Food Chemistry **34** (1989) 15–23

Liberation of Free Fatty Acids during Production of Potato Granules

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(Received 3 October 1988; accepted 14 November 1988)

ABSTRACT

The amount of free fatty acids and their composition were studied during an add-back process of potato granules. Samples were taken at several different stages in the production, from raw tubers to final potato granules.

Lipids were extracted and lipid classes separated by liquid chromatography. Free fatty acids were separated from the neutral lipid fraction by high performance liquid chromatography (HPLC). The fatty acid composition was analysed by gas chromatography (GC).

The amount of free fatty acid increased in the first stages of the process and rose to its highest level in the samples from the steam-cooking step. During further processing the amounts of free fatty acids slowly decreased.

Since enzymatic hydrolysis is one explanation for liberation of fatty acids, lipase activity was measured by a spectrofluorimetric method in the raw tubers, blanched slices and steam-cooked slices. After the blanching step, some enzyme activity was found but after steam-cooking there was none.

INTRODUCTION

Dry food products such as potato granules easily became rancid during storage, due to oxidation of lipids. The lipid content of potato tubers is very low, only 0·5% on a dry matter basis (Galliard, 1973). Nevertheless, lipid oxidation is a major problem, since most of these fatty acids are highly unsaturated. The fatty acids are mainly components of complex membrane lipids.

The manufacturing process is probably very critical to the oxidative

Food Chemistry 0308-8146/89/$03·50

stability of the product, since lipid oxidation and other changes can take place even at this stage. We have previously reported on lipid changes during the production of potato granules by an add-back process (Lilja & Lingnert, 1988). We found the potato granules to contain considerably larger amounts of free fatty acids (FFA) than the raw tubers. This may be a factor that influences the stability of the potato granules during further storage.

This investigation was performed in order to learn at which stage in the process FFA are mainly liberated and to try to find explanations for this liberation. For this reason the FFA content and composition were analysed at several stages during the manufacturing process. Since potato tubers are known to contain large amounts of lipid degrading enzymes, such as lipase and lipoxygenase (Galliard & Matthew, 1973), enzymatic hydrolysis could be an effective mechanism for FFA liberation. Therefore, lipase activity was also measured at various steps during the process.

MATERIALS AND METHODS

Potato granules were produced in a pilot plant as well as in a full scale plant at AB Felix, Sweden, using the add-back process. A scheme of the process is shown in Fig. 1. The pilot plant production has been described previously (Lilja & Lingnert, 1988). Samples for free fatty acid (FFA) analysis were taken from the pilot plant at the stages marked in Fig. 1 and samples for analysis of enzyme activity were taken from both the pilot and full scale plants at the first three marked steps; that is, the raw tubers, the blanched slices and the steam-cooked slices. The slices were a maximum of 20 mm thick and varied in width. In the mixing step the steam-cooked slices were mixed with previously produced potato granules and pulverised into granules. All the following samples were in the form of granules.

The various samples were packed in foil bags and stored at $-40°C$ until analysis, except for the raw tubers which were analyzed directly.

All chemicals used for extraction, separation and analyses were of analytical grade (Merck, FRG, pro analysis). The hexane (Fisons plc, Scientific Equipment Division, England) and propane-2-ol (FSA, Laboratory Supplies, England) were of HPLC grade.

The standards, linoleic acid (C18:2), heptadecanoic acid (C17:0) and fatty acid methyl esters (C6:0–C18:2), were all from Nu Chek Prep., Inc., USA. The substrate for measuring lipase activity was 4-methylumbelliferyl-heptanoate (MUH, Sigma No. M-2514), and linoleic acid emulsified in phosphate buffer was used as the substrate in the analysis of lipoxygenase activity. Lipase, Type 1 from Wheat Germ (Sigma No. L3001) and lipoxidase, Type 1 from soybeans (Sigma No. L7127) were used as standards.

