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ORIGINAL PAPER

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Control of polyphenol oxidase in whole potatoes by low temperature blanching

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Abstract Russet Burbank potatoes can be blanched up to 60 min at 50 °C without any loss in firmness and without appearance of browning on their peels, eyes or infected areas. Low temperature blanching (LTB) for 45 min did not cause a significant reduction in crude polyphenol oxidase (PPO) activity (12%). However, when heating time was extended to 60 min, the activity and specific activity of the enzyme were reduced by 27–45% and 22–43% respectively. The remaining enzyme extracted from heated potatoes was partially purified 3.3–3.75-fold by 0–95% ammonium sulphate precipitation and dialysis, and its kinetic parameters were determined. The comparison of the kinetic parameters of PPO in control (K_m=10.3 mM and V_{max}/K_m=0.15) and in 60 min heated potatoes (K_m=13 mM and V_{max}/K_m=0.054) indicated the reduced affinity of the remaining enzyme to its substrate. Thus, LTB at 50 °C for 60 min not only inactivated part of the PPO activity but also reduced the kinetic capacity of remaining enzyme. Extending heating time to 75 min caused the appearance of slight browning on the peels and eyes of potatoes and reduced their firmness. The observed browning was due to the sharp drop in the K_m that caused the activation of the PPO. Heating at 50 °C did not affect lipoxygenase activity, but the K_m of the enzyme dropped from 0.4 to 0.15 mM and the enzyme became kinetically more reactive at low substrate concentrations. Covalently bound pectin methylesterase considerably activated (38%) by heating and this caused the drop of pH in potato tissues.

Keywords Blanching · Potatoes · Polyphenol oxidase · Lipoxygenase · Pectin methylesterase

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Introduction

Enzymatic browning catalysed by polyphenol oxidase (PPO) is a major problem in the minimal processing of pre-peeled or sliced potatoes. To eliminate this problem, sulphites have been used successfully, but due to their adverse health effects, various alternative chemicals such as ascorbic or erythorbic acids, citric acid, L-cysteine, etc. have been employed in place of sulphites to control browning [1, 2, 3]. Although, these chemicals retard the browning and extend the shelf life of processed potatoes, they are less effective than sulphites. Therefore, to obtain the desired shelf life, many workers have suggested the application of sulphite alternatives in combination with some supportive treatments. Some of these treatments include: removing PPO and phenolic-rich surface tissues of pre-peeled potatoes by lye digestion before inhibitor application [4], packaging under nitrogen atmosphere in some acidic cover solutions [1], reducing inhibitor solution pH by inorganic acids [2] or dipping into heated inhibitor solutions [5].

Low temperature blanching (LTB) of whole potatoes prior to minimal processing may also be a potential treatment to control enzymatic browning in sliced or pre-peeled potatoes and to support sulphite alternatives. LTB between 40–50 °C was applied to whole apples by Kim et al. [6] and it was reported to reduce the tendency of browning in apple slices successfully. These workers did not investigate the effect of LTB on PPO activity. However, assuming that the enzyme cannot be inactivated at such low heating temperatures, they attributed the reduced browning rate in apple slices to the in situ modification of PPO's kinetic properties during heating. Valle et al. [7] also applied LTB to apple pieces between 40 and 65 °C prior to minimal processing and effectively reduced their PPO activity (30–87%). Yemenicioğlu et al. [8] investigated the heat stability of PPO from six different apple cultivars in buffer solutions and reported that the enzyme was extremely heat stable between 68 and 78 °C and activated by heat. Thus, it seems that the in situ effect of low temperatures on PPO enzyme is different and cannot be explained simply by thermal inactivation.

Compared to apples, potatoes are more resistant to heat injury. Therefore, LTB of whole potatoes has been applied to improve their firmness and to increase their resistance to physical breakdown during further processing [9]. Also, LTB may be applied to enhance the storability of potatoes and to prevent their sprouting and spoilage due to invasion by bacterial and fungal pathogens [10]. This study was carried out primarily to investigate the effects of LTB on the activity and kinetic properties of potato PPO. The effects of heating on lipoxygenase (LOX) and pectin methylesterase (PME) were also investigated, to estimate possible changes in the flavour and firmness of heated tubers. The results of this work are a contribution to the studies on extending the shelf life of minimally processed potatoes.

