Enlarged Processing Window of Plasticized Wheat Gluten Using Salicylic Acid

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The temperature window for the extrusion of glycerol-plasticized wheat gluten was increased by the use of salicylic acid, a known scorch retarder and radical scavenger. It was possible to extrude 30 wt % glycerol−wheat gluten films with a die-head temperature as high as 135 °C, rather than 95 °C, by incorporating only 1 wt % salicylic acid. Small effects of shear-induced heating during extrusion at the higher temperatures suggested that the acid acted as a lubricant and viscosity reducer. The latter was suggested to originate primarily from the salicylic-acid-induced reduction in the degree of protein aggregation/cross-linking, as indicated by size-exclusion high-performance liquid chromatography and chemiluminescence. Electron paramagnetic resonance spectroscopy on extruded films indicated that the beneficial effect of salicylic acid was due to its radical scavenging effect. Tensile tests on extrudates revealed that the materials produced at the substantially higher processing temperature were still ductile. The complex shear modulus increased more slowly with increasing salicylic acid content above 110−120 °C, indicating that the aggregation/cross-linking rate was slower with salicylic acid, that is, that it did have a scorch-retarding effect, besides yielding a lower final degree/complexity of aggregation.

Introduction

To make wheat-gluten-based polymers a competitive choice to commodity plastics, their processing properties have to be improved. Solid-state processing, including extrusion and compression molding, is, in general, faster and consequently more commercially interesting than solution-cast processes. In contrast to solution casting, where solvent type and pH are important parameters, the solid-state processability is affected by the mechanical energy input, shear stress level, and pressure.[1−10] The gluten processing window in compression molding and extrusion is narrow and needs to be expanded in order to make it possible to coextrude and laminate it with other polymers.[11] The lower temperature limit, ∼90 °C for glycerol-plasticized gluten, is set by the denaturation temperature. The upper limit is set by the increase in viscosity associated with extensive aggregation.[12,13] An important part of the aggregation is a reorganization of the intramolecular disulfide bonds to intermolecular disulfide bonds via thiol−disulfide exchange reactions.[14] Consequently, it should be possible to increase the upper processing temperature limit by limiting or delaying the disulfide reactions. Traditionally, this has been achieved in the rubber industry by the use of scorch retarders or prevulcanization inhibitors.[15] These are compounds, for example, radical scavengers, that interfere with the cross-linking reactions and either delay the onset of cross-linking or yield a slower cross-linking. In the present study, the scorch retarder technique has been transferred to the protein processing. Salicylic acid was chosen because it has been used in the rubber industry and it is a known radical scavenger.[16,17] It is also on the positive list for packaging applications according to EU Directive 2002172EEC. Further, it is a natural product existing in, for example, blackberries and raspberries.[18] Its germicide properties also make it promising in retaining the quality of the protein throughout its service life.[19,20] This issue was, however, outside the scope of the study.

Wheat gluten with glycerol was mixed with small amounts of salicylic acid, and the mixture was flat-film extruded. The rheological properties of the mixtures and packaging related properties of the films were determined. Size-exclusion high-performance liquid chromatography (SE-HPLC), chemiluminescence, and electron paramagnetic resonance (EPR) spectroscopy were used to reveal whether salicylic acid had any influence on the chemistry and aggregation/cross-linking of gluten during the processing.

Experimental Section

Materials. The wheat gluten (WG) powder was kindly supplied by Reppe AB, Lidköping, Sweden. The powder consisted of 84.8 wt % wheat gluten proteins, 8.1 wt % wheat starch, 5 wt % water, 1.34 wt % fat, and 0.76 wt % ash. The moisture content, as reported by the supplier, is measured on as received dried powder from the plant according to the Nordic Committee on Food Analysis standard method: NMKL no. 23 (1991). This implies measuring the mass loss during 16−18 h of heating at 102−105 °C. Glycerol with a purity of 99.5% was supplied by Karlshamns Tefac AB, Karlshamn, Sweden. Salicylic acid (SA) (99%) was obtained from VWR International. Wheat gliadin was purchased from Sigma Aldrich (G3375, CAS 9007-90-3).