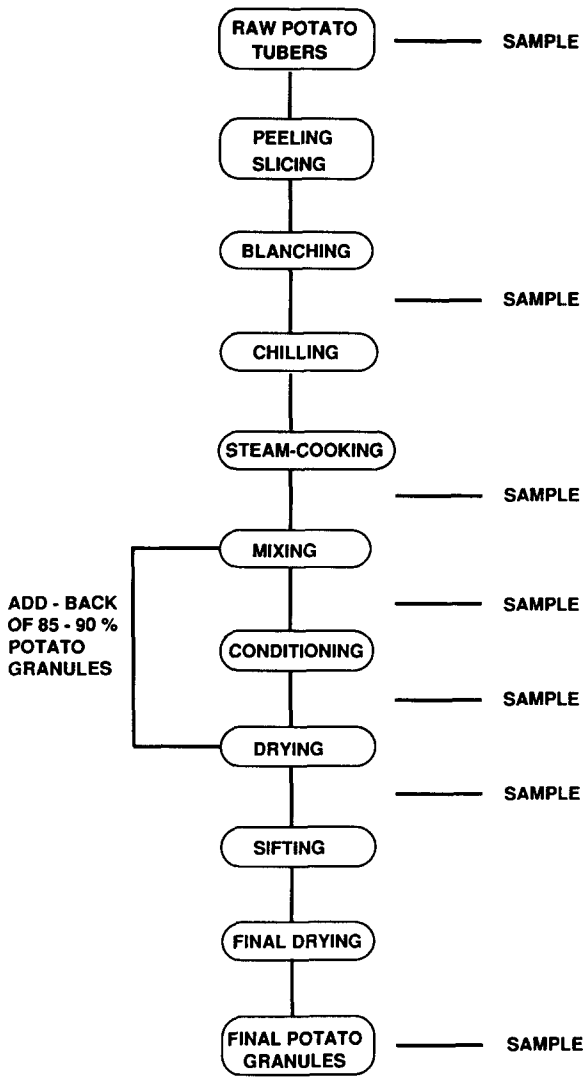

Fig. 1. The steps in the add-back process and the points at which samples were taken.

FFA analysis

Four samples from each marked step in Fig. 1 and twelve samples of raw tubers were analysed for FFA. The lipids were extracted using the Bligh & Dyer method (1959) as previously described (Lilja & Lingnert, 1988).

The fraction of neutral lipids was separated from the extracted lipids on a silicic acid column (Mallincrodt CC4 Special) as described by Rouser *et al.* (1967). The FFA were separated from the neutral lipids by High

Performance Liquid Chromatography (HPLC) on a Shimadzu SCL6A equipped with two silica acid columns (ChromsphereSi, 30 × 100 mm, 5 μm part., Chrompack, Holland) tightly connected together. Hexane/propane-2-ol/HAc (100:0·5:0·01) was used as the mobile phase described by Hamilton & Comai (1984), with a flow rate of 0·8 ml/min.

Linoleic acid (C18:2) was used as the standard for identification of the retention time of the FFA peak. The FFA from five runs were combined for analysis of fatty acid composition by gas chromatography (GC). The fatty acids were methanolysed, analysed and quantified using the same methods as reported previously (Lilja & Lingnert, 1988).

The water content of the different samples was measured by drying samples of about 5 g at 110°C until constant weight was attained. The blanched and steam-cooked slices had to be dried overnight to reach constant weight, while drying for 1 h was enough for the rest of the samples.

Lipase activity measurements

The lipase activity in raw, blanched and steam-cooked tubers was analysed by the MUH (4-methylumbelliferyl-heptanoate) hydrolase method described by Heltved (1984). When measuring the lipase activity in the samples from the pilot plant process, small pieces were cut out from a cross-section at the middle of the potato slices (total amount 3 g) and mixed with 8 ml of 0·2M Tris-HCl-buffer, pH 8·5. The samples were then homogenised for 2 min (in an ice bath) with a Sorvall Omnimixer. The mixture was centrifuged at 3000 *g* for 10 min. The supernatant was recovered and filtered.

When analysing the samples from the commercial production of potato granules, several potato slices were first shredded together in a Philips food processor (HR 2373/B, 0·6 l) to attain homogeneity, before the samples of 3 g were taken.

At zero time, 25 μl 0·1M MUH in 96% ethanol was added to a 3 ml cuvette with sample solution. The change in fluorescence intensity per unit of time, at 20°C, was recorded at excitation and emission wavelengths of 330 and 450 nm, respectively.

The lipase activity of the samples was calculated from a standard curve based on measurements with wheat germ lipase.

Lipoxygenase activity measurements

Measurements of lipoxygenase activity were done with samples from the full scale process using the spectrophotometry assay of Ben-Aziz *et al.* (1970). The same extracts as the ones used in the lipase analysis were analysed.

RESULTS AND DISCUSSION

The free fatty acid (FFA) content was analysed in samples from the pilot plant production of potato granules at the steps shown in Fig. 1.

