

Maisons-Alfort, June, 2<sup>nd</sup> 2003

## **Avis (Opinion)**

Of AFSSA, the French Food Safety Agency

On the authorization of using a lactoperoxidase system  
as a processing aid for the treatment of fresh-cut, ready-to-eat salads

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Further to a mail received on January, 9<sup>th</sup> 2003, AFSSA has been requested on January, 6<sup>th</sup> 2003 by DGCCRF, the French Authority for Competition, Consumer protection and Fraud Prevention for an “avis” (opinion) concerning the authorization of using a lactoperoxidase system as a processing aid for the treatment of fresh-cut, ready-to-eat salads.

Two “avis” (opinions) concerning this request have been written by AFSSA, respectively on March, 7<sup>th</sup> 2002 (saisine n° 2001-SA-0169) and on November, 6<sup>th</sup> 2002 (saisine n° 2002-SA-0167); the second one particularly stating that the lack of some toxicological and microbiological data (detailed in this opinion) did not enable to demonstrate the innocuousness for consumers nor the efficiency of the process, object of the request.

On the basis of a new file written by the petitioner, and after having consulted the specialized Expert Committees “Additives, Aromas and Processing Aids” brought together on April, 1<sup>st</sup> 2003 and “Microbiology” brought together on April, 22<sup>nd</sup> 2003, AFSSA gives the following opinion.

### **Principle and interest of the process**

#### **Principle**

Considering that the principle of the process consists in producing antimicrobial isothiocyanate ions (OSCN<sup>-</sup>), formed water enriched by potassium thiocyanate and hydrogen peroxide, and running on a reactor containing lactoperoxidase fixed on a support (clay particles) ;

Considering that the submitted system enables the production, in industrial conditions, of water containing isothiocyanate , which can be used to wash salads on a fresh-cut salad production line ;

Considering that the assessed process aims at producing isothiocyanate ions at a concentration of 0,6 mM, from a hydrogen peroxide and potassium thiocyanate maximum concentration of 2mM ;

#### **Interest versus the use of chlorine**

Considering that chlorine can be used, with a target value of 80 ppm of active chlorine<sup>1</sup>, to treat fresh-cut, ready-to-eat vegetables so as to enhance their microbial quality ;

Considering that the salads treated with the process behave just as the salads treated with chlorinated water ;

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<sup>1</sup> *Guide of good practice for fresh-cut, ready-to-eat vegetables, 1996*

## **Microbiological efficiency of the process**

Considering that, on microbiological aspects, the submitted file has been notably enhanced compared to the previously assessed files, and provides answers to the various questions regarding the microbiological efficiency of the product ; and that these elements comprise :

### **1. A measurement of the process efficiency against the relevant pathogenic bacteria, and some viruses**

#### **Mesophilic aerobic flora**

Considering that the process actually seems to have the same effect as chlorine as a processing aid, in fresh-cut, ready-to-eat production conditions, and this for the microbiological indicators used in this sector (mesophilic aerobic flora), providing the operating conditions are the same as in the submitted file;

#### **Pathogenic bacteria**

Considering that in laboratory conditions, the isothiocyanate generated by the process at concentrations around 0,6 mM has a bactericidal activity against the pathogenic bacteria which are likely to contaminate vegetable raw materials and become a hazard ; considering nevertheless that this bactericidal activity (although significant) is inferior (1 log in average) to that of chlorine, and shows that it cannot be strictly considered as a chlorine substitute ;

Considering that it is nevertheless advisable to highlight that chlorine activity is considerably reduced in presence of organic material (principally in presence of salad leaves) ; and in that way, a contamination reduction superior to 1 or 2 logs on salad leaves artificially contaminated with *Listeria* and treated with a 50 ppm chlorine solution has never been observed ;

#### **Viruses**

Considering that publications on the antimicrobial effect of the lactoperoxidase system against herpes virus and HIV are presented in the file ; considering nevertheless that the choice of these viruses does not seem relevant ;

Considering in other respects that few of these publications specify the amount of isothiocyanate produced, making the comparison with the petitioner's work difficult ; that when the comparison is possible, the results are comparable to the ones presented in the file ;

Considering that it seems therefore difficult to conclude on the efficiency of the process against viruses;

### **2. A measurement of the disinfecting power of fresh-cut salad washing water**

Considering that the trials undertaken demonstrate that the process enables a reduction of the washing water mesophilic aerobic flora equivalent to the reduction achieved with 100 ppm chlorine, which is a dose superior to the one (80 ppm) usable for the treatment of fresh-cut, ready-to-eat salads;

### **3. Information on the neutralising agent used during analyses**

Considering that isothiocyanate is neutralised before analysis by adding cysteine, and that the petitioner has provided the proof that cysteine, at the concentrations used, actually neutralises the antibacterial activity before bacteriological analysis ;

Considering therefore that the demonstration of the efficiency of the neutralising process is provided ;

#### **4. A presentation of industrial trials, enabling the visualization of the reduction of the microbial population achieved by the considered treatment**

Considering that two industrial trials on line, the first of which includes the treatment of several salad types, are presented ;

Considering that for each trial, raw data are presented with standard deviations, and the reduction achieved with the active principles (submitted process and chlorine as a reference) is clearly demonstrated, and that it is obvious from this that 0,6 mM of isothiocyanate in washing water is equivalent to 120 ppm of chlorine and enables a reduction between 1,5 and 2 log of the mesophilic aerobic flora ;

#### **5. A presentation of the variability between the three analyses done after the trials undertaken**

Considering that raw microbiological counts, and standard deviations most of the time calculated from 3 samples, are presented in the file ;

#### **Toxicological assessment of the process**

Considering that, during the industrial trials in normal operating conditions :

- the analytical data presented show the absence of hydrogen peroxide and the absence of lactoperoxidase enzymatic activity in the washing tank, during and at the end of the production ;
- the analytical data presented show a steady production of isothiocyanate ions during and at the end of the production and that it is planned to do regular analyses during the industrial process operation ;

Considering that the assessed process includes a turbidity measurement and filtering system, in order to control that no matters in suspension are released from the reactor into the washing water ;

Considering that the assessed process includes a final stage consisting in rinsing with non recycled drinkable water ;

Considering that the analytical data presented show that thiocyanate residual concentration on salads is inferior to 0.5 mg/kg after the final rinsing ;

Considering that, according to the analytical data presented by the petitioner, the calculations at the maximum rate, on the basis of a 95<sup>th</sup> percentile consumption of leaf vegetables and fresh herbs of 62g/day<sup>2</sup> and of a 0.5 mg/kg thiocyanate residual concentration on rinsed salads, would lead to an exposure to thiocyanate ions of 0.03 mg/day ;

Considering that the calculation of the average human exposure coming from saliva ingestion results in a normal theoretical input of isothiocyanate ions that can reach 25 mg/day ;

Seeing the set of the above-mentioned considerations, AFSSA considers the consumption of the fresh-cut, ready-to-eat salads (“IV° gamme”) treated with the assessed process, under the operating conditions described in the file, does not present any risk for the health of the consumer.

AFSSA nevertheless states that the presented set of elements does not permit to conclude on the virucidal efficiency of the process.

Martin HIRSH  
Managing Director of the French Food Safety Agency

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<sup>2</sup> Consumption of fruits and vegetables and other food products. Updating dietary patterns enabling the fixation of a limitation regarding the maximum pesticides residues. Complement to the note n°118. Estimation of high levels of consumption. AFSSA-DERNS-Observatoire of Food Consumption. J. Maffre and J-L Volatier. July, 2000.