

**CHARACTERISATION OF THE GEOGRAPHICAL
ORIGIN OF MUSHROOMS
(*AGARICUS BISPORUS*) :**

**REPORT OF PHASE N°II :
SCREENING OF ANALYTICAL VARIABLES**

1. Study objectives

The objective of this phase is to determine the minimum number of analytical parameters (chosen among the most discriminatory) allowing a satisfactory differentiation of the countries of origin involved (China, France, Holland, and Spain). Thus the cost of further analyses will be optimised. This phase therefore consists in choosing the most efficient methodology in order to create a databank (which is planned in phase III).

Analytical parameters involved in the study:

- Isotopic ratios of Carbon ($^{13}\text{C}/^{12}\text{C}$) and Nitrogen ($^{15}\text{N}/^{14}\text{N}$) of the whole mushroom (after grinding and lyophilisation)
- Isotopic ratios of Carbon ($^{13}\text{C}/^{12}\text{C}$), and Oxygen ($^{18}\text{O}/^{16}\text{O}$) of mannitol after extraction and purification
- Isotopic ratios of Hydrogen (D/H) of mannitol after esterification of the exchangeable sites (hydroxyl groups)
- Isotopic ratio of Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) (after extraction of Sr)
- Isotopic ratios of Hydrogen (D/H) and Oxygen ($^{18}\text{O}/^{16}\text{O}$) in the water (for canned products)
- Chromatographic analysis of sugars and polyols
- Trace elements profile by ICP-MS

2. Samples description

The sampling plan for phase II was :

- 10 samples from France
- 10 samples from Holland
- 10 samples from Spain
- 10 samples from China
- 50 samples in total

2.1 French samples

In June 2003, a representative of Eurofins (Eric Jamin) visited three factories around Saumur (the most important production region in France) and took the following 13 samples directly from the lines/warehouses:

Eurofins n°	Description	type
0154/1922	Champignons entiers 1er choix Origine : France Usine : Thouars (79)	Fresh
0154/1923	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Thouars (79)Code emballeur : T38CDLC : 16/06/2007	canned
0154/1924	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Thouars (79)Code emballeur : T38CDLC : 10/06/2007	canned
0154/1925	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Thouars (79)Code emballeur : T38C32DLC : 28/05/2007	canned
0154/1926	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Doué-la-Fontaine (49)Code emballeur : D38CDLC : 31/03/2007	canned
0154/1927	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Doué (49)Code emballeur : D38CDLC : 09/05/2007	canned
0154/1928	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Doué (49)Code emballeur : D38CDLC : 16/06/2007	canned
0154/1929	Champignons entiers 1er choixOrigine : FranceUsine : Doué (49)	Fresh
0154/1930	Champignons entiers 1er choixOrigine : FranceUsine : Beaufort (49)	Fresh
0154/1931	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Beaufort (49)Code emballeur : B38CDLC : 10/06/2003	canned
0154/1932	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Beaufort (49)Code emballeur : B38CDLC : 12/06/2007	canned
0154/1933	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Beaufort (49)Code emballeur : B38C6DLC : 25/10/2006	canned
0154/1934	Champignons entiers 1er choix appertisésIngrédients : champignon, eau, sel, acide citrique, acide ascorbique.Origine : FranceUsine : Beaufort (49)Code emballeur : B38CDLC : 11/06/2007	canned

Among those, 10 samples were analysed and the following ones were stored : 0154/1924, 1927, 1934.

2.2 Dutch samples

The following samples were received by Eurofins from the Dutch association in July 2004:

Eurofins n°	Description	Type
0167/1757	Champignons de Paris entiers 1 er choix appertisés Marque : Lutèce BV Horst-Holland DLUO : 15/04/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1758	Champignons de Paris entiers 1 er choix appertisés Marque : Lutèce BV Horst-Holland DLUO : 02/07/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1759	Champignons de Paris entiers 1 er choix appertisés Marque : Bivita, Biniko foodservices DLUO : 23/06/2008	Canned
0167/1760	Champignons de Paris entiers 1 er choix appertisés Marque : Brakes DLUO : 24/06/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1761	Champignons de Paris entiers 1 er choix appertisés Marque : Brakes DLUO : 01/06/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1762	Champignons de Paris entiers 1 er choix appertisés Marque : Brakes DLUO : 14/06/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1763	Champignons de Paris entiers 1 er choix appertisés Marque : Brakes DLUO : 17/06/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1764	Champignons de Paris entiers 1 er choix appertisés Marque : Brakes DLUO : 15/03/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1765	Champignons de Paris entiers 1 er choix appertisés Marque : Brakes DLUO : 15/06/2008 Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxygène : acide ascorbique.	Canned
0167/1766	Champignons de Paris entiers 1 er choix appertisés Sample of fresh (fait par G. klein) Marque : Dutch mushrooms Pas d'étiquette	Canned
0167/1767	Champignons de Paris entiers 1 er choix surgelés	Frozen

Among those, 10 samples were analysed and the following one was stored : 0167/1767.

