ELSEVIER

Food Chemistry 71 (2000) 9–36

Food Chemistry

www.elsevier.com/locate/foodchem

Review

# Methods for the study of starch retrogradation

A. Abd Karim *, M.H. Norziah, C.C. Seow

*Food Biomaterials Science Group, Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia*

Received 6 August 1999; received in revised form 24 March 2000; accepted 24 March 2000

**Abstract**

The wealth of current knowledge on starch retrogradation is due in large measure to the wide array of analytical methods at the disposal of food scientists. Since retrogradation is a complex process affected by many factors, it is unlikely that any single method would be able to give a complete picture of the retrogradation properties of starch gels at both the macroscopic and molecular levels. Independent evidence derived from two or more methods allows cross comparisons that can provide a fuller understanding of this phenomenon. For quantitative measurement of rates of retrogradation, the "ideal" method should be simple, rapid, non-destructive, precise, and inexpensive. Comparisons of kinetic data from different sources should be made with caution; various factors (thermal history, in particular) that can lead to unjustifiable comparisons and erroneous conclusions should be carefully considered first. This review covers the general principles, capabilities, advantages, and limitations of various methods available to study starch retrogradation. 

**Contents**



* Corresponding author. Tel.: +60-4-657-7888; fax: +60-4-657-3678.
*E-mail address:* akarim@usm.my (A.A. Karim).

0308-8146/00/$ - see front matter 
PII: S0308-8146(00)00130-8

## 1. Introduction

Starches are the major storage polysaccharides in foods of plant origin. The major botanical and commercial sources of starches are cereals, tubers, roots, and pulses. Native and modified starches serve as important ingredients of many fabricated foods. Starches are α-glucans composed basically of two different homopolymers of D-glucose — amylose and amylopectin. Amylose has traditionally been considered to be a linear polymer composed of glucopyranose units linked through α-D-(1→4) glycosidic linkages. Although there is now evidence that amylose is not completely linear (Curá, Jansson & Krisman, 1995), its behaviour approximates that of a linear polymer. Amylopectin is a branched polymer with one of the highest molecular weights known among naturally occurring polymers. It is composed of glucopyranose units linked by α-D-(1→4) glycosidic linkages. For approximately every 20–30 glucopyranose residues, a branch point occurs, where a chain of α-D-(1→4)-glucopyranosyl units is linked to the C-6 hydroxymethyl position of a glucose residue through an α-D-(1→6) glycosidic linkage. Thus, about 4% of the glucopyranose residues in amylopectin are involved in branch points.

Starches exist naturally in the form of discrete granules within plant cells. These granules may be viewed as partially crystalline and partially amorphous polymeric systems (Blanshard, 1987; Slade & Levine, 1989). The crystalline character of the granules of common starches arises from the organization of the amylopectin molecules within the granules, while amylose largely makes up the amorphous regions which are randomly distributed between the amylopectin clusters (Blanshard; Zobel, 1988).

Cooking or processing normally causes starch gelatinization, i.e. irreversible swelling or even disruption of the starch granules, depending upon the severity of the treatment applied. The behaviour of gelatinized starches on cooling and storage, generally termed as retrogradation, is of great interest to food scientists and technologists since it profoundly affects quality, acceptability and shelf-life of starch-containing foods (Biliaderis, 1991). Starch molecules in pastes or gels are known to associate on aging, resulting in effects such as precipitation, gelation, and changes in consistency and opacity. Crystallites begin to form eventually, and this is accompanied by gradual increases in rigidity and phase separation between polymer and solvent (syneresis). It is important to distinguish between the short-term development of gel structure via amylose crystallization and long-term reordering of amylopectin which is a much slower process involving recrystallization of the outer branches (DP = 15) of this polymer (Miles, Morris, Orford & Ring, 1985; Ring et al., 1987). For common starches containing both amylose and amylopectin, a composite gel network forms, consisting of swollen amylopectin-enriched granules (provided granule integrity is maintained) filling an interpenetrating amylose gel matrix (Miles, Morris, Orford et al.). During long-term storage, amylopectin recrystallizes, thus increasing the rigidity of the swollen granules which, in turn, reinforces the continuous amylose phase.

The effects of retrogradation in starch-based products can be desirable or, more usually, undesirable. There is general consensus that starch retrogradation contributes significantly to staling or undesirable firming of bread and other starch-based products (D'Appolonia & Morad, 1981; Knightly, 1977; Kulp & Ponte, 1981; Maga, 1975; Seow & Thevamalar, 1988; Willhoft, 1973). Similarly, the susceptibility of legume starch gels to retrogradation and syneresis makes these types of starches unsuitable for products requiring low-temperature storage. However, retrogradation is sometimes promoted to modify the structural, mechanical or organoleptic properties of certain starch-based products. This is true, for example, in the production of breakfast cereals and parboiled rice, since retrogradation results in hardening

and reduced stickiness (Colonna, Leloup & Buléon, 1992). Freezing/thawing, which accelerates retrogradation, is applied to cooked potato mash in the production of dehydrated mashed potatoes to decrease the amount of soluble starch and to improve the consistency of the reconstituted product (Ooraikul, Parker & Hadziyev, 1974). The production of Japanese 'harusame' noodles also involves a freeze–thaw cycle to reduce stickiness and to obtain a characteristic chewiness (Watanabe, 1981). Similarly, Chinese rice vermicelli (a type of rice noodle) strands are conditioned after complete steam-gelatinization of starch in order to attain the desired textural characteristics (Seow & Teo, 1996).

Because of its industrial significance, many methods for the study of starch retrogradation have been developed. Changes in physical and chemical properties, attributable to changes in the starch component of model starch systems or actual starch-based products during aging, form the usual bases for these methods. These time-dependent changes may directly contribute to or correlate with sensory perception or digestibility of starchy foods. However, it is important to emphasize that, in most cases, following changes in a single parameter with time may not provide an adequate description of retrogradation. Furthermore, retrogradation kinetics, determined using different methods, may also not be in total agreement (Roulet, MacInnes, Wursch, Sanchez & Raemy, 1988). Correct interpretations of results would, therefore, depend on an exact knowledge of the physical and/or chemical basis, as well as an appreciation of the limitations, of any given method. The adoption of proper procedures is, of course, critical in ensuring validity of results. While several critical reviews on starch retrogradation have been published over the years (Biliaderis, 1998; Collison, 1968; Dengate, 1984; Hoover, 1995; Kulp & Ponte, 1981; Morris, 1990; Olkku & Rha, 1978; Sterling, 1978; Slade & Levine, 1986, 1989; Willhoft, 1973), none has focused on methodology. Our objective is to provide a comprehensive coverage of objective methods developed for the study of starch retrogradation, without going into details on instrumentation (for which many standard texts are available) or procedures (which may be obtained from the individual references). Emphasis is given to the principles and comparative advantages and limitations of the major or more popular techniques. The present review should thus serve as a useful complement to the existing reviews on the subject.

Methods to study starch retrogradation can be conveniently classified as: (i) macroscopic techniques, i.e. those methods which monitor alterations in certain physical properties as manifestations of retrogradation, for example, mechanical or textural changes, and (ii) molecular techniques, i.e. those methods which study changes in starch polymer conformation or water mobility in starch gels at molecular levels. Thus, rheological techniques, sensory evaluation of texture, differential scanning calorimetry (DSC), light scattering, turbidometry, and measurement of syneresis may be used to study the macroscopic manifestations of retrogradation. On the other hand, X-ray diffractometry, nuclear magnetic resonance spectroscopy (NMR), vibrational spectroscopy (e.g. Raman spectroscopy) and Fourier transform infra-red (FTIR) spectroscopy may be classified as molecular techniques. In all cases, the inclusion of microcomputer technology of ever-increasing sophistication into the designs of instruments should continue to increase the precision, resolution, speed of analysis, and range of capability of any technique.

## 2. Rheological methods

Direct detection of the development of structure of a full three-dimensional polymer network spanning the system, and the measurement of the properties of this network as it matures, are best conducted using macroscopic techniques. Since a dramatic change in the mechanical behaviour of starch gels is what is actually to be measured, and since it is a 'solid' or 'solid-like' material that is generated by the recrystallization process, it is natural that the course of this structure development should be monitored by rheological or mechanical testing. Rheological measurements may involve the application of large forces or shearing stresses to a starch gel or dispersion that can cause permanent structural damage or shear thinning, thus making it difficult to study the viscoelastic properties of the system. In recent years, small deformation dynamic mechanical devices which enable viscoelastic properties to be studied non-destructively have become increasingly popular.

### *2.1. Large deformation studies*

#### *2.1.1. Uniaxial compression and texture profile analysis*

Starch gel firmness or rigidity increases markedly with retrogradation (Collison, 1968). These changes have traditionally been followed using large deformation fundamental tests such as uniaxial compression or empirical tests such as penetration which provide data on mechanical properties known to show good correlations with sensory textural attributes (Axford, Colwell, Cornford & Elton, 1968; Jankowski & Rha, 1986; Keetels, van Vliet, Jurgens & Walstra, 1996; Keetels, Visser, van Vliet, Jurgens & Walstra, 1996). Whereas dynamic testing can be performed on very soft gels because they can be formed in the rheometer, uniaxial compression tests usually require relatively firm gels. A large number of replicated samples of uniform dimensions are required to obtain acceptable reproducibility using such

methods. Heterogeneity of the rheological profile within and between samples could seriously affect the validity of the results obtained.

An idealised stress–strain curve obtained from a uniaxial compression test is illustrated in Fig. 1. The shape of such a curve is dependent on the conditions used for testing; consequently, these must be specified if a meaningful comparison of data is to be made. Mechanical parameters that can be derived from a uniaxial compression test include Hencky's strain ($\epsilon_h$), and Young's modulus ($E$). The initial portion of the curve is linear and the Young's modulus, $E$, is obtained from its slope. Several workers (Conde-Petit & Escher, 1994; Keetels, van Vliet, Jurgens et al; Keetles, Visser et al.) have used these parameters to express the results of a uniaxial compression test on starch gels. Results derived from the uniaxial compression test can also be expressed in terms of apparent modulus of elasticity (Jankowski & Rha, 1986; Knudsen, Børresen & Nielsen, 1987) or recoverable work (Rao, Nussinovitch & Chinachoti, 1992). Peleg (1987), Bagley (1987), and Keetels, van Vliet, Jurgens et al. (1996) and Keetels, Visser et al., (1996) have discussed the principles and calculations involved in uniaxial compression testing.

A great deal of information has been obtained on bread staling by following the increase in firmness of bread crumbs during storage using uniaxial compression (Dragsdorf & Varriano-Marston, 1980; Ghiasi, Hoseney, Zeleznak & Rogers, 1984; Martin, Zeleznak & Hoseney, 1991; Rogers, Zeleznak, Lai & Hoseney, 1988). Herz (1965) listed many changes in crumb properties associated with staling, including increased crust moisture, crumbliness, opacity and firmness. A strong negative correlation between consumer acceptance and compressibility or firmness has been well documented (Axford et al., 1968).

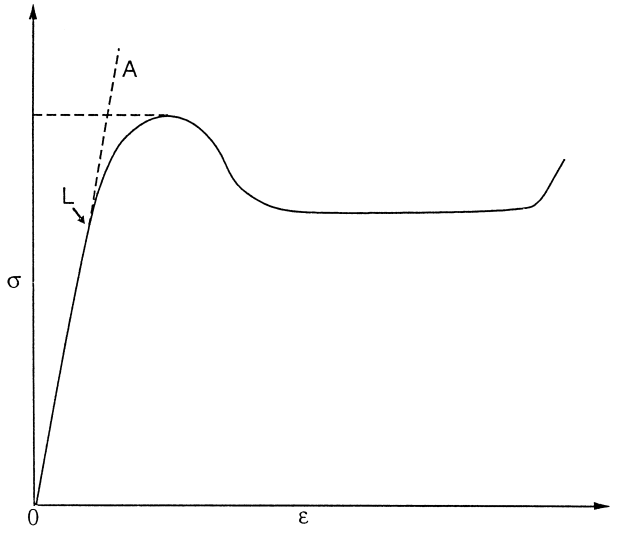


Fig. 1. Idealized stress–strain curve. The slope of line OA is a measure of the Young's modulus.

Instrumental texture profile analysis (TPA), developed by Bourne and co-workers (Bourne, Moyer & Hand, 1966; Bourne, 1968, 1978) using an Instron Universal Testing Machine, has been widely adapted to the study of starch retrogradation in actual food and model starch gel systems (Jankowski, 1992; Seow & Thevamalar, 1988). In a TPA test, a sample of specific dimensions is compressed uniaxially; the compressive force is then removed and the sample is re-compressed. Such a compressive sequence represents two "bites". During the test, compressive force is recorded as a function of the amount of compression (distance). Thus, two force vs distance plots or TPA curves would be derived. Fig. 2 shows the generalized TPA curve obtained using the Instron Universal Testing Machine (Bourne, 1978). Several instrumental texture profile parameters may be derived from the TPA curves: the maximum force ($H$), which occurs at the end of the first compression, equates to "hardness"; the force of the first maximum ($F$) is called "fracturability" (not all foods show this peak); the work done to compress the sample on the "first and second bites" is given as the area under the respective curves ($A_1$ and $A_2$), and the ratio $A_2/A_1$ is related to cohesiveness ($C$); the distance $S$ is called "springiness"; and the negative area 3 is the "adhesiveness" or "stickiness". Textural characteristics such as gumminess (hardness × cohesiveness) and chewiness (hardness × cohesiveness × springiness) are derived functions. A comprehensive review on instrumental TPA, with particular reference to gelled systems, was recently presented by Pons and Fiszman (1996).

A wide range of experimental conditions, encompassing variations in sample size and shape, ratio of compressing probe size vs sample, extent of deformation, cross-head speed, number of "bites", and replicates per

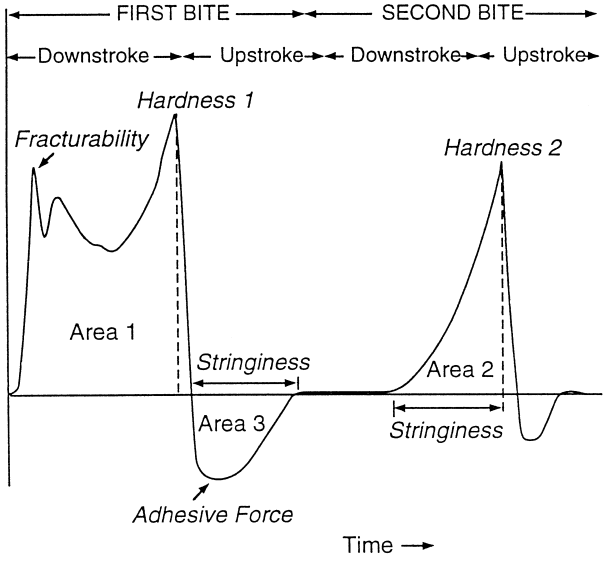


Fig. 2. Generalized texture profile curve obtained using the Instron Testing Machine (Bourne, 1978; with permission).

mean value, has been used for obtaining TPA parameters by different researchers (Table 1). Non-standardization of experimental procedures and conditions used in instrumental TPA makes it difficult to compare TPA data obtained by different investigators. It is also important that a full description of the test procedures and conditions employed be given since textural parameters are known to be greatly influenced by these factors.

With computer-assisted instruments (such as the TA-XT2 Texture Analyser which is becoming increasingly popular), it is possible to perform TPA tests and obtain all TPA parameters directly by means of the available software, without any previous selection of curve values for calculations (Pons & Fiszman, 1996). Usually the user can apply the macro created for specific applications (e.g. TPA) to the selected curve(s) and all the parameters are calculated automatically. This obviously saves time and reduces error in calculations. However, depending on the type of sample, such a degree of automation is not always advisable (Pons & Fiszman). For example, a macro which is written to calculate TPA parameters, including fracturability, will give correct results for samples exhibiting fracturability but not for samples without fracturability.

Using Instron TPA, Jankowski (1992) showed that starch retrogradation markedly influenced texture of cooked potatoes during post-cooking conditioning. Decrease of adhesiveness was the most distinctive feature and was attributed to the association of free amylose leached from starch granules in the cooking process. Increase of cohesiveness and hardness and decrease of fracturability of cooked tubers were slower than changes in adhesiveness; such effects were attributed to the development of a polymeric network within gelled starch in potato cells.

### 2.1.2. Measurement of pasting properties

The tendency of a given starch to retrograde can also be studied from its pasting behaviour, usually by observing changes in viscosity during programmed heating and cooling of a starch suspension, using a variety of instruments. Some of these instruments (e.g. Brabender Amyloviscograph) do not record the absolute viscosity of a starch paste, but the torque as a viscosity signal (expressed as arbitrary Brabender units). This is influenced by a number of factors such as rotational speed, geometry of the measuring device and other methodological factors (Shuey & Tipples, 1980). Most of the common techniques are handicapped by long time requirements for pasting and measuring. Considerable quantities of samples are also required. In spite of these shortcomings, viscosity measurements of starch pastes are well established and approved by different organizations (e.g. Corn Refiners Association).

Dengate (1984) pointed out that the results of Brabender amylography are not repeatable or comparable unless critical details such as time and temperature regime to which the starch is subjected are reported. Frequently, the torsion spring range in use is not reported, or the starch concentration (in terms of wet or dry basis) is poorly defined, or the total weight or volume of suspension is not specified. The minimum information that should accompany any report is the amylograph model type, bowl speed, volume of slurry used, torsion spring in use, exact method of expressing slurry concentration, and starting and holding temperatures (Dengate).

Despite potential errors from geometric and methodological factors (Shuey & Tipples, 1980), the Brabender Amyloviscograph has been used widely for studying starch pasting behaviour. Five characteristic parameters are usually measured from the pasting curve (Fig. 3) (Dengate, 1984): (i) the peak viscosity ($P$), which is the highest apparent viscosity obtained during pasting, i.e. programmed heating to 95°C at 1.5°C $min^{-1}$, and peak viscosity temperature ($PT$); (ii) the ease of cooking, indicated by the apparent viscosity at 95°C in relation to the peak viscosity; (iii) the paste stability

Table 1
References on testing conditions used in uniaxial compression of bread staling and starch retrogradation[a]

| System | Sample/probe diameter or size (mm) | Instrument[b] | % deformation | Cross-head speed (mm $min^{-1}$) | Reference |
|---|---|---|---|---|---|
| Bread | 1 $in^3$/ns | Instron (ns) | 25 | 100 | Ruan et al. (1996) |
| Cooked potato tuber | 12ø × 12/ns | Instron (ns) | 75 | 50 | Jankowski (1992) |
| Starch gels | 4 $cm^2$ square piece/0.5 $cm^2$ | Compression Tester (ns) | 15, 30, 60 | 1.2 | Inaba, Hoshizawa, Adachi, Matsumura and Mori (1994) |
| Starch gels | 30ø × 20/ns | Zwick Universal Testing Machine (1000 N) | 60 | 50 | Conde-Petit and Escher (1994) |
| Bread | 1.25in ø/ns | Instron (ns) | 20–50 | 10 | Rao et al. (1992) |
| Bread | 6 (ø?)/35 | Instron (ns) | ns | ns | Rogers et al. (1988) |
| Bread | 25 (ø?)/36 | Instron (ns) | 25 | 50 | Martin et al. (1991) |
| Bread | ø? × 25/36 | Instron (2 kg) | 16 | 50 | Inagaki and Seib (1992) |

[a] ns, not specified.
[b] Number in bracket denotes load cell.