Figure 2 shows the total amount of FFA, expressed as microgram per gram of dry weight, in the various steps. As can be seen in Fig. 2, the increase of FFA had already appeared in the blanched potato slices but the amounts varied considerably between different samples. The largest amounts of FFA were found in the steam-cooked slices but here also the standard deviation was substantial. In the subsequent steps a continuous decrease in the FFA content was noticed. The most probable mechanism of FFA formation is enzymatic hydrolysis. It is not surprising, therefore, that the FFA content increased from the raw potato to the blanched samples, since hydrolysis can take place before the enzymes are inactivated. However, the further increase of FFA content in the steam-cooked samples indicates that lipases were also active after the blanching step.

To investigate this, the lipase activity was analysed in raw, blanched and steam-cooked tubers. Samples were taken from the pilot plant at the same time as the samples for FFA analyses, but, for comparison, samples were also taken from the full scale plant. In Fig. 3 the lipase activity is presented as units per gram of dry weight. In the steam-cooked potato slices this enzyme was totally inactivated, but in the blanched potato slices there was still some activity.

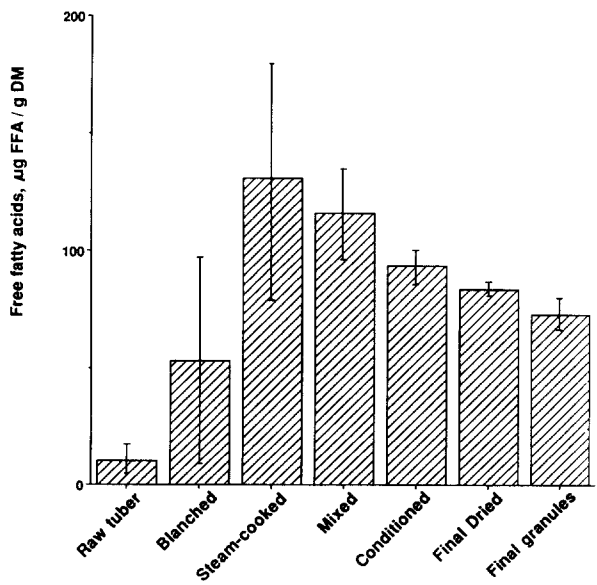

Fig. 2. Amounts of FFA in the different steps of the add-back process.

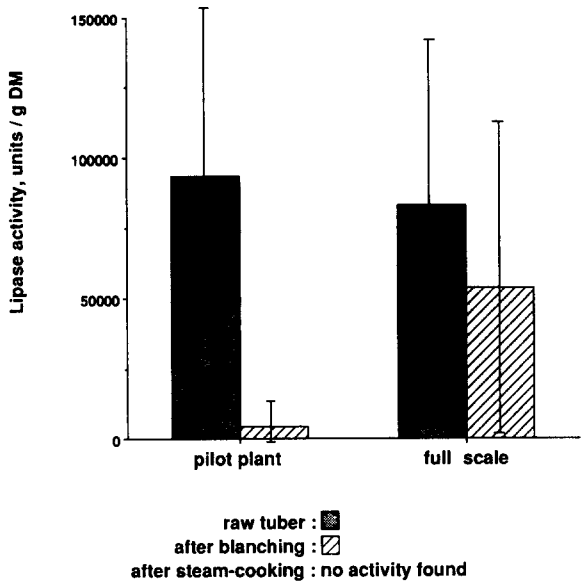

Fig. 3. The lipase activity in raw tubers and blanched slices in two add-back processes, one pilot plant and one full scale process.

In the blanched potato slices from the commercial process the remaining lipase activity was greater than in the pilot plant process. As with the FFA-amounts, there were considerable variations in the lipase activity. This may be due to natural variation of the potato tubers and to variation in size of the tuber slices. Small pieces would be heated enough to inactivate the enzymes in a shorter time than larger ones, and so the lipid degradation would be less in smaller pieces. The large standard deviations were also due to the small number of analyses, but the important thing is to establish that lipolytic activity can actually be found after the blanching step.

Preliminary analyses of the lipoxygenase activity showed results similar to those for the lipase activity. There was no activity of lipoxygenase in the steam-cooked tubers but in the blanched tubers there was still some activity.

There is a delicate balance between different purposes regarding the blanching step in the potato granule process. Most of the enzymes are intended to be inactivated but one, pectin methylesterase, is meant to be activated. This enzyme is activated between 50°C and 70°C (Bartolome & Hoff, 1972) Pectin methylesterase catalyses the hydrolysis of methoxyl groups from carboxyl groups on pectin and, during the following chilling period (10°C, 9–12 min), calcium ions are bonded in bridges between the pectic acids.

