The Liberation of Free Fatty Acids in Potato Slices at Low Temperature

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Free fatty acids (FFA) are liberated in the first stages of potato granule production. In this study, the liberation of FFA was examined in model experiments. Potato slices were exposed to conditions resembling the first stages of the industrial process, which was of the "add-back type." The slices were kept at 9°C in the dark and, after various periods of time (up to two hours), the amount and composition of the FFA liberated at the surface and in the middle of the potato slices were analyzed by gas chromatography.

The liberation of FFA was more pronounced at the surface of the slices than in the middle. The polyunsaturated fatty acids, linoleic acid and linolenic acid, were liberated to a greater extent than the saturated fatty acids. The experiment was repeated at 4°C and the results obtained were very similar. The lower temperature did not decrease the rate of FFA liberation. But, in thin potato strips, which have a larger total surface of damaged membranes than the potato slices, considerably more FFA were liberated.

The amount and composition of FFA also were analyzed in potato slices from the industrial process for potato granule production. Before the blanching bath (76°C for 12–15 min) only minor amounts of FFA were liberated but during the blanching there was a substantial liberation of FFA at the surface of the potato slices. After the blanching bath there were no more FFA liberated at the surface of the slices but in the middle of the potato slices the liberation of FFA continued until the potato slices reached the steam-cooking bath.

KEY WORDS: Free fatty acids, gas chromatography, lipolysis, potato granule process, potato lipids.

The storage time of dry food products is limited due to lipid oxidation. These products are usually powders which have a large surface exposure to oxygen and are, therefore, easily oxidized. However, the lipid oxidation can start much earlier, before and during the manufacturing process of a final product, and can be initiated in various ways. One way is by the influence of lipid-degrading enzymes (1).

The lipid content of potato tubers is low—5.8–9.0 mg/g of dry weight (2)—but about 75% of the fatty acids are linoleic and linolenic acid, C18:2n-6 and C18:3n-3, which are polyunsaturated. The tubers also contain a large amount of lipolytic enzymes (3). In an intact potato tuber, the lipids are very stable but when the membranes are damaged, by cutting for example, the lipolytic enzymes are immediately activated (4,5). The enzyme, lipolytic acylhydrolase, catalyzes the hydrolysis of free fatty acids (FFA) from the glycerides and the enzyme, lipoxygenase, catalyzes the oxidation of these FFA. Galliard (6) showed that the enzymatic degradation of phosphatidylcholine and phosphatidylethanolamine in a homogenate of potato tubers was stimulated by FFA. A phospholipase in potato tubers has been reported to be active down to a temperature of 2°C (7).

We previously showed that FFA are liberated from the lipids in the first stages of potato granule production, probably due to lipolytic enzymes (8). The process was of the "add-back type," a common industrial potato granule process. In the first part of this process, the raw potato tubers are peeled, cut into 2-cm thick slices and blanched in water held at 76°C for 12–15 min. From the time they are peeled until they are in the blanching bath, they are carried on a conveyor belt and by water held at 9–12°C. The speed of FFA liberation at this low temperature was studied with potato slices similar in size to those used in the commercial process, and the reaction rate at the surface of the potato slices was compared with that in the center of the slices. The slices were kept in air at 9°C in darkness and samples were taken after various periods of time. The amounts and composition of FFA were analyzed by gas chromatography at the surface and in the middle of the potato slices.

One way to improve the process and get a more stable product might be to lower the temperature of the processing water. Therefore, similar experiments also were performed at 4°C. The results of these model experiments were compared with analyses of samples from the commercial process for potato granules.

MATERIALS AND METHODS

The raw potato tubers were of the Swedish cultivar Bintje. This is the cultivar mainly used for commercial production of potato products in Sweden. After harvest they were stored for six months in air of 95–98% relative humidity (RH) at 8°C until use.

All chemicals used for extraction, separation and analysis were of analytical grade (Merck, West Germany). For preparation of the free fatty acid fraction a 1-mL Chromabond aminopropyl-column (100 mg, Macherey-Nagel, West Germany) was used in an SPE vacuum elution system (Baker Chemical Co., Phillipsburg, NJ).