(*H*) or resistance to breakdown, indicated by the apparent viscosity after cooking for a period of time (20–60 min) at 95°C; it illustrates the stability of paste during cooking; (iv) setback or cold paste viscosity (*C*), indicated by the apparent viscosity of the paste after programmed cooling to 50°C, and (v) stability of the cooked paste, indicated by the apparent viscosity after stirring at 50°C for periods of up to 1 h. Note that the term "setback" is used in different ways by different authors to mean either ($C - P$) or ($C - H$), the latter sometimes being referred to as "total setback" (Dengate). In any case, the setback values are indicative of the retrogradation tendency of starch. Since the initial gel network development is dominated by amylose gelation (Miles, Morris, Orford et al, 1985), setback is more likely related to the retrogradation tendency of amylose.

The Rapid ViscoAnalyser (RVA) has several advantages over the viscoamylograph (Deffenbaugh & Walker, 1989; Ross, Walker, Booth, Orth & Wrigley, 1987; Walker, Ross, Wrigley & McMaster, 1988). These include small sample sizes and ability to set temperature profiles. Results are commonly reported in Rapid Viscoanalyser units (RVU) which are approximately equal to cP × 10, but may also be reported in cP. However, use of the latter method for data reporting may give the incorrect impression that the measurement is an absolute viscosity (Zhou, Robards, Glennie-Holmes & Helliwell, 1998). The RVA differs from the Brabender Amyloviscograph in two important features: a more rapid rate of heating and a stronger mixing action. Nevertheless, when heating rate is controlled at 1.5°C $min^{-1}$, the results obtained on an RVA have been observed to be similar to those on the amyloviscograph (Deffenbaugh & Walker, 1989).

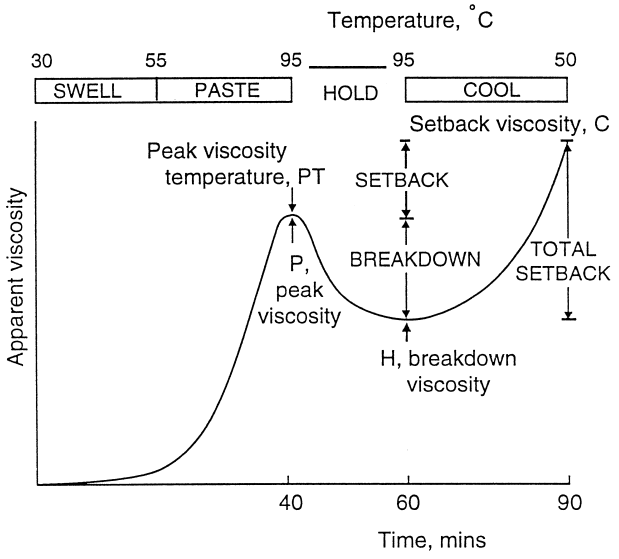


Fig. 3. A pasting cycle curve, typical of wheat starch, showing definition of pasting parameters (Dengate, 1984; with permission).

Amylography has been employed by several researchers (Kim & D'Appolonia, 1977; Morad & D'Appolonia, 1980; Xu, Chung & Ponte, 1992; Xu, Ponte & Chung, 1992; Yasunaga, Bushuk & Irvine, 1968) to study the pasting characteristics of aging bread crumbs. Peak viscosity of bread crumb slurries were reported to decrease with aging of the crumbs (Yasunaga et al.). Differences were also apparent among amyloviscograms of bread crumb, wheat starch and bread flour, particularly in terms of the occurrence of a minor peak before the major peak and a bump during the setback stage (Fig. 4). The minor peak and the bump were attributed to the interactions of solubilised amylose with flour lipids, mainly polar lipids. In the cooling stage, a transition of amylose from random coil to lipid-inclusion helices may be responsible for the observed increase in viscosity. Subsequent crystallization of the helices probably resulted in a decrease in viscosity, thus forming a bump (Xu, Ponte et al.). Sometimes a plateau, attributed to melting of retrograded amylopectin, was observed before the onset of the viscosity increase (Xu, Chung et al.).

### 2.2. Low deformation studies

Rheological studies on biopolymer systems using small deformation dynamic techniques have been extensively reviewed by Clark and Ross-Murphy (1987), Clark (1991, 1992), Rao and Steffe (1992), and Ross-Murphy (1995). Reviews on the subject focused specifically on starch gelation and retrogradation have been presented by Hansen, Hoseney and Faubion (1990, 1991), Biliaderis (1992), Evans and Lips (1992), Lii, Sha and Tseng (1995), Keetels, Oostergetel and van Vliet (1996) and Keetels, van Vliet and Walstra (1996a,b).

There are a number of tests which may be used to study viscoelastic properties of gelatinized starch dispersions to determine the relationships between stress, strain, and time for a given type of deformation or

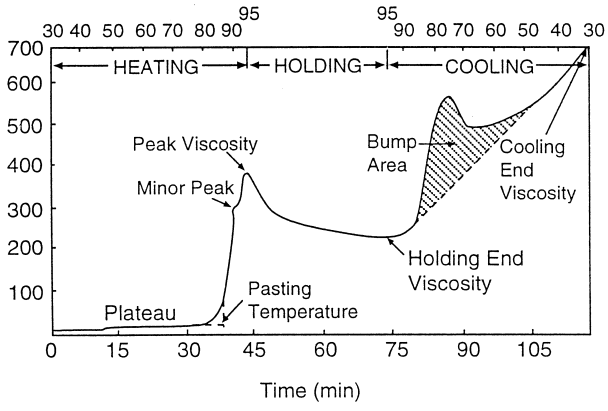


Fig. 4. A typical bread crumb amylogram (Xu, Chung et al., 1992; with permission).

loading pattern. The most important tests include the dynamic oscillatory test, creep compliance/recovery test, and stress relaxation. The last two tests are also known as static experiments.

*2.2.1. Dynamic oscillatory rheometry*

Dynamic oscillatory rheometry has proved useful in monitoring structure development during aging of starch gels (Biliaderis & Zawistowski, 1990; Clark, Gidley, Richardson & Ross-Murphy, 1989; Hansen et al., 1990, 1991; Miles, Morris, Orford et al., 1985; Miles, Morris & Ring, 1985; Ring et al., 1987). It allows continuous assessment of dynamic moduli without breaking structural elements formed in the sample upon aging. By careful measurement of the geometry of the measured sample, stress, and strain, the results are stated in absolute physical units (i.e. Pa $s^{-1}$ or Pa) rather than arbitrary units (e.g. Brabender units). This allows direct comparison of results obtained by various testing instruments and researchers (Weipert, 1990). In addition, there is a great opportunity to utilise various strains (or deformation forces) to obtain a more complete view of a material's physical properties. Very low strains, which allow measurements but do not disturb or destroy inherent gel structure, are of great value in describing the time- and temperature-dependent changes in starch gels during aging. A range of commercial controlled-stress rheometers that provide for numerous operating modes are available.

Starch pastes or gels can have both viscous (liquid-like) and elastic (solid-like) properties; i.e. they are viscoelastic. Quantitatively these two properties may be resolved by the technique of mechanical spectroscopy. Basically, the gel specimen is subjected to a periodic, small amplitude sinusoidal torque (stress), the applied stress being altered at a given frequency (cycles $s^{-1}$ or $\omega$, radians $s^{-1}$). If the behaviour of a viscoelastic material is linear, the strain will also vary sinusoidally with the stress, but will be out of phase with it. This behaviour is intermediate between an ideally elastic material and a true Newtonian liquid where the stress is in phase ($\delta = 0^\circ$) and $90^\circ$ out of phase, respectively, with the strain. Just as modulus is defined as the stress/strain ratio in any constant deformation experiment, then, for a dynamic sinusoidal experiment it follows that two moduli can be defined: (i) stress in-phase/strain or storage modulus ($G'$) and (ii) stress out-of-phase/strain or loss modulus ($G''$).

Storage modulus ($G'$) is a measure of the energy stored in the material and recovered from it per cycle. On a molecular basis, the magnitude of $G'$ is dependent upon what rearrangements can take place within the period of oscillation (Ferry, 1980), and is taken as an indication of the solid or elastic character of the material. For example, an agar gel, which is essentially permanently crosslinked (Bell, 1989), shows a high degree of elastic behaviour, i.e. $G'$ is high. Loss modulus ($G''$) is defined as the stress $90^\circ$ out-of-phase with the strain divided by the strain and is a measure of the energy dissipated or lost (as heat) per cycle of sinusoidal deformation. It is, therefore, taken as an indication of liquid or viscous behaviour (Bell). This type of behaviour is usually exhibited by non-permanently cross-linked systems such as hyaluronate solutions which interact by simple entanglement of the polysaccharide chains (Clark, 1992), and leads to high levels of molecular rearrangement, and a high degree of energy loss (i.e. $G''$ predominates). Another parameter which is often useful in indicating the physical behaviour of a system is the loss tangent ($\tan \delta$). It is the ratio of the energy lost to the energy stored for each cycle of the deformation, i.e. $\tan \delta = G''/G'$. It is a useful indicator of the relative contributions of the viscous ($G''$) and elastic ($G'$) components to the viscoelastic properties of a material. The logarithmic plot of the loss tangent gives rise to several characteristics. For example, for a dilute solution, $\tan \delta$ is high, as $G''$ is a function of both the solvent and the solute, while $G'$ is representative of only the solute, which is a relatively minor component (Bell). However, for a highly cross-linked system, e.g. agar gel, $G'$ becomes the major component and $G''/G'$ falls markedly.

Before any kinetic measurements are made, the starch gels should first be tested over a range of shear strains to determine appropriate conditions for nondestructive testing. This may be preceded by a frequency sweep to test the dependence on frequency of the gel moduli, and a strain sweep to examine the extent of the so-called linear viscoelastic range. Linear viscoelastic range is defined as the zone where the strain measured is in direct proportion to the stress applied (i.e. the range over which the moduli are independent of strain) (Clark, 1991). To determine this range in oscillation mode, increasing cyclic levels of stress and strain are applied at a constant frequency (e.g. 1 Hz). The point at which a dynamic viscoelastic modulus deviates by more than 10% from a constant (plateau) value indicates departure from linear viscoelastic behaviour (it should be noted that the value of 10% deviation is just a matter of convenience). It is worth noting that the linear viscoelastic range is strongly frequency-dependent, so if a frequency sweep is to be performed, the linear region at the extremes of the frequency range covered should be known. The mathematics in the commercial software (which is normally bundled with the rheometer) for calculating the moduli are based on this assumption (i.e. the measurement is done in the linear range). In the region where linear behaviour does not operate, the mathematics for the same type of calculation are ill-defined. Consequently, any calculation of $G'$ or $G''$ is not absolute, and thus only relative comparisons can be made.

Once the linear range limits have been established, it is possible to characterize the structure itself by running the experiment at a suitable frequency (e.g. 1 Hz) and at a strain lower than the 'critical' strain. Typically, the strain is set at the middle value in the linear region to give the best experimental results. For concentrated starch gels (>30% solids), testing at strains < 2.0%, 0.2 Hz appears to meet the requirement for linear viscoelasticity (Biliaderis & Juliano, 1993; Biliaderis & Tonogai, 1991; Biliaderis & Zawistowski, 1990). Typical shear strain and frequency sweep plots for cooked noodles are shown in Figs. 5 and 6, respectively. Fig. 5 shows that below 1% strain, the samples exhibited linear or nearly linear viscoelastic response. It is apparent that the dynamic moduli showed little dependence on frequency (Fig. 6). This is characteristic of a true gel network system with stable physical cross-links (Clark & Ross-Murphy, 1987).

Fully computerised rheometers are available to follow the complex processes outlined above. They are basically of two types: one which operates in a controlled stress mode and the other which measures shear rate. In recent years, the controlled-stress rheometer has become the preferred choice for most laboratories. Modern rheometers, however, are able to operate in both modes. Measurements on the sample are made using the appropriate test fixture (geometry). The dimensions and shape of the geometry control the stress range applied by the motor, and the shear rate experienced by the sample. Several geometries are commonly used. These include parallel plate, cone and plate, and concentric cylinders. Temperature can be controlled accurately to±0.1°C.

Sample preparation for rheological studies can be done in one of two ways: (i) gels are prepared separately in a mould, cut and then loaded on the rheometer, or (ii) gels are prepared directly on the rheometer platform itself. The first method has been described in detail by Biliaderis and Zawistowski (1990), Biliaderis and Tonogai (1991), and Biliaderis and Juliano (1993). In the first method, starch slurries in hermetically sealed stainless steel containers (80 mm i.d. × 1 mm thickness; picture of the device shown in Biliaderis and Tonogai) are heated in a boiling water bath and subsequently quench-cooled in a water bath at 25°C. Using this technique, 1 mm-thick gels can be formed without loss of water and mechanical damage of the network. Gel disks of the desired diameter are cut to fit the parallel geometry of the rheometer. A thin layer of paraffin oil is applied to cover the sample to prevent loss of water by evaporation during the course of rheological measurements. Gel samples are left for 10 min to relax before measurements are taken. Biliaderis and Tonogai reported that the coefficients of variation for all determinations using this method did not exceed 15%. A variation of this method of sample preparation was used by Keetels, van Vliet and Walstra (1996b).

In the second method of gel preparation, the starch slurry is loaded on the ram (rheometer platform). The gap between the measuring geometry and the ram is then adjusted. Paraffin oil is then applied on the geometry's periphery to prevent evaporation. The starch suspension is then subjected to programmed heating and cooling. The development of modulus is followed continuously over a period of time. This method has been described in detail by Lii et al. (1995) and Tsai, Li and Lii (1997). This approach appears to be simpler and more attractive because the heating and cooling regime

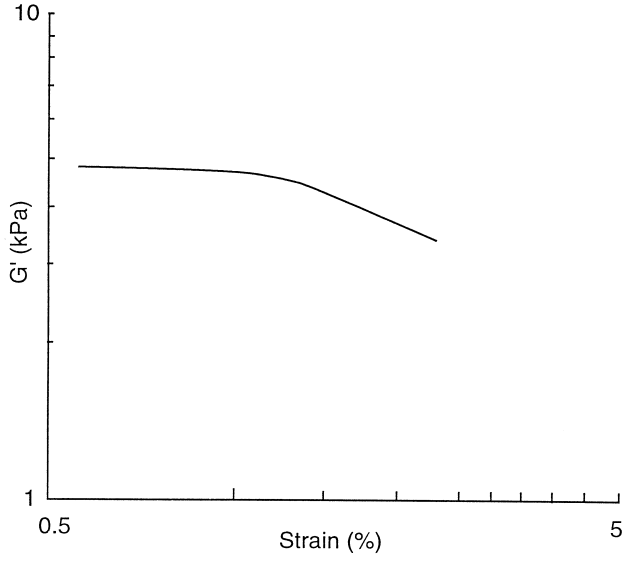


Fig. 5. Typical shear strain sweep of cooked thin noodles. The values of the storage modulus, $G'$ were measured at 1.0 Hz as a function of increasing strain.

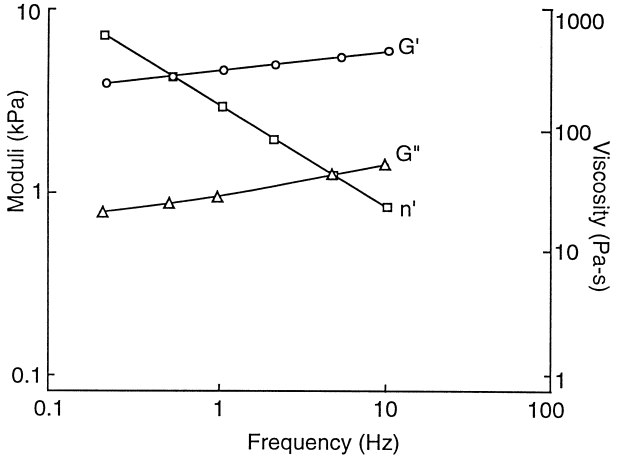


Fig. 6. Typical dynamic viscoelastic mechanical spectra of optimally cooked noodles $G'$ = storage modulus, ○; $G''$ = loss modulus, △; $\eta'$ = dynamic viscosity, □ (Edwards, Izydorczyk, Dexter & Biliaderis, 1993; with permission).

can be controlled accurately. It also involves less handling of the gel once it has formed and is thus more suited for the study of soft and sticky samples such as amylopectin gels. Nevertheless, there are some aspects of this method which require careful consideration. The amount of starch suspension should be kept constant for all determinations and should be just enough to fill the gap. There may also be a problem of shrinkage after the starch is gelatinized, leading to underfilling of the gap. This would affect measurements of modulus, especially if a parallel plate geometry were used because the strain and stress at the edge of the geometry are taken into account in the calculation. However, for comparison purposes, this drawback can probably be tolerated. The problem of starch sedimentation probably is not crucial, since the gap setting usually is very small (500 μm) and the whole operation (sample loading) can be accomplished in a short time. Thus, where this method is concerned, it is critical to standardise all aspects of sample preparation in order to obtain reproducible results.

$G'$–time profiles of concentrated (> 30%, w/w) cereal and legume starch gels revealed a biphasic gelation process (Biliaderis & Tonogai, 1991; Biliaderis & Zawistowski, 1990): an initial rapid rise in modulus followed by a phase of much slower $G'$ development (Fig. 7). The initial rapid development in modulus was attributed to rapid establishment of a cross-linked network of amylose chains at concentrations above the coil overlap concentration, $c^* \sim 1.5\%$ (Miles, Morris & Ring, 1985). Subsequent increases in rigidity of starch gels were linked to recrystallization of amylopectin short DP chain clusters (Ring et al., 1987).

