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The Role of Oxylipins and Antioxidants on Off-Flavor Precursor Formation during Potato Flake Processing

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The impact of processing on nonenzymatic antioxidant degradation and lipid oxidation leading to off-flavor development in potato flakes during storage was investigated. Lipoxygenase activity measurements in parallel with the analysis of lipid oxidation products (oxylipins) profiles using HPLC showed that the processing conditions used inhibited efficiently enzymatic lipid oxidation. However, nonenzymatic lipid oxidation products were found throughout processing and in fresh potato flakes. Furthermore, these autoxidative processes cannot be inactivated by the main endogenous nonenzymatic antioxidants in potato tubers (ascorbic acid, phenolic compounds and carotenoids), as these antioxidants are degraded during processing. Indeed, leaching and thermal treatments taking place during processing lead to a decrease of about 95%, 82% and 27% in the content of ascorbic acid, phenolic compounds and carotenoids, respectively. Therefore, storage is a critical step to prevent off-flavor development in potato flakes. Specific attention has thus to be paid on the use of efficient exogenous antioxidants as well as on storage conditions.

KEYWORDS: Antioxidants; hexanal; lipid oxidation; off-flavors; potato flakes; processing

INTRODUCTION

In the potato industry, dehydrated potato flakes are a crucial byproduct obtained with a raw material that cannot be valorized by other industrial processes. They are used in many foodstuffs such as mashed potatoes, gnocchi or snacks for example. Unfortunately, off-flavors frequently appear during storage of potato flakes leading to economical losses. Mainly the oxidation of polyunsaturated fatty acids (PUFAs) present in potato tubers leads to the formation of volatile compounds responsible for off-flavor development in potato flakes (*1, 2*). These oxygenated PUFAs and their metabolites are collectively named oxylipins (*3*). Oxylipins are formed either by chemical oxidation of PUFAs that is often named autoxidation or by enzymatic oxidation of PUFAs. The main enzyme responsible for oxylipin formation in plants is lipoxygenase (LOX). It specifically introduces dioxygen in a C-18 fatty acid at either carbon 9 (9-LOX) or 13 (13-LOX) in *S* configuration (*4*). Moreover, oxylipins exist in both free and esterified forms. Initial products of enzymatic and chemical oxidation of linoleic acid are 9- and 13-hydroperoxides of linoleic acid (9- and 13-HPOD), respectively. Chemical oxidation led in addition to the formation of 10- and 12-HPOD (*5*). Therefore one can distinguish between chemical and enzymatic fatty acid oxidation by comparison of (i) the positional isomers of linoleic acid and (ii) in the case of 9- and 13-HPOD by analyzing the enantiomeric ratio of these compounds since chemical oxidation always led to formation of a racemic mixture, while LOX always formed pure *S* enantiomers.

Previously, hexanal was identified as the main volatile compound in potato flakes during storage and its formation by nonenzymatic processes was concluded from the analysis of the configuration of its precursors (*6*). Though off-flavor is usually a function of several volatile compounds, Boggs et al. (*7*). showed that the concentration of hexanal measured by static headspace sampling correlated with the degree of off-flavor of the dehydrated potato in sensory panel studies. In addition, detection threshold of hexanal in water is of about 5 nmol/g FW (*8*) and this concentration in hexanal is already reached in potato flakes after 6 weeks of storage (*6*). Therefore, hexanal and its precursors were used here as indicators of off-flavor development in flakes.

The precise origin of hexanal precursors remains unclear (*6*). Hexanal is produced from linoleic acid either enzymatically, through the LOX pathway, and/or nonenzymatically by autoxidation. In both cases, the starting point of hexanal formation is 13-HPOD (*9*).

Peeling, slicing, blanching, cooking, and drying as well as the common use of potato slivers from the French Fries production chain are essential components of potato flake processing. However, these essential steps are thought to promote lipid oxidation in potato

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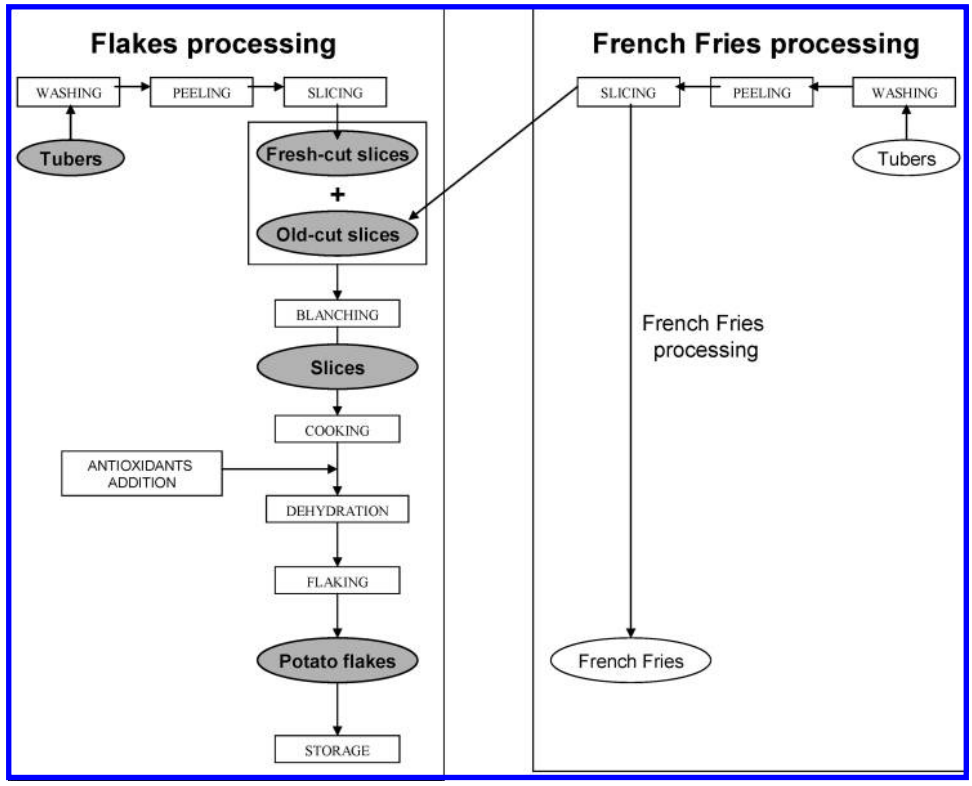