2.3 Spanish samples

The following 10 samples were received by Eurofins from the Spanish association in June 2005:

Eurofins n°	Description	Type
0178/5387	Projet GEPC - Phase II Champignons frais Origine : Espagne Fournisseur : Cidacos	Fresh
0178/5388	Projet GEPC - Phase II Champignons frais Origine : Espagne Fournisseur : Cidacos	Fresh
0178/5389	Projet GEPC - Phase II Champignons frais Origine : Espagne Fournisseur : Eurocamp SAT (AUTOL)	Fresh
0178/5390	Projet GEPC - Phase II Champignons frais Origine : Espagne Fournisseur : Ayecue	Fresh
0178/5391	Projet GEPC - Phase II Champignons en conserve Origine : Espagne Marque : Cidacos Code d'envoi : L516800 09:38AU Ingrédients : acide citrique : 500 ppm, acide ascorbique : 200 ppm, sel : 0.8%. Acides citrique et ascorbique d'origine chinoise.	Canned
0178/5392	Projet GEPC - Phase II Champignons en conserve Origine : Espagne Marque : Cidacos Code d'envoi : L515000 08:44AU Ingrédients : acide citrique : 500 ppm, acide ascorbique : 200 ppm, sel : 0.8%. Acides citrique et ascorbique d'origine chinoise.	Canned
0178/5393	Projet GEPC - Phase II Champignons en conserve Origine : Espagne Marque : Ayecue - AUTOL (LA RIOJA) Code d'envoi : 08:21 US U4A Ingrédients : eau, sel (pas de détail sur la liste des ingrédients et proportions).	Canned
0178/5394	Projet GEPC - Phase II Champignons en conserve Origine : Espagne Marque : Ayecue - AUTOL (LA RIOJA) Code d'envoi : 10:53 US U4A Ingrédients : eau, sel (pas de détail sur la liste des ingrédients et proportions).	Canned
0178/5395	Projet GEPC - Phase II Champignons en conserve Origine : Espagne Marque : Eurocamp SAT (AUTOL) LA RIOJA Code d'envoi : 3104011855CB07 Ingrédients : champignons : 53.20%, eau : 45.80%, sel : 0.90%, acide citrique : 0.09%, acide ascorbique : 0.01%.	Canned
0178/5396	Projet GEPC - Phase II Champignons en conserve Origine : Espagne Marque : Eurocamp SAT (AUTOL) LA RIOJA Code d'envoi : 310401465CA05 Ingrédients : champignons : 53.20%, eau : 45.80%, sel : 0.90%, acide citrique : 0.09%, acide ascorbique : 0.01%.	Canned

2.4 Chinese samples

As agreed with GEPC, it was decided to use samples available in Germany, either from importers or as retail products labelled as coming from China. Thanks to the help of Eurofins Germany colleagues, it was finally possible to gather 20 different samples:

Eurofins n°	Description	Type
0176/2911	Champignons Origin of raw material : Longhai, Zhangzhou, Fujian Province	Fresh
0176/2912	Champignons Origin of raw material : mostly sourced from Yongchun, Quanzhou, Fujian and a very small part was from Putian, Fujian	Fresh
0178/1694	Champignons WEJ n°5422620503053500 01067314 29	Fresh
0178/1695	Champignons WEJ n°5422630503143500 01067315 03	Fresh
0178/2999	Champignons 1. Wahl in scheiben Kings Crown Date de production : 03/03/05 Lot 3500/01067 Batch 40580 Source : importation Origine : Chine, province Fujian	Canned
0178/3000	Champignon 1. Wahl in scheiben King's crown Date de production : 02/03/05 Lot 3500/01067 Batch : 40586 Source : importation Origine : Chine, province Fujian	Canned
0178/3001	Champignons 1. Wahl in scheiben Happy harvest DLUO : 31/12/2008 Date de fabrication : 12/03/05 Lot 3500/01067 Batch 40590 Source : importation Origine : Chine, province Fujian	Canned
0178/3002	Champignons 1. Wahl, ganze Köpfe Kings Crown DLUO : 31/12/07 Supermarché A (Aldi) Origine : Chine	Canned
0178/3003	Champignons 1. Wahl, ganze Köpfe Spar DLUO : 31/12/07 L19399040130 3500/01046 Supermarché B (Spar) Origine : Chine	Canned
0178/3004	Champignon 1. Wahl, geschnitten DLUO : 31/12/08 Lot : L22274041205 35010/01046 Supermarché B (Spar) Origine : Chine	Canned
0181/2421	Champignons entiers I. Wahl. Origine : Chine Lot : 3400/01028 041023 DLUO : 31/12/2007 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique.	Canned
0181/2423	Champignons émincés I. Wahl. Origine : Chine Lot : L22274041206 3500/01046 DLUO : 31/12/2008 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique.	Canned
0181/2424	Champignons entiers I. Wahl. SPAR Origine : Chine Lot : 063 050409 805B 3500/01088 DLUO : 31/12/2008 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique.	Canned
0181/2425	Champignons entiers I. Wahl. SPAR Origine : Chine Lot : 063 050411 805B 3500/01088 DLUO : 31/12/2008 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique.	Canned
0181/2426	Champignons entiers King's Crown I. Wahl. Origine : Chine Lot : 01 1 031017 005 DLUO : 31/12/2007 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique.	Canned
0181/2431	Champignons entiers King's Crown I. Wahl. Origine : Chine Lot : 3700/01140 041010 805 DLUO : 31/12/2007 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique.	Canned
0183/4698	Champignons entiers King's Crown I. Wahl, ganze Köpfe Origine : Chine Lot : 041023 805 DLUO : 31/12/2008 Ingrédients : Champignons, eau, sel, acidifiant : acide citrique.	Canned
0184/1828	Champignons I. Wahl, ganze Köpfe (1er choix, entiers) Ingrédients : champignons, eau, sel, acidifiant : acide citrique. Origine : Chine Fabricant : I. Schroeder - Hamburg DLUO : 31.12.2007 Lot 3700/01140 041022 805	Canned