This firms the cell walls before the steam cooking (100°–102°C steam, 20 min) and further processing. In our case the blanching was performed at

76°C for 12–15 min. Obviously, this was not enough for the inactivation of lipase and lipoxygenase and we conclude that they were able to enzymatically hydrolyse and oxidise the lipids during the chilling step. Furthermore, FFA have also been found to increase the rate of hydrolysis (Galliard, 1971) by disrupting the membrane structure, which allows access of the enzyme to the substrate.

According to Galliard & Rayward-Smith (1977) the enzyme lipolytic acylhydrolase (LAH) in potato tubers (average weight, 57 g) is inactivated after between 20 and 25 min at 70°C and after between 9 and 12 min at 85°C, but the lipoxygenase (LOX) is even somewhat more thermostable. It is not inactivated until after between 12 to 15 min at 85°C. However, in smaller pieces of potato tubers (potato strips, 10 × 8 mm) Fretzdorff *et al.* (1988) found less than 1% enzyme activity of LAH and LOX after as short as 4 min at 70°C and 2 min at 80°C, respectively. This supports the finding that these enzymes may not be fully inactivated after the blanching step and could explain the great variation in enzyme activity between different samples.

As shown in Fig. 1, the steam-cooked tubers were in the subsequent step, mixed with previously produced potato granules to get a more gentle pulverisation. Since the potato enzymes at this stage are totally inactive in this step, there will be no enzymatic hydrolysis or oxidation of the lipids but, after this, the potato granules are exposed to conditions where autoxidation may occur (elevated temperature, oxygen availability) which may affect the free fatty acids. This is a probable explanation for why the amount of FFA

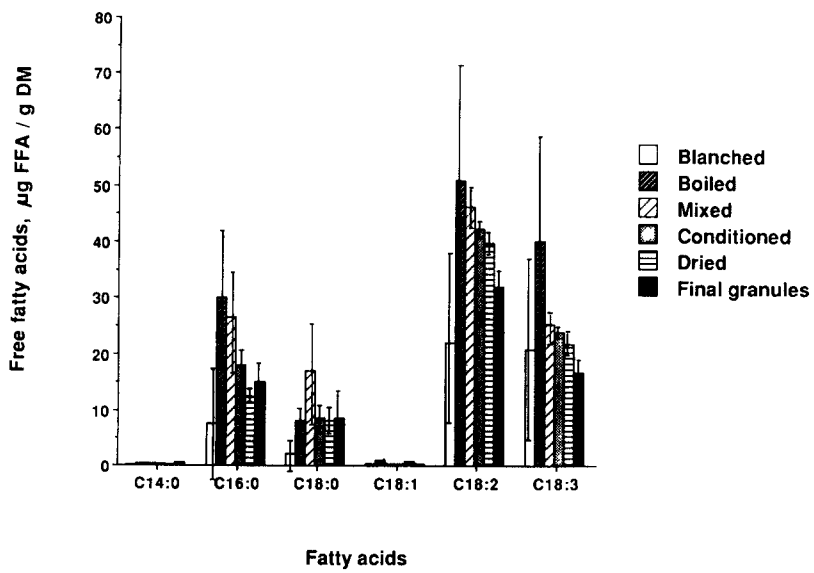

Fig. 4. The composition of the FFA in the different steps of the add-back process.

decreases in every subsequent step during the production, as can be seen in Fig. 3.

Figure 4 shows the composition of the free fatty acids at the various steps in the pilot plant process. They are presented as micrograms FFA per g of dry weight. The standard deviation was large in all the fatty acids of the blanched and steam-cooked slices (for reasons discussed before) but there was an increase in each of the fatty acids in the steam-cooked slices. Linolenic acid (C18:3) was the fatty acid that decreased the most during the manufacturing process after the steam-cooking step.

The unsaturation ratio was slightly decreased. This supports the assumption that oxidation is a cause of the decreasing FFA content.

CONCLUSIONS

Free fatty acids (FFA) were formed during the production of potato granules. An increased amount of FFA was found as early as the blanching step but the largest amount was found in the steam-cooked slices, so liberation of FFA starts directly from the beginning of the manufacturing process, probably by enzymatic hydrolysis. It was also found that there was some lipase and lipoxygenase activity left after the blanching step but there was no activity after the steam-cooking step.

During further processing the amounts of FFA decreased, a likely explanation being autoxidation, primarily of unsaturated FFA.

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