Figure 1. Potato flake production process and its relation with the French Fries production process. Samples collected through the production process are in gray.

tubers and slices (*10*). Moreover, processing degrades the natural antioxidants of potato tubers whereas antioxidants can increase the shelf life of potato flakes by preventing or delaying lipid oxidation leading thus to a better preservation of sensorial quality (*11*). The high temperature required for blanching, cooking and drying as well as exposure to oxygen resulting from the use of French Fries slivers, can activate the enzymatic and the nonenzymatic oxidation of PUFAs and contribute to the degradation of natural nonenzymatic antioxidants (*10, 12*). Moreover, water-soluble antioxidants can diffuse into washing or blanching water (*13*). Furthermore, cellular damage resulting from peeling and slicing increases LOX activity and can thus enhance the formation of hydroperoxides by enzymatic oxidation of PUFAs (*3, 14*).

The main natural nonenzymatic antioxidants in potato tubers are ascorbic acid, phenolic compounds and carotenoids (*15*). These three categories of compounds show different behaviors in response to processing, and their sensitivity to the production process is highly dependent on the commodity (*16, 17*). Nevertheless, natural antioxidants are usually of poor stability in comparison with synthetic antioxidants. Therefore, sulfites, ascorbyl palmitate and synthetic phenolic antioxidants, such as butylated hydroxyanisole (BHA), are widely used in the food industry. However, as some of them are suspected to pose a human health risk, the use of synthetic antioxidants is strictly limited in many countries (*18, 19*). Therefore, potato industries are looking for efficient natural alternatives such as endogenous antioxidants contained in potato tubers.

The aim of the current study is to identify critical processing steps leading to the formation of hexanal precursors and/or to antioxidant degradation in order to reduce off-flavor appearing during the storage of potato flakes. Moreover, the determination of the sensitivity of natural antioxidants to the different process steps is crucial to identify efficient natural alternatives to synthetic antioxidants. For these purposes, we took some samples directly from the process in a potato flake producing plant. In order to monitor lipid oxidation and antioxidant degradation throughout processing, both potato slivers from French Fries production, called old-cut potato slices, and potato slices especially cut for potato flake production, called fresh-cut potato slices, were sampled at different steps of flake production. In these samples, we analyzed at first LOX activity as the major enzymatic reaction leading to the formation of fatty acid hydroperoxides which are potential candidates of hexanal precursors. Additionally, we analyzed profiles of enzymatic and chemical-derived lipid oxidation products. Finally, total antioxidant activity and the content of total phenolic compounds, carotenoids and ascorbic acid were measured.

MATERIALS AND METHODS

Materials. *Solanum tuberosum* L. cultivar Bintje was grown in Belgium under standard agricultural conditions. Potato tubers were directly used for French Fries and flake production after harvest in October 2007. Samples obtained directly from a company were taken during the process of potato flake production. Potato flakes were produced from slices coming from two different production chains: French Fries chain and potato flakes chain itself. Potato flake processing is schematized in **Figure 1**. For the two chains, the first steps were similar: sorted potato tubers were washed, the peels were removed with vapor (between 12 and 14 bar) and the tubers were sliced. Potato slices which were out of grade for French Fries production were transferred to the potato flake production chain in a water stream. These potato slices are called old-cut potato slices. On the other hand, fresh-cut potato slices are potato tubers especially sliced for potato flake production. When all slices were on the potato flake chain, they were blanched for 15 min at 80 °C. Afterward, slices were cooked in steam ($T = 100$ °C) and transformed into mash before adding additives. The resulting mash was distributed by a screw mechanism onto chrome drying cylinders with steam (100 °C $< T <$ 160 °C) passing through them. Mash was then dehydrated and successively stretched to obtain a thin homogeneous film. The film of dehydrated potato from the drying drum was shredded and flaked to obtain a finished product in the form of flakes.

We sampled washed potato tubers, old-cut slices, and fresh-cut slices before and after blanching, as well as fresh potato flakes. All samples were directly ground in liquid nitrogen and stored at −80 °C. The commercial potato flakes formulation analyzed was produced from potato tubers cv. Bintje and contained ascorbyl palmitate as antioxidant and curcuma as coloring agent.

Chemicals. Methanol, *n*-hexane, isopropanol and acetic acid were HPLC grade and were acquired from Thermo Fisher Scientific (Geel, Belgium). (6*Z*,9*Z*,11*E*,13*S*)-13-hydroxy-6,9,11-octadecatrienoic acid was enzymatically synthesized from linolenic acid with lipoxygenase from *Glycine max* (Sigma-Aldrich, Taufkirchen, Germany). Triricinoleate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were from Fluka (Buchs, Switzerland). Linoleic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), *N*-ethylmaleimide (NEM), 2,2′-dipyridyl and Na_2EDTA were purchased from Sigma-Aldrich (Bornem, Belgique). Folin-Ciocalteu reagent was from Merck (Darmstadt, Germany), and dithiothreitol (DTT) was from GE Healthcare (Little Chalf-ont, U.K.).

