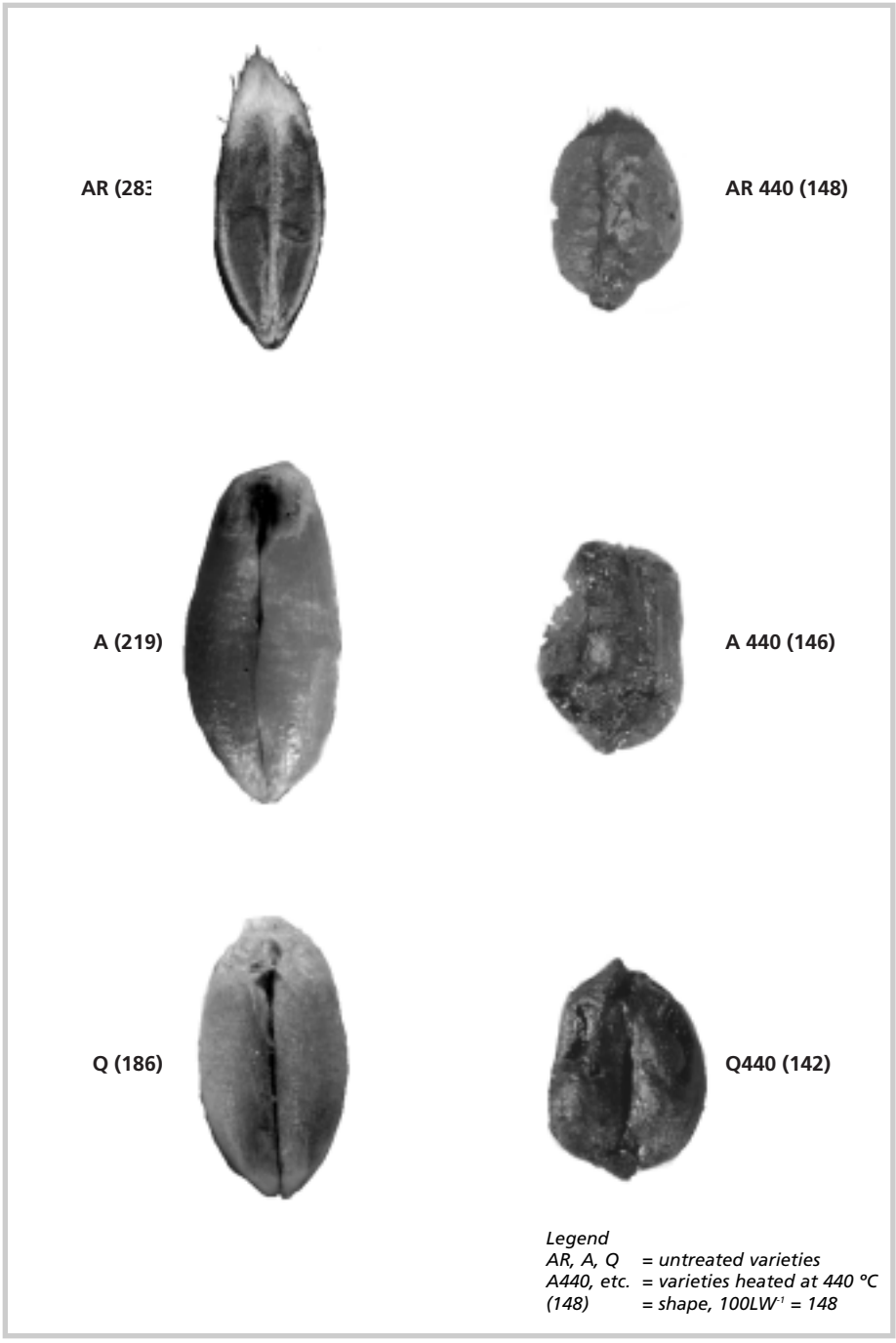

Fig. 6.7
Samples of one untreated grain of the following wheat varieties: AR (emmer wheat), A (macaroni wheat) and Q (bread wheat) indicating variety and shape (100 LW^{-1}): AR (283), A (219) and Q (186), respectively and one grain of the same varieties heated at 440 °C: AR440 (148), A440 (146) and Q440 (142), respectively. Heating time: 120 minutes.

is almost similar: AR440 is 148, A440 is 146 and Q440 is 142. When the grains of a species of wheat are heated the change of the size as a function of the shape factors will follow a path that is similar for each investigated species, but the starting point of the path of each species is different. Three species have been investigated and three paths are thus distinguished (Fig. 6.6). Wheat grains exposed to heat found in the archaeological record are difficult to separate as the heating has distorted the grains and size and shape will have changed (Hillman et al., 1996). As each species has its own characteristic starting point the original differences between the untreated species should be also present in the heated residues. The results show that this is only partly true (Fig. 6.6). The differences in size are generally still the same, however there are also considerable differences in size between the varieties of one species and thus the starting point of the conversion path (Fig. 6.5). Reasons for the difference in size could be variations in climate, soil, weather, etc. This implies that size poses problems when used for separation purposes. At temperatures higher than 290 °C the heated grains of the three species have all more or less the same shape (LW^{-1} = 140-160). Thus shape is also not very useful for the separation of heated wheat grains. It explains and confirms the difficulties in separating heated grains of different wheat species without the presence of characteristic additional plant parts.

HEATED EMMER WHEAT GRAINS ENCLOSED BY CHAFF

The presence of chaff on the change of the size and shape of wheat grains as a result of heating was investigated. For this purpose three samples of 100 grains emmer wheat without chaff were prepared and heated at 290, 340 and 440 °C, respectively. In this way a sample was heated in one of the three groups as described in a previous section. The area, as the size factor, was plotted as a function of the shape factor $100LW^{-1}$ (Fig. 6.8). Subsequently three samples of 100 emmer wheat grains, enclosed by chaff, were heated at these three temperatures. After removal of the chaff the heated grains were measured, the mean of the length, width and area calculated and plotted in the same way as the grains without chaff (Fig. 6.8). It shows that the presence of chaff has no effect on the size and a minimal effect on the shape of the heated grains at 290 and 340 °C. At 440 °C the grains heated with the chaff have an on the order of 10 % smaller shape, but the size remains unaffected.

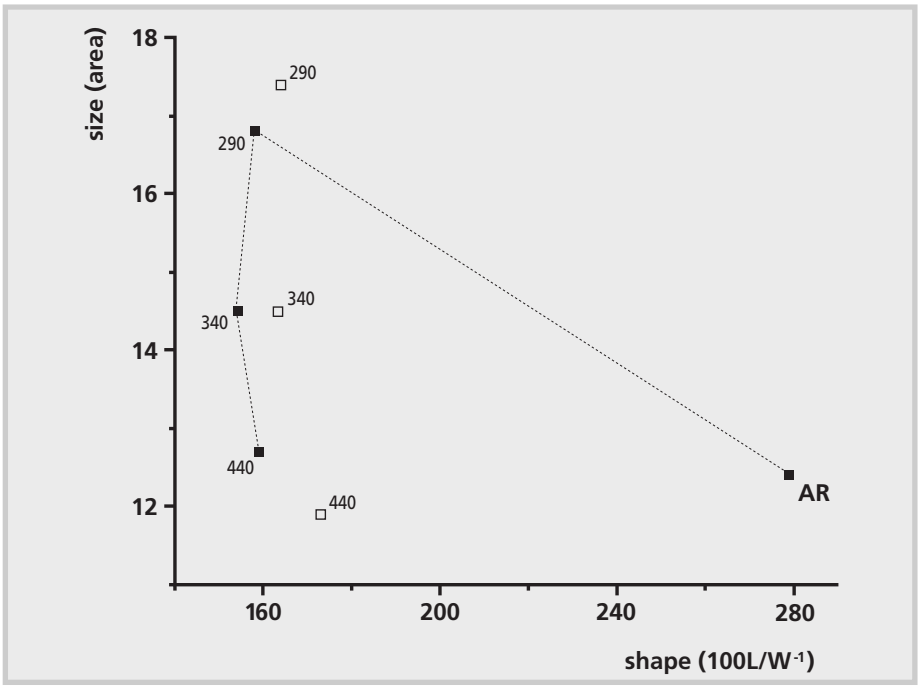

Fig. 6.8
Size (area) as a function of the shape ($100LW^{-1}$) of emmer wheat grains (var. AR) with and without chaff heated at the indicated temperatures (°C) for 120 minutes: (■) emmer wheat (var. AR) without chaff and (□) emmer wheat (var. AR) heated with chaff. Sample size 100 grains. Dotted line added to aid the reader.

CONCLUSIONS

Upon heating under anoxic conditions the obtained properties of wheat grains and peas can be divided in identical stages, as a function of the temperature. The division is based on their physical and molecular properties. In general these stages are also recognized in the changes of size and shape, as defined in this study. However, the changes of wheat grains and peas different. It is suggested that the presence of the pericarp in wheat grains and the lack of it in peas is the reason for this difference.

The experiments with three species of wheat show that the size of the untreated grains varies too much between and within the species as a result of different growing conditions. Therefore size is not a suitable tool for the separation between the species of both untreated and heated wheat grains. Based on the shape it is possible to separate untreated grains of the three species. However, as a result of heating at temperatures higher than 290 °C, the grains of the three investigated species attain an almost identical shape of $100LW^{-1}$ = 140-160. This suggests that after heating of modern varieties of wheat under the present controlled conditions separation between the three species is hardly possible, when solely size and shape are known.

No significant difference in size and minimal changes in the shape is observed when wheat grains enclosed by chaff are heated and compared with grains heated without chaff.

This laboratory study shows why and how size and shape of modern wheat grains and peas change as a result of heating. However, the conclusion that shape, under the present experimental conditions, is not a suitable tool for the separation of different species of wheat is not in accordance with the results obtained with carbonized wheat grains retrieved from the archaeological record. This poses a problem. A reason for this discrepancy might be that the heating conditions prevailing in the past are not in accordance with the conditions chosen for the present experiments. Thus to obtain more insight into this problem it is therefore necessary to carry out further experiments which are focused on the influence of the heating conditions on the carbonized grains.

Acknowledgements

The authors are grateful to Miss. Beatrice Marino (FOM Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands) for her suggestions regarding digital imaging analysis. Practical help with the microphotographs of wheat grains by Mr. E. Mulder (Faculty of Archaeology, Leiden University) is gratefully acknowledged.

CHAPTER 7

Propagules exposed to different heating conditions

ABSTRACT

Samples of peas have been carbonized for 60 minutes in the laboratory under controlled conditions and compared with carbonization experiments under less controlled conditions. To achieve this the heating was carried out at 270, 340, 440 and 500 °C in a tube oven, on a porcelain plate heated above a gas burner and on potsherds in an open fire. The weight loss of each sample was calculated and peas from each sample were subjected to measurement of C-content, vitrinite reflectance and the molecular composition (DTMS-EI). After a maximum of approximately 30 minutes of heating, the temperature of the carrier gas in the tube oven remains constant and the temperature within the peas remains at a level of about 4 °C lower. This difference assures a constant transfer of heat to the peas, which is mainly consumed by the chemical reactions as is shown by the increasing weight loss. The available energy for these chemical reactions depends on the rate of heat transferred (in $Jsec^{-1}$) and the time of exposure (in sec) and not on the temperature within the peas, the mode of heat transfer or a limited supply of air. The properties of propagules exposed to different heating conditions depend solely on the amount of energy acquired by the samples. The energy is determined by the rate of heat transferred and the time that the samples have been exposed to the heat source. For archaeological research this implies that the results from the simulation of the carbonization process under well controlled conditions in a laboratory are also valid for studies on propagules carbonized under less controlled conditions as for example in an open fire. At relatively low temperatures and a longer time of exposure the propagules show a molecular composition that is similar to propagules heated at higher temperatures with a shorter time of exposure. This implies that carbonized remains retrieved from the archaeological record with a different thermal history may show identical properties.