Note that the viscoelastic behaviour of common starch gels, which are often considered as composite systems, would differ from that of individual amylose and amylopectin gels; i.e. the magnitude of the dynamic moduli of starch gels would depend not only on the density of cross-links in the continuous phase but also on the rigidity, spatial distribution, and effective contacts between the granules (Biliaderis & Tonogai, 1991). For gelation of amylose (polymer concentration > 1.0%), it has been suggested that rapid formation of a cross-linked network arises from the adoption of ordered double-helical chain segments, acting as "junction zones", which are interconnected by more mobile amorphous single-chain segments (Gidley, 1989). On the other hand, amylopectin gelation (polymer concentration > 10.0%) is a slow process involving intra- and inter-molecular chain associations. The rate of $G'$ development for starch gels is generally also much faster than the rate of staling endotherm ($\Delta H$) development in an aging gel as determined by DSC (Biliaderis & Zawistowski, 1990). For amylopectin gels, however, the development of modulus can lag behind the development of crystallites detectable by both DSC and X-ray diffraction, depending on concentration (Ring et al., 1987). The slow crystallization rate of amylopectin more closely reflects kinetics of the staling events associated with aging of baked products (Kulp & Ponte, 1981).

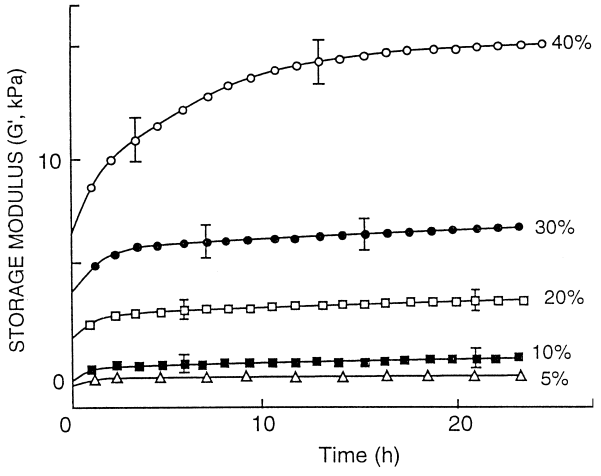


Fig. 7. Storage modulus vs. time (25°C) for gels of various concentration (w/w) of wheat starch: ○, 40%; ●, 30%; □, 20%; ■, 10%; Δ, 5%. Data were obtained at 0.2 Hz and 2.0% strain (Biliaderis & Zawistowski, 1990; with permission).

### 2.2.2. Creep compliance and recovery

A creep test is a static experiment used to investigate the viscoelastic structure of materials over medium and long time scales. In a creep experiment, a constant stress is applied to the sample and the resultant displacement or deformation is measured against time (retardation time). If required, the stress can be removed, and the relaxation of the sample measured. The results are generally expressed in terms of the creep compliance, $J(t)$, where:

$$J(t) = \frac{\text{strain}(t)}{\text{stress}}$$

The creep/recovery response may be classified into several categories, as depicted in Fig. 8 (Barbosa-Canovas et al., 1996). For a perfectly elastic solid, compliance rises instantaneously to the equilibrium value. When the stress is released, there is an instantaneous recovery (Fig. 8b). All the energy is stored in the solid and there is no energy dissipation. For a newtonian liquid, on the other hand, flow occurs in response to the applied stress. As a result, the compliance increases linearly with time with a slope of $1/\eta$, where $\eta$ is the viscosity. The input energy is totally dissipated due to the motion of the liquid and there is no energy storage (Fig. 8b). When the stress is released, the compliance does not decrease (since there is no energy release) but stays constant at the final value (Fig. 8c).

The response of a viscoelastic material lies between these two extremes. When a constant shear stress is

applied, there is an instantaneous rise in compliance. The compliance then increases with time to the equilibrium value. When the stress is released, there is an instantaneous drop in the compliance followed by a time-dependent decrease. For a viscoelastic 'solid', all the energy is stored and, hence, there is a total energy release upon removal of the shear stress. As a result, the final equilibrium compliance is zero (Fig. 8d). For a viscoelastic 'liquid', however, viscous flow takes place and there is only a partial recovery when the stress is removed (Fig. 8e).

Creep-compliance data can provide valuable information on the viscoelastic behaviour of starch gels. When shear stress is applied to an unperturbed structure, it takes a finite amount of energy to perturb the network, after which breaking and reforming of bonds takes place. In a viscoelastic solid, there is a dynamic equilibrium between breaking and reforming of bonds. However, in a viscoelastic liquid, there is a net breaking of bonds resulting in viscous flow. The compliance response of viscoelastic materials in a creep/recovery test may be ascribed to three mechanisms: instantaneous elastic, retarded elastic, and viscous flow (Barbosa-Canovas et al., 1996). Thus, instantaneous elastic compliance, $J_o$ (Fig. 8b), may be attributed to the unperturbed network structure. The retarded elastic contribution to the compliance involves the breaking and reforming of bonds, while the viscous contribution is due to the breakdown of structure.

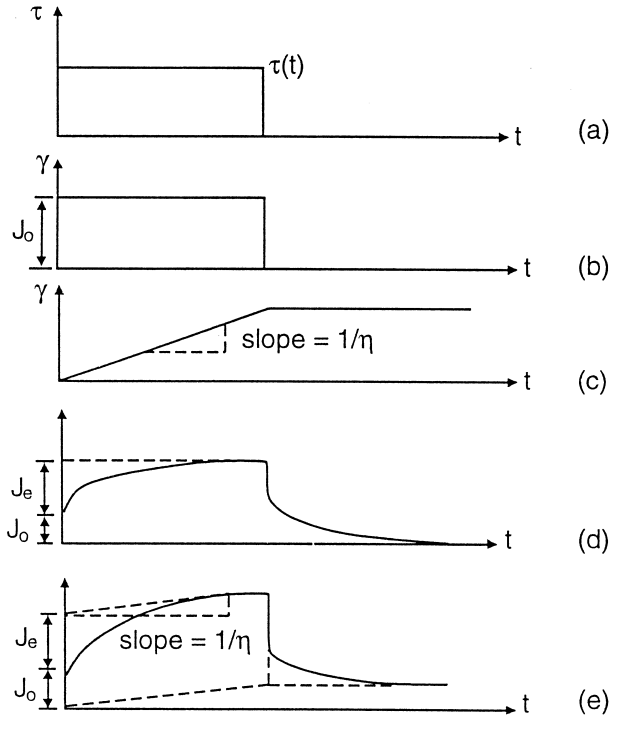


Fig. 8. Creep recovery response of different types of material to the shear stress (Gladwell, Rahalkar & Richmond, 1985; with permission).

In comparison with dynamic tests, creep experiments have received much less attention in the study of the compliance response of aging starch gels. However, this technique has found wide applications for other biopolymeric gels (Gamero, Fiszman & Durán, 1993; Gross, Rao & Smith, 1980; Nussinovitch, Normand & Peleg, 1990; Nussinovitch, Normand & Peleg, 1989). Giboreau, Cuvelier & Launay (1994) showed that, for modified starch pastes, values of instantaneous modulus ($G_o$) determined from creep experiments were in good agreement with $G'$ values from dynamic rheological tests. Miura, Nishimura and Katsuka (1992) investigated the influence of polyols and emulsifiers on hardening of non-glutinous rice starch gels by measuring the creep compliance of the gels stored at 0°C for up to 3000 min. Similar studies have been conducted to observe the effects of saccharides on the compliance of rice starch gels (Katsuka, Nishimura & Miura, 1992) and the effects of polyols on the hardening of wheat starch gels (Amano, Miura & Hayashi, 1997; Amano Takada, Miura, Ishida & Ohshima). Creep behaviour of starch gels at the earlier stage of retrogradation has also been reported by Amano, Hayashi, Miura, Ishida and Ohshima (1995). Other examples of creep studies on aging starch gels include those by Lee and Kim (1983), Shiraishi, Lauzon, Yamazaki, Sawayama, Suigiyama and Kawabata (1995) and Akuzawa, Aikawa, Kawabata and Nakamura (1997).

### 2.2.3. Stress relaxation

The second type of static experiment to measure viscoelasticity is stress relaxation, which is usually conducted at relatively low deformation. Here, instead of applying a constant stress to a sample and measuring strain as a function of time, the polymer is deformed (usually for food samples by compression) to a given value of the strain and the stress necessary to maintain that strain is measured as a function of time. As the sample relaxes (i.e. as the chains change their conformations, disentangle or slide over one another, and so on), the stress decreases. Stress-relaxation experiments are somewhat easier to perform than creep experiments. A typical stress relaxation curve is shown in Fig. 9.

The stress relaxation curves of gels have traditionally been described in terms of the generalised Maxwell model (Mitchell, 1976). Parameters are obtained by non-linear regression which can be quite tedious. In addition, where a large number of parameters is involved, difficulty in physical interpretation and comparison among samples will result. Several alternative models have been developed to facilitate calculation and interpretation of stress relaxation data (Gamero et al., 1993; Nussinovitch et al., 1989; Peleg, 1979). The linearised form of the stress relaxation model proposed by Peleg (1979) is particularly simple and easy to use. As

noted for creep experiments, reported studies on aged starch gels using stress relaxation measurements are also relatively scarce. Some examples include those by Pappas and Rao (1989) on viscoelastic behaviour of cowpea gel, Bashford and Hartung (1976) on freshness of bread, and Akuzawa, Sawayama and Kawabata (1995) on cassava and potato starch.

### 2.3. Large deformation vs small deformation studies

It is worth noting that non-destructive dynamic rehological testing is a fundamental method for determining rheological properties of viscoelastic materials. The amplitude of strain is usually kept small to stay within a linear viscoelastic region. This type of rheological test can provide valuable information on gelation mechanisms, molecular interactions during gel formation and development of gel modulus during aging (storage). However, the dynamic shear test is considered to correlate poorly with sensory evaluation of gel texture (Bourne, 1982).

In real production processes, on the other hand, viscoelastic materials are subjected, most of the time, to high shear deformation conditions, which are linked to the nonlinear viscoelastic behaviour of the material. Likewise, the process of masticating and ingesting food materials involves subjecting the food to a range of deformations whose purpose is to break down the structure into a suitable form for swallowing. Thus, in TPA, the aim has been to simulate, as closely as possible, the mechanical conditions which prevail during mastication. Instrumental and empirical testing methods with large stresses and strains for viscoelastic foods have been correlated with sensory textural attributes (Brady, McKeith & Hunecke, 1985; Dickie & Kokini, 1982). To date, however, very little work has been done to systematically correlate large deformation and small deformation measurements. A number of researchers have attempted to correlate uniaxial and dynamic testing and have shown varying levels of association (Navarro, Martino & Zaritzky, 1997; Amemiya & Menjivar, 1992; Wium & Qvist, 1997).

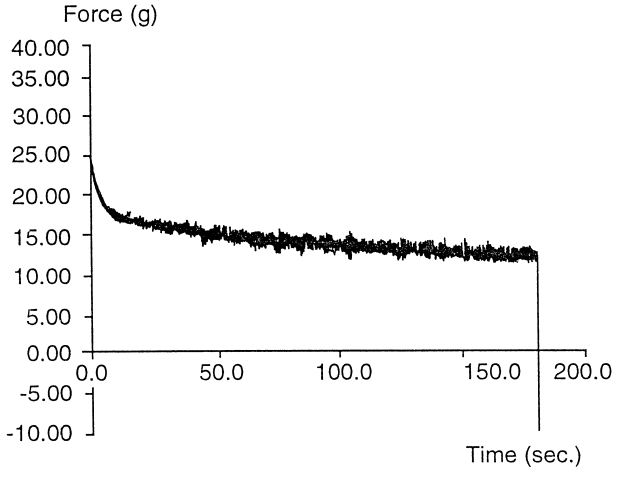


Fig. 9. Typical stress relaxation curve of sweet potato starch gel.

## 3. X-ray diffraction

A starch granule normally consists of concentric layers that contain crystalline micelles arranged perpendicularly to the plane of the layer. Starch granules, being partially crystalline, give distinct X-ray diffraction patterns (Sarko & Wu, 1978). X-ray diffraction shows the regularly repeating nature of double helices of molecular structures, but it does not detect irregularly packed structures. An A-type pattern is exhibited by cereal starches (rice, wheat, and corn) while a B-type pattern is shown by tubers, fruit, high amylose corn (> 40%) starches, and retrograded starch. The C-type pattern, which is intermediate between A and B types, is observed for legume seed starches.

Extensive information on the role of starch in bread staling has been gathered using the X-ray diffraction technique. Starch in freshly baked bread is mostly amorphous but slowly recrystallizes during storage. Changes in crystallinity during aging are shown in the X-ray diffraction patterns. Katz (1934) was probably the first to show, by X-ray diffractometry, that both freshly pasted starch and the starch from fresh bread exhibited amorphous X-ray patterns. However, on storage, each developed crystallinity. Katz termed this return, from the amorphous to the crystalline state, retrogradation. He suggested that all starches, irrespective of whether they give an A or B pattern in the natural state, formed gels which developed a B pattern on aging. Hellman, Fairchild and Senti (1954), however, found that the type of crystals developed in aged cereal starch gels depended on water content. Samples containing more than 43% moisture developed B-patterns on aging while those containing less than 29% moisture gave A patterns. At intermediate moisture contents, a mixture of A- and B-patterns (referred to as C-pattern) was observed. They concluded that there was a critical moisture content which was necessary before a given heat treatment can cause loss of the original starch granule crystallinity or A-pattern and induce the development of a B-pattern. Wright (1971) confirmed the above observations on bread crumbs. Aged gels from cereal starches containing lipids also exhibit an additional V-pattern that has been attributed to amylose-lipid complexes (Mikus, Hixon & Rundle, 1946). The V-pattern is relatively amorphous with a few weak lines that show crystallinity (Willhoft, 1973).

More recent X-ray diffraction studies on cooled amylose gels and amylose precipitated from aqueous solutions have also shown that amylose retrogradation

basically involves a gelation-via-crystallization process, giving rise to a B-type X-ray diffraction pattern (Gidley, 1989; Marsh & Blanshard, 1988; Miles, Morris, Orford et al.,1985; Miles, Morris & Ring 1985). X-ray diffraction studies, supplemented with data from other techniques, clearly show that the development of crystallinity, in an aging non-waxy starch gel, proceeds in a biphasic manner (Miles, Morris, Orford et al; Miles Morris & Ring). Crystallization of amylose is completed very much earlier than that of amylopectin. That being the case, application of the Avrami equation to X-ray data may be considered inappropriate. Note also that, in the case of actual food products (e.g. bread), changes in crystallinity of the starch component may not necessarily parallel the development of rheological properties (e.g. firmness) associated with staling (Dragsdorf & Varriano-Marston, 1980; Zobel & Senti, 1959). Starch–lipid complexes, which exhibit V-patterns, have been found to be metastable and to transform gradually into the more stable B-type crystals on aging of cooked rice (Hibi, Kitamura & Kuge, 1990).

Experimental details in performing X-ray diffraction analysis of starch powders, gels or solutions have been described by several researchers (Dragsdorf & Varriano-Marston, 1980; Miles et al., 1985; Roulet, MacInnes, Wursch, Sanchez & Raemy, 1988). X-ray powder diffraction is usually done on hydrated starch samples. Hydration is accomplished by equilibrating the sample in a desiccator maintained at a certain relative humidity and temperature. Hydration is known to influence X-ray patterns (Buleon, Bizot, Delage & Pontoire, 1987; Wild & Blanshard, 1986), and a certain amount of water is necessary to maintain structural ordering as detected by X-ray diffraction. Hydration was found to improve resolution of the profiles, i.e. the patterns became sharper and more pronounced, without the true patterns being affected (Sievert, Czuchajowska & Pomeranz, 1991). However, the sensitivity of powder X-ray diffraction is relatively low compared with techniques such as NMR and FTIR which are able to detect even minor extents of recrystallization (Smits, Ruhnau, Vliegenthart & van Soest, 1998).

## 4. Thermal analysis

Whenever a material undergoes a change in physical state (e.g. melting), or transforms from one crystalline form to another, or whenever it reacts chemically, heat is either absorbed (endothermic) or liberated (exothermic). Many such processes can be initiated simply by raising the temperature of the material. Among the thermoanalytical methods, differential thermal analysis (DTA) and differential scanning calorimetry (DSC) have proven most useful in providing basic information on starch retrogradation. The theory and applications of these methods can be found in several published texts (Daniels, 1973; Haines, 1995; Harwalkar & Ma, 1990; Wendlandt, 1974; Wunderlich, 1990).

Basically, DSC is a technique whereby the difference in energy input into a substance and a reference material is measured as a function of temperature while both materials are subjected to programmed heating or cooling. DTA, on the other hand, measures the difference in temperature between the sample and the reference. DSC appears to have long-supplanted DTA as the method of choice among food researchers.

In DSC, when a thermal transition occurs, the energy absorbed by the sample is replenished by increased energy input to the sample to maintain the temperature balance. Because this energy input is precisely equivalent in magnitude to the energy absorbed in the transition, a recording of this balancing energy yields a direct calorimetric measurement of the energy transition which is then recorded as a peak. The area under the peak is directly proportional to the enthalpic change ($\Delta H$) and its direction indicates whether the thermal event is endothermic or exothermic. In the case of retrograded starch, the value of $\Delta H$ provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin as well as precise measurements of the transition temperatures (i.e. onset, $T_o$; peak, $T_p$; and conclusion, $T_c$) of this endothermic event.

McIver, Axford, Colwell and Elton (1968) were the first to report the use of DTA to study starch retrogradation. They obtained an endotherm on heating an aged gel and attributed this to the melting of recrystallized starch. They also showed that the kinetics of recrystallization on aging of starch gels can be modelled using the Avrami equation. Using the same technique, Colwell, Axford, Chamberlain and Elton (1969) reported that crystallization of starch was a reversible thermal change. They also observed that gels stored at lower temperatures resulted in the formation of starch crystals which melt at lower temperatures. This was subsequently confirmed by Nakazawa, Noguchi and Takahashi (1985) using DSC. Zeleznak and Hoseney (1987) suggested that this phenomenon was a consequence of annealing of starch crystals at higher storage temperatures.

DSC was apparently first used for measuring gelatinization and retrogradation of starch by Stevens and Elton (1971). Since then, it has proven to be an extremely valuable tool to quantify crystallinity in both native and retrograded starches, to determine retrogradation kinetics, and to study the effects of a myriad of factors influencing retrogradation (Eliasson, 1985; Fearn & Russell, 1982; Jang & Pyun, 1997; León, Duran & de Barber, 1997; Longton & LeGrys, 1981; Nakazawa et al., 1985; Obanni & BeMiller, 1997; Russell, 1983; Seow, Teo, Vasanti Nair, 1996; Zhang & Jackson, 1992).