Instruments. HPLC analyses were performed with a HP 1100 series (Agilent Technologies, Palo Alto, CA) and all spectrophotometric analyses were performed on a UV−visible spectrophotometer (Shimadzu UV-1601, Kyoto, Japan).

LOX Activity. A three-step HPLC method was performed as described in ref *6*. Approximately 0.5 g of sample was added to 1.6 mL of lysis buffer (90 mM Tris/HCl pH 9.5, 10% glycerol, 500 mM NaCl, 0.1 Tween 20) and 2.4 mL of Tris buffer (100 mM Tris/HCl, pH 7.5) on ice. After homogenization with an Ultra Turrax under a stream of nitrogen for 30 s, oxygenation of linoleic acid was carried out by incubating LOX preparations with the substrate (1 μmol of linoleic acid) for 30 min at room temperature. Five micromoles of $SnCl_2$ in methanol was added to the reaction medium. The mixture was incubated at room temperature for 10 min, and 150 μL of acetic acid was added. The reaction products were extracted twice with one volume of chloroform and analyzed by HPLC (see oxylipins profiling). The data are the average of three repetitions.

Oxylipin Profiling. LOX-derived products were extracted in triplicate and analyzed as described in refs *6* and *20*. For the extraction of free oxylipins, (6*Z*,9*Z*,11*E*,13*S*)-13-hydroxy-6,9,11-octadecatrienoic acid was used as internal standard and about 2 g of frozen material was added to 20 mL of extraction medium [isohexane:2-propanol (3:2, v/v) with 0.0025% (w/v) BHT]. For the extraction of esterified oxylipins, triricinoleate was used as internal standard and about 0.5 g of frozen material was added to 20 mL of extraction medium. After homogenization, the extract was centrifuged at 1300*g* at 4 °C for 10 min. The clear upper phase was collected and a 6.7% (w/v) solution of potassium sulfate was added to a volume of 32.5 mL. After vigorous shaking, the extract was centrifuged at 1300*g* at 4 °C for 10 min. The upper hexane-rich layer containing the oxylipin fatty acid derivatives was removed. Subsequently, esterified oxylipins were transmethylated with sodium methoxide.

In a first step, oxylipins were purified by reverse-phase HPLC. This was performed on an EC250/2 Nucleosil 120-5 C18 column (250 × 2.1 mm, 5 μm particle size; Macherey & Nagel, Düren, Germany) with the following binary gradient system: solvent A [methanol:water:acetic acid (75:15:0.1, v/v)] and solvent B [methanol:water:acetic acid (100:0:0.1, v/v)] with the gradient program 20% solvent B for 10 min, followed by a linear increase of solvent B up to 40% within 18 min, by a linear increase of solvent B up to 100% within 2 min and held for 10 min, by a linear decrease up to 20% solvent B within 5 min and finally by an isocratic run at 20% solvent B for 11 min. The flow rate was 0.18 mL/min up to 30 min and increased linearly to 0.36 mL/min within 5 min and held for 10 min followed by a linear decrease up to 0.18 mL/min within 5 min and held for 6 min. The injection volume was 80 μL. Straight-phase HPLC for separation of hydroperoxy fatty acids as well as hydroxy fatty acids was performed on a Zorbax Rx-SIL column (150 × 2.1 mm, 5 μm particle size, Agilent, Waldbronn, Germany) with *n*-hexane/2-propanol/acetic acid (100:1:0.1, v/v/v) as a solvent system at a flow rate of 0.2 mL/min (isocratic mode). The injection volume was 25 μL. For detection of the hydroperoxy and hydroxy fatty acids, the absorbance was recorded at 234 nm. The enantiomeric composition of the hydroxy fatty acids was analyzed by chiral-phase HPLC on a Chiral OD-H column (150 × 2.1 mm, 5 μm particle size; Baker, Griesheim, Germany) with *n*-hexane:2-propanol:acetic acid (100:5:0.1, v/v/v) as a solvent system at a flow rate of 0.1 mL/min (isocratic mode). The injection volume was 10 μL. The data are the average of three repetitions.

Antioxidant Capacity. Free radical scavenging activity was performed using DPPH assays (*21*) with some modifications. DPPH solution was prepared by mixing 0.5 mM of DPPH in 50% aqueous HLPC grade methanol during 1 h in the dark at room temperature. The resulting solution was then filtered (Whatman 595 1/2, 150 mm ∅) and 50 mg of frozen sample powder was added to 50 mL of the filtered DPPH solution. The frozen samples were directly defrosted in the DPPH solution in order to guarantee a rapid interaction between the DPPH and the antioxidant molecules. The reaction flask was placed in a rotating incubator in the dark at 38 °C. After 4 h, the mixture was filtered (Whatman 595 1/2, 150 mm ∅), and the absorption was recorded at 515 nm. Antioxidant activity was expressed as Trolox equivalents (TE, μmol of Trolox/g of samples) using a Trolox calibration curve ranging from 0 to 8.5 μM. All measurements were done in triplicate.

Total Phenolic Compounds. Phenolic compounds extraction was performed as follows: 1 mL of methanol 80% (v/v) was added to 0.05 g of powder. Then the mixture was shaken during 15 min at room temperature and then centrifuged at 17000*g* during 15 min at room temperature. The supernatant was collected and kept on ice. The total phenolics content was determined using the Folin-Ciocalteu method as described in ref *22* with some modifications. Briefly, to 100 μL of supernatant 400 μL of deionized water and 500 μL of Folin-Ciocalteu reagent were added. The mixture was vortexed and kept for 5 min at room temperature. Then, 1 mL of Na_2CO_3 7% (w/v) was added. The mixture was vortexed, and after incubation for 1 h at room temperature the absorption was measured at 760 nm. The amount of total phenolics was expressed as gallic acid equivalents (GAE, mg of gallig acid/g DW) using a calibration curve of a freshly prepared gallic acid solution. The calibration curve ranged from 0 to 10 mg/L. For each process step, two independent extractions were performed and 2 repetitions were performed for each extraction.