0184/1829	Champignons geschnitten, 1. Wahl (émincé, 1er choix) Ingrédients : champignons, eau, sel, acifiant : acide citrique, antioxydant : acide ascorbique. Origine : Chine Fournisseur : I Schroeder KG, Hamburg. DLUO : 31.12.2008 Lot : L22274041207/3500/01046	Canned
0184/1830	Champignons Ganze Köpfe, 1. Wahl (entiers, 1er choix) Ingrédients : champignons, eau, sel, acidifiant : acide citrique, antioxydant : acide ascorbique. Origine : Xiamen, Chine Pour : Agrogenra & Co. Ltd DLUO : 31.12.2007 Lot : 3400/01028 041023	Canned

3. Analytical results

3.1 Isotopic results

Eurofins n°	$\delta^{87}\text{Sr}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰) MANNITOL	$\delta^{18}\text{O}$ (‰) MANNITOL	(D/H) (ppm) MANNITOL HEXAACETATE	(D/H) (ppm) WATER*	$\delta^{18}\text{O}$ (‰) WATER*
0154/1922	-0.6	-24.4	12.8	-24.9	24.7	149.5	-	-
0154/1923	-0.4	-24.0	16.5	-25.6	23.5	143.9	149.6	-5.5
0154/1925	-0.4	-24.3	13.2	-25.5	23.7	143.7	149.5	-5.3
0154/1926	0.0	-24.3	10.7	-25.7	21.2	143.4	149.3	-5.6
0154/1928	-0.1	-24.4	10.5	-25.6	21.7	142.9	150.2	-5.4
0154/1929	0.1	-23.4	9.9	-24.8	25.1	144.8	-	-
0154/1930	-1.5	-23.8	10.1	-24.9	25.3	143.5	-	-
0154/1931	0.3	-24.2	11.2	-24.9	22.5	144.7	149.5	-5.8
0154/1932	0.3	-24.2	11.8	-25.3	22.5	143.1	149.4	-5.8
0154/1933	0.5	-24.4	11.3	-26.3	23.5	143.5	149.6	-5.8
0176/2911	0.6	-23.0	8.0	-23.2	21.7	144.0	-	-
0176/2912	0.2	-23.6	7.8	-23.9	22.7	144.2	-	-
0178/1694	-0.2	-25.5	8.3	-25.6	23.0	143.3	-	-
0178/1695	-0.1	-22.6	6.1	-23.4	23.6	146.1	-	-
0178/2999	-0.3	-24.0	8.6	-24.2	22.0	143.9	150.6	-5.1
0178/3000	-0.4	-22.7	6.8	-22.9	23.1	144.2	150.9	-4.8
0178/3001	-0.6	-23.1	4.2	-24.3	21.6	145.9	150.2	-5.5
0178/3002	4.2	-24.8	13.2	-25.5	22.0	141.9	146.4	-8.2
0178/3003	0.9	-22.8	8.4	-23.3	24.0	144.9	151.9	-4.7
0178/3004	0.0	-22.4	7.2	-22.2	23.4	143.8	151.4	-4.8
0181/2421	3.6	-23.0	13.4	-24.2	19.1	142.4	147.3	-7.7
0181/2423	0.6	-24.0	6.4	-24.1	18.7	143.9	151.0	-4.8
0181/2424	-0.2	-23.0	6.8	-23.4	21.1	145.4	150.9	-4.9
0181/2425	1.2	-24.5	10.1	-24.5	21.9	145.6	150.6	-5.4
0181/2426	4.2	-25.1	13.2	-25.8	18.3	149.4	145.7	-8.7
0181/2431	3.2	-23.4	8.7	-23.6	21.7	149.2	148.6	-9.2
0183/4698	1.3	-23.3	7.1	-22.8	22.0	150.3	149.7	-8.1
0184/1828	3.4	-23.3	5.1	-23.8	17.8	149.8	150.8	-9.2
0184/1829	0.7	-24.9	6.1	-24.7	21.9	151.1	149.6	-4.8
0184/1830	0.9	-23.0	8.6	-24.0	21.6	150.6	150.3	-7.9
0167/1757	3.1	-24.6	9.2	-25.4	22.6	144.5	148.4	-6.3
0167/1758	3.1	-24.8	7.0	-25.6	21.1	142.8	148.2	-6.5
0167/1759	3.3	-25.3	6.9	-26.0	22.0	142.5	148.8	-6.2
0167/1760	3.3	-25.0	8.3	-26.2	21.8	143.3	148.9	-6.3
0167/1761	3.2	-24.8	8.1	-26.0	21.8	143.7	148.5	-6.4
0167/1762	3.3	-25.3	6.1	-26.2	21.7	143.9	149.0	-6.4
0167/1763	3.2	-24.5	6.6	-26.0	22.0	142.6	149.2	-6.2
0167/1764	3.3	-24.6	6.1	-26.1	21.6	144.8	149.4	-6.1
0167/1765	3.2	-24.9	6.8	-25.3	21.5	143.0	148.9	-6.5
0167/1766	2.6	-24.8	8.0	-25.8	21.5	143.7	149.3	-6.3