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Submitted for publication

INTRODUCTION

One of the ways by which plant parts may survive natural biological, physical and chemical decomposition processes is carbonization or heating under anoxic conditions, equivalent to thermochemical conversion. Consequently, carbonized plant remains are often found in the archaeological record (van Zeist, 1970). After being carbonized the material may be deposited in the soil and becomes part the archaeological record. Post-depositional processes will then affect the carbonized material and, finally, archaeologists introduce retrieval techniques, analytical and theoretical processes (Schiffer, 1983; 1987). As a result the character of the carbonized remains will have changed. These effects may, for example, mask or exaggerate patterns in plant resource exploitation or even suggest changes where none occurred. It is therefore of great importance to know how carbonized remains from the archaeological record have acquired the physical and chemical characteristics witnessed at their recovery. For this purpose it is necessary to know the properties of the material directly after the carbonization process.

Polysaccharides, such as cellulose and starch, are present in relatively large amounts in plant material. When microcrystalline cellulose is carbonized for 2.5 hours it was shown that it converts into a range of products with different properties that are mainly determined by the carbonization temperature (Pastorova et al., 1994). To obtain further insights into the carbonization process of whole plant parts, a simulation in the laboratory was carried out by heating modern propagules in a tube oven under anoxic conditions at atmospheric pressure (Braadbaart et al., 2004a; b; d). Seeds and fruits generally carbonize well and are often recovered from the archaeological record (Bakels, 1984). Therefore seeds of peas *(Pisum sativum* L.) and the fruits of emmer wheat *(Triticum dicoccum* Schübl) were chosen for these heating experiments. The seeds and fruits were heated for 60 and 120 minutes, respectively, at temperatures ranging from 130-700 °C. The compositional change as a function of temperature and the physical, bulk chemical and molecular properties were measured.

The earlier experiments took place under controlled laboratory conditions and left open the question whether these results are also achieved when plant parts are exposed to other heat sources under less-controlled conditions. In the case of heating in a tube oven it was observed that the temperature of the carrier gas, which was determined by the oven temperature set for an experiment, attained the temperature of the oven after 30 minutes at low oven temperatures and after less than 10 minutes at high oven temperatures (Braadbaart et al., 2004a). After this time period the temperature within the samples always remained at a level of about 4 °C lower than the temperature of the gas or the oven. This difference assures a constant transfer of thermal energy to the samples.

The total energy released by a heat source is mainly consumed by the external heat transfer, the internal heat and mass transfer and the chemical reactions that cause the conversion of the propagules (Di Blasi, 1996). The energy is determined by the rate of heat transfer (in $Jsec^{-1}$) and the time (in sec) of exposure of the samples to the heat source. This suggests that it is not the temperature at a certain moment within the propagules that determines directly the weight loss or the conversion, but that part of the available energy (rate of heat transfer and time of exposure) that will be consumed by the chemical reactions. It is postulated that the conversion is determined by the available energy and is independent of the mode of heat transfer and, thus, the properties of the heated samples are independent of the heat source. It could also mean that after a longer time of exposure at relatively low temperatures the weight loss or conversion and thus the properties of the converted samples are similar to those that have been heated at higher temperatures for a shorter period of time. It implies that the influence of the post-depositional processes on these samples, although each with a different thermal history, is also similar. By measuring the weight loss, the C content, the vitrinite reflectance and the molecular composition of propagules heated by three different heat sources: (i) tube oven, (ii) gas burner and (iii) open wood fire, these postulations are verified.

METHODS

Samples

Peas were obtained from the Centre for Genetic Resources, Wageningen, The Netherlands (CGN). The variety 'Noord-Hollandse Rozijnerwt' (RE; CGN 10293) was used for the experiments. The variety was grown in the summer of 2002 and about four months after harvesting the peas were used for the experiments without any further pre-treatment. The flowers of this variety are red and the outside colour of the seeds is generally brown with a wrinkled surface. Emmer wheat grains were obtained from ARCHEON, Alphen aan de Rijn, The Netherlands. The wheat was harvested in the summer of 2002. For the experiments the chaff was removed from the grains.

For the experiments both peas and wheat grains could be used, as the main constituent components are similar. As peas are easier to handle near and in open fires these propagules were selected for the heating experiments in the different heat sources. Wheat grains were used for heating experiments with a tube oven to determine the influence of the time of exposure.

Heating conditions

Tube oven

For each experiment ten intact peas were placed in an open pyrex vessel and inserted in a 30 cm long pyrex tube (∅ 2.3 cm) at 18 cm from the inlet. The tube was placed in a pre-heated Carbolite tube oven (model MTF 12/38/250). The heating rate of the oven was set at 2 °C min^{-1}. Subsequently, heating was carried out at 270, 370, 440 and 500 °C for 60 minutes under a constant flow (150 ml min^{-1}) of N_2 at atmospheric pressure. For each temperature a separate experiment was carried out. The temperatures were chosen because they represent carbonized stages with characteristic properties (Braadbaart et al., 2004b; d). Gases and volatiles were vented and not further investigated.

Gas burner

For each experiment a porcelain plate was placed on a tripod over a gas burner. The temperature of the plate could be adjusted by regulating the gas burner. The temperature of the surface of the plate was measured using a thermocouple. Experiments were carried out with the temperature of the plate set at: 270, 370, 440 and 500 °C. The accuracy to which the temperature of the plate could be adjusted was ±10 °C. Ten peas were placed on the heated plate in open air and heated for 60 minutes. However, in this way flaming occurred and the peas were completely consumed to ash. To prevent this a flow of N_2 was directed over the

samples. To measure the temperature within the peas an additional thermocouple was inserted in one of the peas. For this purpose a hole (∅ 0.3 mm) was drilled in the pea. The gases and volatiles were vented.

2.2.3. Open wood fire

A hearth was constructed of Löss soil with a thickness of 0.10 m and a diameter of 0.65 m in one of the prehistoric houses of the archaeological theme park ARCHEON (Alphen aan de Rijn, The Netherlands). An oak wood fire was built on its surface. Potsherds were placed in the fire and the temperatures of the surfaces were measured with a thermocouple. The places in the fire were selected in such a way that the temperatures of the potsherds reached c. 270, 370, 440 and 500 °C. It was not possible to measure the temperature within the peas under these conditions. During the experiments the temperatures of the potsherds had a tendency to deviate ±10 °C. On each potsherd 10 peas were placed and kept in the fire for 60 minutes.

2.3. Methods of investigation

2.3.1. Weight loss

In all experiments the samples of peas were weighed before and after heating, in order to determine the percentage weight loss.

2.3.2. Elemental analyses

C analyses were executed using a NA1500 series 2 NCS analyser from Fisons Instruments. The temperature in the combustion reactor was maintained at 1020 °C, the combustion products were separated on a Porapak QS column with a length of 2 m. All values stated are based on two measurements, not corrected for H_2O and ash content.

2.3.3. Vitrinite reflectance

Entire specimens of peas were heated as described above. From each sample two specimens were embedded in resin blocks and polished. Mean maximum vitrinite measurements *(%Rmax)* were carried out under oil immersion at a wavelength of 546 nm using a Leitz MPV II microscope system. Preparation of polished blocks and reflectance measurements were carried out according to standard methods defined in ISO 7404, part 2 (1985) and part 5 (1994).

2.3.4. Molecular composition by DTMS under EI conditions

The molecular composition of samples of peas was studied using Direct Temperature-Resolved Mass Spectrometry (DTMS) under electron ionization (EI) conditions. Under these conditions a detailed insight can be obtained into the conversion of polysaccharides and proteins as a result of heating at temperatures

higher than 250 °C (Braadbaart et al., 2004b). Samples of untreated peas and peas heated at 270, 370, 440 and 500 °C, were analysed using a JEOL SX-102A double focusing mass spectrometer with B/E geometry. Previous measurements on residues of peas showed that the sampling and experimental variance is normally very small (Braadbaart et al., 2004b). Therefore, the measurements were carried out only once. A powdered sample in water was deposited on the platinum/rhodium (90:10) filament of a probe, which was inserted directly into the ion source of the mass spectrometer. The filament was resistively heated by ramping the current at a rate of 0.5 A min^{-1}. Using this ramp the temperature was linearly increased from ambient to approximately 800 °C in two minutes. Ions were generated by low voltage ionization (16eV) in an ionization chamber kept at 180 °C and accelerated to 10 kV. The mass spectrometer was scanned over an *m/z* range of 20-1000 using a 1 sec cycle time and mass resolution of 1000. The data were acquired using a JEOL MP-7000 data system. Data acquisition and processing were performed in real time.

RESULTS

Average curves from earlier experiments

In earlier experiments samples of peas of different varieties were heated for 60 minutes, at temperatures ranging from 130-700 °C (Braadbaart et al., 2004a). These experiments showed that after a maximum of 30 minutes of heating at low oven temperatures the carrier gas attained the temperature of the oven. From this moment the temperature within the peas is at a level of about 4 °C lower than the temperature of the gas. As this difference is relatively small, although important, it is called the sample temperature to make a comparison possible with experiments carried out using other heat sources. For each experiment the measured weight loss was plotted as a function of the sample temperature and an average curve was constructed (Fig. 7.1a). Similarly average curves of the C content (Fig. 7.1c) and the vitrinite reflectance, on polished sections of peas (Fig. 7.1b), are constructed.

Weight loss

Samples of ten intact peas were heated at 270, 370, 440 and 500 °C for 60 minutes under the three different heating conditions. For each experiment the weight loss was measured. The experimental conditions of the heating of samples 1-4 in the tube oven are identical to previous experiments with similar varieties of peas (Braadbaart et al., 2004a). The present measurements of the weight loss of samples 1-4 (Table 7.1) heated in the tube oven at 270, 370, 440 and 500 °C are plotted in the average curve of Fig. 7.1a. It shows that these results fit the curve well and suggests that the curve can be considered as a standard curve describing the weight loss of peas, heated for 60 minutes, as a function of the sample temperature.

For samples 5-8 heated for 60 minutes on a porcelain plate above a gas burner, the temperature of the surface of the plate was set at 270, 370, 440 and 500°C. The temperature within the peas was measured separately. The results show that for each experiment the temperature within the peas was considerably higher than the temperature of the plate (Table 7.1). The weight loss of the samples was measured for each experiment and plotted as a function of the temperature measured within the peas in the standard curve of Figure 7.1a and they fit the curve well.

The potsherds, on which the peas were heated, were placed in the open fire in such a way that they were heated to 270, 370, 440 and 500°C. In this case the temperature within the peas could not be measured. After 60 minutes of heating the weight loss of each sample was measured and plotted in the standard curve of Figure 7.1a. It is observed that the actual temperature within the sample must

have been considerably higher than the measured temperatures of the potsherds. This suggests that the same phenomenon occurs as witnessed in the experiments with the gas burner.