Total Carotenoids. Total carotenoid content was determined using a spectrophotometric method (*23*). Briefly, 0.3 g of frozen powdered sample was mixed with 1.5 mL of acetone solution containing 1% (w/v) BHT. The mixture was then vortexed and centrifuged at 20000*g*, 10 min at 4 °C. The supernatant was removed and stored on ice. Then, the sample was extracted again and both supernatants were mixed. The absorbance was measured at 450 nm and an extinction coefficient of 2500 mL/(0.01 g·cm) was used. The results were expressed in μg/g DW. For each process step, two independent extractions were performed and two repetitions were performed for each extraction.

Ascorbic Acid. The reduced form of ascorbic acid (L-AA) and the oxidized form of ascorbic acid (DHA) content were determined using a colorimetric method described by ref *24*. The potato tuber powder (0.5 g) was homogenized with two volumes of chilled 5% (w/v) metaphosphoric acid on ice. The homogenate was centrifuged at 18000*g* (15 min, 4 °C), and the supernatant was then used for analysis. Total ascorbic acid was determined after the reduction of DHA to L-AA with dithiothreitol (DTT), and the concentration of DHA was calculated from the difference between the total ascorbate pool (L-AA + DHA) and the L-AA pool. The reaction mixture for the total ascorbate pool contained 100 μL of the supernatant, 250 μL of 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, and 50 μL of 10 mM DTT. After incubation for 10 min at room temperature, 50 μL of 0.5% (v/v) *N*-ethylmaleimide was added to mask excess DTT. L-AA was determined in a similar way except that 100 μL of H_2O was added rather than DTT and *N*-ethylmaleimide. The following reagents were then added: 200 μL of 10% (v/v) trichloroacetic acid, 200 μL of 44% (v/v) ortho-phosphoric acid, 200 μL of 4% (v/v) 2,2′-dipyridyl in 70% (v/v) ethanol and 100 μL of 1% (w/v) $FeCl_3$. After vortexing, the mixture was incubated at 40 °C for 40 min and the absorbance at 525 nm was recorded. Two standard curves were calculated based on L-AA diluted in 5% (w/v) metaphosphoric acid in the 0−1000 μM range.

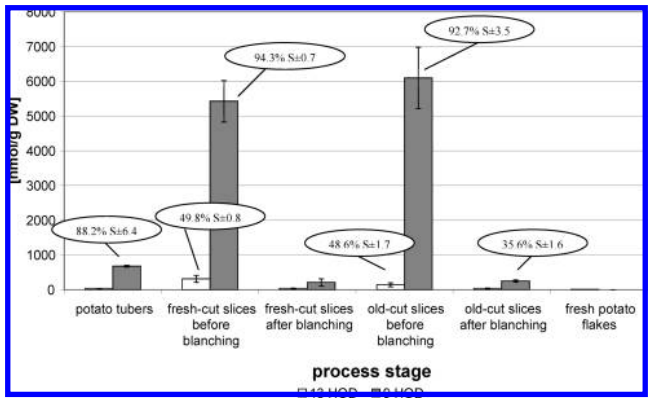

Figure 2. Fatty acid degradation products obtained after incubating of LOX extracts with linoleic acid and percentage of *S* enantiomers of 9- and 13-HOD.

For each process step, two independent extractions were performed and 2 repetitions were performed for each extraction. The results were expressed in terms of mg/g DW.

Data Analysis. In all figures, the values correspond to the mean and the error bars to the standard deviations. After one-way variance analysis means were classified using Student's *t*-test. Differences between means were considered to be significantly different at $P < 0.05$. As water content of potato slices is on average of 82% whereas potato flakes water content is on average 9%, all amounts are expressed per gram of dry weight.

RESULTS AND DISCUSSION

The aim of the current study is to identify production process parameters influencing the formation of hexanal precursors and/or the degradation of natural antioxidants. Here the major focus is on the identification of critical processing steps leading to lipid oxidation and antioxidant degradation.