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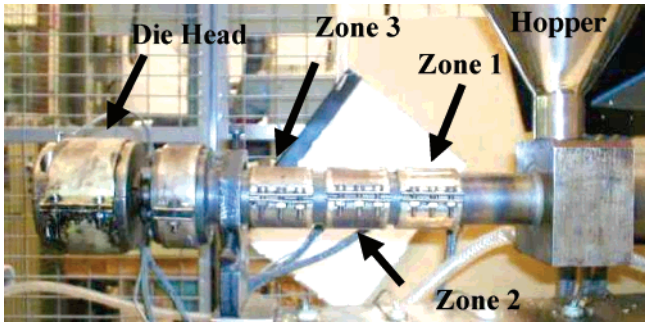

Figure 1. Single screw extruder. The die head and the different zones are equipped with heating mantles, with the set temperatures as described in Table 1.

Table 1. Temperature Profile of the Extruder[a]

	die head (°C)	zone 3 (°C)	zone 2 (°C)	zone 1 (°C)
extrusion of films with 0 wt % SA	95 (90)	90 (70)	75 (70)	60 (30)
extrusion of films with 1 wt % SA	135 (130)	70 (60)	60 (60)	30 (30)

[a] Values within parentheses are set temperatures.

Methods. *Material Preparation for Extrusion.* The WG powder was first conditioned for 3 days over blue gel in an oven (Cawell, Sweden) at 50 °C and 5% RH to yield a moisture content of 3 wt %, as determined by the moisture content measurements described below. The drying was necessary in order to avoid void formation in the extruded films. To obtain a homogeneous distribution of the SA in the material, 3−5 g of SA was first ground in a mortar with an equal amount of gluten powder before it was blended with the remaining WG powder. The powder and glycerol were subsequently blended using a food processor (WATT; DUKA AB, Sweden) at the lowest speed, "speed 1", for 1 min and thereafter at "speed 2" for 2 min. The amount used was 700 g of WG and 300 g of glycerol and an additional 0, 1, and 2 wt % SA. The dough was thereafter stored at room temperature at 30% relative humidity for 3 h in order to make it pelletizable. The moisture content in the dough was 4 wt % after storing. The impact of salicylic acid on the moisture content at these low humidities is insignificant. Pellets were made in a Moretto ML18/10C (Padova, Italy) granulator. In a separate experiment, it was concluded, by visual examination, that the SA aggregates/crystals were dissolved in glycerol at the actual extrusion temperatures.

Extrusion. Extrusion was performed directly after pelletization. The batch size was typically 1 kg. A single screw extruder (BX12, Axon, Sweden) (Figure 1) was used, equipped with a flat sheet die (45 × 0.7 mm) and a gateway screw with a 12.5 mm screw diameter, 6 mm root diameter, 11 mm flight, and L/D ratio of 26:1. The screw speed was 265 rpm, and the temperature profiles are given in Table 1. The actual temperatures were measured with an IR thermometer (MT4, Raytek, U.S.A.).

Tensile Testing. Dumbbell sized specimens (length and width of the narrow section were, respectively, 16.0 ± 1.0 and 4.0 ± 0.1 mm (ISO 37:1994(E)) were punched out along the extrusion direction from the extruded WG films and tensile tested at 50% RH and 25 °C. The specimens were conditioned in this environment for 1 h, 2 days, or 5 days, and the moisture content of the samples was subsequently determined. The tensile test apparatus was an Instron 5566 with a 10 kN load cell (Instron Corp. Ltd., MN), controlled by Merlin software (Merlin Software Service GmbH, Germany). The measurements were performed as described in ISO 527-37/E/120 with a crosshead speed of 100 mm/min. Under each set of conditions, 11−12 replicates were used.