The standards, heptadecanoic acid (C17:0) and fatty acid methyl esters (C6:0–C18:2), were both from Nu Chek Prep, Inc., Elysian, MN.

Studies on potato slices. The raw tubers were washed and chilled to 9°C. In the experiments at 4°C, the tubers were chilled in ice water to a temperature lower than 4°C. After chilling, the tubers were peeled and 2-cm thick slices were cut vertically from the middle of the tubers. The potato slices were kept in darkness in air at 9°C or 4°C. At intervals up to four hours, a layer of 1–2 mm was cut from the surface of the potato slices. At the same time the potato piece was divided into two parts and a 1–2-mm thick slice was removed from the middle. These thin slices were analyzed for free fatty acids (FFA).

Studies on potato strips. To study if a larger sur-

face area of damaged tissue gives a faster liberation of FFA, thin potato strips (1 × 2 mm) were prepared. The raw tubers were chilled in ice water, peeled and cut into strips in a Philips food processor (HR 2373/B, 0.6L). The strips were kept in darkness in air at 4°C and after various periods of time (up to 30 min) samples (2–3 g) were taken for free fatty acid analysis.

Studies on potato slices from the industrial potato granule production. The potato granule process at AB Felix, Sweden, was studied. The process is of the "add-back type." The raw tubers are first washed, peeled and cut into 2-cm thick slices. The raw tubers and the potato slices are brought by a conveyor belt (in the air) and by water (9–12°C) to the blanching bath. The blanching is performed at 76°C for 12–15 min. The blanched potato slices are chilled for 10 min in water (9–12°C) and are then steam-cooked at 102°C for 20 min. The cooked slices are then mixed with already dried potato granules. (This is the reason for the name "add-back process.") The mixed granules are conditioned to level out the water content between the granules and then dried in an air-lift dryer to a water content of approximately 11%. Then about 85–90% of the product is recycled back into the mixer and only 10–15% is further dried and taken out as the final product (water content approximately 7%). In this study, samples were withdrawn from the raw tubers and from potato slices, right before and right after the blanching bath and after the following chilling step. The raw tubers and thin slices from the surface and from the middle of the potato slices were analyzed for FFA.

Preparation of the FFA fraction. The thin slices and strips were immediately weighed and put into refluxing methanol/water (4:1, v/v) to inactivate the enzymes. The lipids were extracted using the Bligh and Dyer method (9) and fractionated into the lipid classes as previously described (10). The fraction of neutral lipids in the chloroform phase was applied to a Chromabond aminopropyl-column, and the FFA were separated from the total lipids by eluting with 2% acetic acid in diethylether using vacuum pressure (11).

Free fatty acid analysis. The FFA were methanolyzed using the method described by Liljenberg and Kates (12). Heptadecanoic acid was used as an internal standard. The methyl esters were analyzed by using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA), using a fused silica capillary column (NB-9C, 0.10 μm, 25 m, 0.20/0.31 mm). The injection was made in split mode (1:50, 200°C) and with helium as the carrier gas (25 mL/min). The gas chromatograph was temperature programmed, 130°–220°C, at a rate of 15°C/min. The methyl esters were detected by a flame-ionization detector (220°C) and identified by comparing the retention times to known standards. A Hewlett-Packard lab data system (HP 3357) (Hewlett-Packard Co.) was used for data collection and evaluation. The fatty acid composition of the galactolipids and the phospholipids were analyzed in the same way.

Water content. The water content of the various samples was measured by drying samples of about 2 g at 110°C until they reached constant weight (approximately one hour).

RESULTS AND DISCUSSION

The liberation of FFA in potato slices. The amount and composition of FFA at the surface and in the middle of the potato slices were analyzed by gas chromatography. Figure 1a shows the liberation of FFA at 9°C during two hours. This is expressed as μg of fatty acids per g of dry weight. The solid line shows the FFA at the surface of the slices and the dotted line represents those at the middle of the slices. Each sampling was repeated two to five times. Despite this, the standard deviation is very large in some of the samples. However, it can be seen that the fatty acids at the surface of the slices were liberated at a higher rate than those in the middle of the slices. Figure 1b shows the results from the experiment carried out at 4°C. This graph shows more clearly that more FFA were liberated at the surface of the potato slices than in the middle.