LOX Activity. A three-step HPLC method including chiral phase analysis was performed to establish a quantitative as well as a qualitative measure of LOX activity in potato slices during flake processing. We measured the production rate and the enantiomeric ratio of hydroxides formed during the incubation of the enzymatic extract from potato samples with its substrate, linoleic acid after subsequent reduction of the initially formed hydroperoxides. The enzymatic formation of fatty acid hydro-(pero)xides is determined by a high *S* enantiomer percentage and was set here as to be higher than 90% (*25*). By using this method, we observed that LOX activity is drastically higher in potato slices before blanching than in tubers (**Figure 2**). Indeed, 9-hydroxide formation from linoleic acid (9-HOD) is, respectively, about 8 and 9 times higher in fresh and old slices before blanching than in potato tubers (5425.9 ± 597.5 nmol/g DW and 6097.4 ± 882 nmol/g DW vs 676.9 ± 23.1 nmol/g DW). In addition the relative amount of *S* enantiomer for 9-HOD was 94.3% ± 0.7 for fresh-slices and 92.7% ± 3.5 for old-slices. This indicates that they are formed enzymatically. Moreover, we observed that formation of 13-hydroxides from linoleic acid (13-HOD) is about 9 and 4 times higher in fresh- and old-slices, respectively, than in potato tubers (312.3 ± 104.6 nmol/g DW and 140.2 ± 57.7 nmol/g DW vs 35.4 ± 0.42 nmol/g DW). However, the relative amount of *S* enantiomer for 13-HOD is 49.8% ± 0.8 for fresh-slices and 48.6% ± 1.7 for old-slices. This indicates that they are formed nonenzymatically. So, first production process steps (peeling and slicing) lead to an increase in 9-LOX activity as well as an increase in autoxidation. However, the formation of both 9- and 13-HOD decreases drastically after blanching (from about 25 times for 9-HOD to about 4 to 8 times for 13-HOD in old-cut and fresh-cut slices, respectively). Furthermore, we observed that residual 9-HOD (248.8 ± 34.1 nmol/g DW) present in old-slices after blanching is formed by autoxidation (relative amount of *S* enantiomer 35.6% ± 1.6), indicating that blanching parameters used (80 °C during 15 min) lead efficiently to denaturation of 9-LOX activity. So, the main objective of blanching, which is to inactivate the enzymes responsible for deterioration reactions such as LOX, is reached (*13*). Finally, the 9-HOD produced in fresh potato flakes is below the detection limit and 13-HOD formation in flakes is almost 5 times lower than in slices after blanching (7.57 ± 0.46 nmol/g DW vs about 36 nmol/g DW), indicating that the cooking and the drying steps also take part in the degradation of residual compounds that may support formation of autoxidation products in potato flakes during processing.

Oxylipin Profiling. Monitoring the capacity to form hydro-(pero)xy-PUFAs during the production process is one indicator to identify those process steps responsible for off-flavor formation. Indeed, hydroperoxides can lead to the formation of volatiles responsible for damageable flavor changes. The major volatile compound responsible for off-flavors in potato flakes is hexanal, which is the degradation product of 13-HPOD, independent whether it is formed enzymatically or chemically. However, other hydroperoxides can lead to the formation of volatile compounds involved in potato flake flavor. Indeed, 9-HPOD can decompose into nonenal (*26*), and 9- hydroperoxide of linolenic acid (9-HPOT) is the precursor of nonadienal (*10*). These two 9-carbon volatile compounds are involved in fresh potato flavor. Therefore, monitoring the accumulation of hydroxides, that represent metabolic relatively stable degradation products of the corresponding hydroperoxides, gives additional information about critical production steps. For example, the formation of the 12- and 16-esterified hydroxides of linolenic acid (12- and 16-HOTMe) formation is indicative for an involvement of autoxidative processes through the production process (*20, 27*).

The nonesterified hydroxy- and hydroperoxy-PUFAs were the main oxylipins identified. Their profiles are shown in **Figure 3A** and **Figure 3B**, respectively, and the profiles of esterified hydroxy- and hydroperoxy-PUFAs are shown in **Figure 3C** and **Figure 3D**, respectively. In general, we observed that potato slices contain more 9- and more linoleic acid-derived oxylipins than 13- and linolenic acid-derived oxylipins. That can be explained by the predominant occurrence of linoleic acid and 9-LOX isoforms in potato tubers (*28*).

Regarding the impact of production process on the formation of off-flavor precursors, it appears that, in general, lipid oxidation product content is not significantly higher in potato flakes than in potato tubers. So, even if some production steps enhance oxylipins formation, other steps, especially thermal treatments, are able to degrade them in most cases.

The degradation of native or produced oxylipins is particularly pronounced for free hydroperoxides and esterified linoleic derived-hydroperoxides (HPODMe), of which content in potato flakes is below the detection limit. So, formation of free hydroperoxides and HPODMe, including hexanal precursors, 13-HPOD(Me), is efficiently inhibited by the production process. We observed a dramatic decrease of nonesterified products since the beginning of the production process indicating that washing makes a significantly important contribution to the degradation of lipid oxidation products. For example, the content of 9-HPOD, which is the predominant oxylipin in potato tubers, is about 17 and 5 times lower in fresh slices before blanching