Moisture Content. The loss of volatile mass was determined according to ASTM D 644-94. The test pieces were weighed and then stored for 24 h at 105 °C in a Nûve FN400 oven, supplied by LabRum Klimat AB, Sweden. The specimens were subsequently cooled in desiccators at 0% RH and 23 °C and then weighed to determine the loss of volatile mass. Five replicates from each sample were used.

Rheological Measurements. The rheological measurements were conducted on doughs prepared as described for the extrusion trials. The instrument used was a dynamic shear rheometer (Rheometrics RDAII, TA Instruments, U.S.A.), with 8 mm circular parallel plates with the temperature ranging from 50 to 200 °C, at a rate of 3 °C/min. The frequency was 0.286 rad/s, the peripheric shear strain was 0.2%, and the gap between the plates was 2 mm.

Chemiluminescence Measurements. The chemiluminescence data were recorded on Tohoku chemiluminescence equipment with a CLD-100 chemiluminescence (CL) detector and a CLC-10 CL counter (Tohokun Industrial Co., Japan). The CL sensitivity ranges from 280 to 650 nm with a maximum sensitivity between 400 and 450 nm. A circular dough sample, prepared according to the extrusion trials (∼4 wt % moisture), with a diameter of 12 mm and a thickness of 2 mm was put on an aluminum plate and placed in the oven. The samples were heated from 40 to 200 °C at a rate of approximately 24 °C/min, with a gas flow of 60 mL/min of either dry air or dry nitrogen. In the case of nitrogen, the samples were conditioned for 1 h before the measurement. Isothermal measurements were also made at 135 °C.

Protein Solubility. Twenty grams of dough, prepared as for extrusion, was hot-pressed into films at 110 and 130 °C for 3 min at 900 bar using a Schwabenthan Polystat 400s (Schwabenthan-Maschinen GmbH & CO. KG, Germany). The choice of pressing rather than extruding was based on the idea of having a better temperature control and also to be able to compare with previous data. The amount and size distribution of proteins in the films were determined using a three-step extraction procedure according to Gällstedt et al.[21] and size-exclusion high-performance liquid chromatography (SE-HPLC). Proteins soluble in dilute sodium dodecyl sulfate (SDS) were extracted in the first step, proteins soluble in SDS after a short sonication were extracted in the second step, and additional proteins were extracted in SDS with repeated sonication.[21]

In the first step, 16.5 mg of each film was suspended in 1.5 mL of 0.5% SDS−phosphate buffer (pH 6.9) and vortexed for 10 s. The suspension was then stirred for 5 min at 2000 rpm and centrifuged for 30 min at 8160 *g* to obtain the supernatant protein. In the second step, the pellet was resuspended in SDS buffer and sonicated in an ultrasonic disintegrator (Soniprep 150, Tamro, Mölndal, Sweden) for 30 s, amplitude 5, fitted with a 3 mm exponential microtip. The samples were then centrifuged (30 min, 8160 *g*) to obtain a supernatant of proteins. For the third step, the pellet was again resuspended in the SDS buffer and sonicated for 30 + 60 + 60 s.

The extracts were filtered through 0.45 mm filters (Millipore, Durapore Membrane Filters) before the SE-HPLC operation. SE-HPLC analyses were performed on a Waters HPLC system using a BIOSEP SEC-4000 Phenomenex column. Separation was obtained during 30 min by loading 20 μL of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluoroacetic acid at a flow rate of 0.2 mL min^{-1}. Proteins were detected by UV absorption at 210 nm. The amount of proteins extracted after each extraction step was normalized to the protein solubility of unprocessed wheat gluten, in which 100% of the proteins were assumed to have been extracted after the last extraction step. The SE-HPLC chromatograms were divided into large "polymeric" protein and small "monomeric" protein fractions, the latter containing also peptides and amino acids.[22]