Elemental analyses

The results of the measurements of the C content (not corrected for H_2O and ash) are summarized in Table 7.1. The C content of untreated peas is on the order 40 wt% (Braadbaart et al., 2004a). The results show an increase of the C content to 60 wt% at 270 °C and 70 wt% at 700 °C. For samples 1-8, the temperature within the sample was measured and by plotting the C content of each sample on the average curve (Fig. 7.1c) it shows that they follow the curve well considering the scale of the vertical axis. This is also true for the values of the C content of samples 9-12, when the temperatures within the peas for each experiment are derived from the curve of Fig. 7.1a.

Vitrinite reflectance

For the results of the vitrinite reflectance measurements (Table 7.1) the same procedure is followed as for the results of the weight loss (Braadbaart et al., 2004a). The present results of the vitrinite reflectance of the peas heated in the

Sample nr.	*Heat source*	*Temperature measured (°C)*	*Temperature sample measured (°C)*	*Temperature sample calculated (°C)*	*Weight loss (%)*	*C content (wt%)*	*%Rmax*	*Type DTMS-EI spectrum*
		oven						
1	*oven*	*270*	*268*		*48.4*	*59.6*	*0.16*	*C*
2	*oven*	*370*	*368*		*67.6*	*65.0*	*0.76*	*D*
3	*oven*	*440*	*438*		*73.5*	*67.1*	*1.40*	*E_1*
4	*oven*	*500*	*498*		*76.3*	*68.6*	*2.26*	*E_2*
		porc.plate						
5	*gas burner*	*270*	*290*		*53.0*	*61.2*	*0.27*	*C*
6	*gas burner*	*370*	*410*		*70.8*	*66.0*	*0.92*	*D*
7	*gas burner*	*440*	*490*		*76.3*	*65.7*	*2.16*	*E_1*
8	*gas burner*	*500*	*570*		*77.9*	*62.2*	*3.14*	*E_2*
		potsherd						
9	*open fire*	*280*		*350-400*	*66.3*	*64.6*	*0.98*	*D*
10	*open fire*	*370*		*490*	*75.8*	*64.9*	*2.08*	*E_1*
11	*open fire*	*440*		*550*	*77.1*	*65.9*	*3.04*	*E_2*
12	*open fire*	*500*		*640*	*78.7*	*69.7*	*3.84*	*E_2*

Table 7.1
Results of measurements on peas (P. sativum), var. RE, heated for 60 minutes in three heat sources: tube oven, gas burner and open fire.

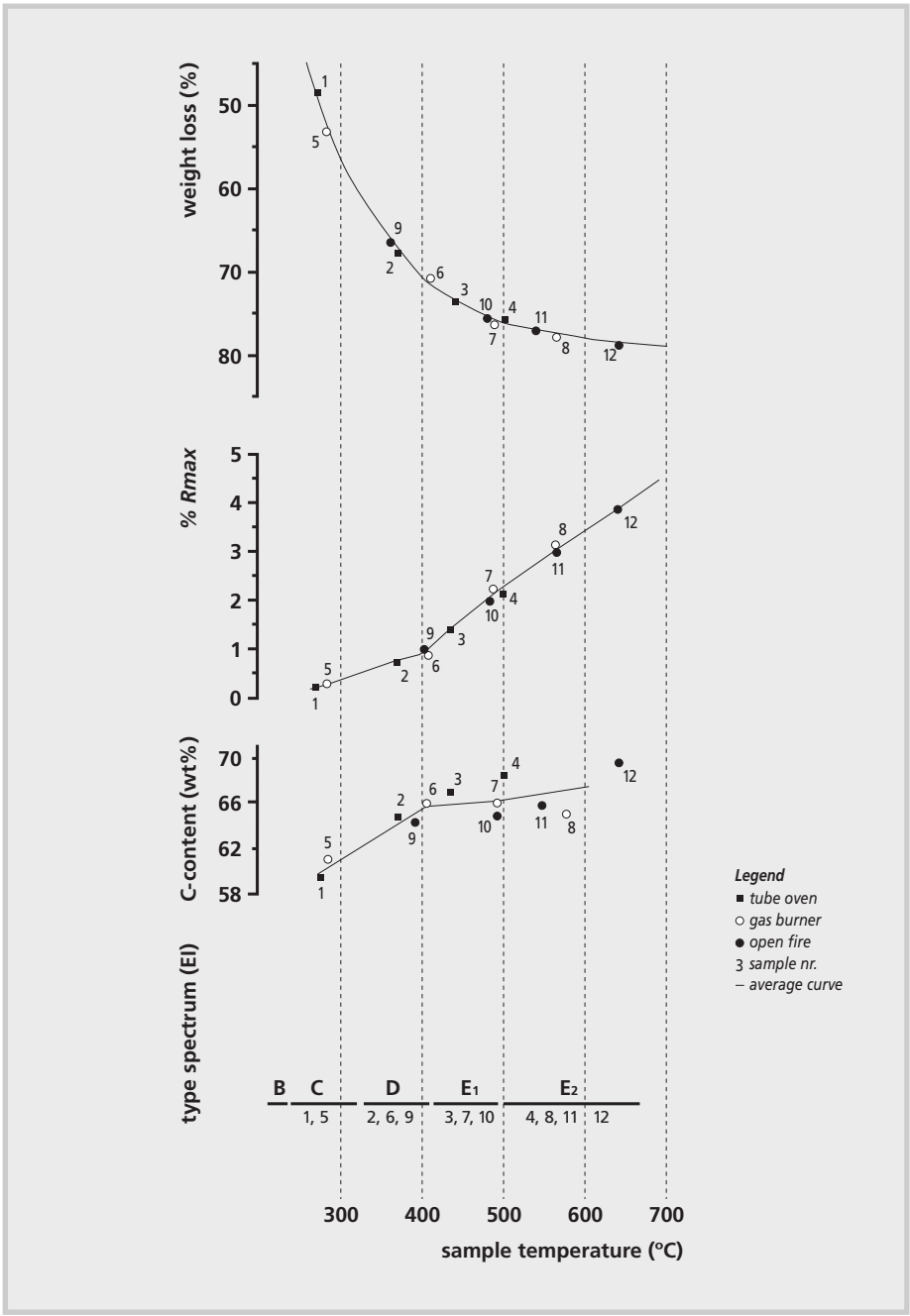


Figure 7.1
Results of measurements on peas (P. sativum), var. RE, heated under three different conditions, for 60 minutes, as a function of the measured or derived sample temperature (°C): (a) weight loss (%); (b) vitrinite reflectance (%Rmax); (c) C content (wt%, not corrected for H_2O and ash) and (d) type B, C, D, E_1 and E_2 of DTMS-EI spectra. For further details see text and legend in Figure.

tube oven correspond well with this curve and the curve is considered as a standard curve, describing the vitrinite reflectance of peas, heated for 60 minutes, as a function of the sample temperature (Fig. 7.1b). The results of the vitrinite reflectance measurements on peas heated with the gas burner (Table 7.1) are plotted in this curve at the temperatures measured within the peas. The vitrinite reflectance of peas from samples heated in an open fire is also plotted on the curve and the actual temperature within the peas can be calculated. Once more it is observed that the temperature within the peas was higher than the temperature measured on the surface of the potsherds.

DTMS-EI measurements

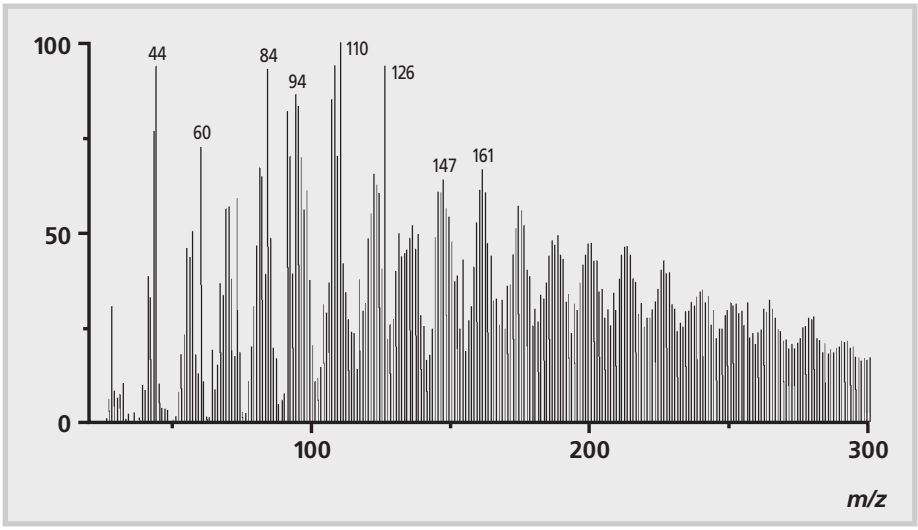
3.5.1. Untreated peas

Polysaccharides, proteins and lipids are common constituents of peas (Belitz and Grosch, 1999). The polysaccharides, the bulk fraction of peas, are represented mainly by starch and hemicelluloses (Daveby et al., 1993). In earlier experiments DTMS-EI experiments were carried out with untreated peas to study their molecular nature (Braadbaart et al., 2004b). The mass spectrum of these peas (not shown) shows the characteristics of polysaccharides (starch and hemicelluloses), proteins and lipids. The masses representing starch are the fragment ions *m/z* 43, 57, 60, 73, 98, 126 and 144, derived from hexosepolysaccharides. Pentosepolysaccharides (hemicelluloses) are represented as anhydroxyloses by *m/z* 85 and 114. Typical protein derived ions observed are *m/z* 69, 70, 84, 91, 131, 138, 154, etc. Lipids are recognized as fatty acids (e.g. *m/z* 262, M-H_2O $C_{18:2}$), sitosterol (*m/z* 396-414, $C_{29:1}$), diglycerides (e.g. *m/z* 576, $C_{16:0,18:1}$) and triglycerides (e.g. *m/z* 862, $C_{16:0,18:0,18:0}$).