Material and methods

Materials. Russet Burbank potatoes were obtained from a potato processing plant in Bolu, Turkey. The average length, thickness and weight of potatoes were 109.2±6.1 mm, 45.9±4.6 mm and 164.9±22.7 g respectively. The dialysis tubing (D-9527, prepared as described in product information), citrus pectin (galacturonic acid content 79%, methoxy content 8%), linoleic acid (99%) and bovine serum albumin were purchased from Sigma. Ammonium sulphate (for biochemistry) and catechol were purchased from Merck. All the other chemicals were reagent grade.

Heating temperature. For the determination of a suitable heating temperature, the potatoes were put into perforated cloth sacks and blanched in tap water at 50, 55 or 60 °C for 45, 60 or 75 min using a circulating waterbath (Polyscience, Model 71). In all studies, almost 2 kg potatoes were blanched in 25 l tap water. The heating profiles of several potatoes were monitored by placing thermocouples in their geometric center and by using a portable temperature recorder (Cole Parmer, DualLogR). The blanched samples were cooled in cold water, drained, halved longitudinally and examined for their firmness by using a Nippon fruit hardness tester-5 kg (Model FHR-5) equipped with a cone type tip (base diameter, 12 mm; height, 10 mm). The penetrations were carried out on the cut surfaces of each potato half at 18 points (≅1 cm spaced points designed to form three rows and six columns). After heating, cooling and halving, some of the potatoes were left for almost 1 h at room temperature and they were examined carefully to detect possible browning due to heat injury.

Enzyme extraction and partial purification. Acetone powder was used as enzyme source in this study. For the preparation of acetone powder, 200 g sliced cold potatoes were added to a Waring blender containing 300 ml acetone at –18 °C and 1 g polyvinylpolypyrrolidone (PVPP). The mixture was homogenised for 2 min and filtered through a Buchner funnel containing a Whatman No 1 filter paper. This procedure was repeated twice using 200 ml cold acetone and the powder obtained was left overnight to evaporate the acetone. This powder was kept in a deep freezer and used in all enzyme extractions. For enzyme extraction, two different extraction mixtures were used. Extraction mixture 1 (EM1) was prepared by mixing 2 g acetone powder, 0.4 g PVPP and 50ml of cold 8.8% NaCl solution. Extraction mixture 2 (EM2) was prepared by mixing 2.5 g acetone powder, 0.5 g PVPP and 60ml of cold 8.8% NaCl solution. The extractions were carried out under magnetic stirring for 3 h at 4 °C and the slurries obtained were filtered through six layers of cheese cloth and centrifuged at 4 °C and 11,000 *g* for 20 min. These crude extracts contained soluble+ionically bound enzymes. Covalently bound enzyme extracts were prepared for the assay of PME enzyme activity only. For this purpose the slurries obtained after enzyme extraction were centrifuged at 1200 g for 20 min and the collected pellets were resuspended in cold deionised water.

For partial purification the following procedure was applied unless otherwise stated. The enzyme extracts were slowly brought to 0–95% saturation with solid $(NH_4)_2SO_4$, mixed gently for 1 h at 4 °C and then centrifuged at 4 °C and 15,000 *g* for 45 min. The collected pellets were dissolved in cold deionised water and dialysed for 24 h at 4 °C against 2×2000 l deionised water.