0178/5387	4.8	-23.7	13.8	-24.2	19.5	149.4	-	-
0178/5388	1.3	-22.3	10.6	-23.7	19.9	149.4	-	-
0178/5389	0.3	-23.3	15.7	-23.6	20.6	142.9	-	-
0178/5390	0.7	-23.9	13.5	-24.0	21.5	143.5	-	-
0178/5391	0.0	-23.4	13.6	-24.1	24.4	143.8	148.6	-6.5
0178/5392	0.0	-23.3	12.9	-24.8	24.1	143.3	148.1	-6.7
0178/5393	-1.1	-23.4	14.1	-23.9	23.8	143.5	149.3	-5.8
0178/5394	-1.2	-23.4	13.8	-23.6	22.4	143.8	148.3	-6.4
0178/5395	-0.3	-23.0	13.6	-24.1	18.4	143.5	147.9	-7.2
0178/5396	-0.3	-23.1	14.6	-23.7	19.5	143.8	148.1	-7.1

*The covering water could be analysed only in canned samples.

3.2 Trace elements results

Trace elements : all results below are expressed in mg/kg (“nd” means not detected):

Eurofins n°	Li	Be	Na	K	V	Mn	Ni	Zn	Rb	Sr	Mo	Cd	Cs	Ba	Pb	P
0154/1922	nd	nd	59	2500	nd	0.49	0.02	5.2	0.47	0.34	nd	nd	nd	0.09	nd	810
0154/1923	nd	nd	2400	520	0.01	0.30	nd	5.4	0.15	0.51	nd	nd	nd	0.08	0.01	370
0154/1925	nd	nd	2650	680	nd	0.34	nd	5.4	0.20	0.42	nd	nd	nd	0.06	nd	440
0154/1926	nd	nd	2400	400	nd	0.29	nd	4.3	0.11	0.75	nd	nd	nd	0.04	0.01	410
0154/1928	nd	nd	2400	600	nd	0.25	nd	3.0	0.15	0.53	nd	nd	nd	0.24	0.01	360
0154/1929	nd	nd	110	1600	nd	0.36	nd	3.4	0.43	0.25	nd	nd	nd	0.08	nd	510
0154/1930	0.11	0.0	65	2600	0.20	0.23	0.25	8.8	1.50	3.60	0.08	nd	0.03	0.09	0.07	720
0154/1931	0.04	nd	2500	670	0.02	0.46	0.02	6.0	0.21	3.00	nd	nd	nd	0.10	0.02	400
0154/1932	0.09	nd	2800	620	nd	0.45	nd	4.6	0.18	2.90	0.04	nd	nd	0.07	0.01	390
0154/1933	0.06	nd	2500	520	nd	0.28	nd	4.1	0.14	2.60	nd	nd	nd	0.06	nd	360
0176/2911	0.98	nd	2800	720	0.02	0.36	0.02	3.3	0.16	0.94	0.02	nd	0.01	0.17	0.02	500
0176/2912	0.13	nd	2650	525	0.01	0.93	0.66	7.0	1.60	0.65	0.04	nd	nd	0.35	0.02	545
0178/1694	0.01	nd	2800	1100	0.05	0.46	0.04	3.5	1.90	1.20	nd	nd	nd	0.36	0.06	120
0178/1695	0.02	nd	2600	490	0.07	0.45	0.03	4.7	2.60	1.20	nd	nd	0.01	0.19	0.06	490
0178/2999	0.01	nd	3000	840	0.07	0.57	0.03	4.6	4.30	0.97	nd	nd	0.02	0.29	0.04	460
0178/3000	nd	nd	2000	1000	0.04	0.55	0.02	3.7	4.10	0.62	nd	nd	0.02	0.30	0.03	480
0178/3001	0.01	nd	2600	240	0.07	0.43	0.03	3.7	1.00	1.10	0.01	nd	nd	0.16	0.04	300
0178/3002	0.02	nd	3700	1600	0.05	0.73	0.04	8.7	0.72	0.48	0.03	0.03	nd	0.32	0.01	820
0178/3003	0.02	nd	2450	890	0.06	0.67	0.05	8.8	3.65	1.05	0.01	nd	0.02	0.20	0.05	595
0178/3004	0.01	nd	3200	760	0.04	0.57	0.04	6.6	2.50	1.10	nd	nd	0.01	0.10	0.04	450