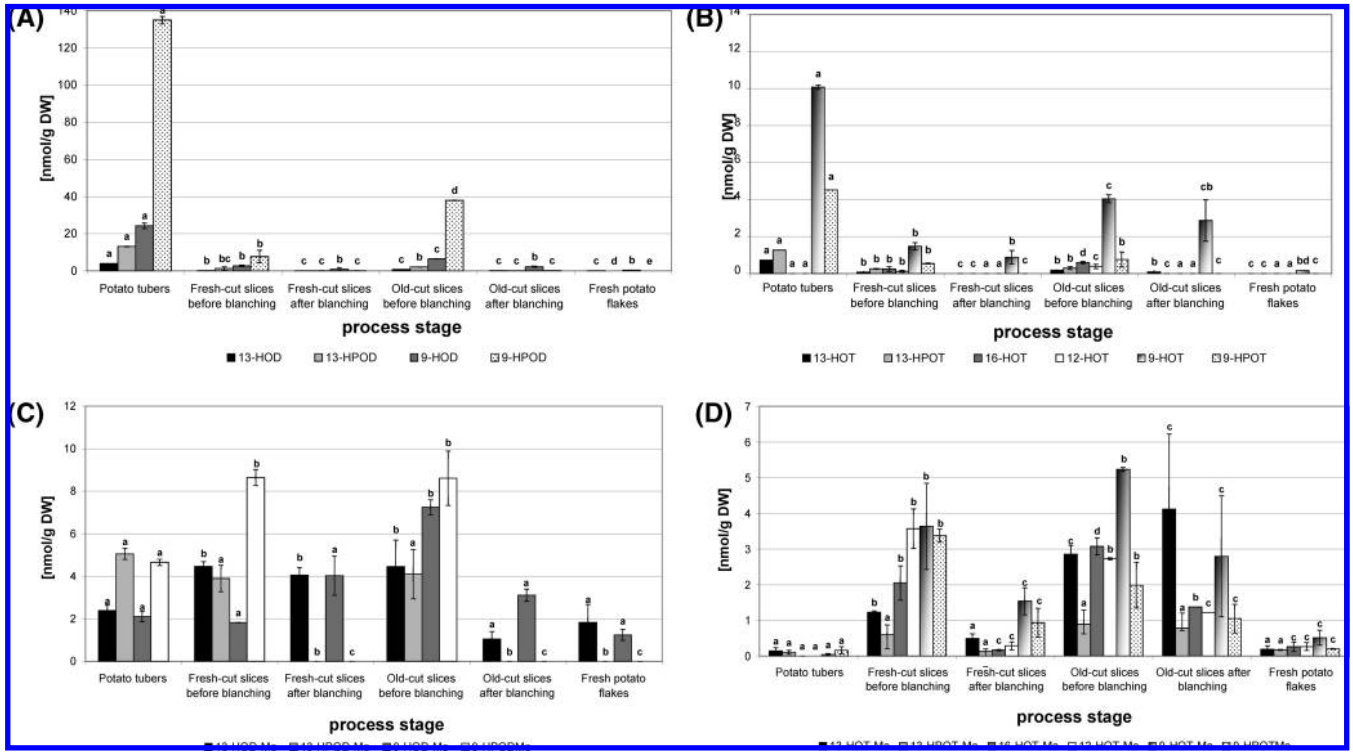

Figure 3. (**A**). Free linoleic-derived oxylipins profiling during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$). (**B**). Free linolenic-derived oxylipins profiling during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$). (**C**). Esterified linoleic-derived oxylipins profiling during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$). (**D**). Esterified linolenic-derived oxylipins profiling during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$).

and old-slices before blanching respectively than in potato tubers (**Figure 3A**). Moreover, thermal treatments degrade the majority of residual nonesterified products. Indeed, only content of 9-hydroxides from linolenic acid (9-HOT) and 9-HOD is not below the detection limit but 9-HOT and 9-HOD content in flakes is significantly lower than in potato tubers.

On the other hand, content of esterified oxylipins (13-HOD-Me, 9-HOD-Me, 13-HPOT-Me, 13-HOT-Me, 9-HPOT-Me, 9-HOT-Me) increases during the first steps of the production process (**Figure 3C** and **Figure 3D**). However, following thermal treatments, their content decreases and is not significantly different in potato flakes than in potato tubers except for 12- and 16-HOT-Me. Indeed, these two autoxidation products were detected in potato slices during processing and in potato flakes (0.26 ± 0.13 nmol/g DW for 16-HOT-Me and 0.26 ± 0.11 nmol/g DW for 12-HOT-Me) whereas they were not detected in potato tuber indicating that autoxidation is activated during processing. Moreover, the presence of 12- and 16-HOT-Me in flakes indicates that thermal treatments such as blanching, cooking and drying steps cannot completely degrade autoxidation products formed during the early stages of processing and/or that thermal treatments cannot inactivate the autoxidation processes leading to their formation.

Total Antioxidant Activity. The measurement of total antioxidant activity throughout flake processing is indicative for the sensitivity to processing of potato tuber antioxidants as a whole. Antioxidants prevent or delay lipid oxidation due to their ability to scavenge free radicals (*11*). It appears that total antioxidant activity is strongly enhanced by adding synthetic antioxidants at the end of the process (**Figure 4**). Indeed, the total antioxidant activity in flakes is about 4 times higher at the end of the production process than in tubers and in the slices during the process. On the contrary, we observed that the total

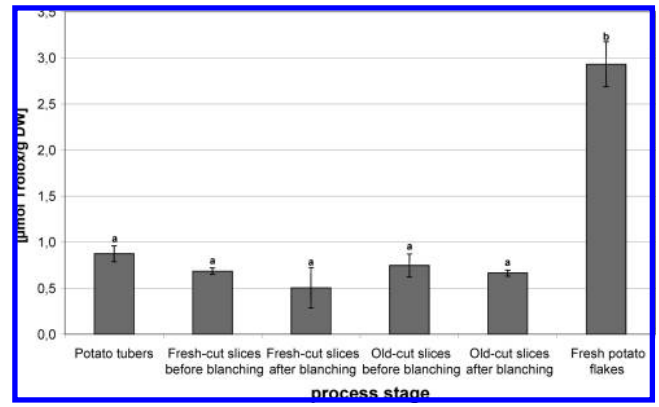

Figure 4. Total antioxidant activity of potato slices during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$).

antioxidant activity is not significantly different through the process before synthetic antioxidant addition. However, free radical scavenging activity measurement leads to an incomplete estimation of total antioxidant activity (*29*). Moreover, DPPH assay does not allow determination of the sensitivity to processing of each intrinsic antioxidants present in potato tubers. For this purpose, we focused on the formation of total phenolic compounds, total carotenoid and ascorbic acid content of potato tubers and slices during potato flake processing.