Enzyme assays. A Shimatzu (Model 1601) spectrophotometer, equipped with a constant temperature cell holder, was used in spectrophotometric assays. All the assays were conducted at 30 °C constant temperature. PPO activity was determined at 420 nm by using the following reaction mixtures: reaction mixture 1 (RM1), 2 ml of 0.01 M sodium phosphate buffer at pH 7.0, 0.2 ml of 0.25 M catechol and 0.3 ml of enzyme extract; and reaction mixture 2 (RM2), 2ml of 0.01 M Na-phosphate buffer at pH 7.0, 0.4 ml of 0.06 M catechol and 0.2 ml enzyme extract. LOX activity was determined at 234 nm using the following reaction mixture: 2 ml of 0.01 M sodium phosphate buffer (pH 7.0), 0.05 ml linoleic acid solution prepared with Tween-20 as described in Rackis et al. [11] and 0.1 ml enzyme extract. This reaction mixture was named as RM1a or RM1b when the concentrations of linoleic acid were 14.3 or 18.9 mM, respectively. PME activity was assayed at 620 nm as described in Hagerman and Austin [12]. The reaction mixture contained 2 ml of 0.35% pectin solution prepared in 0.1 N NaCl, 0.3 ml of 0.01% (w/v) bromothymol blue prepared in 0.003 M sodium phosphate buffer at pH 7.5, and 0.3 ml enzyme extract. The activity of PME was also assayed by the titrimetric method given in Yemenicioğlu and Cemeroğlu [13]. The reaction mixture contained 4 ml (or 8 g suspension in the assay for covalently bound PME) enzyme extract and 18 ml of 0.35% pectin solution prepared in 0.1 N NaCl. The pH of reactants were brought to 7.5 with 0.1 N NaOH solution before each assay and the titrations were carried out manually for 10 min by using 0.01 N (or 0.003 N in the assay for covalently bound PME) NaOH solution. The titrimetric assays were conducted in a double walled, magnetically stirred cell connected to a circulating water bath working at 30 °C. The activities of enzymes were expressed as units (U) and units per milligram protein. In spectrophotometric assays, 1 U was defined as 0.001 change in absorbance/min/ml enzyme extract. In titrimetric assays, 1 U was defined as the number of micromoles carboxyl liberated in 10 min per millilitre (or per gram for the covalently bound PME) of enzyme extract. An average of 10 or 15 measurements were used to find crude enzyme activity when no purification was applied. In partial purification studies an average of 3–5 measurements were used to find crude and partially purified enzyme activities.

Enzyme kinetics. Michaelis constants (K_m) and maximum velocities (V_{max}) of enzymes were determined using the double reciprocal plots. To reduce the final concentrations of substrates in cuvettes into the neighbourhood of the K_m of PPO and LOX, the concentrations of catechol (0.025–1 M) and linoleic acid (2.84–18.6 mM) solutions were selected carefully. At each substrate concentration, 4–5 and 5–7 measurements were conducted at high and low substrate concentrations, respectively.

pH and conductivity measurements. The pH and conductivities of extracts obtained from heated potatoes were determined, to detect the action of PME and electrolyte leakage due to membrane damage, respectively. For the preparation of extracts, 5 g ground tissue, obtained from the centre of a single potato, was mixed with 350 ml of deionised water. The mixture was stirred for 5 min with a magnetic stirrer and filtered through six layers of cheese cloth. For each heating time, this procedure was applied to at least five potatoes. The measurements were carried out by a WTW-multi parameter (Inolab, Level-3).

Protein content. Protein was determined by the method of Lowry using bovine serum as a standard [14]. The protein standards of crude and partially purified extracts were prepared in 8.8% NaCl solution and deionised water, respectively. All assays were carried out at least five times.

Table 1 Determination of time/temperature limits for low temperature blanching

Sample number	Temperature (°C)	Time (min)	Firmness (kg)	Number of penetrations	Browning
1 (Control)	–	–	2.52±0.12	719	–
1	50	45	2.53±0.12	362	No
1	50	60	2.53±0.12	362	No
1	55	45	2.48±0.10	362	Slightly
1	55	60	2.51±0.10	362	Slightly
1	60	45	2.45±0.11	362	Apparent
1	60	60	2.44±0.13	362	Apparent
2 (Control)	–	–	2.48±0.12	575	–
2	50	45	2.50±0.10	575	No
2	50	60	2.50±0.12	575	No
2	50	75	2.44±0.14	575	Slightly
3 (Control)[a]	–	–	2.54±0.13	160	–
3	50	60	2.57±0.13	160	No
3	50	90	2.61±0.11	160	No
4 (Control)[a]	–	–	2.47±0.13	128	–
4	50	45	2.49±0.12	128	No
4	50	60	2.52±0.12	128	No
4	50	90	2.57±0.11	128	No
4	50	120	2.52±0.10	128	Slightly