It has been suggested that the starch fraction responsible for retrogradation, as measured by DSC, is amylopectin (Eliasson, 1985; Eliasson & Ljunger, 1988; Russell, 1983, 1987). This is based on the fact that when retrograded waxy maize starch (or amylopectin) was melted in the differential scanning calorimeter, the temperature location of the endothermic event was similar to that observed for retrograded wheat starch (Eliasson, 1983; Longton & LeGrys, 1981; McIver, Axford, Colwell & Elton, 1968; Russell, 1983). Further support for the role of amylopectin has been obtained by comparing DSC results with results obtained by X-ray diffraction (Miles, Orford et al., 1985). Retrogradation of amylopectin involves a crystallization process of the outer branches (DP14-18). In contrast to what is observed with amylose, the crystallization of amylopectin is a slow process continuing over a period of several days or weeks. Due to the limited dimensions of the chains, the stability of these crystallites is lower than that of amylose crystallites. While recrystallized amylopectin melts in the temperature range 40–100°C, amylose crystallites do so only at much higher temperatures (120–170°C) (Eerlingen, Jacobs & Delcour, 1994; Sievert & Pomeranz, 1989). To study the latter, pressurised DSC would be needed to prevent the pans from leaking before the endothermic transition is reached (Russell, 1987).

The procedure to study starch retrogradation with DSC is relatively simple and does not require highly skilled personnel. The slurry is normally prepared directly in the sample pan (usually aluminium), water being added using a microsyringe, prior to hermetic sealing. A chemical preservative may be added (e.g. 0.02% sodium azide or 0.01% thiomersal) for retrogradation studies which involve long storage periods, especially at room temperature or higher. There is no published report on the possibility of artifacts on the endotherm in the presence of these preservatives. A variation in sample preparation is to prepare the starch slurry in a separate container (e.g. screw-capped bottle) before filling into the hermetic sample pans. In either case, it is recommended that the sample be allowed to stand for 1–2 h or even longer, to ensure complete and uniform hydration of the starch granules, before being heated in the DSC cell. Gelatinization of the starch slurry is known to produce swollen but nondisrupted granules. The conditions of gelatinization in the calorimeter, therefore, more closely approximate those encountered during baking than those encountered during starch cooking for other purposes (Jacobson & BeMiller, 1998). An empty reference pan is usually used to counterbalance, as much as possible, the heat capacity of the sample. It should be pointed out that, since the sample pan is sealed hermetically, the gelatinization process probably occurs under slightly elevated pressure (2–3 atm) (Schiraldi, Piazza & Riva, 1996) caused by the increase in vapour pressure when the samples are heated. According to Schiraldi et al., if open pans are used to study the same kinds of samples, water would be released on heating (as in real baking), with a consequent decrease of the overall heat capacity of the sample and large bending of the baseline of the DSC trace. A typical temperature scanning range is from 30 to 120°C at a heating rate of 5 or 10°C min-1. The heating program may be repeated to ensure complete gelatinization of the starch. The pan is then subjected to retrogradation conditions, and the contents are reanalyzed at periodic intervals, normally using the same heating program.

To study retrogradation by DSC, a starch concentration of >20% (w/w) is required. This concentration dependence of retrogradation has been reported by several workers (Jacobson & BeMiller, 1998; Jang & Pyun, 1997; Longton & LeGrys, 1981; Zeleznak & Hoseney, 1986). They reported a bell-shaped distribution of retrogradation $\Delta H$ as a function of starch concentration with maximum values in the range of 50-60% starch. Longton and LeGrys reported that no retrogradation was observed at 4°C if the starch concentration was below 10% or above 80%. Eliasson (1983) reported similar results, with maximum retrogradation being observed at a starch concentration of ~55%. These DSC data support the X-ray diffraction studies of Hellman et al. (1954) who reported that 50% starch gels produced the most intense X-ray pattern.

Starch retrogradation enthalpies are usually 60–80% smaller ($<8$ Jg$^{-1}$) compared with gelatinization enthalpies (9–15 J g$^{-1}$). However, the retrogradation temperature range ($T_c$–$T_o$) is usually broader than the gelatinization range for a given sample. Furthermore, the endothermic transition temperatures ($T_o$, $T_p$, and $T_c$) associated with melting of retrograded starch occur at temperatures 10–26°C lower than those for gelatinization of starch granules (Baker & Rayas-Duarte, 1998; White, Abbas & Johnson, 1989; Yuan, Thompson & Boyer, 1993), suggesting that retrogradation results in crystalline forms that are different in nature from those present in the native starch granules. This is confirmed by changes in X-ray diffraction pattern from an A-pattern in native cereal starches to a B-pattern in retrograded starches (Collison, 1968). It has been suggested (Cooke & Gidley, 1992; Shi & Sheib, 1992; Nakazawa et al., 1985; Yuan et al.) that during storage at low temperatures (e.g. 4°C), gelatinized starch molecules reassociate, but in less ordered and hence less perfect or stable forms than those existing in the native granules.

The temperature location of the endotherm associated with melting of recrystallized amylopectin also depends upon storage temperature. The higher the storage temperature (within the range 5–50°C), the higher the transition temperatures (Fig. 10) (Eliasson, 1985; Jang & Pyun, 1997; Jankowski & Rha, 1986; Nakazawa et al., 1985). This effect may be attributed to the temperature-

dependency of polymer crystallization which influences the 'perfectness' of the crystals produced. The lower the storage temperature, the less perfect would be the crystallites formed, resulting in lower melting temperatures ($T_m$) and broader endothermic transitions (Biliaderis, 1991). This is in accord with classical polymer crystallization theories (Wunderlich, 1976), which predict formation of less-perfect crystals as the degree of supercooling ($T_m - T$) increases.

DSC, applied to the study of starch retrogradation, has the following advantages (Nakazawa et al., 1985): (i) it is applicable over a wide range of water content, (ii) it allows direct determination of the energy required to melt the retrograded starch, (iii) no change of water content occurs over the course of aging due to perfect sealing of the sample cells, (iv) it is not time consuming and does not require any special technique, and (v) determinations can be made with very small sample sizes, typically 5–10 mg. This reduces the problem of settling of starch in the slurried sample. However, because of the small sample size, it is very important to ensure that representative samples are obtained for analysis. Also, DSC is not amenable to the determination of retrogradation of dilute starch pastes. It can, however, be conveniently used for the study of freeze–thaw stability of starches (Baker & Rayas-Duarte, 1998; Grant, 1998; Jacobson & BeMiller, 1998; Kim & Eliasson, 1993; White et al., 1989; Yuan & Thompson, 1998). Disadvantages of DSC include the high initial capital as well as running costs (in terms of disposable hermetically-sealed pans) required, and its limited sensitivity.

Silverio, Svensson, Eliasson and Olofsson have employed isothermal microcalorimetry (which is very much more sensitive and requires larger sample sizes than conventional DSC) to study the early stages of starch retrogradation. The results were displayed in the form of *P–t* (thermal power vs time) curves (Fig. 11). With this technique, the crystallization of amylose (which predominated over the first 5–10 h) could be easily differentiated from that of amylopectin. The heat produced on starch crystallization ($\Delta H_{MC}$), obtained by integration of the *P--t* trace during the first 24 h, was generally lower than the endothermic melting enthalpy ($\Delta H_{DSC}$) determined by DSC. It was suggested that phase separation on amylose crystallization led to disruption of hydrogen bonds between starch and water, thereby producing an endothermic heat of reaction which lowered the net exothermic heat determined by isothermal microcalorimetry. The usefulness of this technique for the study of the anti-staling effects of lipids and surfactants was also demonstrated.

## 5. Spectroscopic methods

### 5.1. Nuclear magnetic resonance (NMR)

NMR has long been used in the study of water in foods and other biological materials (Berendsen, 1992; Chinachoti, 1995; Hills, Takaes & Belton, 1990; Kuntz & Kauzmann, 1974; Richardson & Steinberg, 1987; Schmidt & Lai, 1991; Steinberg & Leung, 1975) and in the analysis of fats and oils (Gambhir, 1992; Waddington, 1986). In recent years, its application has been extended to several new areas as a result of innovative developments such as magnetic resonance imaging (MRI) and solid-state NMR. The principles of NMR have been thoroughly covered in several texts and articles (Hemminga, 1992; Sanders & Hunter, 1987). In this paper, we shall direct our attention specifically to the applications of NMR in the study of starch retrogradation.

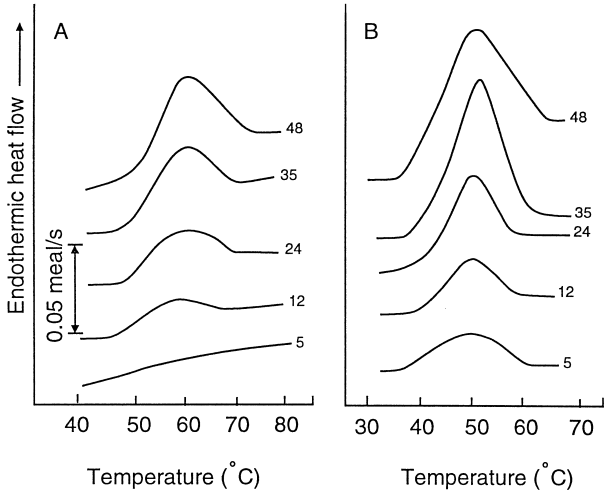


Fig. 10. Thermal transitions of cooked and stored wheat grain: A, grain stored at 20°C; B, grain stored at 4°C. Numbers indicate storage time (h) (Jankowski & Rha, 1986; with permission).

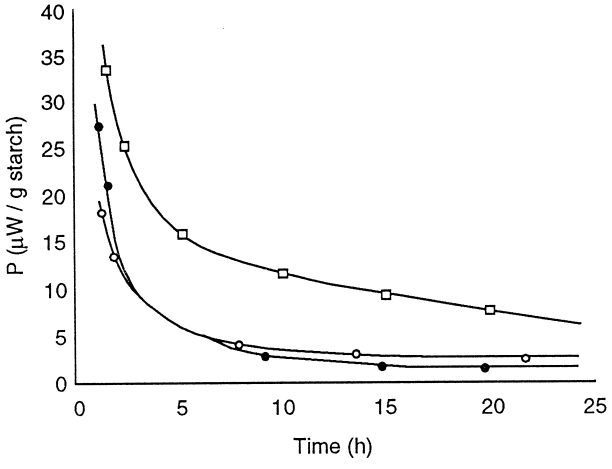


Fig. 11. P-t traces from the isothermal microcalorimetric analysis of wheat (○), potato (●) and maize starches (□) (Silverio et al., 1996; with permission).

The technique most frequently applied to the study of food systems is low-resolution $^1$H NMR which is capable of elucidating physical structure from analysis of the NMR decay signal (Ablett, 1992). The spin-spin relaxation time ($T_2$) is particularly sensitive to changes in molecular mobility. The $T_2$ value of a material in the solid state differs by several orders of magnitude from that in the liquid state. This characteristic serves as the basis for differentiating between starch molecules in the more mobile liquid and in the more 'immobile' solid-like (i.e. retrograded) state. Note, however, that water $^1$H relaxation may not reflect macromolecular mobility. Lechert (1981) approached the study of starch retrogradation using pulsed NMR by replacing the water and hydroxyl protons of starch with deuterons without exchanging the CH protons of the starch. Samples were deuterated and swollen in $D_2O$. Changes in proton resonance of the CH protons of the starch, which reflected alterations in the polymer itself, were monitored during aging. Thus, from the $^1$H spin-echo decays, mobile starch chains or segments could be differentiated from the retrograded starch because of the large difference in $T_2$ in the swollen and in the solid-like state. Using deuterium NMR, Leung, Magnuson and Bruinsma (1983) reported a similar decrease in $T_2$ values during storage of bread. Leung (1981) had earlier suggested that, as bread stales, starch changes from the amorphous state to the more stable cystalline state and water molecules are immobilized by incorporation into the crystalline structure. Lechert found that solid-like starch was abolished by gelatinization. On cooling, the solid-like signal was partially recovered and gradually increased with storage time. While this method provides non-destructive and rapid measurement of starch retrogradation, the need for deuteration may pose an inconvenience.

Nakazawa, Noguchi, Takahashi and Takada (1983) observed little change in $^1$H NMR $T_1$, $T_2$, correlation time, and the fraction of "bound" water during storage of non-glutinous rice starch gel (1:1 starch–water ratio) at 3°C. However, correlation time of water molecules and the fraction of "bound" water in glutinous rice starch gel increased markedly during aging. The reason for the discrepancy between glutinous and non-glutinous rice starch remains unclear.

Teo and Seow (1992) developed a simple, non-invasive, low-resolution, pulsed NMR method for the study of starch retrogradation based on the familiar principle that the NMR proton signals from the solid and liquid components in a system, following a 90° pulse of radio-frequency radiation, may be easily differentiated since they decay at significantly different rates. Fig. 12 shows the free induction decay (FID) curves at 25°C following a 90° pulse for fresh and aged (14 days at 5°C) rice starch gels at a starch to water ratio of 1:1. The signal from protons in the solid phase of the starch gels fell very rapidly and had virtually disappeared at 70 μs after the 90° pulse, the decay being much faster in the aged sample, indicating a shortening of the spin–spin relaxation ($T_2$) time. Thereafter, the signal decayed at a much slower rate due to liquid phase relaxation which does not appear to be affected by retrogradation, as evidenced by the parallel FID curves for the fresh and aged samples beyond 70 μs. As recrystallization of starch proceeds during aging of a starch gel, the proportion of 'solid-like' component in the system increases, thereby resulting in a decrease in the signal from the liquid component, but a concomitant increase in the signal attributed to protons in the 'solid-like' fraction. Such a method dispenses with the need for deuteration of samples. A closely similar technique was employed by Le Botlan and Desbois (1995) to study starch retrogradation in the presence of sucrose.

Wu, Bryant and Eads (1992) applied $^1$H nuclear magnetic cross-relaxation spectroscopy to detect solid-like components in starch. Such a technique is capable of studying the molecular dynamics of the starch polymer itself via observation of the liquid signal. The line shape of the cross-relaxation spectrum obtained is dependent on the properties of the solid-like component. Thus, as a starch gel ages, the spectrum increases in area and width as shown in Fig. 13.

The use of $^{17}$O NMR to examine the mobility of water and the carbohydrate polymer may allow additional information to be obtained about aging starch systems at the molecular level. Richardson (1988) reported an increase in the $^1$H decoupled $^{17}$O NMR $R_2$ of a variety of instant starch pastes (except in the case of

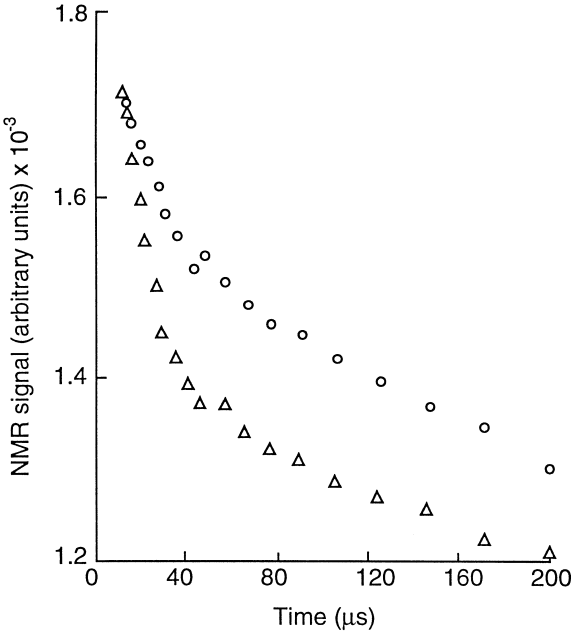


Fig. 12. Free induction decay of the NMR signal from fresh (Δ) and aged (○) rice starch gels (1:1 starch/water) after a 90° r.f. pulse (Teo & Seow, 1992; with permission).

one starch) after room- and low-temperature storage as well as after a six-cycle freeze–thaw treatment. However, it was difficult to relate such changes to visually observable differences in the degree of retrogradation. Kim-Shin, Mari, Rao and Stengle (1991) applied $^{17}O$ NMR to study changes in water mobility during bread staling. The effect of surfactant was also studied. They found that the $T_2$ values of the water in untreated bread (without surfactants) decreased rapidly, by 20–30%, during the first 3–4 days of storage. This is consistent with the earlier findings of Leung et al. (1983) using deuterium NMR. Wynne-Jones and Blanshard (1986) used both DSC and proton magnetic resonance to ascertain the state of water in aging starch gels and concluded that the change in physical state of water occurred primarily in the amylopectin fraction. However, Kim-Shin et al. found no correlation between amylopectin crystallization and $T_2$ during bread staling. Addition of antistaling surfactants, which inhibited amylopectin crystallization, did not significantly affect $T_2$. Therefore, they proposed that the effects observed were not caused by amylopectin crystallization but were more likely due to changes that took place within the amorphous regions.

In recent years, high-resolution solid-state $^{13}C$ NMR has been increasingly applied to the study of starch retrogradation using a special technique referred to as 'cross-polarization and magic-angle spinning (CP/MAS) NMR spectroscopy'. This technique enhances sensitivity and enables resonance peaks to be detected in the NMR spectrum of solid domains which would not otherwise be detected using a conventional liquid-state high-resolution spectrometer. Using an NMR spectrometer operating at 400 MHz for $^1H$ and 100.63 MHz for $^{13}C$, Smits et al. (1998) reported that the $^{13}C$ CP/MAS spectra of freeze-dried gelatinized potato starch showed no significant changes in lineshapes or chemical shifts during storage at different relative humidities (30, 60 and 90% RH) at 20°C. Richardson (1988) had earlier reported that storage did not apparently affect the $^{13}C$ spectra of instant starch pastes, suggesting that starch chains in the retrograded state retained the same mobility as in the gelatinized sol state probably due to sufficient hydration. However, they did find that freezing and thawing caused substantial changes in the $^{13}C$ spectra of an instant starch (Mira-Gel) that was freeze–thaw unstable. Such effects were attributed to polymer conformational changes during freezing. Although, $^{13}C$ spectra may not be sensitive enough to detect small variations in molecular structure, minor extents of recrystallization, or very small crystals, Smits et al. observed that proton rotating frame relaxation times ($T_{1\rho}$) changed dramatically during storage of the freeze-dried gelatinized potato starch samples (Fig. 14). For the material conditioned at 30% RH, which was in the glassy state (i.e. below the glass transition temperature, $T_g$) and thus did not undergo retrogradation, $T_{1\rho}$ values increased steeply during the initial period of storage before levelling off. The effects were attributed to sub-$T_g$ physical aging of the sample. On the other hand, samples stored at 60 and 90% RH displayed inverted bell-shaped $T_{1\rho}$–time curves, the initial decrease in $T_{1\rho}$ being ascribed to absorption of water during moisture equilibration and the rising part of the curve being attributed to the development of crystallinity (i.e. retrogradation) in the rubbery state.