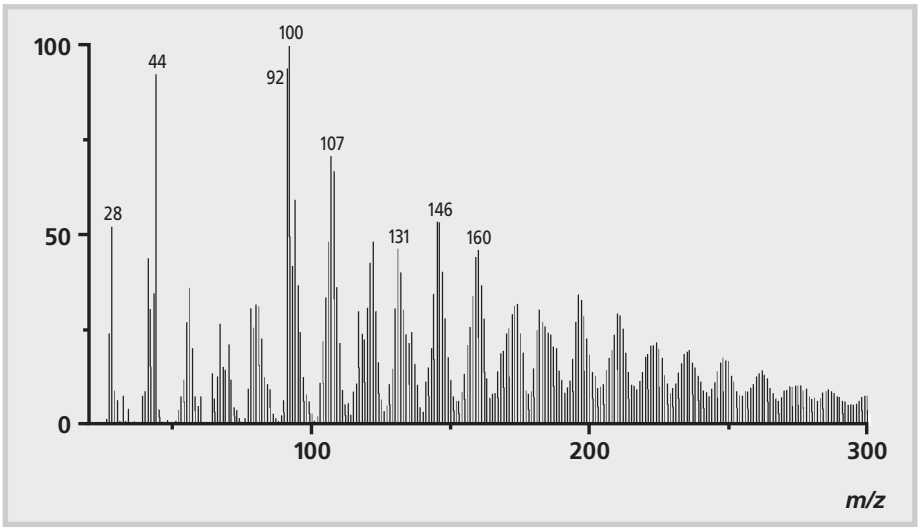
3.5.2. Heated peas

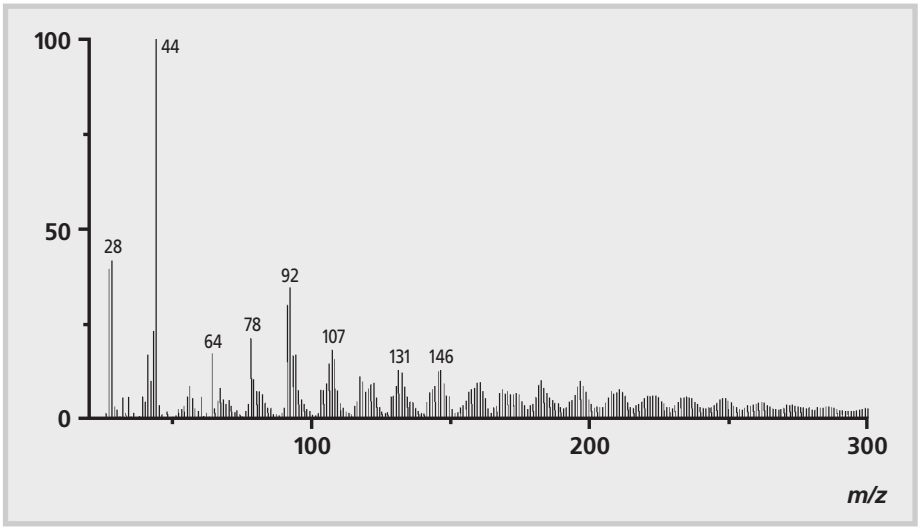
The physical and molecular changes of peas heated under anoxic conditions, for 60 minutes, have been divided into five compositional stages (A, B, C, D and E), as a function of the sample temperature (Braadbaart et al., 2004b). The stages of importance for this study are: stage C from 270-310 °C, stage D from 340-400 °C and stage E higher than 400 °C (Fig. 7.1d). Stage C is still dominated by the presence of original polysaccharides and protein, but also by a series of aromatic compounds, which includes furans, (alkyl)phenols, (alkyl)benzenes and condensed aromatic compounds. The conversion of the polysaccharide rich material into thermally more stable products characterizes this stage. The spectra of samples 1 and 5 (Table 7.1) correspond with these characteristics (Fig. 7.2a) showing the masses *m/z* 60, 98, 126 and 144 (polysaccharide markers); 95, 96 and 110 (furans); 94, 107, 108 (alkyl)phenols; 91, 105, 119 (alkyl)benzenes and the series 146, 160, 174, etc. (condensed aromatic compounds). The protein derived ions such as *m/z* 70 and 84, and lipids can still be recognized in the DTMS data at these temperatures. Moreover two series of odd-numbered ions (i) *m/z* 117, 131, 145, etc. and

a


100
50
0
44
84
110
94
126
60
147
161
100
200
300
m/z

b


100
50
0
100
44
92
107
28
146
131
160
100
200
300
m/z



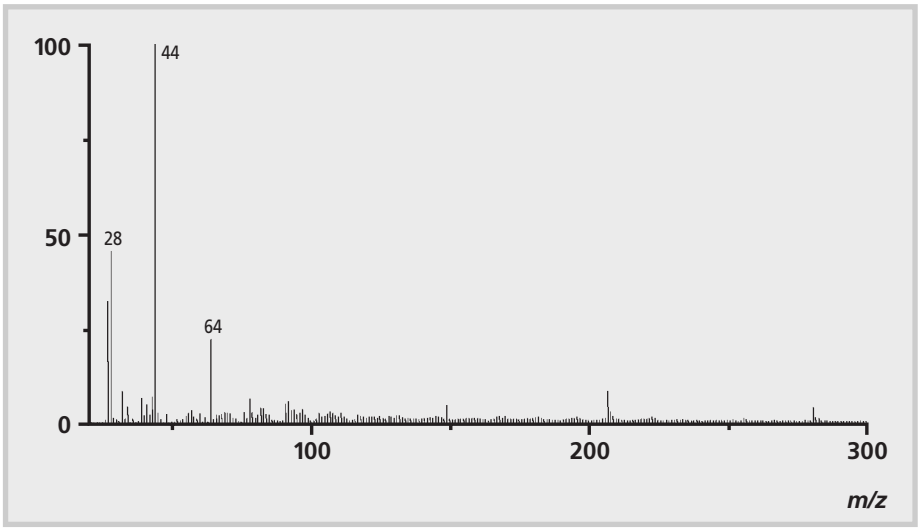


Figure 7.2
DTMS-EI spectra of peas (P. sativum), var. RE, heated under three different conditions, for 60 minutes: (a) peas from sample 1, spectrum type C; (b) peas from sample 9, spectrum type D; (c) peas from sample 10, spectrum type E_1 and (d) peas from sample 8, spectrum type E_2.

(ii) *m/z* 147, 161, 175, etc. are observed, which are attributed to N containing heterocyclic compounds (Braadbaart et al., 2004b). The spectra of the samples 2, 6 and 9 (Fig. 7.2b) correspond with stage D, which is characterized by products formed from secondary reactions. Masses representing polysaccharides, furans, protein markers and lipids as present in untreated peas and stage C, are now no longer observed. The characteristic compounds in the spectra are now alkylphenols (*m/z* 94, 108, 122), alkylbenzenes (*m/z* 91, 92), condensed aromatic compounds (*m/z* 146, 160, 174, etc.) and the above mentioned two series of odd numbered ions. Stage E is a carbon enriched material and its spectrum is characterized by the decrease of the number of ions that is released. The spectra of the samples 3, 7 and 10 (Fig. 7.2c) still show the release of alkylbenzenes (*m/z* 91, 92) and N containing heterocyclic compounds (*m/z* 117, 131, 145, etc.). In addition, the ions *m/z* 27 (HCN), 41 (CH_3CN), 64 (SO_2) and 78 (benzene) are observed. This stage, E_1, can be considered as the transition stage from stage D into a strongly carbon enriched product, stage E_2, represented by samples 4, 8, 11 and 12. The latter samples hardly yield any ions under these conditions. In the spectra of these samples (Fig. 7.2d) only the masses *m/z* 27 (HCN), 28 (CO), 44 (CO_2) and 64 (SO_2) are recognized.

DISCUSSION

The present study investigates whether the results of carbonization experiments with modern propagules under controlled conditions in a laboratory are also valid when similar propagules are exposed to less controlled conditions, for example, in an open wood fire. The experiments in the laboratory were carried out under anoxic conditions in a tube oven with peas and wheat grains heated for 60 and 120 minutes, respectively. Samples of both types of propagules are converted as a function of oven temperature into products that serve as a basis for a classification in compositional stages with identical properties. Because the main constituent components of peas as well as wheat grains are starch and protein, this suggests that these bulk polymer components determine the heat driven conversion process of these propagules into new materials at higher temperatures and thus the properties of their induced conversion products (Braadbaart et al., 2004a; b; d). We have concluded that both propagules can be used interchangeably for carbonization experiments. An exception is made for the dimensional changes of the propagules. The way in which the dimensions will change depends on the anatomical and morphological differences between peas and wheat grains. As peas are easier to handle near or in an open fire, it was preferable to use them for the experiments carried out under the three different heat conditions.

Thermal energy or heat is transferred from the three heat sources to the samples because of a temperature gradient between each heat source and the samples. In the case of the experiments with the tube oven, the temperature of the oven is determined by the rate of heat transferred from the wall of the oven to the sample. In a tube oven the generated heat is mainly transferred to the sample by convection via the constant flow of N_2 in the pyrex tube and to a small extent by conduction through the walls of the pyrex tube and the vessel where the tube is in direct contact with wall of the oven. The presence of the flow of N_2 assures a reducing atmosphere for the sample. Near or in an open fire this rate will depend on the quality and quantity of the fuel and the available air. The heat is transferred mainly by radiation from the fire to the sample and to a small extent by conduction via the porcelain plate or the potsherd on which the sample is placed. The limited supply of air during the experiments with the gas burner was assured by a flow of N_2 over the samples. In an open fire the supply of air is limited due a poor mixing of fuel and air in open fires (Albini, 1993). The time of exposure for all the experiments was 60 minutes and the change of the properties is presented as a function of the sample temperature attained after this particular period of time (Fig. 7.1). Although the experimental conditions, which include mode of heat transfer and reducing environment, differ widely it is observed that the converted samples, which are classified in a similar stage of compositional conversion (A-E), attain values of weight loss, C content and vitrinite reflectance, which correspond with these stages (Fig. 7.1).

The main fundamental physical processes that consume the energy transferred to the samples are the external heat transfer, the heat and mass transfer within the sample and the chemical reactions that cause the thermal conversion of the samples (Di Blasi, 1996). To obtain more insight in the latter processes experiments were carried out in a tube oven using wheat grains. The weight loss of the wheat grains at a constant oven temperature (T_{oven}) is plotted as a function of the time of exposure for each T_{oven} (Fig. 7.3). The results show a continuing decrease in weight with an increasing time of exposure. Earlier experiments showed that the temperature of the carrier gas will attain T_{oven} after a period of time varying from 6 to 30 minutes depending on T_{oven} (Braadbaart et al., 2004a). Within the samples this temperature will never be reached and is always at the level of about 4 °C lower assuring a continuous transfer of heat to the sample. This implies that after the initial period of time the rate of heat transferred from the wall of the oven to the sample is not only consumed to maintain the temperature of the gas and the sample, but that a part is always available for the chemical reactions that cause the chemical conversion of the sample, resulting in a weight loss. For example, after seven days of heating at T_{oven} = 220 °C a 42 % weight loss is measured, but this result could also be reached by heating a sample for 120 minutes at T_{oven} = 250 °C, or for 9 minutes at T_{oven} = 310 °C. Lines of equal weight loss are constructed at 45, 60 and 70 % weight loss (Fig. 7.3). Samples on each line of equal weight loss, marked by a circle, were selected and studied using DTMS-EI to determine the molecular composition. The spectra of the four samples with a weight loss of 45 % correspond with compositional stage B (see Braadbaart et al., 2004b), the five samples of 60 % weight loss with a transition from compositional stage C to D and the three samples of 70 % weight loss with compositional stage D (Fig. 7.1d). This suggests that samples with a similar weight loss or stage of conversion have absorbed a similar amount of energy required for the chemical reactions that drive the conversion of the samples. The lines of equal weight loss can therefore also be interpreted as lines of equal energy. In this case the energy (in J) can be described as that part of the heating rate (in $Jsec^{-1}$), which is used for the conversion transferred from the wall of the oven to the samples during the given exposure time (in sec). It determines the actual weight loss or the stage of conversion and consequently the molecular composition, C content and vitrinite reflectance of the sample. Hence, it is obvious that the time of exposure is an important parameter in the determination of the properties of the carbonizing samples.

The results of the experiments under different heating conditions show that samples, exposed for 60 minutes to these conditions, have reached a similar stage of chemical conversion i.e. 1 and 5 in stage C; 2, 6 and 9 in stage D; 3, 7 and 10 in stage E_1 and 4, 8, 11 and 12 in stage E_2 (Fig. 7.1). Following the above described considerations samples in each stage have received a similar amount of energy – consumed by the conversion reactions - which results in products with similar

properties. However, in each stage samples are present that have been heated under different conditions and apparently the resulting properties are independent of these conditions. Thus both the rate of heat transferred and the time of exposure determine the conversion, which suggests that the conversion is independent of the heating conditions.