[a] The results obtained for local cultivars

Results and Discussion

Heating temperature

Heating at 55 °C and 60 °C caused slight and obvious reductions in the internal firmness of potatoes, respectively (Table 1). Also, after cooling, various degrees of browning were observed on peels, eyes, infected areas and injured areas of these potatoes. In contrast, heating at 50 °C for 45 or 60 min increased the internal firmness of potatoes slightly and caused no browning. This result was confirmed by repeating the heating studies at 50 °C with some new samples (No: 2). Also, this time we tried 75 min heating. However, 15 min more heating at this temperature reduced the internal firmness of potatoes and caused slight browning on their peels. Thus, for Russet Burbank potatoes used in this study, the upper limit of heating at 50 °C was between 60 and 75 min. The effect of heating at 50 °C was also tested for two local cultivars (from Ödemiş, İzmir). In these potatoes, the firming effect pronounced and the appearance of browning and/or softening was not observed before 90 min. Because of the differences in heating conditions, it is hard to compare our results with those of the previously conducted studies. However, the firming effect of 50 °C heating was also reported by Mittal [15] who heated whole potatoes at five different temperatures between 30 and 90 °C. The same author reported extensive thermal softening above 60 °C. Ranganna et al. [10] investigated hot water dipping of whole potatoes to enhance their storability and reported heat damage above 57.5 °C. In contrast, these researchers detected no signs of heat injury in potatoes heated at 57.5° or 52.5 °C for 30 min. Also, in their detailed review, Andersson et al. [9] reported that no reduction in potato tensile strength (minimum longitudinal stress required to pull a section of potato tuber tissue apart) or denaturation of cell wall membranes occurred when potatoes were heated at 50 °C or at lower temperatures. Thus, we decided that 50 °C would be a suitable temperature for our purpose.

Effect of heating on PPO

The effect of LTB at 50 °C on remaining PPO activity in potatoes was compared for different extraction and assay conditions (Table 2). When crude enzyme extracts were prepared using EM1 and assayed using RM1, in which the final substrate concentration (20 mM) was higher than the K_m of control, no significant inactivation occurred in potatoes heated for 45 min. Therefore, we concentrated on 60 min heating which caused 27% loss of PPO activity in potatoes under the same extraction and assay conditions. When enzyme was extracted using EM2 and assayed using RM2, in which final substrate concentration (9.2 mM) was close to the K_m of control, more than 40% inactivation occurred in the activity and specific activity of crude PPO. The observed increase in percentage inactivation was apparently due to the higher and lower PPO activity in control and heated potatoes under the changed conditions, respectively. During heating, it took almost 45 and 60 min to reach 49 and 50 °C at the centre of potatoes, respectively (Fig. 1). Thus, it is apparent that partial PPO inactivation occurred in 15 min when the whole potato temperature was between 49 and 50 °C. The inactivation of part of the PPO activity at such a low heating temperature was not surprising. This is because potatoes contain multiple forms of PPO enzyme [16], of which some are extremely heat labile [17].

To obtain more information about the kinetic characteristics of remaining PPO in heated potatoes, we determined the K_m and V_{max} of partially purified enzymes

Table 2 Activity and kinetic parameters of polyphenol oxidase from control and low temperature blanched potatoes. *EM* Extraction mixture, *RM* reaction mixture

Sample	Heating time at 50 °C (min)	Activity (U)	Specific activity (U mg^{-1})	Recovery (%)	Purity (Fold)
Crude extract-I (EM1, RM1)	Control	391±19.5	416±21.0	–	–
	45	343±14.9 (–12%)[a]	404±17.5 (–3%)	–	–
	60	284±13.6 (–27%)	323±15.4 (–22%)	–	–
	75	329±13.7 (–16%)	354±14.7 (–15%)	–	–
Crude extract-II (EM2, RM2)	Control	451±5	570±7	100	1
	60	249±7 (–45%)	323±10 (–43%)	100	1
	75	279±25 (–38%)	467±42 (–18%)	100	1
0–95% $(NH_4)_2SO_4$ precipitation and dialysis of crude extract-II	Control	772±18	1897±44	171	3.3
		K_m=10.3 mM; V_{max}=1.58Δabs. $min^{-1}ml^{-1}$; V_{max}/K_m=0.15			
	60	304±16	1211±63	122	3.75
		K_m=13.0 mM; V_{max}=0.7Δabs.$min^{-1}ml^{-1}$; V_{max}/K_m=0.054			
	75	438±15	1710±57	156	3.6
		K_m=8.8 mM; V_{max}=0.85Δabs.$min^{-1}ml^{-1}$; V_{max}/K_m=0.1			
0–90% $(NH_4)_2SO_4$ precipitation and dialysis of crude extract-I (EM1, RM2)	Control	874±6	2081±15	–	–
		K_m= 11.5 mM; V_{max}=1.92Δabs.$min^{-1}ml^{-1}$; V_{max}/K_m=0.17			
	75	490±24	1065±52	–	–
		K_m=6.8 mM; V_{max}=0.84Δabs.$min^{-1}ml^{-1}$; V_{max}/K_m=0.12			