The experiment at 4°C was run for four hours, while the experiment at 9°C was run for only two hours since the reaction was expected to be faster at this higher temperature. However, as is seen in Figure

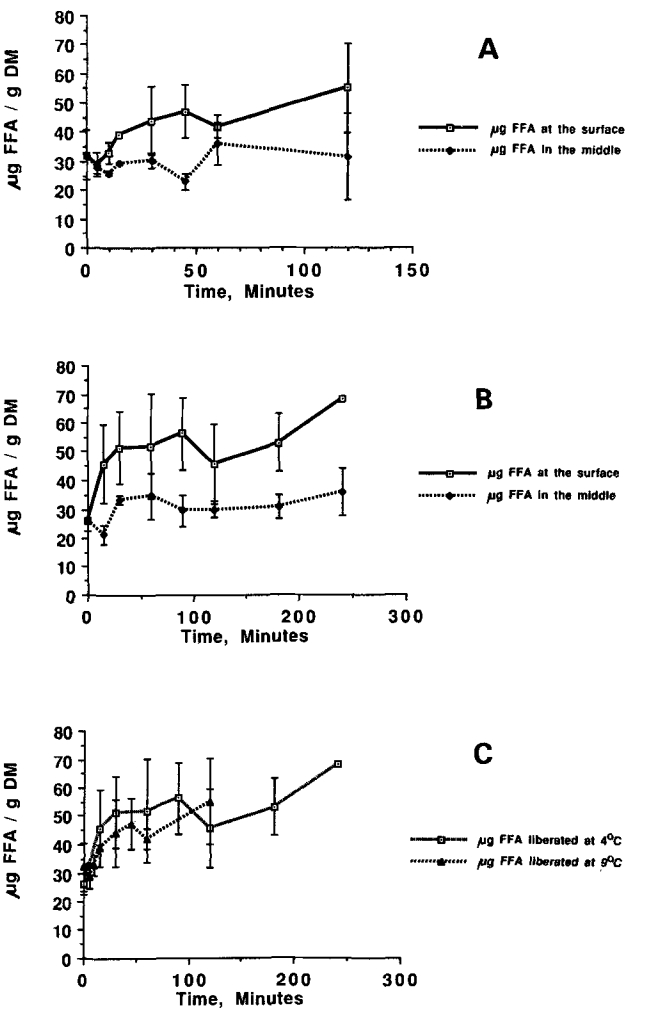

FIG. 1. The liberation of FFA at the surface and in the middle of potato slices, kept in darkness at 9°C and 4°C. a. The amount of FFA liberated at 9°C. b. The amount of FFA liberated at 4°C. c. The amount of FFA liberated at the surface of the potato slices at both 9°C and 4°C.

1c, the liberation of FFA at the surface was similar at 9°C and 4°C. Probably, from this already low temperature, 9°C, further lowering of the temperature did not significantly affect the reaction. The liberation of FFA seems to be fastest during the first 20–30 min. The apparent slower liberation of FFA later on may be due to increased oxidation of the liberated FFA or to limitations in the substrate available to the enzyme.

Figure 2a shows the amount of the polyunsaturated fatty acids (C18:2 + C18:3) in the FFA liberated at 4°C, both at the surface and in the middle of the slices. The polyunsaturated FFA show the same pattern as the total amount of liberated fatty acids presented in Figure 1b. The liberation was more pronounced at the surface of the 2-cm thick potato slices than in the middle of them. At the surface, the concentration of polyunsaturated FFA increased from 7 μg/g to 38 μg/g during the four-hour period. The liberation of saturated FFA (C16:0 + C18:0) during the same time period is presented in Figure 2b. The concentration of saturated FFA was fairly constant during the time period even at the surface of the potato slices, so obviously mainly unsaturated FFA were liberated. The larger amount of FFA liberated at the surface of the potato slices, compared with the center of the slices, is most probably due to damaged membranes which cause either activation of lipolytic enzymes or increased contact between enzyme and substrate (4).