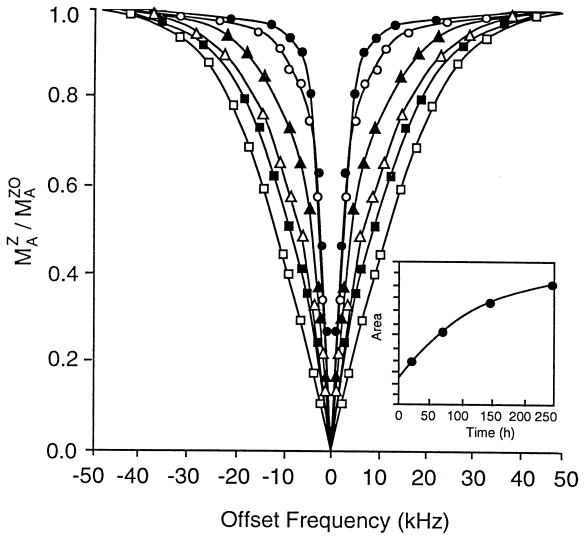


Fig. 13. Time dependence of cross-relaxation line shape of gelatinized 25% waxy maize starch during retrogradation at 5°C: ●, 3 h; ○, 20 h; ▲, 64 h; Δ, 140 h; ■, 10 days; □, 67 days (Wu et al., 1992; with permission).

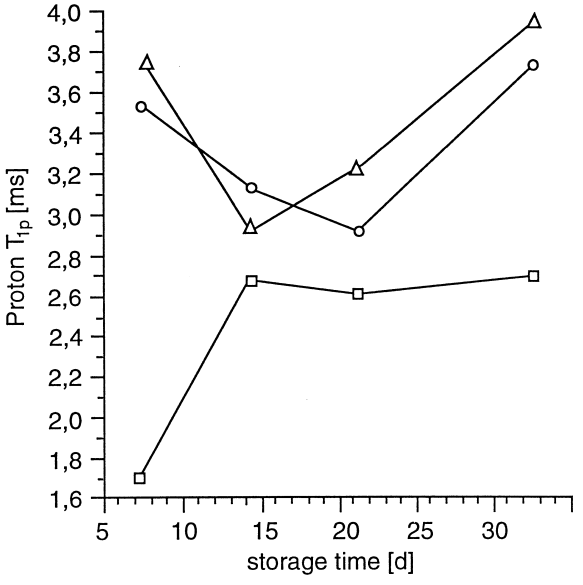


Fig. 14. Proton $T_{1\rho}$ relaxation times for freeze dried gelatinized potato starch conditioned at 30%, 60% and 90% RH: □, 30% RH; ○, 60% RH; Δ, 90% RH (Smits et al., 1998; with permission).

Using $^{13}C$ CP/MAS NMR spectroscopy, Morgan, Furneaux and Stanley (1992) found that, by taking combinations of spectra at different proton rotating frame relaxation times, $T_{1\rho}(H)$, it was possible to differentiate between sub-spectra of the crystalline and amorphous regions of native starch or a substantially aged gel. The spectra of the gel exhibited increasing contributions from the crystalline components during aging (Fig. 15). The kinetics of starch retrogradation could be determined by measuring the increase in total intensity for all peaks in the NMR spectrum relative to an internal standard of polyethylene. The Avrami equation was then used to calculate the rate of recrystallization.

Ruan, Almaer, Huang, Perkins, Chen and Fulcher (1996) used both low-resolution pulsed NMR and MRI to study water mobility, which they found to be highly correlated with the firming process, in starch-based food systems during storage. MRI revealed the spatial redistribution of moisture and water mobility within the samples as they aged.

### 5.2. Infra-red (IR) Spectoscopy

Recently, the technique of fourier transform mid-infra-red (FTIR) spectroscopy in combination with attenuated total reflectance (ATR) has been used to follow starch retrogradation (Goodfellow & Wilson, 1990; Smits et al., 1998; van Soest, de Wit, Tournois & Vliegenthart, 1994a,b; Wilson, Goodfellow, Belton, Osborne, Oliver & Russell, 1991; Wilson, Kalichevski, Ring & Belton, 1987). Conformational changes, due to retrogradation during storage, can be monitored by analysis of the observed band-narrowing process and of the observed intensity changes of conformational-sensitive bands in the 1300–800 $cm^{-1}$ region. In the initial disordered state, the polymer has a spread of conformations. As retrogradation proceeds, the system becomes more ordered and the range of conformations will be reduced, resulting in a smaller distribution of bond energies compared with the initial state, and hence the band-narrowing observed (Wilson et al., 1991). Basically, to monitor the kinetics of retrogradation, measurements are taken from band intensities at different time points during the experiment, using ratio measurements between selected peaks (typically those peaks exhibiting pronounced intensity changes). Of particular interest are the peaks at 1047 $cm^{-1}$ (characteristic of the crystalline regions of a starch system) and 1022 $cm^{-1}$ (characteristic of amorphous starch). Retrogradation has been observed to cause an increase in the ratio of the peak intensities at 1047 and 1022 $cm^{-1}$, which suggests a reduction in amorphousness or an increase in organization of the structure (Smits et al.).

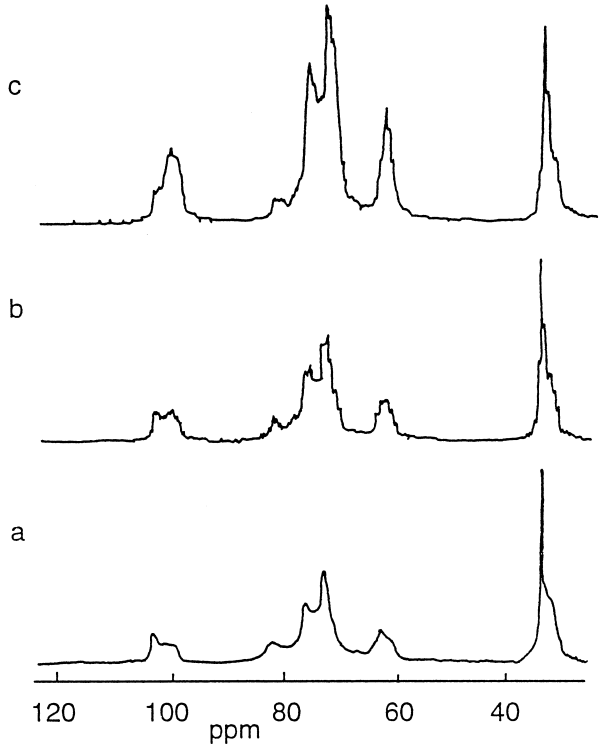


Fig. 15. $^{13}C$ CP MAS NMR spectra of moistened wheat starch (500 μl of water per g of starch) that has been gelatinized by heating. Spectra were recorded after (a) 0, (b) 6, and (c) 140 h. The peak at 33.0 ppm is due to polyethylene which is used as an internal density reference (Morgan et al., 1992; with permission).

Goodfellow and Wilson (1990) monitored the gelation and retrogradation of amylose and amylopectin using FTIR. The results obtained were in consonance with those from other physical and chemical methods. They suggested that gelation of amylose (concentration < 10%) involves rapid formation of double helical structures upon cooling of an amorphous sol. Such coil-to-helix transitions are intermolecular processes which lead to the establishment of a three-dimensional hydrated gel network, with the helical structures acting as junction zones among the polymer chains. Further lateral aggregation of double helices leads to formation of B-type crystalline structures.

It has also been shown that the FTIR/ATR method could provide high-quality spectra of the starch fraction of bread (Wilson & Belton, 1988) from which processes similar to those seen in starch gels could be observed.

The potential of near infra-red reflectance (NIR) spectroscopy to study the disorder–order transition in the starch fraction of bread crumbs has been explored by Wilson et al. (1991). This is based on the assumption that since starch polymers are extensively hydrogen-bonded, both intramolecularly and to solvent water, changes in the hydrogen bonding network of the system may be reflected in the NIR reflectance spectra. They followed the progress of bread staling by recording log $1/R$, where $R$ is the relative reflectance, defined as $P_s/P_o$, i.e. the ratio of the powers of radiation reflected from

the sample ($P_s$) and from a ceramic standard ($P_o$). The observed general decrease in log $1/R$ with storage time suggests an increase in scattering of NIR radiation as the crumb structure changes during staling. This is a result of the physical manifestation of the development of crystallinity in the amylopectin fraction of the bread crumbs.

### 5.3. Raman spectroscopy

The use of vibrational spectroscopy, i.e. Raman spectroscopy, for characterization of crystallinity and retrogradation of starch has been explored by Bulkin, Kwak and Dea (1986). Raman spectroscopy is, similar to IR spectroscopy, a technique which is generally used to probe the internal vibrations of molecules. As such, it measures the stretching and bending of bonds, characterizing these motions in terms of energy required and the change in polarizability (Raman) which occurs during the vibration. Bulkin et al. (1986) noted that changes in band width of the major skeletal mode at 480 $cm^{-1}$ appear to be a good index of starch retrogradation over long time scales. They obtained major differences in Raman spectra on gelatinization and subsequent retrogradation of concentrated waxy corn/water preparations.

## 6. Turbidimetric methods

A physical characteristic of aging gelatinized starch solutions is the increase in turbidity which results from changes in density distribution due to phase separation (Miles, Morris & Ring, 1985). Measurements of light-scattering (Foster & Sterman, 1956; Paschall & Foster, 1952) and reduction in transmitted light (Gidley & Bulpin, 1989; Jacobson & BeMiller, 1998; Jacobson, Obanni & BeMiller, 1997; Maciejewska, Poliszko & Kaczmarski, 1989; Miles, Morris & Ring, 1985; Ring et al., 1987) by spectrophotometry have been used to follow retrogradation in both low concentration ($< 2\%$) starch pastes and solutions of amylose and amylopectin. These methods measure turbidity development which results from molecular associations that occur during the early stages of the retrogradation process, before larger-scale organizations (that are more easily detected by means such as DSC and X-ray diffraction) are formed (Ring et al., 1987).

## 7. Measuring the resistance of starch to hydrolysis

An early indication of retrogradation is an increased resistance of the starch to hydrolysis by acid or amylolytic enzymes (e.g. α-amylase, β-amylase, glucoamylase and pullulanase) (Berry, 1986; Björck, Nyman, Pedersen, Siljestrom, Asp & Eggum, 1987; Matsukura, Matsunaga & Kainuma, 1983; Ring, Gee, Whittam, Orford & Johnson, 1988; Sievert & Pomeranz, 1989). It is well-established that the extent of crystallization and type of crystalline structure are major factors influencing the digestibility of starch (British Nutrition Foundation, 1990; Morris, 1990; Sievert et al., 1991). Measurement of the resistance of starch to enzymatic hydrolysis appears to be a very sensitive tool for following the rate of retrogradation in the early stages.

Results from a number of studies (Berry, 1986; Berry, I'Anson, Miles, Morris & Russell, 1988; Eerlingen, Crombez & Delcour, 1993; Sievert & Pomeranz, 1989) led to a conclusion that resistant starch type III, formed after gelatinization of starch, consists mainly of retrograded amylose. Depending upon how enzyme-resistant starch is defined in vitro, retrograded amylopectin may also play a role in the enzyme resistance of starch. When resistant starch is determined as the fraction of starch not digested to glucose after incubation for 2 h at 37°C with pancreatic α-amylase and amyloglucosidase (Englyst, Kingman & Cummings, 1992), retrograded amylopectin can yield high levels of resistant starch for starch gels stored under specific time and temperature conditions to obtain extensive retrogradation. Eerlingen et al. (1994) also reported that a resistant starch level of 42% was measured when waxy maize starch had been stored for 24 h at 6°C followed by 29 days at 40°C. On the other hand, when resistant starch is determined as the starch fraction surviving incubation with a heat-stable α-amylase at 100°C (Sievert & Pomeranz, 1989, 1990; Siljeström, Eliasson & Björck, 1989), no resistant starch can be detected, because the molecular order in retrograded amylopectin would be lost at this high temperature. Thus, as far as this method is concerned, it is important to select appropriate heating and storage conditions to induce crystallization of amylose or amylopectin in the starch gel.

In general, enzymatic methods used to determine degree of gelatinization of starch (Chiang & Johnson, 1977; Shetty, Lineback & Seib, 1974) may also be employed to determine extent of retrogradation. Kainuma, Matsunaga, Itagawa and Kobayashi (1981) devised an enzymatic method, based on the application of β-amylase and pullulanase (BAP), to determine the degree of gelatinization and retrogradation. Tsuge, Hishida, Iwasaki, Watanabe and Goshima (1990) developed an enzymatic method to determine the degree of starch retrogradation which is applicable to starch-containing foodstuffs. Basically the procedure involved the digestion of gelatinized starch by *Bacillus subtilis* α-amylase, which can only attack the gelatinized starch. The residual non-digestible starch was then determined colorimetrically with iodine. The presence of considerable amounts of other ingredients (30% sucrose, 20% NaCl or 30% casein) did not appear to interfere with

the determination. The α-amylase method was also compared with the glucoamylase method and the BAP method of Kainuma et al.. These methods gave different values for the extent of retrogradation (α-amylase > BAP > glucoamylase) determined on the same sample, implicating the specificity of enzyme activity on the retrograded starch.

## 8. Measurement of syneresis

The ability of starch to withstand the undesirable physical changes during freezing and thawing has been commonly termed "freeze–thaw" stability and can be used as an indicator of the tendency of starch to retrograde (Schoch, 1968). When a starch paste or gel is frozen, phase separation occurs with the formation of ice crystals. On thawing, the paste or gel will continue to be composed of a starch-rich and starch-deficient aqueous phase. The extent of phase separation increases with an increase in the number of freeze–thaw cycles due to an increase in amylopectin retrogradation in the starch-rich phase (Yuan & Thompson, 1998). Upon thawing, the water can be easily expressed from the dense network, a phenomenon known as syneresis. This is usually viewed unfavorably as product deterioration. The amount of syneresis is directly related to the tendency of a starch to retrograde.

Freeze–thaw stability may be simply evaluated by gravimetric measurements of the water of syneresis separated from starch pastes or gels or starch-containing products (Dreher, Tinsley, Scheerens & Berry, 1983; Hood & Seifried, 1974; Schoch, 1968; Wu & Seib, 1990). Typically, this method involves subjecting samples to repeated freezing and intermittent thawing to room temperature over a period of 2–4 h. At the end of the last cycle, the free liquid is separated (usually by centrifugation) and weighed. Alternatively, the weight of the sample after liquid separation may be determined. The extent of syneresis is calculated thus:

$$\text{Syneresis (\%)} = \frac{\text{liquid separated (g)}}{\text{Total weight of sample (g)}} \times 100 \quad (1)$$

Yuan and Thompson (1998) have shown that comparing freeze–thaw stabilities of starch pastes, based on one syneresis measurement taken after a fixed number of freeze–thaw cycles, may lead to improper or misleading conclusions, since most starch pastes subjected to several freeze–thaw cycles would reabsorb most of the separated liquid upon standing for 1 h at room temperature. They suggested that it might be appropriate to define freeze–thaw stability of starch pastes by the number of freeze–thaw cycles taken to detect the first appearance of free liquid above the paste after centrifugation.

At present, the procedure to determine freeze–thaw stability of starches, based on measurement of syneresis, has not been standardized. For example, measurement of syneresis may involve different separation techniques, centrifugal forces, freezing temperature/rate, freezing duration and number of freeze–thaw cycles. Some of these parameters may influence the course of retrogradation significantly while others may be of lesser importance. Eliasson and Kim (1992) have reported that centrifugation conditions, for instance, need to be carefully controlled when using this method, since the extent of syneresis measured depends upon the force applied during centrifugation. They showed that the centrifugal forces influenced the detection of first syneresis as well as the extent of syneresis, i.e. the sensitivity of the method increased with increasing gravitational force. The rate of freezing is also known to affect retrogradation rate (Slade & Levine, 1986). Slower freezing rates would result in more starch molecular associations and precipitation. This can be explained by the fact that, during slower freezing, the starch paste or gel is at temperatures near that of maximum nucleation for a longer time, allowing more molecular associations to occur (Jacobson & BeMiller, 1998). Consequently, different freezing rates used in a freeze–thaw study would be expected to give rise to different extents of syneresis. Such variations in procedure might be expected to lead to some confusion and difficulty in comparing data on freeze–thaw stability of starches reported by different researchers. However, if comparisons are based on data derived from an experiment conducted under a fixed set of conditions, this method appears to be quite reliable.

## 9. Miscellaneous methods

The reaction between starch and iodine has been known for over a century. Rundle and Baldwin (1943) postulated that the iodine component of the complex is present in a unidimensional array within an amylose helix with six glucose residues per turn. The formation of a complex between amylose and iodine gives rise to the typical deep blue colour ($\lambda_{max} = 640$ nm) of starch dispersion stained with iodine and forms the basis of quantitative assessment of amylose; in $I_2$/KI solution, the guest molecules are polyiodide ions. The conformations of amylose chains in the amorphous domains appear to be mainly single helix or random coil (Biliaderis, 1998). It is the ability of iodine (and a variety of polar and nonpolar compounds) to satisfy the solvation requirements of the hydrophobic helical cavity (ca. 0.5 nm in diameter) that enables the polysaccharide chain to adopt a regular conformation (V-helix), where the ligand molecule resides within the helix (Banks &

Greenwood, 1975). It has been observed (Cencic, Rosa, Nicoli & Cherubin, 1989; Collison, 1968) that amylose, on retrogradation, loses its ability to form a blue complex with iodine. On aging, amylose may form double-helical associations of 40–70 glucose units (Jane & Robyt, 1984; Leloup, Colonna, Ring, Roberts & Wells, 1982, Liu, Arntfield, Holley & Aime, 1997) which cannot accommodate the iodine; double helices may associate and organize into crystallites (Miles, Morris, Orford et al., 1985; Miles, Morris & Ring, 1985; Ring et al., 1987), and gelation results under appropriate conditions.