0181/2421	0.02	nd	3000	730	0.03	0.68	0.02	8.0	0.50	0.62	0.06	0.03	nd	0.33	0.02	620
0181/2423	0.01	nd	2400	1200	0.01	0.64	0.02	7.3	4.30	0.82	0.03	nd	0.03	0.08	0.04	1200
0181/2424	0.03	nd	3550	21	0.03	0.15	0.02	1.3	0.03	2.10	0.04	nd	nd	0.16	0.07	375
0181/2425	0.03	nd	3400	21	0.06	0.59	0.03	5.5	0.05	2.10	0.02	nd	nd	0.38	0.10	350
0181/2426	0.03	nd	4200	4200	0.03	0.65	0.02	9.7	0.43	0.89	0.05	0.05	nd	0.35	nd	740
0181/2431	0.03	nd	3800	990	0.03	0.34	0.01	4.3	0.56	0.37	0.05	nd	nd	0.16	nd	550
0183/4698	0.05	nd	2400	815	0.02	0.44	0.07	4.5	0.54	2.40	nd	0.02	nd	0.16	0.01	530
0184/1828	0.04	nd	3350	525	nd	0.50	0.09	6.5	1.75	1.10	nd	0.10	nd	0.24	0.02	390

0184/1829	0.01	nd	2150	930	0.01	0.60	0.20	7.2	1.73	1.12	0.02	0.05	0.01	0.22	0.03	500
0184/1830	0.02	nd	3300	570	0.03	0.74	nd	6.3	2.40	1.40	0.06	nd	0.01	0.17	0.02	680
0167/1757	0.02	nd	2600	960	0.02	0.36	0.04	5.8	0.30	0.95	nd	nd	nd	0.07	nd	545
0167/1758	0.02	nd	2400	640	0.01	0.19	0.02	1.9	0.19	0.80	0.02	nd	nd	0.05	nd	460
0167/1759	0.02	nd	1900	840	0.01	0.27	0.02	3.2	0.25	0.78	0.02	0.01	nd	0.06	nd	460
0167/1760	0.02	nd	2600	1100	0.01	0.28	0.02	4.0	0.25	0.85	0.02	nd	nd	0.07	nd	620
0167/1761	0.02	nd	3200	1100	0.01	0.28	0.03	3.7	0.30	0.96	nd	nd	nd	0.06	nd	620
0167/1762	0.02	nd	2500	770	nd	0.24	0.04	4.2	0.29	0.83	nd	nd	nd	0.07	nd	490
0167/1763	0.02	nd	1900	850	nd	0.24	nd	3.2	0.25	0.71	nd	nd	nd	0.05	nd	430
0167/1764	0.02	nd	2000	770	0.01	0.29	0.02	3.7	0.38	0.83	nd	nd	nd	0.06	nd	480
0167/1765	0.02	nd	1800	740	0.01	0.21	nd	2.7	0.22	0.74	nd	nd	nd	0.05	nd	400
0167/1766	0.02	nd	2200	950	0.01	0.31	0.02	5.3	0.37	0.76	nd	nd	nd	0.05	nd	580
0178/5387	0.03	nd	88	3800	0.06	0.94	0.02	5.6	1.50	0.56	0.03	nd	0.01	0.16	0.02	990
0178/5388	4.60	0.01	75	2850	0.69	0.94	0.58	5.8	1.35	3.50	0.04	nd	0.08	0.09	0.18	760
0178/5389	0.02	nd	50	2700	nd	0.61	nd	6.9	0.80	0.16	0.01	nd	nd	0.02	0.02	740
0178/5390	0.02	nd	34	3700	nd	0.85	nd	7.2	0.66	0.12	0.02	nd	nd	0.02	nd	150
0178/5391	0.06	nd	3100	1000	0.02	0.33	0.02	5.8	0.32	3.30	0.02	nd	nd	0.06	0.02	460
0178/5392	0.06	nd	3450	795	0.02	0.27	nd	4.0	0.20	3.30	0.01	nd	nd	nd	nd	495
0178/5393	0.02	nd	2200	600	0.02	0.19	0.02	2.0	0.18	1.10	nd	nd	nd	0.11	0.01	350
0178/5394	0.01	nd	3400	740	0.01	0.26	0.03	2.9	0.24	2.00	0.05	nd	nd	0.14	0.02	410
0178/5395	0.02	nd	2400	850	nd	0.25	nd	3.5	0.22	1.80	0.02	nd	nd	0.07	nd	480
0178/5396	0.04	nd	2800	740	0.02	0.28	0.01	4.8	2.10	0.22	0.01	nd	nd	0.08	0.01	520