Natural Nonenzymatic Antioxidant Content. *Ascorbic Acid.* Ascorbic acid is the major antioxidant in potato tuber and is highly water-soluble (*17*). During processing, ascorbic acid degradation takes place mainly by leaching into blanching or washing waters for example. However, oxidation and thermal degradation can also lead to the degradation of ascorbic acid (*30*). Diffusion of ascorbic acid into blanching water is increased

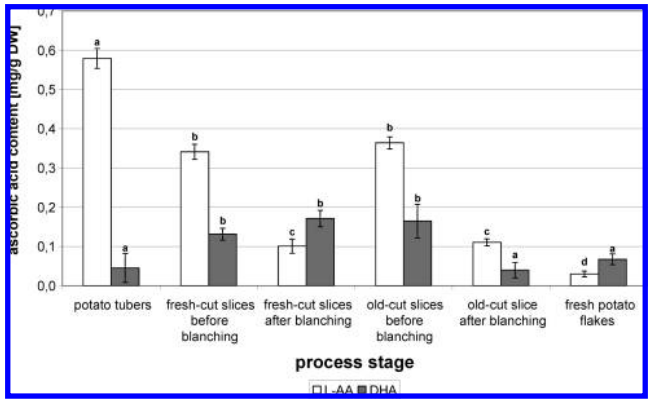

Figure 5. L-AA and DHA contents of potato slices during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$).

Table 1. Total AA Content and Relationship between L-AA and DHA during Potato Flake Processing

process stage	AA [mg/g DW]	L-AA:DHA
tubers	0.63 ± 0.02	1:0.09
fresh-cut slices before blanching	0.47 ± 0.01	1:0.38
fresh-cut slices after blanching	0.27 ± 0.02	1:1.76
old-cut slices before blanching	0.50 ± 0.06	1:0.44
old-cut slices after blanching	0.15 ± 0.02	1:0.36
fresh potato flakes with ascorbyl palmitate	0.10 ± 0.01	1:2.43
fresh potato flakes without ascorbyl palmitate	0.13 ± 0.01	1:1.85

with heating of potato tissue at 55−90 °C. This effect is probably due to the denaturation of cell membranes, which allows molecular solutes, like ascorbic acid, to diffuse freely in the tissues (*13*).

The effect of the production process on reduced ascorbic acid (L-AA) and oxidized ascorbic acid (DHA) contents in potato tuber is shown in **Figure 5**. We observed that losses of total ascorbic acid (AA) were about 80% during production process and that the relationship between L-AA and DHA changes drastically as shown in **Table 1**, indicating a degradation of L-AA toward DHA. Overall, L-AA content decreases drastically (about 95% of loss) during processing of potato flakes whereas DHA content increases (about 40% of increase). It is not surprising as changes in the relationship between L-AA and DHA during French Fries processing follow the same scheme (*30*).

During flake processing, washing, slicing, blanching and drying lead to significant losses of L-AA. Concerning slicing and washing, L-AA content is about 40% lower in fresh-cut slices before blanching than in potato tubers whereas DHA content in fresh-cut slices before blanching is about 59% higher than in potato tubers. The degradation of L-AA in potato slices can mainly be explained by diffusion of ascorbic acid into cleaning water. Indeed, we know that leaching is one of the major causes of ascorbic acid loss during processing (*30*). Moreover, as oxidation due to the transfer of slices between two production chains does not lead to a significant decrease in L-AA content, the decrease in L-AA content in potato slices before blanching should be mainly due to leaching and not to oxidation. On the other hand, the increase in DHA content can be explained by the oxidation of L-AA resulting from slicing. Concerning the blanching step, the loss of L-AA is about 70% in the fresh-cut as well as in the old-cut potato tubers. This huge decrease is due to diffusion of ascorbic acid into blanching water. Moreover, the blanching temperature used (80 °C) during flake processing is included in the temperature range leading

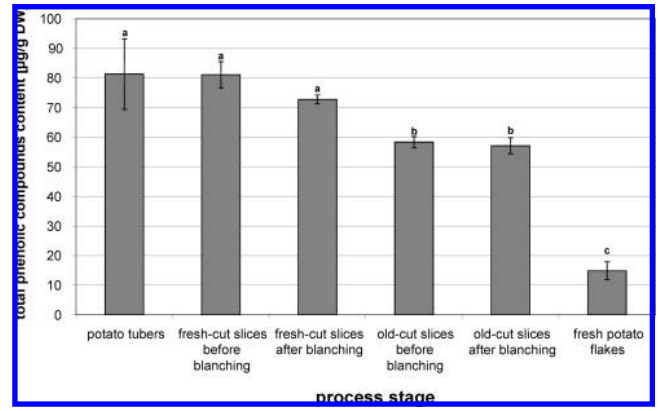

Figure 6. Total phenolic content of potato slices during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$).

to an increased diffusion of ascorbic acid into blanching water. Regarding the last processing steps which are cooking and drying, we observed that L-AA content in fresh potato flakes is about 50% lower than in slices after blanching. Thermal degradation of L-AA during potato mash dehydration is thus also responsible for L-AA loss during the process.

Finally, we quantified L-AA content in potato flakes without ascorbyl palmitate in order to determine if the quantification of L-AA in flakes containing ascorbyl palmitate did not lead to an overestimation of L-AA content in flakes. We found no significant differences between L-AA content in potato with or without ascorbyl palmitate (data not shown).

Total Phenolics. Phenolic compounds, including chlorogenic acid which is the major phenolic compound in potato tubers (*15*), are water-soluble and thus susceptible to leaching. Moreover, since phenolic compounds are antioxidants, they are subjected to oxidation during processing and storage of foodstuff. However, during processing, the decrease in phenolic compounds content is usually largely due to leaching rather than oxidation. Furthermore, changes in phenolic compounds content during thermal treatments appear to be highly variable by commodity. However, thermal treatments such as blanching or cooking, usually appears to increase the extractability and thus the leaching of phenolic compounds (*17*).

The effect of flake processing on total phenolic content in potato tubers is shown in **Figure 6**. Main critical steps for phenolic compounds during flake processing are the cooking and the drying steps. Indeed, total phenolic content of potato flakes is 74 and 80% lower than in old-cut and fresh-cut slices, respectively. Therefore, the high temperature required for cooking and drying are responsible for phenolic compound losses at the end of processing.