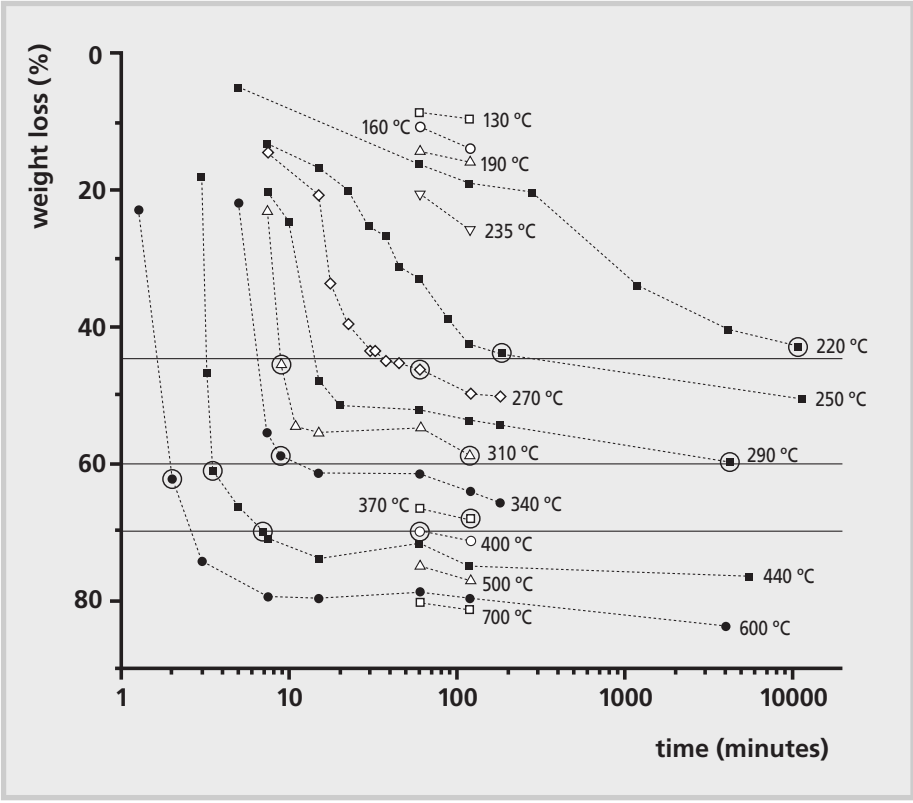


Figure 7.3
Weight loss (%) of in a tube oven heated emmer wheat grains (T. dicoccum), var. AR, as a function of the heating time (minutes) on a logarithmic scale. Oven temperatures are given in °C. The encircled samples are investigated for molecular composition by DTMS-EI. The dashed lines are only meant to clarify the figure.

CONCLUSIONS

When propagules are exposed to different heating conditions the properties of the resulting conversion products depend solely on the amount of energy absorbed by the samples. The energy is determined by the rate of heat transfer and the time that the samples have been exposed to the heat source. In archaeological research this conversion process is frequently called carbonization and the resulting products the carbonized remains. This implies that the results from the simulation of the carbonization process under well controlled conditions in a laboratory are also valid for studies on propagules carbonized under less controlled conditions as for example in an open fire. As long as thermal energy or heat is transferred to the propagules the processes controlling the carbonization continue, the weight loss increases and the other properties change accordingly (Fig. 7.3). Even at relatively low temperatures and a longer time of exposure propagules can be converted into products with a molecular composition that is similar to propagules heated at higher temperatures with a shorter time of exposure. This implies that carbonized remains with a different thermal history may show identical properties.

Acknowledgements

We are grateful to ARCHEON, Alphen aan de Rijn, The Netherlands, whose facilities could be used for the experiments with an open fire and for the practical help of Miss M. de Boer and Mr E. Schouten. The assistance of Miss K. Reimer of Geo-Environment, TNO-NITG, Utrecht, the Netherlands with the vitrinite reflectance measurements and Mr J. van der Horst of the FOM Institute with the DTMS experiments are kindly acknowledged. This research is supported by FOM research program 49 funded by the Foundation for Fundamental Research on Matter (FOM), a subsidiary of the Dutch Organisation for Scientific Research (NWO).

CHAPTER 8

Conclusions and implications for archaeology

CONCLUSIONS

Introduction

Upon deposition in the soil various post-depositional processes will affect carbonized botanical remains. As a result their physical, chemical and molecular properties will change and under certain conditions the propagules may disappear altogether. North-western European Mesolithic sites with only hazelnut shells are an example of the latter (Bakels, 1991). One wonders what has become of other fruits and seeds because hazelnuts cannot have been the only species consumed. Another example is the almost absence of Neolithic crop remains in some areas, while they are present in comparative sites elsewhere. The early Neolithic of Northern France is an example of the latter (Bakels, 1999). As a result the archaeological record of the carbonized remains might be biased, which in turn affects interpretation of past societies, especially their use of vegetable foods. This implies that more insight is required into the effects of the post-depositional processes that determine the fate of carbonized botanical remains. To obtain this further insight the properties of these remains should be known directly after carbonization occurred and consequently, before a possible deposition in the soil. Methods acquired from natural sciences were used to determine these properties. Carbonization of modern counterparts of peas and wheat grains was simulated under controlled conditions in the laboratory and these experiments provided the samples on which the properties were measured. These two species were chosen because they are also found in the archaeological record. The results provided the fundamental information to study the change of modern propagules as a result of exposure to heat under less controlled conditions as for example in or near open fires. This will make a comparison possible with carbonized botanical remains from the archaeological record as, most likely, these will have been exposed to a similar heat source.

Carbonization of modern propagules in the laboratory

Samples of peas and wheat grains were heated, for 60 and 120 minutes respectively, in a tube oven at oven temperatures ranging from 130-700 °C at atmospheric pressure. The reducing conditions were maintained under a constant flow of nitrogen. For each oven temperature a separate experiment was carried out and the properties of the carbonized material were determined. The results have shown that these conditions allow for experiments that can be reproduced for verification.

Investigated properties

From each sample of peas or wheat grains the properties including weight loss, morphology, bulk chemical composition, vitrinite reflectance and molecular composition were measured. For both propagules the results for each property were presented as a function of the oven temperature, which are summarized for peas in Scheme 3.1 and for wheat grains in Scheme 5.1. Various stages were identified using these data. The temperature at which a transition from one stage to the next one occurs is not similar for peas and wheat grains. The difference in the changing morphology of both propagules is the most striking, which is not surprising as both species have a different morphology and anatomy. In spite of these differences between the transition temperatures five compositional stages A, B, C, D and E were identified, as a function of the oven temperature, which are similar for both propagules under the present experimental conditions. The resulting material formed from both peas and wheat grains have an identical molecular composition, but the oven temperatures at which the various products are formed differ on the order of 10 °C between the two types of propagules. In stage A, up to an oven temperature *(T_{oven})* of 220 °C, starch and protein, the main original biomacromolecules, are the main components. The colour changes gradually into brown suggesting possible Maillard reactions. The carbonized propagules recovered from the archaeological record are always black, which corresponds to propagules from the later stages viz. C-E. In stage B (T_{oven} = 220-270 °C) oligo- and monosaccharides and furans, which are derived from the original polysaccharides, are still present. In the spectra of the DTMS measurements the original protein markers are still recognizable. These compounds gradually disappear in stage C *(T_{oven}* = 270-310 °C) to be replaced gradually by aromatic compounds. In stage D *(T_{oven}* = 310-420 °C) only aromatic compounds such as (alkyl)phenols, (alkyl)benzenes, condensed aromatic compounds and N containing heterocyclic compounds are present. Stage E *(T_{oven}* > 420 °C) is characterized by a strongly carbon enriched material. The results show that the residues of both peas and wheat grains can be divided into five stages with different properties. The flow model as presented in Fig. 1.1 shows only the term "carbonization", suggesting one process providing one product. To illustrate the present results the five stages are incorporated into this figure and shown in Fig. 8.1. The presence of five stages implies that post-depositional processes such as biological, physical and chemical processes will affect the residues from each stage differently.

Dimensional changes

The morphology of the two species studied are different and the heating experiments have shown that peas and grains wheat change differently. Not only the changes vary as a function of the oven temperature, but the dimensions of both

propagules also change in different ways. The latter is important for archaeological research as one of the means to separate and identify the different species of wheat is based on the measurement of the dimensions. To study these changes the morphology was described by a size and shape factor. For both types of propagules the area and 100 LW^{-1}, in which L is length and W is width, were selected as size factor and the shape factor, respectively. The definitions are shown in Fig. 6.1. Size and shape were analyzed by digital imaging analysis. The changes were measured, as a function of the temperature. Wheat grains swell, up to an oven temperature of 250 °C, which causes an increase in size while the shape becomes rounder. At higher temperatures the size decreases again and the shape no longer changes. Peas do not swell at all, but the size decreases starting at about 190 °C

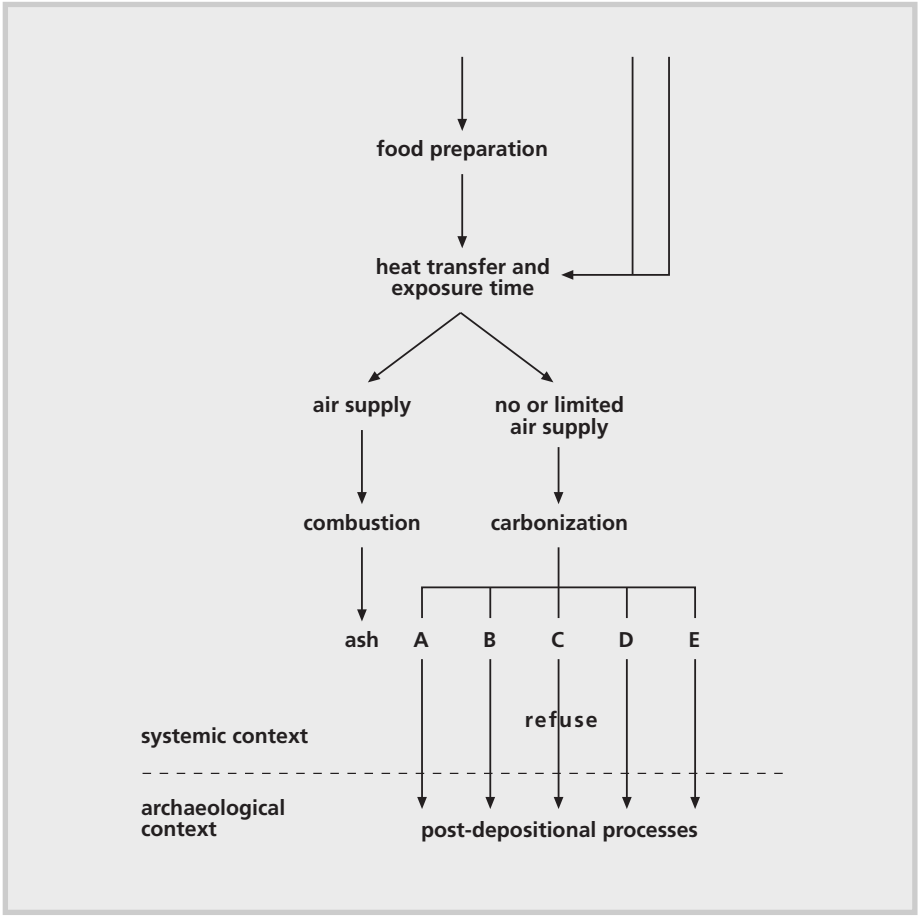


Figure 8.1
Illustration of conclusions regarding the carbonization of propagules. For explanation see text and Figure 1.1

while the shape becomes rounder. The testa or seed coat remains present on the seed of the peas at all the applied temperatures. The presence of the pericarp, the enclosing tissue of wheat grains, and the lack of it in peas most probably explains the difference in these morphological changes. The pericarp is also the reason why distortions of wheat grains can have a different character.