3.3 Mannitol

all results below are expressed in mg/kg of drained mushroom

Eurofins n°	Mannitol
0154/1922	15290
0154/1923	12642
0154/1925	12734
0154/1926	12659
0154/1928	6343
0154/1929	9259
0154/1930	7014
0154/1931	5894
0154/1932	12289
0154/1933	10485
0176/2911	5088
0176/2912	8373
0178/1694	4408
0178/1695	2685
0178/2999	5669
0178/3000	6471
0178/3001	1290
0178/3002	7584
0178/3003	5295
0178/3004	7061
0181/2421	7237
0181/2423	13195
0181/2424	1739
0181/2425	1565
0181/2426	6821
0181/2431	12497
0183/4698	8687
0184/1828	6820
0184/1829	7020
0184/1830	7772
0167/1757	6253
0167/1758	6881
0167/1759	12168
0167/1760	8737
0167/1761	8145
0167/1762	6437
0167/1763	9964
0167/1764	9417
0167/1765	11817
0167/1766	8310
0178/5387	17080
0178/5388	18545

0178/5389	7075
0178/5390	14688
0178/5391	7283
0178/5392	8353
0178/5393	7563
0178/5394	6572
0178/5395	7934
0178/5396	7153

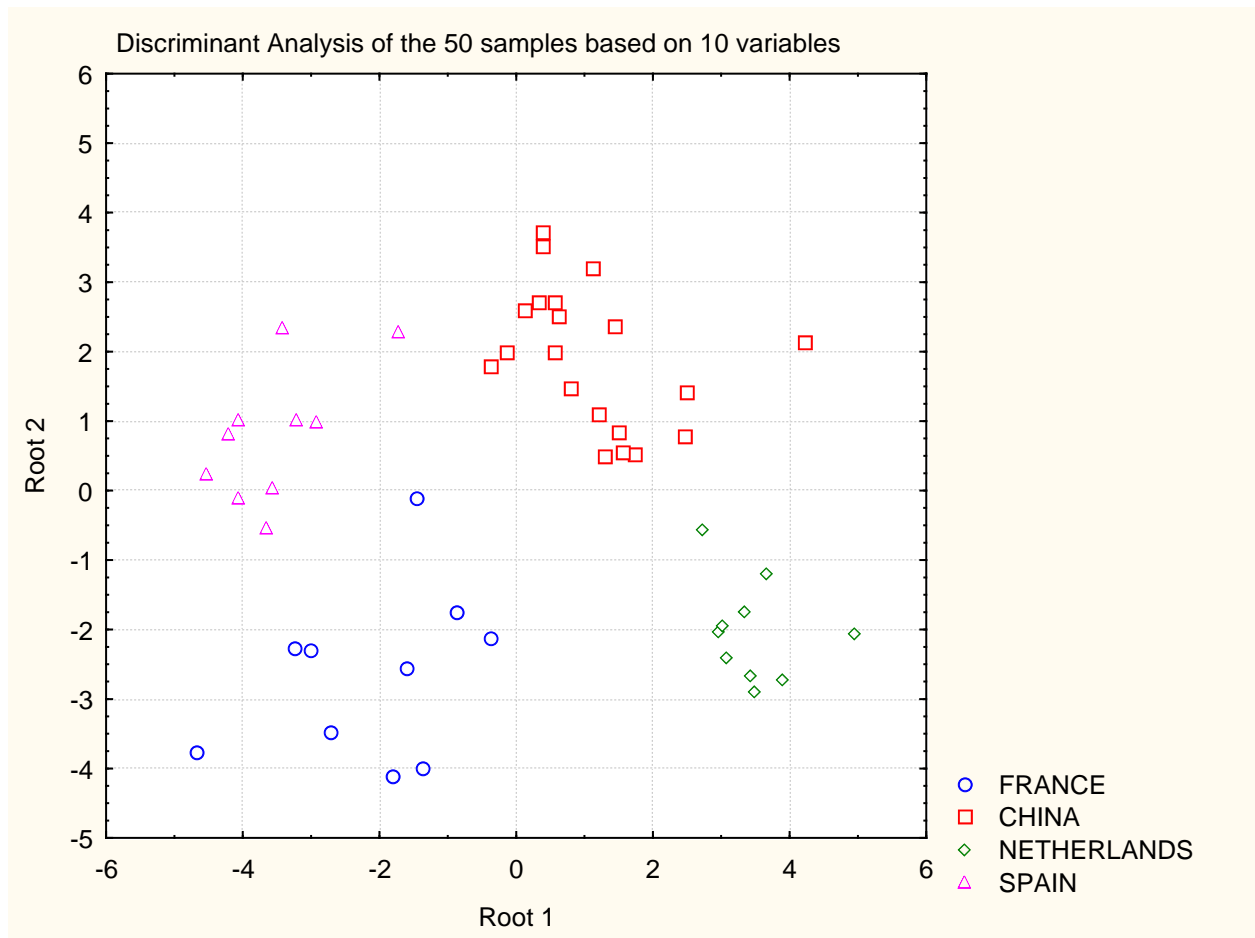
4. Statistical exploitation

A preliminary observation of the results shows some parameters which were found at concentrations below or only slightly above the quantification limit. This concerns sugars and polyols (except mannitol) and some trace metals. This information can still be useful to characterise the samples of phase II against future samples, but it cannot be used to discriminate between the four countries, so they have not been included in the statistical exploitation. Similarly the sodium levels are obviously influenced by the salt used in canned products, so this parameter has also been discarded.

Then, a univariate analysis of each of the remaining analytical parameter was performed through variance analysis (see annex 1) and “box and whiskers” graphical representations (see annex 2). Variance analysis allows to test whether there are significant differences between countries (when $F_{\text{calculated}}$ is higher than F_{critical}), or not. The “box and whiskers” graphs allow to visualise the distribution of a given parameter between countries and therefore to see where the differences are. After this step 10 analytical variables were retained:

- Isotope ratios of carbon (on whole mushroom and mannitol), nitrogen (on whole mushroom), and strontium (on whole mushroom); although its $F_{\text{calculated}}$ is slightly higher than F_{critical} , the oxygen isotope ratio of mannitol was eliminated because of a lack of analytical robustness
- Barium, Rubidium, Manganese, Zinc and Strontium levels (Zn and Sr were not found as significant according to variance analysis but were kept because they might be interesting to differentiate other origins in future studies)
- Mannitol content

These variables were combined to try and find discriminant functions by Principal Component Analysis and Discriminant Analysis. A reasonable discrimination was obtained when including all samples:



This model assigns 100% of the samples correctly.

Interestingly this includes the fresh samples as well, which allows future comparisons between fresh and canned samples. Actually the results variability was found to be similar based on canned mushrooms only.

5. Conclusions regarding phase II

These results confirm the possibility to obtain a powerful origin recognition of the country of origin by using a combination of isotopic and compositional analyses. A selection of 10 significant analytical variables was made, out of the 31 parameters examined during phase II.

Surprisingly the oxygen and deuterium isotope ratios of the organic matter did not contribute significantly to the discrimination, although they have been useful in other studies. The reason is probably the technical difficulty of measuring non-exchangeable atoms in mannitol, which requires many preparation steps and probably leads to an increased analytical uncertainty. On the other hand the carbon and nitrogen isotope ratios were confirmed to be the most discriminant parameters (probably because they are strongly linked to the source of compost used to grow the mushrooms). Although no significant difference was observed between water isotope ratios within this limited sampling, it is foreseen that these parameters can provide additional and useful information about the canning place (when more factories are included in the future).

Several trace metals also show a significant discrimination power, especially barium, Rubidium and Manganese.