Additionally, we observed no significant impact of slicing, washing or blanching on total phenolic content whereas there is a significant impact of the transfer between the two different production chains. Indeed, total phenolic content of old-cut potato tubers (58.3 ± 1.9 µg/g DW) is significantly lower than in fresh-cut potato tubers (81.1 ± 4.4 µg/g DW). Oxidative attacks sustained by slices when they are transferred from the French Fries production chain to the potato flake production chain explain this result.

Total Carotenoids. The main carotenoids in potato are xanthophylls (*15*). Processing can be responsible for carotenoid degradation especially due to their high sensitivity to oxidation by oxygen or peroxides (*31, 32*). The effect of production process on total carotenoid content in potato tuber is shown in **Figure 7** First, it appears that about 27% of carotenoids are

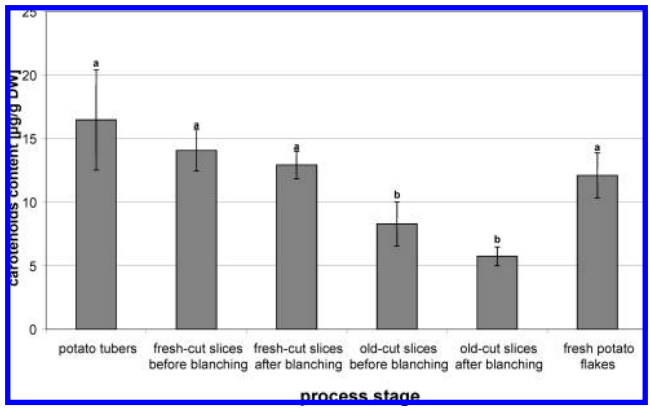

Figure 7. Total carotenoid content of potato slices during potato flake processing. Within the same data set, bars with different letters are significantly different ($P < 0.05$).

lost during production process. This result coincides with the results of ref *32* showing that 25 to 34% of carotenoids are lost during the processing of hot chilli peppers (*Capsicum frutescens* L.). The most critical point is the oxidation sustained by carotenoids during the transport between two production chains. Indeed, we observed that total carotenoid content of old-cut slices before blanching (8.28 ± 1.73 μg/g DW) is significantly lower than total carotenoid content of fresh-cut slices before blanching (14.1 ± 1.6 μg/g DW) and potato tuber (16.48 ± 3.95 μg/g DW). On the other hand, even if carotenoid content decreases with slicing and blanching, this decrease is not significant. Moreover, carotenoid content is not significantly influenced by cooking or by the drying step.

To summarize, we observed overall that flake processing does not contribute to an increase in the content of lipid oxidation products. Thermal treatments performed during flake processing lead to the inhibition of LOX activity and to the degradation of hexanal precursors but they cannot completely degrade autoxidation products and/or inhibit autoxidation processes in flakes. Moreover, processing promotes autoxidation by degradation and/or elimination of intrinsic antioxidants of potato tubers.

So, we can conclude that hexanal is formed by autoxidation during storage and that processing does not contribute to the formation of its precursors. This is consistent with the content in hexanal and its precursors during flake storage. Indeed, at the end of the production process, the content in hexanal (*6*) and in hexanal precursors was below the detection limit in potato flakes containing ascorbyl palmitate and curcuma. After 4 weeks of storage, hexanal content was still below the detection limit but very low content of hexanal precursors was already detected (0.06 ± 0.02 nmol/g DW for 13-HPOD and 0.32 ± 0.05 nmol/g DW for 13-HPOD-Me) (*6*).

In order to prevent lipid oxidation during storage, antioxidants are added at the end of the production process. As some synthetic antioxidants are suspected to pose a human health risk, natural alternatives to synthetic antioxidants are in great demand (*18, 19*). Ascorbic acid and phenolic compounds are the main antioxidants in potato tubers. However, because of their water-solubility, their content decreases drastically due to their diffusion into washing and blanching waters. Therefore, the use of recycled water during washing and blanching could lead to an increase in the final retention of these compounds. Indeed, it has been shown that the diffusivity of ascorbic acid during potato blanching at 80 °C in recycled water was lower than that obtained at the same temperature in distilled water (*30*). Concerning the use of natural antioxidants as exogenous extracts, it must be noted that, in the potato industry, antioxidants are added in mashed potato before the drying step. Therefore, natural antioxidant candidates have to show a good preservation during the drying step. Carotenoids show thus good attributes as natural antioxidants, but further investigations are required to determine the impact of the drying step on natural antioxidant extracts. In addition, some potato species and cultivars contain naturally high carotenoid levels (*23*) or can be engineered to contain substantial carotenoid content (*33*). Therefore, potato tubers containing high carotenoid content and showing appropriate properties for industrial transformation could be tested for their preservation skills during flake processing. Finally, specific attention has to be paid to storage conditions to prevent loss of sensory quality of flakes. Storage temperature and atmosphere packing are key components that can be modified in order to prevent lipid oxidation during flake storage. Indeed, storage temperature below room temperature, as well as nitrogen packing, extends shelf life of stored products by preserving sensory quality (*1, 10*).

ABBREVIATIONS USED

PUFAs, polyunsaturated fatty acids; LOX, lipoxygenase; HOD, hydroxy octadecadienoic acid; HOT, hydroxy octadecatrienoic acid; HPOD, hydroperoxy octadecadienoic acid; HPOT, hydroperoxy octadecatrienoic acid; Me, esterified; AA, total ascorbic acid; DHA, oxidized ascorbic acid; L-AA, reduced ascorbic acid.

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