The measurement of the shape allows for the separation of the untreated grains of the three investigated species of wheat: bread wheat, macaroni wheat and emmer wheat. After carbonization under the present experimental conditions the grains of the three taxa show a very similar shape and separation, solely on size and shape, is therefore no longer possible.

Processes that govern the carbonization process

The main biomacromolecules of both peas and wheat grains are starch and protein. The results of the carbonization experiments show that both starch and proteins are converted into new, thermally more stable, products. Starch and proteins undergo significant dehydration and produce brown products, implying Maillard reactions. This occurs up to an oven temperature of 270 °C. In the thermally altered material both constituents still dominate. A path that produces thermally more stable products determines the conversion at higher temperatures. From 270 up to 310 °C the resulting material has changed from a carbohydrate-protein dominated material into substances with mainly aromatic constituents. Fibrous polysaccharide polymers have changed into a three-dimensional condensed network polymer. The proteins are gradually converted into more stable N containing heterocyclic moieties. Concurrently, from 290 °C, exothermic reactions occur resulting from secondary reactions between converting solids and the volatiles traversing the residue on their way to the external environment. The confined structure of the propagules enhances the production of secondary reaction products. This newly formed thermally stable material dissociates further at higher temperatures and a disproportionation occurs leading to a highly condensed aromatic polymer by loss of CO and CO_2 at 400 °C. From 400-440 °C large quantities of volatiles are released and these appear to be removed so fast from the reaction zone in the propagules that no reactions can take place between the volatiles and the converting material within the propagules. Thus a third kind of chemically distinct material is formed characterized by highly C-enriched substances, the presence of N containing moieties and a high porosity. The presence of HCN *(m/z* 27) in the DTMS spectra of the samples heated at 400 °C and higher is considered as a marker for the latter moieties, although their chemical structure is unknown.

Carbonization in three different heat sources

Is it possible to compare the results from the samples carbonized in a tube oven under controlled conditions with data from field experiments? To answer this question peas were carbonized for 60 minutes under three heating conditions: (i) in a tube oven, (ii) on a porcelain plate above a gas burner and (iii) on potsherds in an open wood fire. In the tube oven the mode of heat transfer is mainly convection and the supply of air can be prevented easily. The mode of heat transfer in the latter two heat sources is mainly radiation and a limited access of air is unavoidable. The results show however, that samples with a similar weight loss have similar properties independent of the heat source.

To obtain further insight into these results a series of experiments using wheat grains was carried out in a tube oven at constant temperature as a function of the time of exposure. The results show an increase of the weight loss as a function of the time of exposure for each oven temperature (Fig. 7.3). Even after an exposure of 7-8 days the conversion, measured as weight loss, appears to continue. For example after seven days of heating at an oven temperature of 220 °C a weight loss was measured on the order of 42 %, but a similar weight loss was measured after nine minutes of heating at 340 °C (Fig. 7.3). The data suggest that the rate of heat (J sec^{-1}) transferred to the sample and the exposure time (sec) determine the rate of conversion or weight loss. Thus the energy (J) that is required for the chemical reactions drives the conversion. Samples situated on lines of equal weight loss have a similar molecular composition, as was confirmed by DTMS-EI measurements and from these results it is inferred that these lines are to be considered lines of equal energy. Consequently stage A ends at 15 % weight loss, stage B is situated between 15-45 %, stage C between 45-60 %, stage D between 60-70 % and stage E > 70 % (Fig. 7.3). These stages correspond to the five stages mentioned in a previous section (Fig. 8.1), which were determined using the properties of samples heated at various temperatures in a tube oven, but whereby the time of exposure, for peas 60 minutes and for wheat grains 120 minutes, was known. The fact that both the temperature and the time of exposure are known, the latter experiments have to be considered as special cases. It is concluded that by measuring physical, chemical and molecular properties of propagules carbonized under less controlled conditions the temperature attained by the propagules cannot be inferred. Only the rate of conversion corresponding to a weight loss can be determined (Fig. 7.3).

IMPLICATIONS FOR ARCHAEOLOGICAL RESEARCH

Introduction

This study is focused on the investigation of the physical, chemical and molecular properties of carbonized modern counterparts of species that are found in the archaeological record. Heating experiments, under controlled conditions in the laboratory, have provided the knowledge about these properties, as a function of the energy, transferred as heat from a heat source to samples of the propagules. It was shown that not the temperature of the propagules governs the carbonization process, but the rate of heat transfer to the propagules together with the time of exposure to the heat source. The properties of the carbonizing propagules are summarized in five compositional stages A, B, C, D and E, defined as a function of the weight loss of the converting material. Although it is not the focus of this thesis some related implications for archaeological research can be inferred.

Heat induced compositional stages

As a result of exposure to a heat source, under a limited supply of air, propagules are converted into new, thermally and probably environmentally more stable, products. In archaeological research this conversion process is frequently referred to as carbonization. The resulting products are often called the carbonized or charred remains, suggesting a similar composition. It was shown that the properties of this material may vary widely as a function of the energy available for the reactions that cause the chemical conversion (Fig. 8.1). The terms carbonized or charred remains are therefore too general and it is important to specify the properties of the remains.

The properties of the chemically converted propagules are divided into five compositional stages A, B, C, D and E, as a function of the weight loss (Fig. 8.1). This will also apply to botanical remains carbonized in the past directly before deposition in the soil. The division into five stages provides a rigorous basis for a further study of the effects of the post-depositional processes on heat induced converted propagules.

Preservation potential

After the deposition of the carbonized propagules in the soil it can be assumed that the polysaccharide and protein characterized material from stages A and B will be readily degraded over time, under normal environmental conditions (Kirk

and Cowling, 1984; Miksicek, 1987; Atlas and Bartha, 1993). It is expected that carbonized propagules with the characteristic properties of stage C and higher will survive the post-depositional processes. However, no studies are known of, which show that under all circumstances propagules with the characteristic molecular composition of stages C, D and E are resistant to post-depositional processes. A first indication that these propagules might be resistant is the fact that the heated samples started to become black from stage C onwards and usually propagules recovered from the archaeological record are black too. Although carbonized propagules are frequently found in the archaeological record it should be stressed that carbonization only occurs when the propagules are exposed to heat in that part of the heat source where reducing conditions prevail. This implies that an unknown number of propagules might have been exposed to heat under oxidizing conditions and consumed to ashes. Consequently, this part of the propagules from the systemic context (Fig. 8.1) will not be transferred to the archaeological record.

Identification of wheat grains and peas after carbonization

Untreated taxa of wheat can be separated based on shape, but it was shown that under the present experimental conditions this is no longer possible. This result is not in accordance with the archaeobotanical practice. Upon thermal conversion the shape and size of peas and wheat grains change in different ways. Peas get smaller and rounder, but wheat grains swell first and get smaller and rounder at a later stage. The presence of the pericarp in wheat grains and the lack of it in peas is the reason for this difference in morphological behaviour. The results of the experiments in the tube oven show that the seed coat of the carbonized peas is always present. In most of the peas recovered from the archaeological record the seed coat is no longer present (Hubbard, 1992).

FURTHER RESEARCH

This thesis has provided physical, chemical and molecular boundary conditions of the properties of carbonized modern propagules. In this way the situation was simulated by which the properties are known of propagules carbonized in the past, directly before they might have been deposited in the soil. Post-depositional processes will subsequently affect these propagules and upon recovery by archaeologists more changes will occur as a result of the C2-transforms (Fig. 1.1). Further research into the changes of the properties of the propagules as a result of these processes would provide archaeologists additional insight into the factors, which gave propagules retrieved from the archaeological record their properties as witnessed to-day. Possible avenues for further exploration are:

1. It was shown that after carbonization under the present experimental conditions shape alone is not a suitable tool to separate carbonized grains of different taxa of wheat. This is not in accordance with the archaeobotanical practice. A reason for this discrepancy could be that carbonization of wheat grains in the past occurred under different circumstances. Thus more research is necessary to answer the questions: what are these circumstances and what are the effects of these circumstances on the shape of the propagules.

2. Investigation of the effects of biological, physical and chemical degradation processes on carbonized modern propagules by enzymic degradation. Using this approach one could determine if black propagules, with the characteristics of stages C, D and E, are under all circumstances resistant to this type of degradation. Also the influence of the environmental conditions, such as type of soil, pH, etc. on the various stages of carbonized propagules has to be investigated using the properties of soils from which carbonized propagules have been excavated or from soils where the absence of carbonized propagules cannot be explained in any other way.

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SUMMARY

Archaeology is concerned with the study of past societies primarily through material remains such as buildings, tools, plant remains and other artefacts that constitute what is the material culture left over from these societies. In most cases the remains have been hidden in the soil for ages before being excavated by archaeologists. Between their deposition in the soil and excavation natural biological, chemical and physical degradation processes may have affected the properties of the remains. It implies that inferences from these remains about past societies may be not correct and it is therefore of great importance to know how the remains have acquired the characteristics witnessed today.

The focus of this study is on plant remains and more in particular on remains that were exposed to heat under reducing conditions before being deposited. This process is frequently called carbonization and is one of the means by which plant remains may survive the natural degradation processes. However, survival does not mean that these processes may not have affected the remains. To understand how these effects have changed the various properties of carbonized plant remains it is necessary to know these properties directly after the carbonization process and consequently, before a deposition into the soil would have been occurred. To determine these properties the carbonization process is simulated by laboratory experiments using the modern counterparts of seeds and fruits found in the archaeological record. For the experiments two species were selected: peas *(Pisum sativum* L.) and emmer wheat grains *(Triticum dicoccum* Schübl).

Chapters 2 and 3 describe the simulation of the carbonization process with samples of modern peas heated for 60 minutes in a preheated tube oven at temperatures ranging from 130 up to 700 °C under reducing conditions at atmospheric pressure. Separate experiments were carried out at selected temperatures and from each of the in total sixteen carbonized samples weight loss, morphology, bulk chemical composition, vitrinite reflectance and molecular composition were measured. Based on these data the change of these properties versus oven temperature is determined and five stages (A, B, C, D and E) are identified. As far as the molecular composition is concerned the main constituents in peas are starch and protein and both compounds are converted into new, thermally more stable, products. Up to a temperature of 270 °C both compounds still dominate (stages A and B), but from 270 up to 310 °C the reactant changes gradually into a material with mainly aromatic compounds (stage C). No change in the composition of the samples is observed when the samples are heated at temperatures ranging from 310 up to 400 °C (stage D). From 400-440 °C and higher a chemically distinct material is formed characterized by a strong C-enriched material and the presence of N containing moieties (stage E). This classification provides a rigorous basis for

studies regarding the effects of post-depositional processes on carbonized peas. Under the present conditions vitrinite reflectance provides a fast and cheap tool to determine the temperature to which the specimens have been exposed.