[a] Indicates the percentage change in activity or specific activity as compared to the corresponding control

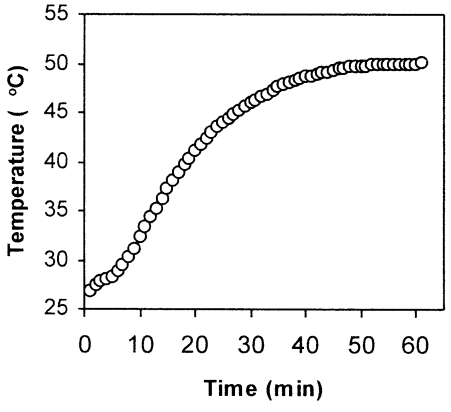

Fig. 1 Heat penetration curve of potatoes at 50 °C

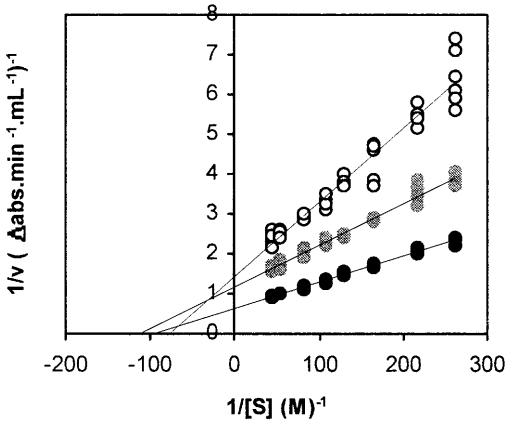

Fig. 2 Double reciprocal plots for partially purified polyphenol oxidase from control and low temperature blanched potatoes. *Grey circles* 50 °C/75 min, *white circles* 50 °C/60 min, *black circles* control

(Table 2). LTB potatoes at 50 °C for 60 min caused some desired changes in the kinetic parameters of the enzyme. The increase in the K_m value and the reduction in the V_{max}/K_m value clearly indicated the reduced affinity of the residual enzyme to its substrate (Fig. 2). Thus, LTB at 50 °C for 60 min not only inactivated part of the PPO activity, but also reduced the kinetic capacity of the remaining enzyme. These positive effects of LTB may increase the shelf life of minimally processed potatoes by reducing their tendency of browning and/or by delaying the exhaustion of added antioxidants such as ascorbic acid and its derivatives. Our results confirmed the assumptions of Kim et al. [6] who subjected whole apples to LTB at 40, 45 and 50 °C and obtained less tendency of browning in apple slices. These researchers did not investigate the effect of LTB on PPO activity, but attributed the reduced browning rate in apple slices to the in situ kinetic changes in the PPO enzyme. The change occurred in the kinetic properties of enzyme at a relatively low temperature may be explained simply by the inacti-

Table 3 Activity and kinetic parameters of lipoxygenase from control and low temperature blanched potatoes. EM Extraction mixture, RM reaction mixture

Sample	Heating time at 50 °C (min)	Activity (U)	Specific activity (U mg^{-1})	Recovery (%)	Purity (Fold)
Crude extract-I (EM1, RM1a)	Control	4853±220	5168±234	–	–
	45	4938±218 (+2%)[a]	5816±256 (+13%)	–	–
	60	4395±180 (–9%)	5000±205 (–3%)	–	–
	75	4559±189 (–6%)	4907±204 (–5%)	–	–
Crude extract-II (EM2, RM1b)	Control	5213±125	6591±158	100	1
	60	4718±166 (–9%)	6135±216 (–7%)	100	1
0–95% $(NH_4)_2SO_4$ precipitation and dialysis of crude extract-II	Control	5064±516	12442±1268	97	1.88
		K_m=0.4 mM; V_{max}=10.1Δabs. $min^{-1}ml^{-1}$; V_{max}/K_m=25.3			
	60	3766±183	9276±451	80	1.51
		K_m=0.15 mM; V_{max}=5.0 Δabs. $min^{-1}ml^{-1}$; V_{max}/K_m=33.3			