Previously, we reported (10) that the unsaturation ratio—UR = (C18:2 + C18:3)/(C16:0 + C18:0)—of the total lipids of potato tubers (Swedish cultivar Bintje) was 3.45. The liberated fatty acids in the present study never reached this high UR which could be due to a simultaneous oxidation of the polyunsaturated fatty acids.

The liberation of FFA in potato strips. The raw tubers also were cut into thin strips to further investigate the influence of the surface area of damaged tissue. The liberation of FFA in the strips during 30 min in darkness at 4°C was followed.

Figure 3 shows the results of this experiment. There were considerably more FFA liberated in the strips than in the slices. After 30 min there was a total amount of 125 μg of FFA per g of dry weight in the strips, which is about 2% of the lipid content. The standard deviation between the replicates was smaller in this experiment compared with the results from the experiment with potato slices. This is probably due to the strips being a more homogenous sample material than the potato slices.

Analysis of individual lipid classes showed that approximately 7% of the total galactolipids and phospholipids in the strips were lost after 20 min, the galactolipids being degraded to a somewhat greater extent than the phospholipids. Galliard (4) reported that 13% of the phospholipids and galactolipids were degraded in a homogenate of potato tubers during the first 30 sec at 0°C. After 10 min the total amount of lipids was reported to be decreased by 26% in the homogenate. This shows that the degradation of lipids is highly affected by the surface area.

The amount of FFA in potato slices from the industrial process. The results from the model studies were compared with analyses of samples from the commercial production of potato granules. Samples were taken from the early steps of the industrial process. The amount and composition of FFA were analyzed in the raw potato tubers and at the surface and in the middle of the potato slices. Figure 4a shows the total amount of FFA expressed as μg of fatty acids per g of dry weight. Figure 4b shows the content (%) of the polyunsaturated fatty acids (C18:2 + C18:3) in these FFA.

The time period from when the raw potato tubers were peeled until the potato slices fell down in the blanching bath was about seven minutes. The tubers were sliced just before the blanching. During this period of seven minutes, there was only a small amount of FFA liberated at the surface of the potato slices, less than ten μg/g. There was slightly more FFA liberated in the middle of the slices than at the surface, but

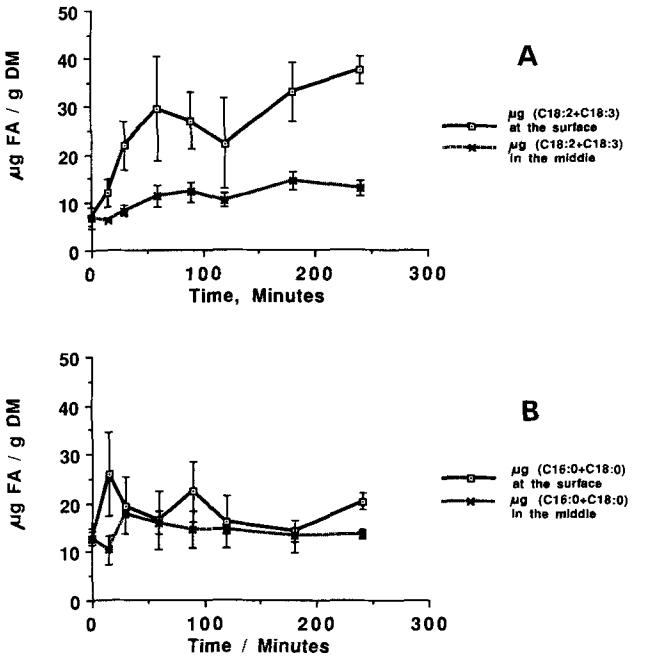

FIG. 2. a. The amount of linoleic acid and linolenic acid (C18:2 + C18:3) in the FFA liberated at 4°C. b. The amount of palmitic acid and stearic acid (C16:0 + C18:0) in the FFA liberated at 4°C.