Electron Paramagnetic Resonance (EPR) Spectroscopy. Samples were collected immediately after the flat extrusion die, cut into small rectangles, and placed into EPR glass containers. These were immediately transferred to an isopentane bath kept close to its freezing point (−160 °C) and then transferred to a liquid nitrogen container. The complete cycle lasted for approximately 2 min. EPR spectra (first derivatives) were recorded for the frozen samples using a Bruker ELEXSYS 500 instrument at 90 K (2 mW microwave power and 0.5 mT modulation amplitude). The g value is calculated from the relationship $h\nu = g\beta B$, where h is Plank's constant (6.63×10^{-34} J s), ν is the microwave frequency (9.4 GHz, measured by a frequency counter), β is the Bohr magneton (9.27×10^{-24} A m^2), and B is the

Figure 2. Wheat gluten film extrudate without salicylic acid. The bar is 5 cm long.

Figure 3. Wheat gluten film extrudate with 1 wt % salicylic acid. The bar is 5 cm long.

magnetic field (G). Spectra were recorded on three film samples cut from extrudates from two different extrusion runs.

Results and Discussion

Extrusion. With only glycerol and gluten present, the extruded films were uneven and contained discontinuities such as holes and cracks (Figure 2). Nevertheless, the extrudability, that is, the smoothness and film continuity, increased with increasing glycerol content up to a level of 32 wt %. Higher concentrations yielded granulates which were too sticky to pass through the hopper.

As shown in Table 1, the actual temperatures were significantly higher than the set values; that is, the shear-induced heating led to a temperature increase of 20−30 °C. In fact, Morel et al.[13] and we ourselves have shown that the shear effect makes it possible to extrude gluten with no or only a small heat input.

The films became softer, more flat, and more continuous when salicylic acid was added (Figure 3). The frictional heat generated inside the barrel decreased, and thus, the differences between the actual and set temperatures decreased as well (Table 1). For the materials with 2 wt % SA, or higher, the actual temperature was approximately the same as the set temperature. The die-head temperature was set to 130 °C to increase the viscosity of the extrudate. If this was omitted, the film leaving the extruder was a soft dough. It is possible that the lowering of the pH in the presence of salicylic acid is responsible for the observed effects. SA was therefore replaced by the same amount of sodium acid pyrophosphate ($Na_2H_2P_2O_7$), which is acidic but not a radical scavenger. The extrudate quality was poorer with $Na_2H_2P_2O_7$; in fact, it was similar to that of the first films without SA. In addition, $Na_2H_2P_2O_7$ films were significantly stiffer and more brittle then the SA films. The reason for the differences was not due to moisture, since the SA and $Na_2H_2P_2O_7$ film moisture contents were similar. Thus, it is ruled out that a low pH, by itself, is responsible for the easier processing with SA.

Tensile Properties of WG Film Extrudates. It is important to know the mechanical integrity of the extrudate to be able to succeed with subsequent converting operations in a future wheat gluten plastic processing plant. The tensile properties of the extruded films were therefore measured directly after extrusion (1 h) and after 2 and 5 days (Table 2). The aging at 50% RH led to a significant loss in stiffness and strength and an increase in ductility, during the 5 day period. The extruded samples had been dried before processing, and they therefore absorbed moisture when stored at 50% RH (Table 2). Any change in the degree of aggregation during the 5 day period would therefore probably be hidden behind the effect of moisture uptake. The presence of SA also led to a small but significant increase in the moisture content (Table 2). This was unexpected, since SA is less hygroscopic than glycerol, which it partly replaced.[23] A small part of this increase originated from a loss of SA during the drying at 105 °C when performing the moisture content measurement. Measurements on pure salicylic acid and salicylic acid dispersed in glycerol and/or water indicated that its loss during 24 h of drying was between 10 and 20% of its initial weight. A part of the moisture increase was possibly due to a more extensive gelling and water binding capacity of the starch component in the SA-containing samples, since they were extruded at a significantly higher temperature than the SA-free samples.[24] The reason why the SA-free extrudates had a higher Young's modulus and fracture stress, than the SA-containing materials, was probably due to the lower moisture content in the former. The most important information in Table 2 was that the large improvement in gluten processability induced by SA was not accompanied by any critical loss in mechanical integrity. It must be remembered that the SA-containing extrudates were exposed to higher processing temperatures than the SA-free materials, resulting in a higher degree of aggregation. This may have been the reason for the lower fracture strain of the SA-containing films.