Finally, as already observed in the first pilot study, the mannitol content is confirmed as an interesting information as well, Chinese samples especially being characterised by a lower level. In a few cases, low levels of sorbitol and inositol were also observed, but this is not country-dependant. The same is true for sugars analysis. Xylitol and maltitol were not found in any of the 50 samples.

In conclusion, the remaining 10 parameters, although none of them is sufficient to differentiate all four origins, are very efficient when combined together. Therefore the combination of different techniques is still required, but the number of determinations for each technique can be reduced.

6. Proposal for phase III

The above results confirm the possibility to characterise the geographic origin of mushrooms by combining complementary analytical techniques. The next step is to establish a database which will take into account all the possible variability of the production within one country (to be defined by participants) and which can also include new origins. A reduction of the number of parameters will allow to reduce costs and turn-around time. Our recommendation is to focus on industrial samples of canned mushrooms (with as few additives and other ingredients as possible). As mentioned in the initial proposal, a reasonable goal would be to reach a total of 50 reference samples by country. Then it will be possible to check the declared origin of unknown samples.

The results of phase II leads use to propose the following list of recommended parameters for phase III (consolidation of an enlarged database):

- Isotope ratios of carbon, nitrogen, and strontium in the bulk matter
- Isotope ratios of carbon in extracted mannitol
- Isotope ratios of oxygen and hydrogen in the covering water*
- Trace elements by ICP-MS (limited screening of 5 elements: Barium , Rubidium, Manganese, Zinc* and Strontium*)
- Sugar alcohols (for mannitol content) by ionic chromatography

** parameters marked with an * did not provide additional discrimination during phase II but from our experience they could help to characterise other origins. Especially the isotope ratios of the canning water can provide information about some specific canning places.*

The price of the above testing for phase III (based on current Eurofins price list) is 1296 € by sample. Assuming that a minimum number of 100 samples will be analysed in phase III, a discount of 25% can be offered to the members of the GEPC consortium who have already contributed to phase II, which would lead to a cost by sample of 972 €.

Annex 1: Variance analysis

	Number of samples	$\delta^{87}\text{Sr}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{18}\text{O}$ (‰)	(D/H) (ppm)	(D/H) (ppm)	$\delta^{18}\text{O}$ (‰)	Mannitol
					MANNITOL	MANNITOL	MANNITOL HEXAACETATE	WATER ^a	WATER ^a	mg/kg
France (mean)	10	-0.16	-24.1	11.8	-25.4	23.4	144.3	149.6	-5.6	10461
CHINA (mean)	20	1.16	-23.6	8.2	-24.0	21.6	146.0	149.7	-6.5	6364
NETHERLANDS (mean)	10	3.16	-24.9	7.3	-25.8	21.8	143.5	148.8	-6.3	8813
SPAIN (mean)	10	0.41	-23.3	13.6	-24.0	21.4	144.7	148.4	-6.6	10225
Fcalculated		12.2	12.3	24.7	24.6	3.4	2.8	2.4	1.0	4.8
Fcritical (95% confidence level)		2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Conclusion		significant	significant	significant	significant	significant				significant
Discrimination potential		++	++	+++	+++	+	-	-	-	+

	Number of samples	Li	Be	Na	K	V	Mn	Ni	Zn	Rb	Sr	Mo	Cd	Cs	Ba	Pb	P
		mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
France (mean)	10	0.03	^b	^b	1071	0.02	0.35	0.03	5.02	0.35	1.49	0.012	^b	^b	0.09	0.013	477
CHINA (mean)	20	0.07	^b	^b	916	0.04	0.55	0.06	5.63	1.74	1.11	0.022	^b	^b	0.23	0.031	541
NETHERLANDS (mean)	10	0.02	^b	^b	1377	0.08	0.27	0.07	3.77	0.28	0.82	0.011	^b	^b	0.06	0.020	583
SPAIN (mean)	10	0.49	^b	^b	1113	0.01	0.49	0.01	4.85	0.76	1.61	0.014	^b	^b	0.08	0.008	361
Fcalculated		1.2	^b	^b	0.5	1.0	6.7	0.6	2.4	7.0	1.6	0.9	^b	^b	21.8	1.5	2.4
Fcritical (95% confidence level)		2.8	^b	^b	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	^b	^b	2.8	2.8	2.8
Conclusion			^b	^b			significant			significant			^b	^b	significant		
Discrimination potential		-	^b	^b	-	-	+	-	-	+	-	-	^b	^b	+++	-	-

^a For these parameters the number of results are 7, 16, 10 and 6 (see table in 3.1)

^b For these parameters the variance analysis is not applicable (too few detected results, or influence of salt in the case of Na)

Annex 2: Box & Whiskers plots of individual parameters