In chapter 4 the selection is discussed of one variety to be used for the carbonization experiments out of seven varieties of emmer wheat. Compression tests applied on individual specimens showed the higher the protein content the higher the resistance against deformation. The pericarp, the enclosing tissue, and the difference in nitrogen content determine the difference in behaviour between the varieties. Based on these results variety AR was selected. Different types of distortion as a result of heating are observed. The two main types are grains with protrusions and grains with an open crease.

Chapter 5 describes the simulation of the carbonization of modern emmer wheat grains, var. AR. Similar experiments were carried out as presented in chapters 2 and 3 for peas. The main constituents in both wheat grains and peas are starch and protein, but the anatomy and morphology differ between the two species. The results show that the changes, as a function of the temperature, of the measured properties are similar to those observed in peas and the same stages A, B, C, D and E are identified. The changes in morphology are different.

Chapter 6 describes how and why the morphology of carbonized peas and wheat grains changes as a function of the temperature. The morphology is described by a size and a shape factor, which are measured by digital imaging analysis. These changes correspond well to the physical and molecular changes, as described by the five compositional stages. Wheat grains swell, up to an oven temperature of 250 °C, which causes an increase in size while the shape becomes rounder. At higher temperatures the size decreases again and the shape no longer changes. The experiments in the laboratory show that solely on size and shape grains of carbonized species of wheat and its varieties grains cannot be separated. Peas do not swell at all, but the size decreases starting at about 190 °C while the shape becomes rounder. The testa or seed coat remains present on the seed of the peas at all the applied temperatures. The presence of the pericarp in wheat grains, and the lack of it in peas, most probably explains the difference in these morphological changes.

In chapter 7 an answer is given to the question if the results of experiments in the laboratory are also valid for propagules carbonized under less controlled conditions such as in an open wood fire. For this purpose carbonization experiments were carried out under three heating conditions: (i) in a tube oven, (ii) on a porcelain plate above a gas burner and (iii) on potsherds in an open wood fire. In the former the mode of heat transfer is mainly convection and the supply of air can be prevented easily; in the latter two heat sources heat transfer occurs mainly through radiation and a limited access of air is unavoidable. Although the exper-

imental conditions differ the results show that samples with a similar weight loss have similar properties independent of the heat source. To elaborate on the influence of time a series of separate experiments at a constant temperature was carried out as a function of the time of exposure. It showed that carbonized propagules exposed for a long time at a low temperature have identical properties as propagules heated for a short time at a higher temperature. The conclusion is that the rate of heat (J sec^{-1}) transferred to the sample and the exposure time (sec) determine the rate conversion or weight loss. Thus the energy (J) that is required for the chemical reactions drives the conversion. These results imply that the division into five stages based on the oven temperature has to be reconsidered and based on the acquired energy or the weight loss. Thus stage A covers samples with a weight loss of up to15 %, stage B from 15-45 %, stage C from 45-60 %, stage D from 60-70 % and stage E for samples with a weight loss of over 70 %. Consequently, the division into these five stages of the carbonized samples of peas and wheat grains based on the oven temperature, as described in the chapters 2, 3 and 5, has to be considered as a special case whereby apart from the temperature also the time of exposure, for peas 60 minutes and for wheat grains 120 minutes, was known. Thus by measuring physical, chemical or molecular properties of propagules carbonized under less controlled conditions the temperature attained by the propagules cannot be inferred and only the rate of conversion corresponding to the weight loss can be determined. The earlier suggestion that vitrinite reflectance measurements could be used for the measurement of the temperature at which the carbonization took place has to be limited to the measurement of the weight loss. The results also show that carbonized propagules with identical properties may have a different thermal history.

Although the focus of this study is on the determination of the properties of seeds and fruits directly after they have been carbonized, which in a future project will allow to investigate the influence of the post-depositional processes on these carbonized remains, some implications for archaeological research can already be inferred. These include: (i) carbonized or charred remains is a frequently used expression for entities retrieved from the archaeological record suggesting a similar composition, but it is shown that their properties may vary widely; (ii) the division into five compositional stages shows that the influence of the natural degradation processes will be different on carbonized propagules belonging to different stages; (iii) the thermal history of samples of carbonized propagules with identical properties does not have to be identical and (iv) the conclusion that carbonized wheat grains cannot be separated based solely on size and shape is not in accordance with the archaeobotanical practice, a fact that is not understood yet.

In conclusion, the experiments conducted in this thesis have made it possible to divide the carbonized propagules into five compositional stages, each stage with its own characteristic physical, chemical and molecular properties. This rigorous

base will allow for a further study of the possible influences of the post-depositional processes on freshly carbonized modern propagules. Along with these results a number of implications for archaeological research are inferred. It was shown that interdisciplinary research, which made it possible to introduce methods to determine the properties, is pivotal to obtain the necessary insight to solve certain problems related to archaeological studies.

SAMENVATTING

Archeologie bestudeert de menselijke samenlevingen uit het verleden voornamelijk aan de hand van materiële resten zoals gebouwen, gereedschappen, plantenresten en andere artefacten die deel uit maakten van de betreffende materiële cultuur. In de meeste gevallen zijn de resten eeuwenlang begraven gebleven voordat ze werden opgegraven door archeologen. Na depositie in de grond en voor opgraving kunnen de in de natuur aanwezige biologische, chemische en fysische afbraakprocessen de eigenschappen van de resten hebben veranderd. Dit houdt in dat gevolgtrekkingen over de samenlevingen aan de hand van deze resten niet juist kunnen zijn. Het is daarom van grote betekenis om te weten hoe en in welke mate deze resten de eigenschappen hebben gekregen zoals ze worden waargenomen nadat ze zijn opgegraven.

Dit proefschrift concentreert zich op plantenresten en in het bijzonder op die resten die aan een warmtebron, waarbij de aanwezigheid van lucht beperkt is, zijn blootgesteld voordat ze in de grond werden gedeponeerd. Dit proces wordt vaak verkoling genoemd en is een van de manieren waarbij plantenresten de in de natuur voorkomende afbraakprocessen kunnen overleven. Echter, overleven wil nog niet zeggen dat de resten niet worden aangetast. Om te begrijpen hoe en in welke mate deze processen de eigenschappen van het verkoolde plantenmateriaal hebben veranderd is het noodzakelijk om deze direct na het verkolingsproces te bepalen en dus voordat het verkoolde plantenmateriaal in de grond zou worden gedeponeerd. Om deze eigenschappen te kunnen bepalen is middels experimenten in het laboratorium het verkolingsproces nagebootst door gebruik te maken van de moderne tegenhangers van de zaden en vruchten die in archeologische opgravingen worden gevonden. Voor de experimenten werden twee soorten gekozen: erwten *(Pisum sativum* L.) en emmertarwe *(Triticum dicoccum* Schübl).

De hoofdstukken 2 en 3 geven een beschrijving van het nabootsen van het verkolingsproces in het laboratorium. Hiervoor zijn monsters van moderne erwten gebruikt die werden verhit in een buisoven onder reducerende omstandigheden bij atmosferische druk gedurende 60 minuten bij oventemperaturen die varieerden van 130 tot 700 °C. Voor iedere gekozen temperatuur werd een afzonderlijk experiment uitgevoerd wat resulteerde in zestien verkoolde monsters. Van deze monsters werden gewichtsverlies, morfologie, bulk chemische samenstelling, vitrinietreflectie en moleculaire samenstelling gemeten. Gebaseerd op deze gegevens is de verandering van iedere eigenschap, als functie van de temperatuur, vastgesteld en werden vijf klassen (A, B, C, D en E) herkend. Met betrekking tot de moleculaire samenstelling kan men in erwten als de belangrijkste bestanddelen zetmeel en eiwitten onderscheiden. Ten gevolge van de verhitting worden beide stoffen omgezet in nieuwe, thermisch meer stabiele, producten. Tot een temperatuur van

270 °C komen beide stoffen nog steeds het meest voor in verkoolde erwten (klassen A en B), maar tussen 270 en 310 °C worde beide stoffen geleidelijk omgezet in aromatische verbindingen (klasse C). Wanneer de monsters verder worden verhit tot 400 °C vindt er geen verdere verandering van samenstelling plaats (klasse D). Bij temperaturen hoger dan 400-440 °C ontstaat er chemisch gezien een ander materiaal dat wordt gekenmerkt door een hoog koolstofgehalte en de aanwezigheid van stikstof bevattende verbindingen (klasse E). Deze classificatie geeft een goede basis om de invloed van post-depositionele processen op de verkoolde erwten te bestuderen. Onder de toegepaste experimentele omstandigheden is gebleken dat het meten van de vitrinietreflectie een snelle en goedkope methode is om de temperatuur te bepalen waaraan de erwten tijdens de verkoling zijn blootgesteld.

In hoofdstuk 4 wordt de manier besproken waarop de selectie plaats vond van een variëteit emmertarwe die voor de verdere verkolingsexperimenten kan worden gebruikt. Hiertoe zijn zeven variëteiten onderzocht. Samendrukkingsproeven uitgevoerd op individuele verkoolde tarwekorrels laten zien, dat hoe hoger de eiwitconcentratie is, hoe hoger de weerstand tegen deformatie wordt. De pericarp, het omhullende weefsel, en het verschil in eiwitgehalte tussen de variëteiten, bepalen het verschil in gedrag ten gevolge van de verkoling. Gebaseerd op deze resultaten is variëteit AR geselecteerd voor de verdere experimenten. Verschillende vormen van deformatie als gevolg van de verkoling van tarwekorrels kunnen worden onderscheiden. De belangrijkste nieuwe vormen zijn korrels met een uitstulping en korrels met een open groef (crease).

De nabootsing van de verkoling van de moderne emmertarwekorrels, var. AR, is in hoofdstuk 5 aan de orde. De experimentele omstandigheden zijn gelijk aan die voor erwten, zoals besproken in de hoofdstukken 2 en 3. Tarwekorrels en erwten hebben een verschillende anatomie en morfologie, maar in beide soorten zijn zetmeel en eiwitten de belangrijkste bestanddelen. De veranderingen als gevolg van de verkoling laten dan ook zien dat de morfologie van beide verschillend wordt. Daarentegen is de verandering van de overige eigenschappen gelijk aan de veranderingen zoals die bij erwten zijn geconstateerd en dezelfde vijf klassen kunnen worden onderscheiden.

Hoofdstuk 6 geeft een beschrijving van hoe en waarom de morfologie van verkoolde erwten en tarwekorrels verandert als functie van de temperatuur. De morfologie wordt beschreven aan de hand van een grootte- en vormfactor en gemeten met een programma voor digital image analysis. De opgetreden veranderingen komen goed overeen met de veranderingen van de overige eigenschappen, zoals die zijn samengevat in de eerder genoemde vijf klassen. Tarwekorrels zetten uit bij verhitting tot een temperatuur van 250 °C, waardoor de grootte toeneemt en de vorm ronder wordt. Bij hogere temperaturen neemt de grootte weer af, maar de vorm verandert niet meer. De experimenten in het labo-

ratorium laten zien dat alleen gebaseerd op grootte en vorm verkoolde tarwekorrels en hun variëteiten niet kunnen worden gedetermineerd naar soort. Erwten zetten daarentegen niet uit en de grootte neemt dan ook af vanaf 190 °C, terwijl de vorm ronder wordt. De testa of zaadhuid van erwten blijft onder alle toegepaste temperaturen aanwezig op verkoolde erwten. De aanwezigheid van de pericarp bij tarwekorrels en het ontbreken van dit weefsel bij erwten is hoogst waarschijnlijk de verklaring voor het verschil in gedrag tussen erwten en tarwekorrels.