Protein Solubility. The improvement in processability could be due to lubricating, plasticising, and/or chemical effects. The chemical effects were first investigated by determining the degree of protein aggregation/cross-linking. Figure 4 shows that the amount of soluble proteins, both monomers and polymers, decreased with increasing process temperature as a consequence of heat-induced aggregation.[21] Figure 4 also shows that SA increased significantly the amount of soluble proteins at 135 °C, whereas its effect was small at 110 °C. The latter is explained by the fact that extensive aggregation in the SA-free samples occurs above 110 °C, as observed by, for example, the rheological measurements below. Consequently, SA is expected to be effective mainly above 110 °C. The fact that the protein solubility already after the SDS treatment was higher in the presence of SA suggests that SA reduced primarily the degree/complexity of aggregation and/or the amount of disulfide

Table 2. Tensile Properties and Moisture Contents of Wheat Gluten Film Extrudates, Stored at 25 °C and 50% RH for 1 h, 2 Days, and 5 Days[a]

	Young's modulus (MPa)			fracture stress (MPa)			fracture strain (%)			moisture content (wt %)		
wt % SA	0	1	2	0	1	2	0	1	2	0	1	2
1 h	36.7 A	30.5 B	27.0 B	3.0 A	1.8 B	2.0 B	50.8 D	53.9 D	47.3 D	9.4 F	11.2 E	12.5 D
2 days	8.7 C	2.5 D	5.6 C D	1.5 C	0.9 E	1.2 D	134.5 B	141.7 B	98.3 C	12.2 D E	14.1 C	15.5 B
5 days	5.5 C D	2.4 D	3.2 D	1.3 D	0.9 E	1.0 E	159 A	147 A B	104.1 C	14.3 C	15 B	17.6 A

[a] The numbers connected with the same letter are, based on an all-paired student's t test ($p \leq 0.05$), not significantly different.

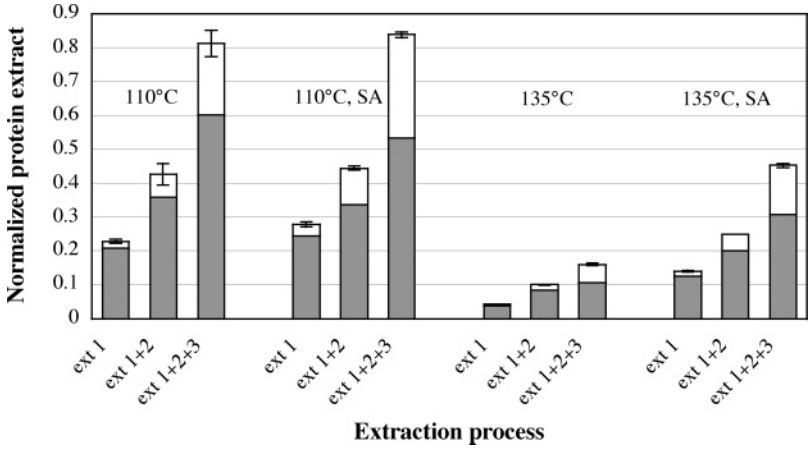

Figure 4. Normalized protein solubility, that is, the ratio of the protein solubility of the film to the protein solubility of the unprocessed wheat gluten exposed to a solution of SDS (ext 1), SDS solution and 30 s sonication (ext 2), and SDS and 150 s (30 s and 60 + 60 s) sonication (ext 3) treatments.[20] The gray and white parts represent, respectively, soluble monomers and polymers. The amount of SA was 2 wt %.