[a] Indicates the percentage change in activity or specific activity as compared to the corresponding control

vation of heat labile fractions of PPO, which have high substrate specificity. The modification of the enzyme's quaternary or tertiary structures during heating may also be possible. In the literature, data related to the effects of low temperatures on PPO configuration are scarce. However, Vamos-Vigyazo [16]have reported that oligomeric forms of PPO are generally more active than its monomeric forms, and that these forms may transform each other by association-dissociation reactions. This kind of modification in oligomeric enzymes is observed very frequently when relatively low pressures (100 and 200 MPa) are applied to them during processing [18, 19]. High pressure disrupts hydrophobic and ion-pair bonds between the subunits of oligomeric enzymes and causes depolymerisation [19]. Cho and Ahn [20] showed that potato PPO may exist as a dimer of identical subunits (2×43 kDa). Thus, it is also likely that mild heating caused the dissociation of some oligomeric PPO forms to less active monomers with a mechanism similar to that of high pressure processing. Further research is needed to reveal the mechanisms of in situ kinetic changes and in situ inactivation of PPO at low temperatures.

A considerable reduction was observed in the K_m of PPO when potatoes were heated at 50 °C for 75 min. The reduction in K_m was sharper when enzyme extracts were obtained by changing the extraction mixture (EM1), partial purification (0–90% $(NH_4)_2SO_4$ precipitation) and dialysis (44 h) conditions. This drastic change in K_m clearly explained the increase in PPO activity when heating time was extended. The V_{max} and V_{max}/K_m values of PPO from potatoes heated for 75 min were also higher than those of PPO from potatoes heated for 60 min, but lower than those of PPO from controls. The appearance of internal softening and browning on peel during heating at 50 °C and activation of PPO occurred at the same time. Thus, softening possibly allowed the diffusion of some activators from vacuoles or from other cell components to the locations of PPO and contact of the enzyme with those agents under mild heating conditions caused the modification of the enzyme's structure and related kinetic properties. It seems that the activation was not due to the release of some other latent PPO forms. This is because we did not observe a significant increase in PPO's V_{max} value that is related to enzyme concentration. The drop in our K_m value was also very sharp and such a drastic change needs the activation of a considerable amount of latent forms with relatively low K_m values. These results and explanations were in line with those of Rodriguez-Lopez et al. [21] who also reported a sharp drop in the K_m of crude mushroom PPO activated during heating. The apparent activation of enzyme suggested that in a LTB process, which aims to control PPO activity, the limits of heating should be determined by monitoring the activity of this enzyme and finding the time of its activation.

Effect of heating on LOX

Heating at 50 °C for 45 min caused a slight activation of LOX (Table 3). Thus, unlike PPO activation, LOX activation was not related to the thermal softening which had appeared by 75 min heating. Heating for 60 or 75 min caused only a slight reduction in crude enzyme activity. However, we detected considerable changes in the kinetic characteristics of enzyme obtained from 60 min heated potatoes. The sharp reduction of its K_m by heating made LOX more reactive to its substrate at its low concentrations (Fig. 3). However, the reduction of the enzyme's V_{max} indicated that activation and inactivation of LOX occurred at the same time. This explains why LOX activation did not cause an apparent increase in enzyme activity. In general, plant LOX is a monomeric enzyme located in the cytoplasm and/or in subcellular

Table 4 Activities of pectin methylesterases (*PME*) from control and low temperature blanched potatoes

Heating time at 50 °C (min)	Ionically+Soluble PME				Covalently bound PME Activity[a] (U)
	Activity[a] (U)	Specific activity[a] (U mg^{-1})	Activity[b] (U)	Specific activity[b] (U mg^{-1})	
Control	268±10	284±11	152±15	161±16	26±0.8
45	243±8 (–9%)[c]	287±10 (+1%)	128±14 (–16%)	151±16 (–6%)	29±1.2 (+12%)
60	231±9 (–14%)	263±11 (–7%)	130±23 (–14%)	148±27 (–8%)	36±1.4 (+38%)
75	221±4 (–18%)	238±4 (–16%)	142±24 (–7%)	153±26 (–5%)	30±2 (+15%)

