Pilot scale biotransformation of vegetal oil into natural green notes flavor using sugar beet leaves as sources of hydroperoxide lyase

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Introduction
Natural green note aromas (GLVs) are highly attractive flavors commonly used in the food industry. These are produced in extremely low levels upon physiological stress in plant organs of any sort. This weak sporadic presence entails a very expensive extraction step to obtain pure GLVs. Therefore catalytic biotransformations of fatty acid sources, the initial substrate for GLVs, have been developed. Enzymatic defense pathways and particularly the LOX pathway produce the major part of GLVs. Unlike GLV molecules that are emitted in the atmosphere, the enzymes are extractable from the plant material. Thus, a combination of plant enzyme extracts and substrate preparations provides all the ingredients for GLV production. Besides, sugar beet leaves present high levels of hydroperoxide lyase among plant sources and are available in large amounts during three months. In this enzymatic pathway, fatty acids are successively transformed by lipoxygenase, lipoxygenase and hydroperoxide lase into aldehydes and alcohols, final compounds of GLVs pathway. Limiting and problematic steps occur with the action of hydroperoxide lase, when enzymatic catalysis is followed by an enzyme denaturation. Alternative substrates lead invariability to the same group of the enzyme and end the reaction. This post briefly describes the development of a complete bioprocess for natural GLV production, from hydrolyses to purification. A high level of biotransformation could be achieved using optimum experimental conditions and a cheap source of plant materials.

Objective and purpose
Establishing a simple production bioprocess of green leaf flavors using unwanted plant materials like sugar beet leaves. The process has to be efficient and leads to the purest flavor composition.

Material and Methods
All reactions have been performed in pilot scale vessels, including mixed bioreactor of 100L. Reaction order simply follows the natural lipoxygenase pathway present in most of the superior plants.

Main substrate source
Sugar beet leaves do not present high levels of fatty acids content (only 0.6%). Therefore, the use of an external source of initial substrate is necessary. Vegetable oils are cheap sources of unsaturated fatty acids which are easily obtained, and stable enough for flavor production. Final aroma composition will depend on concentration of -linolenic or -linolenic acid present in the oil.

Fatty acids hydrolysis
Following plant characterization, laked oil has been chosen as substrate source, on account of its high linolenic acid content. Hydrolysis is a common chemical reaction in industry. We hydrolyze the oil enzymatically with lipoxygenase to keep neutral conditions at room temperature. Hydrolysis is performed by immobilized lipoxygenases from a Bacillus strain. Enzymes are recycled 3 times and a high yield (more than 90%) of free fatty acids has been recovered after centrifugation. No emulsifying compounds have been used to avoid loss of free fatty acids by centrifugation.

Fatty acids oxidation
The lack of GLV activity (less than 0.1 IUg/g of fresh weight) in sugar beet leaves requires an external source of this enzyme. Actually, certain cleavage seeds are the best sources of -13-lipoxygenase activity. This reaction is fast and completed within 10 minutes at 4°C and pH 6.3.

Aldehyde synthesis
This is the most critical step of the lipoxygenase pathway which is due to the instability of hydroperoxide lase activity. The aldehyde synthesis has to be carefully controlled. After the setup of the optimum conditions (pH and temperature) for the hydroperoxide lase of sugar beet, ground plant materials are quickly added to the production juice containing previously synthesized hydroperoxides.

Proposed pilot-scale biotransformation process of vegetal oil into green note flavor using sugar beet leaves

Following the mixing of substrates and enzymes, only productions of 2-Hexenal and E-2-Hexenal were measured. Maximum reaction levels are obtained within 5 minutes and thereafter decrease slowly due to the aldehyde consumption by other enzymic activities from the plant materials. The maximum concentration can be maintained for 30 minutes after a pH regulation to inhibit the enzymatic degradation.

Conclusion
At the end of the process, pure aldehydes (Hexanal and E-2-Hexenal) can be recovered. These compounds can be labeled as “natural flavor” because they are vegetal oil derivatives. The price value is more than 10006/pot for these compounds and they could be easily used in food and beverage sectors. This long and complex biotransformation process has many reasons in expensive flavors may be a solution to convert unwanted crop byproducts, such as sugar beet leaves.