In hoofdstuk 7 wordt een antwoord gegeven op de vraag of de resultaten van experimenten uitgevoerd in het laboratorium ook van toepassing zijn op erwten en tarwekorrels die zijn verkoold onder minder controleerbare omstandigheden zoals in een open houtvuur. Voor dit doel zijn er verkolingsexperimenten uitgevoerd waarbij erwten zijn blootgesteld aan drie verschillende warmtebronnen: 1. in een buisoven, 2. op een porseleinen plaatje boven een gasbrander en 3. op plaatjes aardewerk in een open houtvuur. In het eerste geval vindt de warmteoverdracht naar de erwten voornamelijk plaats via convectie en de toevoer van lucht kan gemakkelijk worden voorkomen; in de laatste twee gevallen vindt de warmteoverdracht voornamelijk plaats door straling en een geringe toevoer van lucht is niet te voorkomen. Ofschoon de experimentele omstandigheden sterk verschillen, laten de resultaten zien dat monsters met gelijk gewichtsverlies dezelfde eigenschappen hebben onafhankelijk van de warmtebron. Om de invloed van de tijd op dit proces te bepalen zijn afzonderlijke experimenten uitgevoerd bij een constante temperatuur als functie van de tijd van blootstelling aan de warmtebron. De conclusie is dat de mate van warmteoverdracht (J sec^{-1}) en de tijd van blootstelling (sec) samen de mate van omzetting of het gewichtsverlies bepalen. De energie (J) die nodig is voor de chemische reacties bepaalt dan de mate van omzetting. De verdeling in vijf klassen op basis van de temperatuur dient dus te worden herzien als functie van de ter beschikking staande hoeveelheid energie of het gewichtsverlies. Klasse A omvat dan de monsters met een gewichtsverlies tot 15 %; klasse B van 15-45 %; klasse C van 45-60 %; klasse D van 60-70 % en klasse E monsters met een gewichtsverlies hoger dan 70 %. De verdeling in vijf klassen zoals die plaats vond bij de verkoling van erwten en tarwekorrels, zoals beschreven in de hoofdstukken 2, 3 en 5, dient te worden beschouwd als een speciaal geval waarbij naast de temperatuur ook de tijd van blootstelling, voor erwten 60 minuten en voor tarwekorrels 120 minuten, bekend was. Het is dus niet mogelijk de temperatuur die heerste tijdens de verkoling te bepalen door de fysische, chemische of moleculaire eigenschappen van verkoolde erwten en tarwekorrels te meten, maar alleen de mate van omzetting, die overeen komt met het gewichtsverlies. Dit wil zeggen dat de eerdere suggestie dat middels metingen van de vitrinietreflectie de temperatuur tijdens de verkoling kan worden bepaald moet worden beperkt tot de bepaling van het gewichtsverlies. De resultaten laten ook zien dat verkoolde erwten en tarwekorrels met dezelfde eigenschappen een verschillende thermische geschiedenis kunnen hebben.

Ofschoon dit proefschrift is gericht op de bepaling van de eigenschappen van zaden direct nadat deze zijn verkoold om op die manier de invloed van post-depositionele processen op deze verkoolde monsters te kunnen bestuderen is het toch reeds mogelijk enkele gevolgtrekkingen voor archeologisch onderzoek te maken. Deze omvatten: 1. “carbonized or charred remains” is in de archeologie een veel gebruikte uitdrukking voor verkoolde resten die zijn opgegraven, dit suggereert dezelfde samenstelling die er echter niet is; 2. de verdeling in vijf klassen laat zien dat de invloed van de natuurlijke afbraakprocessen verschillend zal zijn voor resten die tot verschillende klassen behoren; 3. de thermische geschiedenis van verkoolde resten met gelijke eigenschappen hoeft niet dezelfde te zijn en 4. de conclusie, dat verkoolde tarwekorrels en hun variëteiten niet kunnen worden gedetermineerd op soort op basis van grootte en vorm alleen, wordt niet onderschreven door de archeobotanische praktijk. Deze discrepantie is onderwerp van nader onderzoek.

De algemene conclusie is dat de experimenten uitgevoerd in dit proefschrift het mogelijk maken verkoolde erwten en tarwekorrels in vijf klassen, als functie van de ter beschikking staande hoeveelheid energie, te verdelen. Hierbij wordt iedere klasse gekenmerkt door specifieke fysische, chemische en moleculaire eigenschappen. Deze basis maakt het mogelijk een verdere studie te doen naar de invloed van de post-depositionele processen op verkoolde moderne zaden. Het onderzoek leverde ook enige implicaties op voor archeologisch onderzoek. Het onderschrijft dat interdisciplinair onderzoek, waardoor het mogelijk was de methoden te introduceren om de verschillende eigenschappen te kunnen bepalen, van groot belang is om bepaalde problemen in het archeologisch onderzoek op te lossen.

DANKWOORD

Na vier jaar onderzoek kom ik weer tevoorschijn uit de wereld van de verkoolde erwten en tarwekorrels. De zwarte knikkertjes waarvan iedereen dacht: wat moet die man daar nou mee? Die vraag heb ik vele malen gekregen en tot op heden ben ik nog niet in staat gebleken om op feestjes en partijtjes daar een zodanig antwoord op te geven dat men het enthousiast ging verder vertellen. Nee, meestal liep men hoofdschuddend weer verder. Bovendien is het een gecompliceerde zaak met al die instituten, waar men ook vreemd opkeek als ik begon uit te leggen wat de bedoeling was. Het verkolen gaat gepaard met vreemde luchtjes en tot mijn verdriet vond men het vaak stinken. Op die manier werd ik dan toch nog bekend: Ik ruik het al, Freek is weer bezig!

Het onderzoek begon met een idee van Corrie Bakels, die vond dat er nou eindelijk eens uitgezocht moest worden waarom er langs de rivier de Aisne in Noord-Frankrijk nauwelijks of geen verkoolde zaden werden gevonden, terwijl dat in andere vergelijkbare streken wel het geval was. Na enig vooronderzoek kwam ik tot de conclusie dat voor een dergelijke studie toch eerst de eigenschappen van verkoolde zaden bekend moesten zijn voordat deze in de grond verdwenen. Corrie sprak toen de gedenkwaardige woorden: “Weet wel waar je aan begint!” en liep hoofdschuddend weg. Nu wist ik dat natuurlijk ook niet, maar het resultaat ligt er nu.

Hoewel Corrie zo haar bedenkingen had, bleef ze me steunen en na enige tijd begon ze ook enthousiast te worden en vele discussies waren het gevolg. Zonder de bijdragen van ondermeer de volgende leden van de Faculteit der Archeologie: Wim Kuijper (altijd aanwezig), Eric Mulder (foto’s van zaden) en Medy Oberendorff (scanner voor digital analysis) was een en ander niet gelukt. Veel dank.

Verkolen is een chemisch proces en voor dat soort onderzoek is een chemisch laboratorium onontbeerlijk. Waar haal je dat vandaan als beginnend archeoloog? Bij een bezoek aan het Department of Anthropology van de Washington University in St.Louis, USA ontmoette ik John Kelly die direct reageerde en zei dat daar op dit onderwerp kort geleden iemand gepromoveerd was. Dat bleek Patty Wright te zijn en haar belangrijkste conclusie was: je moet eerst meer onderzoek doen naar het verkolingsproces proces zelf en de eigenschappen van het vers verkoolde materiaal kennen. Thank you Patty and John. Zo kwam ik dan in Carbonville, Illinois terecht, de juiste naam voor de juiste plek. Daar ontmoette ik toevallig Tomasz Kuder die mij vertelde dat ik voor dergelijk onderzoek bij Jaap Boon, Jan de Leeuw of Pim van Bergen in Nederland moest zijn. Zo ben ik dus bij jullie terechtgekomen. Eerst naar Jaap (FOM Instituut, Amsterdam) gegaan die direct heel positief reageerde en voorstelde de moleculaire samenstelling van de verkoolde materialen met DTMS te onderzoeken. De enige conditie was dat ik geen

salaris kreeg. DTMS-metingen wil zeggen Jerre van der Horst. Jerre bedankt voor alle geduld en het feit dat je altijd klaar stond om de problemen aan te pakken en op te lossen. Hoewel ik relatief weinig aanwezig was wil ik toch ook de andere leden van de groep bedanken en in bijzonder Ester Ferreira, Beatrice Marino, Katrien Keune en Gert Eijkel.

Dan de verkoling zelf met de bulk chemie, de microscopie, de vitrinietreflectie, etc. De eerste gesprekken met Jan en Pim (Departement der Aardwetenschappen, Universiteit Utrecht) waren ook al zeer positief en Pim werd mijn begeleider. De eerste twee jaar heb ik veel tijd doorgebracht in Utrecht en steun gehad van veel mensen, waarvan ik er hier toch enkele wil noemen en bedanken: Kathrin Reimer, Petra David, Harry Veld en Bertil van Osch van TNO-NITG en Pieter Kleingeld, Arnold van Dijk, Gijs Nobbe en Ton Zalm van Aardwetenschappen. En niet te vergeten: Marianne Baas van het NIOZ op Texel, die mij de eerste beginselen van het verkolen bijbracht.

Voorts was er nog de Hortus Botanicus in Leiden waar mij werd geleerd om erwten en tarwe te laten groeien. Wessel de Priester van het Clusius Laboratorium in Leiden, die de mooie SEM-plaatjes maakte, ik bedank je voor de prettige wijze waarop je steeds klaar stond. Het Archeon in Alphen aan de Rijn, waar Pieter en ik veel hebben gelachen en het ons is gelukt om niet in berenvellen te hoeven werken, ook vriendelijk bedankt en vooral Miriam de Boer en Erik Schouten.

Ik zal mogelijk hier en daar iemand vergeten zijn, dan hiervoor alvast mijn excuses. Het mag duidelijk zijn dat al jullie steun en hulp niet vergeefs zijn geweest zoals je nu kunt zien.

Dit was dan de technische kant van het werk. Thuisgekomen moet er dan iemand zijn die je verhalen aanhoort en dat was altijd Mieke. Haar begrip en vooral haar geduld zijn onuitputtelijk. Gelukkig kon ze ook genieten van de bezoeken aan de opgravingen langs de Aisne en heeft ze vele vrienden gemaakt. Ook onze kinderen en kleinkinderen hebben altijd geduldig geluisterd en gelezen en zo ook hun bijdrage geleverd.

CURRICULUM VITAE

De auteur werd op 8 augustus 1935 in Oisterwijk geboren, deed in 1954 eindexamen VWO in Breda en behaalde in 1962 het diploma van Mijnbouwkundig Ingenieur aan de Technische Universiteit in Delft, met als specialisatie geofysica. Hij werkte daarna tot 1995 in het bedrijfsleven, waarvan het grootste gedeelte buiten Nederland werd doorgebracht. Mede hierdoor ontstond belangstelling voor de archeologie en dat leidde in 1996 tot een studie aan de Faculteit der Archeologie van de Universiteit Leiden. Het uiteindelijke resultaat is het voorliggende proefschrift.

The work described in this thesis was performed at the following institutes:

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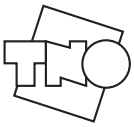

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