Many techniques employing the starch–iodine reaction have been used to determine the amount of amylose in starch, where amylose is present in a natural mixture with amylopectin. In general, the methods used to estimate amylose can be conveniently adopted to follow the starch retrogradation process. One such method is colorimetric assay in which iodine binds with amylose to produce a blue-coloured complex (Knutson & Grove, 1994; Martinez & Prodolliet, 1996; McGrance, Cornell & Rix, 1998; Morrison & Laignelet, 1983). For example, in the Blue Value determination, the Blue Value was defined by Morrison and Laignelet as the absorbance at 635 nm of 10 mg anhydrous starch in 100 ml dilute $I_2$–KI solution at 20°C, and calculated according to the following formula:

$$\text{Blue value}^T = \frac{(m_1 + m_2).A.10}{m_3.m_1.\left(\frac{100-h}{100}\right).1000}$$

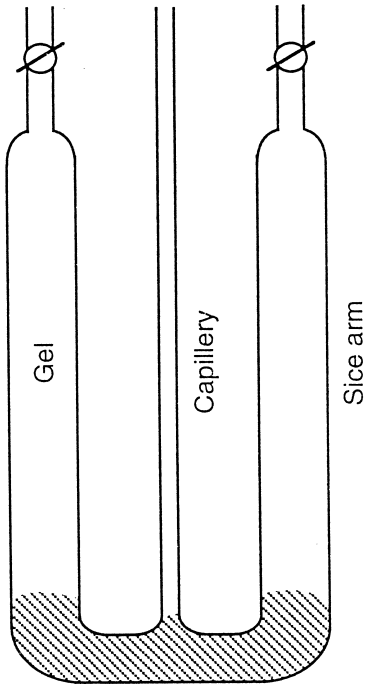


Fig. 16. Schematic diagram of dilatometer (Miles, Morris & Ring, 1985; with permission).

where $m_1$ = mass of the test portion (g); $m_2$ = mass of the solvent (urea + dimethylsulfoxide); $m_3$ = mass of the solution aliquot (g); $A$ = absorbance at 635 nm, measured at temperature $T$, and $h$ = moisture content (%). It should be noted that, since the complex is formed mainly with amylose, the iodine binding or the blue complex method reflects only retrogradation of amylose and not amylopectin.

The Blue Value Index, reflecting the amount of soluble amylose in cooked potatoes, was found to decrease rapidly with time of storage for the first 8 h and to become constant after 24 h at 20°C as retrogradation progressed (Jankowski, 1992). The rapid decrease of soluble amylose during storage was attributed to aggregation of the linear amylose fraction into insoluble complexes.

Miles, Morris and Ring (1985) used dilatometry to monitor volume changes during gelation of amylose. The apparatus used is shown in Fig. 16. The lower part of the dilatometer was filled with mercury; one side-arm contained water, the other the amylose solution. Experiments were conducted by filling the dilatometer at 60°C with the amylose solution. The sample-arm tap was closed and the dilatometer was mounted in the water bath at 32±0.0001°C. After 20 min, sufficient mercury was introduced through the other side-arm and its tap closed. The level of mercury in the capillary tube was then measured as a function of time. The dilatometry results indicated slow positive volume changes which were completed after approximately 5 h. It was suggested that the randomly-coiled amylose separated into a polymer-rich network phase, leaving polymer-deficient, i.e. more water-rich, regions within the gel.

## 10. A general comparison of the various methods reviewed

It is evident that the different methods used to study starch gelation and retrogradation operate on different principles and may measure different properties of a starch paste or gel. For example, turbidity measures distribution of refractive index (hence density); DSC measures the latent heat of melting of crystalline regions; X-ray diffraction measures long-range three-dimensional order in crystalline starch domains; vibrational (Raman) spectroscopy monitors conformation- and crystallinity-dependent vibrational frequencies of chemical bonds; NMR monitors chain segmental motions, conformation-dependent chemical shifts (resonance frequencies) and degree of crystallinity; and rheology monitors the development of supramolecular structure of a full three-dimensional polymer network as the gel matures. All these physical studies show that, in essence, the time course of change depends on the material and property being measured (Wu & Eads,

1993) and, therefore, some changes may precede or lag behind others. For example, it has been observed that the rate of $G'$ increase for non-waxy starch gels is generally much faster than the rate of staling endotherm ($\Delta H$) development in an aging gel (Biliaderis & Zawistowski, 1990). For amylopectin gels, however, the development of modulus can lag behind the development of crystallites detectable by both DSC and X-ray diffraction, depending on concentration (Ring et al., 1987).

X-ray diffraction is commonly used together with DSC to assess starch retrogradation. In analysing and interpreting the results of such studies, it should be noted that DSC and X-ray diffraction, as probes of structural order, do not necessarily measure the same type of structure in retrograded starch (Miles, Morris & Ring, 1985; Russell, 1987). The X-ray technique detects regular and repetitive ordering of helices, thereby reflecting the three dimensional order of starch crystallinity. The technique is less sensitive to irregularly packed structures, small chain aggregates, or isolated single helices (Gidley & Cooke, 1991). The DSC enthalpy changes are generally considered to correspond to order-disorder transitions of crystallites (i.e. helices present in extended ordered arrays) and regions of lesser crystalline order. According to Russell (1987), DSC is evidently sensitive to the amylopectin fraction of the gels (and perhaps a small fraction of the amylose that co-crystallises with amylopectin domains) and not to the major proportion of amylose. In contrast, X-ray diffraction gives a measure of the combined crystallinity of amylopectin and amylose.

A limited number of comparative studies (Kim, Kim & Shin, 1997; Roulet et al., 1988; Seow & Teo, 1996; Wilson et al., 1991) have been conducted to compare several methods of measuring the rate and extent of starch retrogradation. Wilson et al. (1991) followed the progress of bread staling by FTIR spectroscopy, DSC and NIR reflectance spectroscopy. These different techniques yielded data which, when fitted by an exponential equation, gave calculated rate constants in the region (12.8–16.3) $\times$ $10^{-3}$ $h^{-1}$. With these techniques, the results may be interpreted in terms of the development of crystallinity in the amylopectin fraction of bread. Each of the techniques monitors different aspects of amylopectin crystallization. DSC measures the actual melting of the crystallites in the bread sample and directly measures crystallization. NIR reflectance measures the scattering by the sample, which depends upon the physical state of the bread resulting from the crystallization of the amylopectin. FTIR measures the degree of short-range ordering in the system which is directly related to conformational changes at a molecular level. Consequently, these multiple techniques allow a more complete picture of the bread staling process to be obtained at both microscopic and macroscopic levels (Wilson et al., 1991).

Seow and Teo (1996) demonstrated that measurements by pulsed NMR (based on the increase in signal from the solid phase of a gel on ageing) gave very highly significant correlation ($P < 0.001$) with Instron firmness measurement. Fig. 17 shows the increase in firmness and in the normalised NMR solid phase signal (S) during storage of corn starch gel at 15°C. Both parameters increased rapidly in the early stages of storage before levelling off, after approximately the same period of time. The calculated rate constants ($k$) and Avrami exponents ($n$) obtained by compression are in good agreement with the corresponding values derived from NMR measurements. Ruan, Zou, Wadhawan, Martinez, Chen and Addis (1997) also reported that the increase in firmness of rice during 10 days' storage at 5°C correlated well with changes in NMR parameters. NMR techniques are, therefore, rapid and non-destructive means of monitoring changes at the molecular level, which manifest themselves at the macroscopic level as an increase in firmness during aging of starch gels and starch-based products within the same time frame.

A comparative study on retrogradation of rice starch gels by DSC, X-ray and α-amylase, conducted by Kim et al. (1997), indicated that the α-amylase-iodine method was the most sensitive and X-ray diffraction the least sensitive in determining the extent of retrogradation. Nevertheless, the relatively more complex or tedious methodology involved and possible interference by other food constituents may be considered as serious disadvantages of enzymatic hydrolysis methods (Jankiewicz & Michniewicz, 1986; Tsuge et al., 1990).

More recently, Smits et al. (1998) compared FTIR and solid state NMR spectroscopy to study the retrogradation and physical aging of model starch systems. High resolution solid state NMR spectroscopy is sensitive

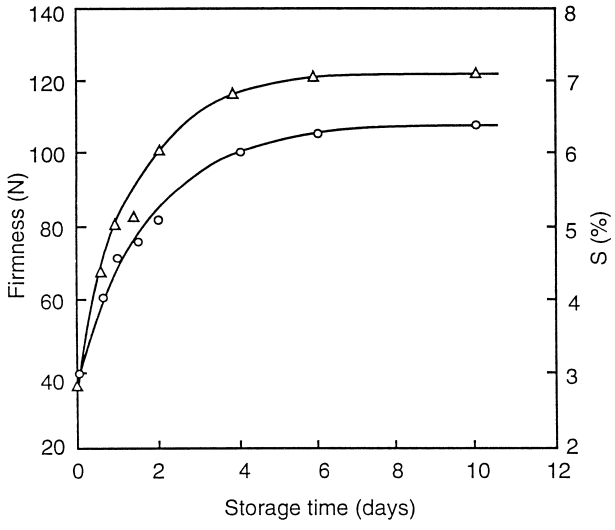


Fig. 17. Changes in firmness (Δ) and normalised NMR solid phase signal [s] (○) during storage of corn starch gel at 15°C (Seow & Teo, 1996; with permission).

Table 2
Rate and extent retrogradation of starches from different botanical sources

| Pasting conditions | Retrogradation conditions | Method | Order of initial retrogradation rates | Order of extent of retrogradation | Reference |
|---|---|---|---|---|---|
| 2.5% paste, atmospheric cooking under mild shear | Freeze–thaw cycle | Turbidometry | Potato > corn > wheat > rice > tapioca > waxy maize | | Jacobson and BeMiller (1998) |
| 2% paste, atmospheric cooking under mild shear | 4°C for 56 days | Turbidometry | Wheat, common corn > rice, tapioca, potato≫waxy maize | Common corn, rice > wheat≫tapioca > potato≫ waxy maize | Jacobson et al. (1997) |
| 40% gels; gelatinized in oven at 95°C for 110 min without shearing | Stored at 20°C | Rheological measurements and DSC | Pea > potato > rice > manioc, wheat > waxy rice (rheological method)<br>Potato, pea > rice, wheat > manioc, waxy rice (DSC) | Potato > pea, rice, manioc > wheat > waxy rice (rheological method)<br>Manioc, rice > potato > pea, waxy rice > wheat (DSC) | Roulet et al. (1990) |
| Starch gels at various starch to water ratio (1:1, 1:2, 1:4, 1:6, 1:8) | Stored at 5°C | Pulsed NMR | Mung bean > potato > corn > sago > rice > waxy rice | Mung bean > potato > corn > sago > rice > waxy rice | Teo and Seow (1992) |
| 30% starch gel | | X-ray and rheology | Pea > corn > wheat > potato | Pea > potato > corn > wheat | Orford et al. (1987) |

to structural organization at the molecular level and should therefore complement information obtained from FTIR or X-ray diffraction. Smits et al. suggested that both FTIR and NMR spectroscopy are good techniques for observing physical aging and retrogradation by means of spectral changes in lineshapes and linewidths and by the determination of relaxation times.

In terms of convenience, simplicity (particularly in sample preparation), and precision, NMR and DSC methods are probably the methods of choice. Sample sizes for NMR are usually far larger than for DSC, thus minimising sample variation. NMR and DSC methods are amenable to measurements over a wide range of temperatures and starch concentrations. For NMR measurements, precise weighings of samples are not usually required. Readings over a period of time may be carried out using the same sample because of the non-destructive nature of the method (Teo & Seow, 1992). DSC, however, provides a measure of the enthalpy associated with retrogradation. Rheological methods are also relatively simple, especially if an in situ gel preparation technique is used. In addition, structure development during retrogradation can be followed in 'real time'.

It is interesting to note that, in comparing the retrogradation tendencies of different types of starches in relatively concentrated (30–40% starch) systems, different methods of analysis gave generally similar results (Table 2). At 30% starch concentration, Orford, Ring, Carol, Miles & Morris (1987) reported that the order, as determined using a rheological method was: pea > potato > corn > wheat. Retrogradation rates in 33% gels determined by NMR (Teo & Seow, 1992) followed the order: mung bean > potato > corn > sago > rice > waxy rice. Using DSC, Roulet, MacInnes, Gumy and Wü (1990) found that retrogradation rates of 40% starch gels fell in the order: potato > wheat > rice > tapioca > modified waxy maize. There are some indications that starch concentration could affect this retrogradation rate order (Jacobson & BeMiller, 1998).

## 11. Concluding remarks

An attempt has been made to review the current state of knowledge and development of the various methods available to study starch retrogradation. The types of information, advantages and disadvantages of each method, techniques, and precautions have been compared and discussed. Evidently, a researcher has an array of methods available to him/her and the choice would naturally depend on the facilities available as well as the objective to be achieved in the experiment. The researcher should be aware of the limitations of the chosen method. In any case, it might be wise to choose at least two methods so that the results derived from one method can be verified and compared with other methods.

## References

Ablett, S. (1992). Overview of NMR applications in food science. *Trends in Food Science and Technology, 3*, 246–250.

Akuzawa, S., Aikawa, R., Kawabata, A., & Nakamura, M. (1997). Rheological properties of katakuri starch gel at the extremely small and large deformations. *Journal of Applied Glycoscience, 44*(4), 479–487.

Akuzawa, S., Sawayama, S., & Kawabata, A. (1995). Dynamic viscoelasticity and stress relaxation in starch pastes. *Journal of Texture Studies, 26*, 489–500.

Amano, T., Hayashi, A., Miura, M., Ishida, K., & Ohshima, K. (1995). Creep behaviour of starch gels at the earlier retrogradation stage. *Journal of Applied Glycoscience, 42*(4), 355–363.

Amano, T., Miura, M., & Hayashi, S. (1997). Retardation of the hardening of starch gels by polyols. III. Retardation effects of sugar

alcohols on hardening of wheat starch gels. *Journal of the Japanese Society of Food Science and Technology, 44*(7), 485–493.

Amano, T., Takada, S., Miura, M., Ishida, K., & Ohshima, K. (1997). Retardation of the hardening of starch gels by polyols. II. Retardation effects of saccharides on the hardening of wheat starch gels. *Journal of the Japanese Society of Food Science and Technology, 44*(2), 93–101.

Amemiya, J. I., & Menjivar, J. A. (1992). Comparison of small and large deformation measurements to characterize the rheology of wheat flour doughs. *Journal of Food Engineering, 16*(1/2), 91–108.

Axford, D., Colwell, K., Cornford, S., & Elton, F. (1968). Effect of loaf-specific volume on the rate and extent of staling in bread. *Journal of the Science of Food and Agriculture, 19*, 95–98.

Bagley, E. B. (1987). Mechanical properties of solid foods — deformation, fracture and stress relaxation. In R. Jowitt, F. Escher, M. McKenna, B. McKenna, & M. Roques, *Physical properties of foods* — 2 (pp. 265–345). London: Elsevier Applied Science.

Baker, L. A., & Rayas-Duarte, P. (1998). Freeze-thaw stability of amaranth starch and the effects of salt and sugars. *Cereal Chemistry, 75*(3), 301–303.

Banks, W., & Greenwood, C. T. (1975). *Starch and its components*. Edinburgh: Edinburgh University Press.

Barbosa-Canovas, G. V., Kokini, J. L., Ma, L., & Ibarz, A. (1996). The rheology of semiliquid foods. *Advances in Food and Nutrition Research, 39*, 1–69.

Bashford, L. L., & Hartung, T. E. (1976). Rheological properties related to bread freshness. *Journal of Food Science, 41*, 446–447.

Bell, A. E. (1989). Gel structure and food biopolymer. In T. M. Hardman, *Water and food quality* (pp. 251–275). London: Elsevier Applied Science.

Berendsen, H. J. C. (1992). Rationale for using NMR to study water relations in foods and biological tissues. *Trends in Food Science and Technology, 3*, 202–205.

Berry, C. S. (1986). Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *Journal of Cereal Science, 4*, 301–314.

Berry, C. S., I'Anson, I., Miles, M. J., Morris, V. J., & Russell, P. L. (1988). Physical chemical characterization of resistant starch from wheat. *Journal of Cereal Science, 8*, 203–206.

Biliaderis, C. G. (1991). The structure and interactions of starch with food constituents. *Canadian Journal of Physiology and Pharmacology, 69*, 60–78.

Biliaderis, C. G. (1992). Characterization of starch networks by small strain dynamic rheometry. In R. J. Alexander, & H. F. Zobel, *Developments in carbohydrate chemistry* (pp. 87–135). StPaul, MN: American Association of Cereal Chemists.

Biliaderis, C. G. (1998). Structures and phase transitions of starch polymers. In R. H. Walter, *Polysaccharide association structures in food* (pp. 57–168). New York: Marcel Dekker, Inc.

Biliaderis, C. G., & Juliano, B. O. (1993). Thermal and mechanical properties of concentrated rice starch gels of varying composition. *Food Chemistry, 48*, 243–250.

Biliaderis, C. G., & Tonogai, J. R. (1991). Influence of lipids on the thermal and mechanical properties of concentrated starch gels. *Journal of Agricultural and Food Chemistry, 39*, 833–840.

Biliaderis, C. G., & Zawistowski, J. (1990). Viscoelastic behaviour of aging starch gels: Effects of concentration, temperature, and starch hydrolysates on network properties. *Cereal Chemistry, 67*, 240–246.

Björck, I., Nyman, M., Pedersen, B., Siljestrom, M., Asp, N. G., & Eggum, B. O. (1987). Formation of enzyme resistant starch during autoclaving of wheat starch. *Journal of Cereal Science, 6*, 159–172.

Blanshard, J. M. V. (1987). Starch granule structure and function: a physicochemical approach. In T. Galliard, *Starch: Properties and potential* (pp. 16–54). Chichester: John Wiley & Sons.

Bourne, M. C. (1968). Texture profile of ripening pears. *Journal of Food Science, 33*, 223–226.

Bourne, M. C. (1978). Texture profile analysis. *Food Technology, 32*(7), 62–66,72.

Bourne, M. C. (1982). *Food texture and viscosity. Concept and measurement*. New York: Academic Press.

Bourne, M. C., Moyer, J. C., & Hand, D. B. (1966). Measurement of food texture by a universal testing machine. *Food Technology, 20*(4), 522–526.

Brady, P. L., McKeith, F. K., & Hunecke, M. E. (1985). Comparison of sensory and instrumental texture profile techniques for the evaluation of beef and beef–soy loaves. *Journal of Food Science, 50*, 1537–1539.

British Nutrition Foundation (1990). *Complex carbohydrates in foods*. London: Chapman and Hall.