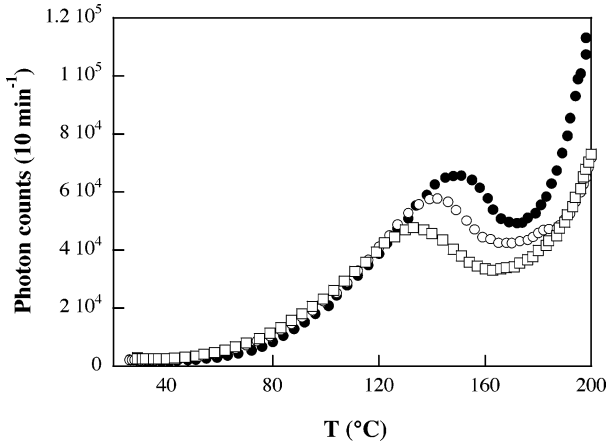

Figure 5. Chemiluminescence signal as a function of temperature for wheat gluten−glycerol samples in air containing 0 wt % SA (●), 1 wt % SA (○), and 2 wt % SA (□).

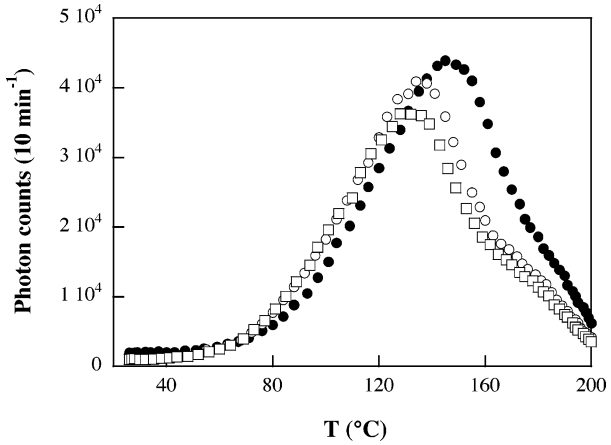

Figure 6. Chemiluminescence signal as a function of temperature for wheat gluten−glycerol samples in nitrogen containing 0 wt % SA (●), 1 wt % SA (○), and 2 wt % SA (□).

exchange reactions involved in the cross-linking. This is concluded since it is known that SDS does not break covalent bonds but rather breaks primarily hydrophobic and hydrophilic secondary bonds.[25] As expected, sonication, which is expected to break, apart from secondary bonds, mainly disulfide bonds,[25] increased the protein solubility (both monomers and polymers) in all materials. To conclude, SE-HPLC revealed that SA reduced the degree/complexity of protein aggregation at temperatures where extensive aggregation would otherwise occur.

Chemiluminescence Measurements. If salicylic acid acts as an antioxidant or radical scavenger in the protein and captures, for example, thiyl radicals, it would be possible to observe this with chemiluminescence. Unfortunately, the WG system is very complex and several reactions may be chemiluminescent.[26,27] These include fat peroxidation and maillard reactions. It is known that several amino acids in gluten oxidize easily, including tyrosine, tryptophan, methionine, proline, and cysteine.[26−28] Of these, tyrosine and tryptophan are strongly phosphorescent during oxidation. Cysteine, which is involved in protein polymerization/cross-linking, is only weakly chemiluminescent during oxidation at room temperature, but the oxidation may be sufficiently chemiluminescent to be detected during heating.

The wheat gluten film chemilumiscence intensity during heating is shown in Figures 6 and 7. For samples without SA, there is a peak in CL intensity in the vicinity of 150 °C. This low-temperature peak is observed both in air and after 1 h in nitrogen (Figures 5 and 6). However, the increase in CL intensity above 170 °C was suppressed in nitrogen. This indicated that the observed increase above 170 °C, which seems to be the upper part of a continuous exponential type of CL signal−temperature curve, overlapping the low-temperature peak, was due to a thermooxidative process in the wheat gluten film.

