(11) **EP 1 310 562 A1**

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication: 14.05.2003 Bulletin 2003/20

(21) Application number: 01960746.4

(22) Date of filing: 02.08.2001

(51) Int Cl.7: C12P 7/22, A23L 1/30

(86) International application number: PCT/ES01/00314

(87) International publication number: WO 02/016628 (28.02.2002 Gazette 2002/09)

(84) Designated Contracting States:

AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

Designated Extension States:

AL LT LV MK RO SI

(30) Priority: 11.08.2000 ES 200002073

(71) Applicant: CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS 28006 Madrid (ES)

(72) Inventors:

 Espin de Gea, Juan Carlos Ctro. E. y B.A. del Seg. 30080 Murcia (ES) De Tomas Barberan, Francisco A.
 C.E.y B.A.del Seg.
 30080 Murcia (ES)

- Garcia Viguera, Maria C. Ctro. E. y B.A. del Seg. 30080 Murcia (ES)
- Ferreres de Arce, Federico Ctro. E. y B.A. del Seg 300080 Murcia (ES)
- Soler Rivas, Cristina 6700 Wageningen (NL)
- Wichers, Harry J.
 6700 Wageningen (NL)
- (74) Representative: Ungria Lopez, Javier et al Avda. Ramon y Cajal, 78 28043 Madrid (ES)

(54) ENZYMATIC SYNTHESIS OF ANTIOXIDANT HYDROXYTYROSOL

(57) The reaction occurs in a medium buffered with phosphate in an aqueous medium, at neutral pH and at room temperature. Hence, the reaction medium consists of: tyrosol, as precursor; (commercial) mushroom tyrosinase to catalyze the process and vitamin C in excess. Reaction starts after initial shaking. The reaction stops when the concentration of the initial tyrosol is exhausted. In order for the reaction to continue, it will suffice to add more tyrosol (always ensuring that the vita-

min C/tyrosol ratio is more than 1). Once the desired concentration of hydroxytyrosol is obtained, it is filtered. The enzyme with molecular weight over twice the size of the pore is retained in the filter and can be used once again. This first extract, which is very enriched in antioxidant hydroxytyrosol with a high antioxidant capacity (in the present case, in combination with vitamin C), can already be used as a food additive.

EP 1 310 562 A1

INTERNATIONAL SEARCH REPORT International application No. . . PCT/ES 01/314 CLASSIFICATION OF SUBJECT MATTER IPC 7 C12P 7/22, A23L 1/30 According to International Patent Classification (IPC) or to both national classification and IPC FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12P, A23L Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CIBEPAT, EPODOC, WPI, HCAPLUS, FSTA C. DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. Category* ESPIN, J.C., ET AL., "SINTHESIS OF THE ANTIOXIDANT HIDROXYTYROSOL USING TYROSINASE AS BIOCATALYST"; J.AGRIC.FOOD CHEM. 2001; VOL 49,N°3,PAG.1187-1193 PX 1-5 W00036936A1 (BOOM et al.) 29.06.00 X 5 CAPPASSO, R., et al, "A HIGHLY CONVENIENT SYNTHESIS OF HIDROXYTYROSOL AND ITS RECOVERY FROM AGRICULTURAL WASTE WATERS", J.AGRIC.FOOD CHEM.1999; VOL,47; \mathbb{N}° 4; PAG.1745-1748 À X See patent family annex. Further documents are listed in the continuation of Box C. later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "O" document referring to an oral disclosure, use, exhibition or other document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report 21 December 2001 (21.12.01) 10 December 2001 (10.12.01) Name and mailing address of the ISA/ Authorized officer S.P.T.O. Telephone No. Facsimile No.

Form PCT/ISA/210 (second sheet) (July 1992)