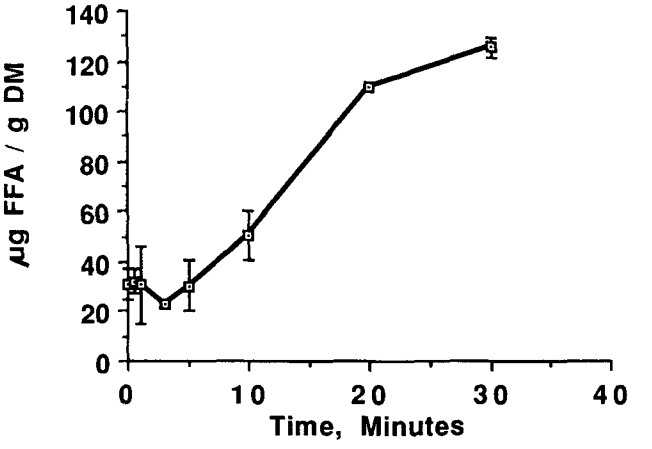

FIG. 3. The liberation of FFA in potato strips kept in darkness at 4°C.

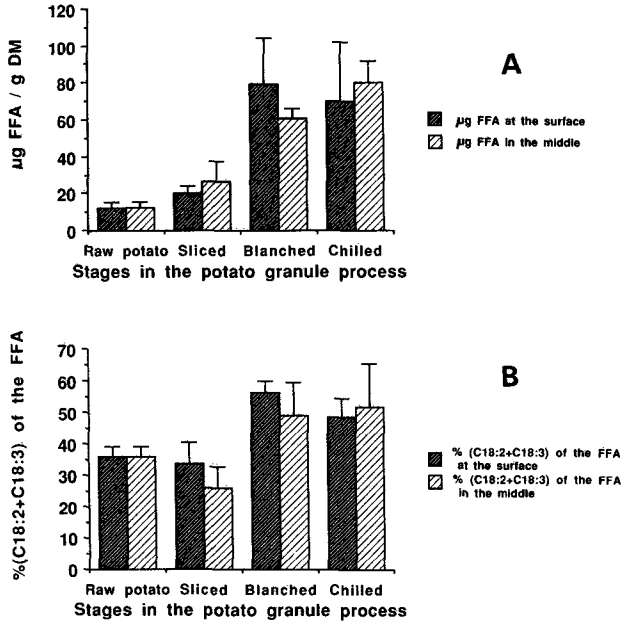

FIG. 4. The amount of FFA liberated at the surface and in the middle of potato slices, which were withdrawn from the first stages in the industrial process for potato granule production. a. The total amount of FFA. b. The percentage of linoleic acid and linolenic acid—% (C18:2 + C18:3)—in the FFA.

the FFA content also varied more between these industrial samples than between potato slices in the model experiment. At both temperatures in the model experiment, the liberation of FFA also was less than ten µg/g during the first seven minutes.

The blanching bath had a temperature of 76°C, and the potato slices passed through the bath in 12–15 min. During blanching, the amount of FFA increased considerably, especially at the surface of the potato slices. This fast liberation of FFA could be due to higher activity of the enzymes during the first part of the blanching bath. The activity of lipolytic acylhydrolase in potato tubers, particularly phospholipase, has been shown to increase with increasing temperature up to 50°C (7). Between 15°C and 25°C the activity increases most, especially in the presence of Ca^{2+}. The optimum temperature of lipolytic acylhydrolase in potato leaves has been reported to be 30°C for reaction with phosphatidylcholine (13).

In the commercial process, the enzymes were apparently inactivated at the surface of the potato slices after blanching since no more fatty acids were liberated. After chilling, the amount of FFA was slightly reduced at the surface of the slices and, as can be seen in Figure 4b, it was mainly the unsaturated fatty acids that were degraded, probably by being oxidized. In the middle of the slices, the liberation of fatty acids was less than at the surface during the blanching, but during the chilling step the amount of FFA from the middle continued to increase. This indicates that there was still enzyme activity left in the middle of the potato slices after the blanching bath, which could cause lipolysis during the subsequent chilling step. Remaining activity of lipolytic acylhydrolase after blanching also was reported previously (8).

Obviously, it is not the very first stages in the potato granule process that cause the greatest liberation of FFA. A lowering of the temperature from 9°C to 4°C does not influence the activity of the lipolytic enzymes and will probably not decrease the lipid degradation. Instead, the main problem seems to be that it takes a long time to heat the potato slices in the blanching bath, during which enzymatic hydrolysis is favorable.

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