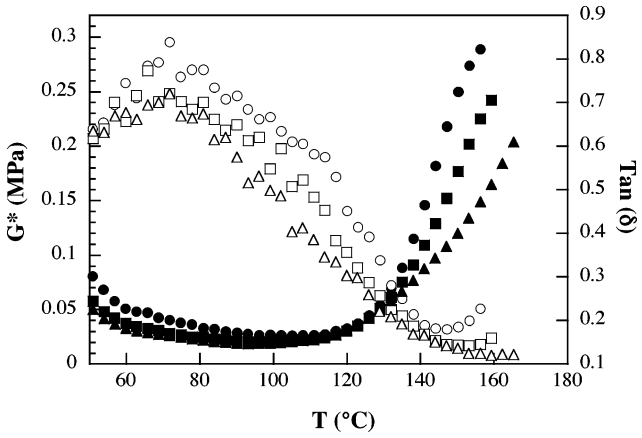

Figure 7. Complex shear modulus (G^*) as a function of temperature for wheat gluten−glycerol samples containing 0 wt % SA (●), 1 wt % SA (■), and 2 wt % SA (▲). Also given is the tan(δ) for wheat gluten films with 0 wt % SA (○), with 1 wt % SA (□), and with 2 wt % SA (△). The standard deviations based on duplicate runs were for G^* ±0.02 MPa and for tan(δ) ±0.04.

Interestingly, the effect of adding the salicylic acid was to reduce both the low-temperature peak and the thermooxidative process at higher temperature (cf. Figures 5 and 6). Isothermal CL experiments in air, performed on wheat gluten doughs inserted at 135 °C into the CL chamber, revealed a narrow peak at short times (within 2 min), similar to the low-temperature peak in Figure 5, and an overlapping broad peak/shoulder with a maximum/plateau at longer times, associated with the high-temperature peak in Figure 5. The origin of the low-temperature peak was difficult to explain. The peak was reduced significantly after 24 h and even more after 48 h of nitrogen treatment. Consequently, it appeared to depend on residual oxygen in the film. Treatment for 24 h in air and 1 h in nitrogen yielded a smaller peak than treatments for only 1 h in nitrogen. Thus, aging after film preparation seemed to lower the peak. The position of the peak, its reduction with salicylic acid, and its dependence on aging suggested that this peak corresponded to aggregation and polymerization/cross-linking following the protein denaturation. It also showed that the process, at least to a certain extent, required oxygen. Hence, chemiluminescense also showed that SA reduces the degree of aggregation and cross-linking. The most important oxidative process that leads to protein polymerization and cross-linking is the disulfide rearrangement through thiyl and thiol intermediates.[13] It is probable that SA traps the intermediate sulfur products, at least for some time. The large effect of only 1−2 wt % SA is not surprising considering that it equals 120−240 μmol of SA molecules per gram of pure wheat gluten and that the corresponding total amount of thiols and disulfides is on the order of 90−170 μm/g.[13,29] To rule out that the CL signal originates from lipid peroxidation, pure gliadin was tested in nitrogen and the same type of CL signals was observed as for wheat gluten. Thus, the observed CL behavior was not attributed to the small amount of lipids present in wheat gluten.

Rheological Measurements. The rheological data of pure and SA-containing gluten are shown in Figure 7. The complex shear modulus (G^*) decreased with increasing temperature between 50 and approximately 100 °C. A maximum in damping, in the same interval, was observed at ∼70 °C, corresponding to the denaturation temperature.[9] The increase in G^* starting at 110−120 °C was associated with the onset of protein aggregation/cross-linking. Interestingly, the slope in G^* after 120 °C decreased with increasing SA content. This is attributed to a reduced rate of aggregation/cross-linking induced by SA, and thus a scorch-retarding effect.