[a] Assayed by the titrimetric method
[b] Assayed by the spectrophotometric method
[c] Indicates the percentage change in activity or specific activity as compared to the corresponding control

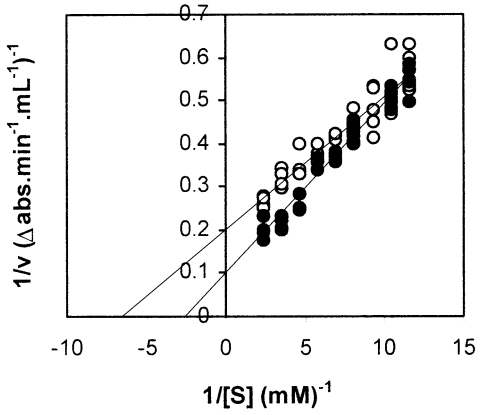

Fig. 3 Double reciprocal plots for partially purified lipoxygenase from control and low temperature blanched potatoes. *White circles* 50 °C/60 min, *black circles* control

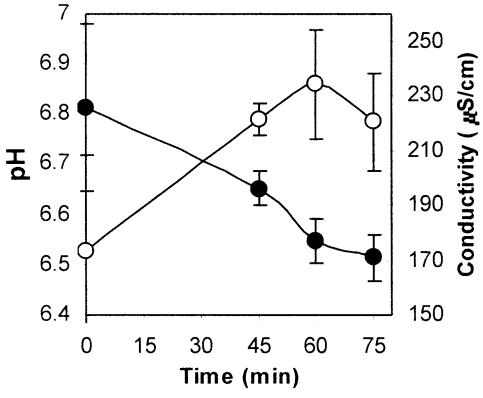

Fig. 4 The effect of low temperature blanching on conductivity (*white circles*) and pH (*black circles*) of potato extracts

organelles and it can be activated readily by Ca^{2+} ions [22, 23]. Thus, the increased reactivity of LOX might be attributed to its contact with Ca^{2+}, which may be released from starch during heating [9]. The activation of LOX may cause the formation of off flavours, degradation of some nutrients or acceleration of the exhaustion of anti-browning agents (antioxidants) during the storage of minimally processed potatoes. Therefore, it may be a handicap for the control of potato PPO by LTB. However, It is worth noting that the activation of potato LOX may be desirable for biotechnological purposes. Potato LOX is different from the other plant LOX in its unique capability to oxidise 20 carbon atom polyunsaturated fatty acids. Thus, biotechnologists consider this enzyme as an alternative to mammalian LOX which may be used for the production of hydroperoxide precursors of some biologically active compounds [24].

Effect of heating on PME

Heating at 50 °C for 45 min slightly activated covalently bound PME, whereas 60 min heating caused significant activation of enzyme and increased its activity almost 1.4-fold (Table 4). Bartolome and Hoff [25] reported that heat activation of covalently bound cell wall PME occurred by the contact of enzyme with intracellular electrolytes. However, these researchers believed that this contact occurred in the 60–70 °C range where cellular membranes (plasmalemma) lose their integrity. Our conductivity measurements in potato extracts showed that heating caused almost 27–35% increase in the conductivities of heated potato extracts as compared to those of controls (Fig. 4). Heating apparently increased the permeability of cell wall membranes and this caused the leakage of more ions from potato cells. Thus, our results showed that activation of covalently bound cell wall PME is possible at lower temperatures. The activity of ionically bound+soluble PME in potatoes is considerably higher than the activity of covalently bound PME. However, heating at 50 °C did not activate this enzyme fraction. The apparent pH drop in potato extracts in the first hour of heating indicated the contact of PME with pectic compounds. Thus, it seemed that heat treatment at 50 °C might be beneficial in improving the texture of minimally processed potatoes.

In conclusion, LTB of whole potatoes at 50 °C may be used to reduce PPO activity and to reduce the affinity of PPO to its substrate. Overheating caused the significant activation of PPO and softening. Thus, limits of heating should be determined by monitoring the activity of PPO enzyme and potato firmness. Heating at 50 °C also activated covalently bound PME enzyme and this may be beneficial to improve the texture of potatoes. The activation of LOX during heating is a handicap of LTB.

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