Buleon, A., Bizot, H., Delage, M. M., & Pontoire, B. (1987). Comparison of X-ray diffraction patterns and sorption properties of the hydrolysed starches of potato, wrinkled and smooth pea, broad bean and wheat. *Carbohydrate Polymer, 7*, 461.

Bulkin, B. J., Kwak, Y., & Dea, I. (1986). Raman spectroscopic study of starch retrogradation. In S. S. Stivala, V. Crescenzi, & I. C. M. Dea, *Industrial polysaccharides* (pp. 267–281). New York: Gordon and Breach.

Cencic, L., Rosa, D. M., Nicoli, M. C., & Cherubin, S. (1989). Estimation of the degree of gelatinization of starch during processing of pasta and bakery products. In W. E. L. Spiess, & H. Schubert, *Engineering and food. Vol. 1. Physical properties and process control* (pp. 551–564). London: Elsevier Applied Science.

Chiang, B. Y., & Johnson, J. A. (1977). Measurement of total and gelatinized starch by glucoamylase and o-toluidine reagent. *Cereal Chemistry, 54*, 429–435.

Chinachoti, P. (1995). New techniques to characterize water in foods. In G. V. Barbosa-Cánovas, & J. Welti-Chanes, *Food preservation by moisture control* (pp. 191–207). Lancaster: Technomic Publishing Company.

Clark, A. H. (1991). Structural and mechanical properties of biopolymer gels. In E. Dickinson, *Food Polymers, gels and colloids* (pp. 322–337). London: Royal Society and Chemistry.

Clark, A. H. (1992). Gels and gelling. In H. G. Schwartzberg, & R. W. Hartel, *Physical chemistry of foods* (pp. 263–305). New York: Marcell Dekker Inc.

Clark, A. H., Gidley, M. J., Richardson, R. K., & Ross-Murphy, S. B. (1989). Rheological studies of aqueous amylose gels: the effects of chain length and concentration on gel modulus. *Macromolecules, 22*, 346–351.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. *Advances in Polymer Science, 83*, 55–192.

Collison, R. (1968). Starch retrogradation. In J. A. Radley, *Starch and its derivatives* (pp. 198–202). London: Chapman and Hall.

Colonna, P., Leloup, V., & Buléon, A. (1992). Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition (Suppl. 2), 46*, S17–S32.

Colwell, K. H., Axford, D. W. E., Chamberlain, N., & Elton, G. A. H. (1969). Effect of storage temperature on the ageing of concentrated wheat starch gels. *Journal of the Science of Food and Agriculture, 20*, 550–555.

Conde-Petit, B., & Escher, F. (1994). Influence of starch–lipid complexation on the ageing behaviour of high concentration starch gels. *Starch/Stärke, 46*(5), 172–177.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinization: origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112.

Curá, J. A., Jansson, P. E., & Krisman, C. R. (1995). Amylose is not linear. *Starch/Stärke, 47*(6), 207–209.

D'appolonia, B. L., & Morad, M. M. (1981). Bread staling. *Cereal Chemistry, 58*(3), 186–190.

Daniels, T. C. (1973). *Thermal analysis*. New York: John Wiley & Sons.

Deffenbaugh, L. B., & Walker, C. E. (1989). Comparison of starch pasting properties in the Brabender Viscograph and the Rapid Visco-Analyzer. *Cereal Chemistry, 66*, 493–499.

Dengate, H. N. (1984). Swelling, pasting, and gelling of wheat starch. In Y. Pomeranz, *Advances in cereal science and technology, (Vol. 6)* (pp. 49–82). Minnesota: American Association of Cereal Chemists.

Dickie, A. M., & Kokini, J. L. (1982). Use of the Bird–Leider equation in food rheology. *Journal of Food Process Engineering, 5*, 157–174.

Dragsdorf, R. D., & Varriano-Marston, E. (1980). Bread staling: X-ray diffraction studies on bread supplemented with α-amylases from different sources. *Cereal Chemistry, 57*, 310–314.

Dreher, M. L., Tinsley, A. M., Scheerens, J. C., & Berry, J. W. (1983). Buffalo gourd root starch. Part II. Rheological behaviour, freeze–thaw stability and stability for use in food products. *Starch/Stärke, 35*(5), 157–162.

Edwards, N. M., Izydorczyk, M. S., Dexter, J. E., & Biliaderis, C. G. (1993). Cooked pasta texture: comparison of dynamic viscoelastic properties to instrumental assessment of firmness. *Cereal Chemistry, 70*(2), 122–126.

Eerlingen, R. C., Crombez, M., & Delcour, J. A. (1993). Enzyme-resistant starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chemistry, 70*, 339–344.

Eerlingen, R. C., Jacobs, H., & Delcour, J. A. (1994). Enzyme-resistant starch. V. Effect of retrogradation of waxy maize starch on enzyme susceptibility. *Cereal Chemistry, 71*(4), 351–355.

Eliasson, A.-C. (1983). Differential scanning calorimetry studies on wheat starch gluten mixtures. II. Effect of gluten and sodium stearoyl lactylate on starch crystallization during ageing of wheat starch gels. *Journal of Cereal Science, 1*(3), 207–213.

Eliasson, A.-C. (1985). Retrogradation of starch as measured by differential scanning calorimetry. In R. D. Hill, & L. Munck, *New approaches to research on cereal carbohydrates* (pp. 93–98). Amsterdam, Netherlands: Elsevier Science Publishers.

Eliasson, A.-C., & Kim, H. R. (1992). Changes in rheological properties of hydroxypropyl potato starch pastes during freeze–thaw treatments. 1. A Rheological approach for evaluation of freeze–thaw stability. *Journal Texture Studies, 23*, 279–295.

Eliasson, A.-C., & Ljunger, G. (1988). Interactions between amylopectin and lipid additives during retrogradation in a model system. *Journal of the Science of Food and Agriculture, 44*, 353–361.

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition (Suppl. 2), 46*, S33–S50.

Evans, I. D., & Lips, A. (1992). Viscoelasticity of gelatinized starch dispersions. *Journal of Texture Studies, 23*, 69–86.

Fearn, T., & Russell, P. C. (1982). A kinetic study of bread staling by differential scanning calorimetry. The effect of loaf specific volume. *Journal of the Science of Food and Agriculture, 33*, 537–548.

Ferry, J. D. (1980). *Viscoelastic properties of polymers* (3rd. ed). New York: John Wiley.

Foster, J. F., & Sterman, M. D. (1956). A light scattering investigation of the retrogradation of amylose. *Journal of Polymer Science, 21*, 91–101.

Gambhir, P. N. (1992). Applications of low-resolution pulsed NMR to the determination of oil and moisture in oilseeds. *Trends in Food Science and Technology, 3*, 191–196.

Gamero, M., Fiszman, S. M., & Durán, L. (1993). Stress relaxation of fruit gels. Evaluation of models and effects of composition. *Journal of Food Science, 58*(5), 1125–1134.

Ghiasi, K., Hoseney, R. C., Zeleznak, K., & Rogers, D. E. (1984). Effect of waxy barley starch and reheating on firmness of bread crumb. *Cereal Chemistry, 61*, 281–285.

Giboreau, S., Cuvelier, G., & Launay, B. (1994). Rheological behaviour of three biopolymer/water systems, with emphasis on yield stress and viscoelastic properties. *Journal of Texture Studies, 25*, 119–137.

Gidley, M. J. (1989). Molecular mechanisms underlying amylose aggregation and gelation. *Macromolecules, 22*, 351–358.

Gidley, M. J., & Bulpin, P. V. (1989). Aggregation of amylose in aqueous systems: the effect of chain length on phase behavior and aggregation kinetics. *Macromolecules, 22*, 341–346.

Gidley, M. J., & Cooke, D. (1991). Aspects of molecular organization and ultrastructure in starch granules. *Biochemistry Society Transactions, 19*, 551–555.

Gladwell, N., Rahalkar, R. R., & Richmond, P. (1985). Creep/recovery behaviour of oil-water emulsions: Influence of disperse phase concentration. *Journal of Food Science, 50*, 1477–1481.

Goodfellow, B. J., & Wilson, R. H. (1990). A fourier transform IR study of the gelation of amylose and amylopectin. *Biopolymers, 30*, 1183–1189.

Grant, L. A. (1998). Effects of starch isolation, drying, and grinding techniques on its gelatinization and retrogradation properties. *Cereal Chemistry, 75*(5), 590–594.

Gross, M. A., Rao, V. N. M., & Smith, C. J. B. (1980). Rheological characterization of low-methoxyl pectin by normal creep and stress relaxation. *Journal of Texture Studies, 11*, 271–290.

Haines, P. J. (1995). *Thermal methods of analysis- principles, applications and problems*. London: Blackie Academic & Professional.

Hansen, L. M., Hoseney, R. C., & Faubion, J. M. (1990). Oscillatory probe rheometry as a tool for determining the rheological properties of starch–water systems. *Journal of Texture Studies, 21*, 213–224.

Hansen, L. M., Hoseney, R. C., & Faubion, J. M. (1991). Oscillatory rheometry of starch-water systems: effect of starch concentration and temperature. *Cereal Chemistry, 68*(4), 347–351.

Harwalkar, V. R., & Ma, C.-Y. (1990). *Thermal analysis of foods*. London: Elsevier Applied Science.

Hellman, N. N., Fairchild, B., & Senti, F. R. (1954). The bread staling problem. Molecular organization of starch upon aging of concentrated starch gels at various moisture level. *Cereal Chemistry, 31*, 495–504.

Hemminga, M. A. (1992). Introduction to NMR. *Trends in Food Science and Technology, 3*, 179–190.

Herz, K. O. (1965). Staling of bread. A review. *Food Technology, 19*, 1828–1841.

Hibi, Y., Kitamura, S., & Kuge, T. (1990). Effect of lipids in the retrogradation of cooked rice. *Cereal Chemistry, 67*, 7–10.

Hills, B. P., Takaes, S. F., & Belton, P. S. (1990). A new interpretation of proton NMR relaxation time measurements for water in food. *Food Chemistry, 16*, 95–111.

Hood, L. F., & Seifried, A. S. (1974). Effect of frozen storage on the microstructure and syneresis of modified tapioca starch–milk gels. *Journal of Food Science, 39*, 121–124.

Hoover, R. (1995). Starch retrogradation. *Food Reviews International, 11*(2), 331–346.

Inaba, H., Hoshizawa, M., Adachi, T., Matsumura, Y., & Mori, T. (1994). Characterization of texture and mechanical properties of starch gels. *Food Hydrocolloids, 8*(1), 33–44.

Inagaki, T., & Seib, P. A. (1992). Firming of bread crumb with cross-linked waxy barley starch substituted for wheat starch. *Cereal Chemistry, 69*(3), 321–325.

Jacobson, M. R., & BeMiller, J. N. (1998). Method for determining the rate and extent of accelerated starch retrogradation. *Cereal Chemistry, 75*(1), 22–29.

Jacobson, M. R., Obanni, M., & BeMiller, J. N. (1997). Retrogradation of starches from different botanical sources. *Cereal Chemistry, 74*(5), 511–518.

Jane, J. L., & Robyt, J. F. (1984). Structure studies of amylose V-complexes and retrograded amylose by action of alpha amylases, and a new method for preparing amylodextrins. *Carbohydrate Research, 132*, 105–118.

Jang, J. K., & Pyun, Y. R. (1997). Effect of moisture level on the crystallinity of wheat starch aged at different temperatures. *Starch/Stärke (7/8), 49*, 272–277.

Jankiewicz, M., & Michniewicz, J. (1986). The effect of soluble pentosans isolated from rye grain and staling of bread. *Food Chemistry, 25*, 241–249.

Jankowski, T. (1992). Influence of starch retrogradation on the texture of cooked potato tuber. *International Journal of Food Science and Technology, 27*, 637–642.

Jankowski, T., & Rha, C. K. (1986). Retrogradation of starch in cooked wheat. *Starch/Stärke, 38*(1), 6–9.

Kainuma, K., Matsunaga, A., Itagawa, M., & Kobayashi, S. (1981). New enzyme system — β-amylase-pullulanase to determine the degree of gelatinization and retrogradation of starch or starch products. *Journal of the Japanese Society of Starch Science, 28*, 235–240.

Katsuka, K., Nishimura, A., & Miura, M. (1992). Effects of saccharides on stabilities of rice starch gels. II. Oligosaccharides. *Food Hydrocolloids, 6*(4), 399–408.

Katz, J. R. (1934). The physical chemistry of starch and bread baking XX. *Z. Physical Chemistry, A169*, 321.

Keetels, C. J. A. M., Oostergetel, G. T., & van Vliet, T. (1996). Recrystallization of amylopectin in concentrated starch gels. *Carbohydrate Polymers, 30*, 61–64.

Keetels, C. J. A. M., van Vliet, T., Jurgens, A., & Walstra, P. (1996). Effects of lipid surfactants on the structure and mechanics of concentrated starch gels and starch bread. *Journal of Cereal Science, 24*, 33–45.

Keetels, C. J. A. M., van Vliet, T., & Walstra, P. (1996a). Gelation and retrogradation of concentrated starch systems: 1 Gelation. *Food Hydrocolloids, 10*(3), 343–353.

Keetels, C. J. A. M., van Vliet, T., & Walstra, P. (1996b). Gelation and retrogradation of concentrated starch gels. 2. Retrogradation. *Food Hydrocolloids, 10*(3), 355–362.

Keetels, C. J. A. M., Visser, K. A., van Vliet, T., Jurgens, A., & Walstra, P. (1996). Structure and mechanics of starch bread. *Journal of Cereal Science, 24*(1), 15–26.

Kim, S. K., & D'Appolonia, B. L. (1977). Bread staling studies. I. Effect of protein content on staling rate and bread crumb pasting properties. *Cereal Chemistry, 54*, 207–215.

Kim, H. R., & Eliasson, A.-C. (1993). Changes in rheological properties of hydroxypropyl potato starch pastes during freeze–thaw treatments. II. Effect of molar substitution and cross-linking. *Journal of Texture Studies, 24*, 199–213.

Kim, J. K., Kim, W. S., & Shin, M. S. (1997). A comparative study on retrogradation of rice starch gels by DSC, X-ray and α-amylase method. *Starch/Stärke, 49*(2), 71–75.

Kim-Shin, M. S., Mari, F., Rao, P. A., & Stengle, T. R. (1991). $^{17}O$ Nuclear magnetic resonance studies of water mobility during bread staling. *Journal of Agricultural and Food Chemistry, 39*(11), 1915–1920.

Knightly, W. H. (1977). The staling of bread. *Baker's Digest, 51*(5), 52–56,144.

Knudsen, L., Børresen, T., & Nielsen, J. (1987). A compression test for measuring the gel forming ability of fish protein. In R. Jowitt, F. Kent, M. Kent, B. McKenna, & M. Roques, *Physical properties of foods* — 2 (pp. 479–484). London: Elsevier Applied Science.

Knutson, C. A., & Grove, M. J. (1994). Rapid method for estimation of amylose in maize starches. *Cereal Chemistry, 71*(5), 469–471.

Kulp, K., & Ponte, J. G. (1981). Staling of white pan bread: fundamental causes. *Critical Reviews in Food Science and Nutrition, 15*, 1–48.

Kuntz, I. D., & Kauzmann, W. (1974). Hydration of proteins and polypeptides. *Advances in Protein Chemistry, 28*, 239–345.

Le Botlan, D., & Desbois, P. (1995). Starch retrogradation study in presence of sucrose by low-resolution nuclear magnetic resonance. *Cereal Chemistry, 72*(2), 191–193.

Lechert, H. (1981). Water binding on starch: NMR studies on native and gelatinized starch. In L. B. Rockland, & G. F. Stewart, *Water activity: Influence on food quality* (pp. 223–245). New York: Academic Press.

Lee, C. H., & Kim, C. W. (1983). Studies on the rheological property of Korean noodles. II. mechanical model parameters of cooked and stored noodles. *Korean Journal of Food Science and Technology, 15*(3), 295–301.

Leloup, V. M., Colonna, P., Ring, S. G., Roberts, K., & Wells, B. (1992). Microstructure of amylose gels. *Carbohydrate Polymers, 18*, 189–197.

León, A., Durán, & deBarber, C. B. (1997). A new approach to study starch changes occurring in the dough-baking process and during bread storage. *Zeitschrift-fuer-Lebensmittel-Untersuchung-und-Forschung, 204*, 316–320.

Leung, H. K. (1981). Structure and properties of water. *Cereal Foods World, 26*, 350–352.

Leung, H. K., Magnuson, J. A., & Bruinsma, B. L. (1983). Water binding of wheat flour doughs and breads as studied by deuteron relaxation. *Journal of Food Science, 48*, 95–99.

Lii, C. Y., Shao, Y. Y., & Tseng, K. H. (1995). Gelation mechanism and rheological properties of rice starch. *Cereal Chemistry, 72*(4), 393–400.

Liu, Q., Arntfield, S. D., Holley, R. A., & Aime, D. B. (1997). Amylose-lipid complex formation in acetylated pea starch–lipid systems. *Cereal Chemistry, 74*, 159–162.

Longton, J., & LeGrys, G. A. (1981). Differential scanning calorimetry studies on the crystallinity of ageing wheat starch gels. *Starch/Stärke, 33*, 410–414.

Maciejewska, W., Poliszko, S., & Kaczmarski, M. (1989). Alcohol as an initiator in amylose retrogradation. In W. E. L. Spiess, & H. Schubert, *Engineering and food. Vol. 2. Preservation processes and related techniques* (pp. 863–872). London: Elsevier Applied Science.

Maga, J. A. (1975). Bread staling. *Critical Reviews in Food Technology, 5*, 443–486.

Marsh, R. D. L., & Blanshard, J. M. V. (1988). The application of polymer crystal growth theory to the kinetics of formation of the B-amylose polymorph in a 50% wheat starch gel. *Carbohydrate Polymers, 9*, 301–317.

Martin, M. L., Zeleznak, K. J., & Hoseney, R. C. (1991). A mechanism of bread firming. I. Role of starch swelling. *Cereal Chemistry, 68*(5), 498–503.

Martinez, C., & Prodolliet, J. (1996). Determination of amylose in cereal and non-cereal starches by a colorimetric assay: collaborative study. *Starch/Stärke, 48*(3), 81–85.

Matsukura, U., Matsunaga, A., & Kainuma, K. (1983). Structural studies of the retrogradation of gelatinized starch. *Journal of the Japanese Society of Starch Science, 30*, 106–109.

McGrance, S. J., Cornell, H. J., & Rix, C. J. (1998). A simple and rapid colorimetric method for the determination of amylose in starch products. *Starch/Stärke, 50*(4), 158–163.