Electron Paramagnetic Resonance (EPR) Spectroscopy. Typical EPR spectra for SA-free and SA-containing extrudates are presented in Figure 8. It was striking that the spectra from the different runs were basically identical and that they had the same features. Samples heated to room temperature and kept there for 5 min and cooled to 90 K again showed the same EPR spectra as the unheated samples. Evidently, the EPR signals corresponded to relatively stable free radicals. Any rapidly recombining free radicals would obviously have been lost before freezing the extrudates after extrusion. It was not possible to determine the total amount of free radicals in the extrudates, since it could not be ensured that all EPR signals were actually in the g range investigated. Nevertheless, in the g range studied, it was observed that the largest EPR signal was due to R−N• or R−NO• radicals ($g \approx 2.005$). The signal intensity was larger for the SA-free extruded films, indicating that SA acted as a nitrogen or nitroxyl radical scavenger. The SA-free extrudate showed several small signal "peaks" in the interval $g = 2.03$−2.09, whereas the SA extrudate signals were less pronounced.

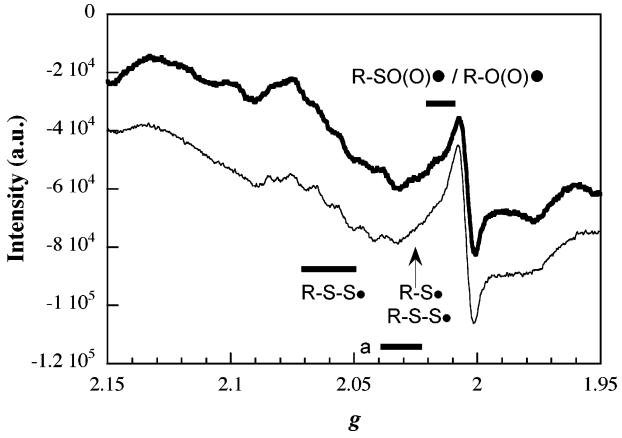

Figure 8. Electron paramagnetic resonance (EPR) spectra of extrudates with (thick line) and without (thin line) 2 wt % SA. "a" refers to R−S•−S−R, (R−S−S−R)*+, (R−S−S−R)*−, or R−S•−SH−R.[30]

It is known that sulfur centered radicals have signals in this interval.[30] Signals from thiyl radicals formed by hydrogen abstraction from cysteine or scission of disulfide bonds should be observed at $g \approx 2.025$.[30] Figure 8 shows that these radicals were either absent or their signal was hidden in the broad R−N•/R−NO• signal. This was also the case for peroxyl or sulfoxyl radicals at $g = 2.01$−2.02.[30] Signals at $g = 2.024$ and 2.05−2.07 are attributed to R−S−S• species which can be formed by scission of the C−S bond during extrusion. Signals at $g = 2.024$−2.038 correspond to species formed by recombination of two R−S• radicals, R−S•−S−R, oxidation or reduction of disulfide compounds, (R−S−S−R)*+ or (R−S−S−R)*−, or splittings of a single line from protonated disulfides, R−S•−SH−R.[30] The extrudate without salicylic acid showed more pronounced signal peaks in the latter region (marked by an "a" in Figure 8) and also in the R−S−S• region compared to the SA-containing extrudate. It is thus suggested that salicylic acid scavenges, besides nitrogen or nitroxyl radicals, also sulfur radicals and that this prevents extensive cross-linking during extrusion.

Conclusion

The less drastic increase in complex shear modulus with increasing temperature above 110−120 °C, as well as the increase in upper process temperature, indicated that salicylic acid had a scorch-retarding effect on gluten. SE-HPLC and chemiluminescence also indicated that the final degree of aggregation/cross-linking was lower in the presence of salicylic acid in those films that were exposed to a temperature (135 °C) where extensive aggregation would normally occur. Consequently, the effect of SA was small at 110 °C. EPR spectroscopy indicated that a salicylic-acid-induced lowering in cross-link density was due to radical scavenging effects. Mechanical data revealed that the films extruded at the higher temperature in the presence of salicylic acid were still ductile.

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Glossary

CL = chemiluminescence
EPR = electron paramagnetic resonance
RH = relative humidity
SA = salicylic acid
SDS = sodium dodecyl sulfate
SE-HPLC = size-exclusion high-performance liquid chromatography
WG = wheat gluten

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