McIver, R. G., Axford, D. W. E., Colwell, K. H., & Elton, G. A. H. (1968). Kinetic study of the retrogradation of gelatinized starch. *Journal of the Science of Food and Agriculture, 19*, 560–563.

Mikus, F. F., Hixon, R. M., & Rundle, R. E. (1946). The complexes of fatty acids with amylose. *Journal of the American Chemical Society, 68*, 1115–1123.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research, 135*, 271–278.

Miles, M. J., Morris, V. J., & Ring, S. G. (1985). Gelation of amylose. *Carbohydrate Research, 135*, 247–269.

Mitchell, J. R. (1976). Rheology of gels. *Journal of Texture Studies, 7*, 313–339.

Miura, M., Nishimura, A., & Katsuka, K. (1992). Influence of polyols and food emulsifiers on the retrogradation rate of starch. *Food Structure, 11*(3), 225–236.

Morad, M. M., & D'Appolonia, B. L. (1980). Effect of baking procedure and surfactants on the pasting properties of bread crumb. *Cereal Chemistry, 57*, 239–241.

Morgan, K. R., Furneaux, R. H., & Stanley, R. A. (1992). Observation by solid state $^{3}C$ CP MAS NMR spectroscopy of the transformations of wheat starch associated with the making and staling of bread. *Carbohydrate Research, 235*, 15–22.

Morris, V. J. (1990). Starch gelation and retrogradation. *Trends in Food Science and Technology, 1*(1), 2–6.

Morrison, W. R., & Laignelet, B. (1983). An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *Journal of Cereal Science, 1*, 9–20.

Nakazawa, F., Noguchi, S., & Takahashi, J. (1985). Retrogradation of gelatinized potato starch studied by differential scanning calorimetry. *Agricultural and Biological Chemistry, 49*(4), 953–957.

Nakazawa, F., Noguchi, S., Takahashi, J., & Takada, M. (1983). Pulsed NMR study of water behaviour in retrogradation process of rice and rice starch. *Kaseigaku Zasshi, 34*, 566–570.

Navarro, A. S., Martino, M. N., & Zaritzky, N. E. (1997). Correlation between transient rotational viscometry and a dynamic oscillatory test for viscoelastic starch-based system. *Journal of Texture Studies, 28*, 365–385.

Nussinovitch, A., Ak, M. M., Normand, M. D., & Peleg, M. (1990). Characterization of gellan gels by uniaxial compression, stress relaxation and creep. *Journal of Texture Studies, 21*, 37–49.

Nussinovitch, A., Peleg, M., & Normand, M. D. (1989). A modified Maxwell and a nonexponential model for characterization of the stress relaxation of agar and alginate gels. *Journal of Food Science, 54*(4), 1013–1016.

Obanni, M., & BeMiller, J. N. (1997). Properties of some starch blends. *Cereal Chemistry, 74*(4), 431–436.

Olkku, J., & Rha, C. K. (1978). Gelatinization of starch and wheat flour starch — a review. *Food Chemistry, 3*, 293–317.

Ooraikul, B., Parker, G. J. K., & Hadziyev, D. (1974). Starch and pectin substances as affected by a freeze–thaw potato granule process. *Journal of Food Science, 39*, 358–364.

Orford, P. D., Ring, S. G., Carol, V., Miles, M. J., & Morris, V. J. (1987). The effect of concentration and botanical source on the gelation and retrogradation of starch. *Journal of the Science of Food and Agriculture, 39*, 169–177.

Pappas, G., & Rao, V. N. M. (1989). Effect of temperature and moisture content on the viscoelastic behaviour of cowpeas. *Journal of Texture Studies, 20*, 393–407.

Paschall, E. F., & Foster, J. F. (1952). An investigation by light scattering of the state of aggregation of amylose in aqueous solutions. *Journal of Polymer Science, 9*, 73–84.

Peleg, M. (1979). Characterization of the stress relaxation curves of solid foods. *Journal of Food Science, 44*, 277–281.

Peleg, M. (1987). The basics of solid food rheology. In H. M. Moskowitz, *Food texture* (pp. 3–33). Basel: Marcel Dekker.

Pons, M., & Fiszman, S. M. (1996). Instrumental Texture Profile Analysis with particular reference to gelled system. *Journal of Texture Studies, 27*, 597–624.

Rao, M. A., & Steffe, J. F. (1992). *Viscoelastic properties of foods*. London: Elsevier Applied Science.

Rao, P. A., Nussinovitch, A., & Chinachoti, P. (1992). Effects of selected surfactants on amylopectin recrystallization and on recoverability of bread crumb during storage. *Cereal Chemistry, 69*(6), 613–618.

Richardson, S. J. (1988). Molecular mobilities of instant starch gels determined by oxygen-17 and carbon-13 nuclear magnetic resonance. *Journal of Food Science, 53*(4), 1175–1179.

Richardson, S. J., & Steinberg, M. P. (1987). Application of nuclear magnetic resonance. In L. Rockland, & L. R. Beuchat, *Water activity: Theory and applications in food* (pp. 235–285). New York: Marcel Dekker.

Ring, S. G., Gee, J. M., Whittam, M., Orford, P., & Johnson, I. T. (1988). Resistant starch: its chemical form in foodstuffs and effect on digestibility in vitro. *Food Chemistry, 28*, 97–109.

Ring, S. R., Colonna, P., I'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J., & Orford, P. D. (1987). The gelation and crystallization of amylopectin. *Carbohydrate Research, 162*, 277–293.

Rogers, D. E., Zeleznak, K. J., Lai, C. S., & Hoseney, R. C. (1988). Effect of native lipids, shortening, and bread moisture on bread firming. *Cereal Chemistry, 65*, 398–401.

Ross, A. S., Walker, C. E., Booth, R. I., Orth, R. A., & Wrigley, C. W. (1987). The Rapid ViscoAnalyser: a new technique for the evaluation of sprout damage. *Cereal Foods World, 32*, 827–829.

Ross-Murphy, S. B. (1995). Rheological characterization of gels. *Journal of Texture Studies, 26*, 391–400.

Roulet, P., MacInnes, W. M., Gumy, D., & Würsch, P. (1990). Retrogradation kinetics of eight starches. *Starch/Stärke, 42*(3), 99–101.

Roulet, P., MacInnes, W. M., Wursch, P., Sanchez, R. M., & Raemy, A. (1988). A comparative study of the retrogradation kinetics of gelatinized wheat starch gel and powder form using X-rays, differential scanning calorimetry and dynamic mechanical analysis. *Food Hydrocolloids, 2*, 381–396.

Ruan, R. R., Almaer, S., Huang, V. T., Perkins, P., Chen, P., & Fulcher, R. G. (1996). Relationship between firming and water mobility in starch-based food systems during storage. *Cereal Chemistry, 73*(3), 328–332.

Ruan, R. R., Zou, C., Wadhawan, C., Martinez, B., Chen, P. L., & Addis, P. (1997). Studies of hardness and water mobility of cooked wild rice using nuclear magnetic resonance. *Journal of Food Processing and Preservation, 21*, 91–104.

Rundle, R. E., & Baldwin, R. J. (1943). *Journal of American Chemical Society, 65*, 544–557.

Russell, P. L. (1983). A kinetic study of bread staling by differential scanning calorimetry and compressibility measurements. The effect of added monoglyceride. *Journal of Cereal Science, 1*, 297–303.

Russell, P. L. (1987). The ageing of gels from starches of different amylose/amylopectin content studied by differential scanning calorimetry. *Journal of Cereal Science, 6*, 147–158.

Sanders, J. K., & Hunter, B. K. (1987). *Modern NMR spectroscopy. A guide for chemists*. London: Oxford University Press.

Sarko, A., & Wu, H.-C. H. (1978). The crystal structures of A-, B- and C-polymorphs of amylose and starch. *Starch/Stärke, 30*, 73–78.

Schiraldi, A., Piazza, L., & Riva, M. (1996). Bread staling: a calorimetric approach. *Cereal Chemistry, 73*(1), 32–39.

Schmidt, S. J., & Lai, H.-M. (1991). Use of NMR and MRI to study water relations in foods. In H. Levine, & L. Slade, *Water relationships in foods* (pp. 405–452). New York: Plenum Press.

Schoch, T. J. (1968). Effects of freezing and cold storage on pasted starches. In D. K. Tressler, W. B. van Arsdel, & M. J. Copley, *The Freezing preservation of foods, (Vol. 4)* (pp. 44–56). Westport, CT: AVI.

Seow, C. C., & Teo, C. H. (1996). Staling of starch-based products: a comparative study by firmness and pulsed NMR measurements. *Starch/Stärke, 48*(3), 90–93.

Seow, C. C., & Thevamalar, K. (1988). Problems associated with traditional Malaysian starch-based intermediate moisture foods. In C. C. Seow, *Food preservation by moisture control* (pp. 233–252). London: Elsevier Applied Science.

Seow, C. C., Teo, C. H., & Vasanti Nair, C. K. (1996). A DSC study of the effects of sugars on thermal properties of rice starch gels before and after aging. *Journal of Thermal Analysis, 47*, 1201–1212.

Shetty, R. M., Lineback, D. R., & Seib, P. A. (1974). Determining the degree of starch gelatinization. *Cereal Chemistry, 51*, 364–375.

Shi, Y.-C., & Seib, P. A. (1992). The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydrate Research, 227*, 131–145.

Shiraishi, K., Lauzon, R. D., Yamazaki, M., Sawayama, S., Sugiyama, N., & Kawabata, A. (1995). Rheological properties of cocoyam starch paste and gel. *Food Hydrocolloids, 9*(2), 69–75.

Shuey, W. C., & Tipples, K. H. (1980). *The amylograph handbook*. St. Paul, MN: American Association of Cereal Chemists.

Sievert, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermomechanical, and microscopic methods. *Cereal Chemistry, 66*, 342–347.

Sievert, D., & Pomeranz, Y. (1990). Enzyme-resistant startch-II. Differential scanning calorimetry studies on hear-treated starches and enzyme-resistant starch residues. *Cereal Chemistry, 67*(3), 217–221.

Sievert, D., Czuchajowska, Z., & Pomeranz, Y. (1991). Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII starch and enzyme-resistant starch residues. *Cereal Chemistry, 68*(1), 86–91.

Siljeström, M., & Eliasson, A.-C., & Björck, I. (1989). Characterization of resistant starch from autoclaved wheat starch. *Starch/Staerke, 41*, 147–151.

Silverio, J., Svensson, E., Eliasson, A.-C., & Olofsson, G. (1996). Isothermal microcalorimetric studies on starch retrogradation. *Journal of Thermal Analysis, 47*, 1179–1200.

Slade, L., & Levine, H. (1986). Recent advances in starch retrogradation. In S. S. Stivala, V. Crescenzi, & I. C. M. Dea, *Industrial polysaccharides* (pp. 387–430). New York: Gordon and Breach.

Slade, L., & Levine, H. (1989). A food polymer science approach to selected aspects of starch gelatinization and retrogradation. In R. P. Millane, J. N. BeMiller, & R. Chandrasekaran, *Frontiers in carbohydrate research* (pp. 215–270). London: Elsevier Applied Science.

Smits, A. L. M., Ruhnau, F. C., Vliegenthart, J. F. G., & van Soest, J. J. G. (1998). Ageing of starch based systems as observed with FT-IR and solid state NMR spectroscopy. *Starch/Stärke, 50*, 478–483.

Steinberg, M. P., & Leung, H. (1975). Some applications of wide-line and pulsed nuclear magnetic resonance in investigations of water in foods. In R. B. Duckworth, *Water relations of foods* (pp. 233–248). London: Academic Press.

Sterling, C. (1978). Starch. In Y. Pomeranz, *Advances in cereal science and technology, (Vol. 2)* (pp. 119–151). St. Paul, MN: American Association of Cereal Chemists.

Stevens, D. J., & Elton, G. A. H. (1971). Thermal properties of starch/water system. I. Measurement of heat of gelatinization by differential scanning calorimetry. *Starch/Stärke, 23*, 8–11.

Tang, Q., McCarthy, O. J., & Munro, P. A. (1997). Effects of pH on whey protein concentrate gel properties: comparisons between small deformation (dynamic) and large deformation (failure) testing. *Journal of Texture Studies, 26*, 255–272.

Teo, C. H., & Seow, C. C. (1992). A pulsed NMR method for the study of starch retrogradation. *Starch/Stärke, 44*, 288–292.

Tsai, M. L., Li, C. F., & Lii, C. Y. (1997). Effects of granular structures on the pasting behaviours of starches. *Cereal Chemistry, 74*(6), 750–757.

Tsuge, H., Hishida, M., Iwasaki, H., Watanabe, S., & Goshima, G. (1990). Enzymatic evaluation for the degree of starch retrogradation in foods and foodstuffs. *Starch/Stärke, 42*(6), 213–216.

van Soest, J. J. G., de Wit, D., Tournois, H., & Vliegenthart, J. F. G. (1994). Retrogradation of potato starch as studied by Fourier Transform Infrared spectroscopy. *Starch/Stärke, 12*, 453–457.

van Soest, J. J. G., de Wit, D., Tournois, H., & Vliegenthart, J. F. G. (1994). The influence of glycerol on structural changes in waxy maize starch as studied by Fourier transform infra-red spectroscopy. *Polymer, 35*(22), 4722–4727.

Waddington, D. (1986). Applications of wide-line nuclear magnetic resonance in the oils and fats industry. In R. J. Hamilton, & J. B. Rossell, *Analysis of oils and fats* (pp. 341–399). London: Elsevier Applied Science.

Walker, C. E., Ross, A. S., Wrigley, C. W., & McMaster, G. J. (1988). Accelerated starch-paste characterization with the Rapid Visco-Analyzer. *Cereal Foods World, 33*, 491–494.

Watanabe, T. (1981). Traditional techniques in Japan for food preservation by freezing, thawing and drying. In L. B. Rockland, & G. F. Stewart, *Water activity: influences on food quality* (pp. 733–742). New York: Academic Press.

Weipert, D. (1990). The benefits of basic rheometry in studying dough rheology. *Cereal Chemistry, 67*(4), 311–317.

Wendlandt, W. W. (1974). *Thermal methods of analysis* (2nd. Ed). New York: John Wiley & Sons.

White, P. J., Abbas, I. R., & Johnson, L. A. (1989). Freeze–thaw stability and refrigerated-storage retrogradation of starches. *Starch/Stärke, 41*(5), 176–180.

Wild, D. L., & Blanshard, J. M. V. (1986). The relationship of the crystal structure of amylose polymorphs to the structure of the starch granule. *Carbohydrate Polymers, 6*, 121.

Willhoft, E. M. A. (1973). Mechanism and theory of staling of bread and baked goods, and associated changes in textural properties. *Journal of Texture Studies, 4*, 292–322.

Wilson, R. H., & Belton, P. S. (1988). A Fourier Transform Infrared study of wheat starch gels. *Carbohydrate Research, 180*, 339–344.

Wilson, R. H., Goodfellow, B. J., Belton, P. S., Osborne, B. G., Oliver, G., & Russell, P. L. (1991). Comparison of Fourier Transform MID infrared spectroscopy and near infrared spectroscopy with differential scanning calorimetry for the study of the staling of bread. *Journal of the Science of Food and Agriculture, 54*, 471–483.

Wilson, R. H., Kalichevski, M. T., Ring, S. G., & Belton, P. S. (1987). A Fourier Transform IR study of the gelation and retrogradation of waxy maize starch. *Carbohydrate Research, 166*, 182.

Wium, H., & Qvist, K. B. (1997). Rheological properties of UF-Feta cheese determined by uniaxial compression and dynamic testing. *Journal of Texture Studies, 28*, 435–454.

Wright, W. B. (1971). Starch: granule structure and technology. *Chemistry and Industry, 22*, 568–570.

Wu, J. Y., & Eads, T. M. (1993). Evolution of polymer mobility during ageing of gelatinized waxy maize starch: a magnetization transfer $^{1}$H NMR study. *Carbohydrate Polymers, 20*, 51–60.

Wu, J. Y., Bryant, R. G., & Eads, T. M. (1992). Detection of solidlike components in starch using cross-relaxation and fourier transform wide-line $^{1}$H NMR methods. *Journal of Agricultural and Food Chemistry, 40*(3), 449–455.

Wu, S. H., & Seib, P. A. (1990). Acetylated and hydroxypropylated distarch phosphates from waxy barley: paste properties and freeze–thaw stability. *Cereal Chemistry, 67*(2), 202–208.

Wunderlich, B. (1976). *Macromolecular physics, Vol. 2. — Crystal nucleation, growth and annealing*. New York: Academic Press.

Wunderlich, B. (1990). *Thermal analysis*. New York: Academic Press.

Wynne-Jones, S., & Blanshard, J. M. V. (1986). Hydration studies of wheat starch amylopectin, amylose gels and bread by proton magnetic resonance. *Carbohydrate Polymers, 6*, 289.

Xu, A., Chung, O. K., & Ponte, J. G. Jr (1992). Bread crumb amylograph studies. I. Effects of storage time, shortening, flour lipids, and surfactants. *Cereal Chemistry, 69*(5), 495–501.

Xu, A., Ponte, J. G. Jr, & Chung, O. K. (1992). Bread crumb amylograph studies. II. Cause of unique properties. *Cereal Chemistry, 69*(5), 502–507.

Yasunaga, T., Bushuk, W., & Irvine, G. N. (1968). Gelatinization of starch during bread-baking. *Cereal Chemistry, 45*, 269–279.

Yuan, R. C., & Thompson, D. B. (1998). Freeze–thaw stability of three waxy maize starch pastes measured by centrifugation and calorimetry. *Cereal Chemistry, 75*(4), 571–573.

Yuan, R. C., Thompson, D. B., & Boyer, C. D. (1993). Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wx-containing genotypes in two inbred lines. *Cereal Chemistry, 70*(1), 81–89.

Zeleznak, K. J., & Hoseney, R. C. (1987). Characterization of starch from bread aged at different temperatures. *Starch/Stärke, 39*, 231–233.

Zhang, W., & Jackson, D. S. (1992). Retrogradation behaviour of wheat starch gels with differing molecular profiles. *Journal of Food Science, 57*(6), 1428–1432.

Zhou, M., Robards, K., Glennie-Holmes, M., & Helliwell, S. (1998). Structure and pasting properties of oat starch. *Cereal Chemistry, 75*(3), 273–281.

Zobel, H. F. (1988). Molecules to granules: a comprehensive starch review. *Starch/Stärke, 40*(2), 44–50.

obel, H. F., & Senti, F. R. (1959). The bread staling problem. X-ray diffraction studies on breads containing a cross-linked starch and a heat-stable amylase. *Cereal Chemistry, 36*, 441–451.